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## YOLK–NUCLEUS AND POLAR RINGS.

KATHARINE FOOT, EVANSTON, ILL.

POLAR rings have been observed in *Clepsine* by Grube, Leuckart, Robin, and Whitman,[1] in *Rhynchelmis,* by Vejdovsky,[2] and in *Allolobophora foetida*[3] by the author.

In the present paper I hope to prove that the polar rings and the so-called yolk-nucleus are one and the same substance, and that therefore the material which forms these structures is by no means confined to the three forms just mentioned. In the fall of 1894, I identified the granular masses of cytoplasm found in the ovarian egg with the polar rings of later stages, and traced the substance step by step during the growth of the maturing and fertilized egg; but the publication of this work was reserved to form part of a later paper on the maturation and fertilization of the egg of *Allolobophora foetida.*

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[1] C. O. Whitman, "The Embryology of Clepsine." *Quart. Journ. of Micr. Sci.*, vol. XVIII, 1878, p. 234.

[2] F. Vejdovsky, "Entwicklungsgeschichtliche Untersuchungen." Prag, 1892.

[3] "Preliminary Note on the Maturation and Fertilization of the Egg of *Allolobophora foetida.* JOURNAL OF MORPHOLOGY, vol. IX, 1894.

In the following winter, the ovarian egg of *Lumbricus* was studied by Mr. Calkins [1] in the laboratory of Professor E. B. Wilson of Columbia College. His results differ so radically from those obtained by the study of *Allolobophora foetida* that I think it best to give a brief account of my results. Calkins's results are, briefly, as follows: "The yolk-nucleus is chromatin in the form of granules." "This granular mass disintegrates and the parts form the yolk plates of the egg."

In *Allolobophora foetida* the "yolk-nucleus" can be sharply differentiated from the chromatin, and in normal eggs I find no structures answering to the "great yolk plates" described and figured by Calkins for *Lumbricus*. In a few cases, how-



FIG. 1. — Section of a degenerating egg from ovary of All. foe. Abbe camera.
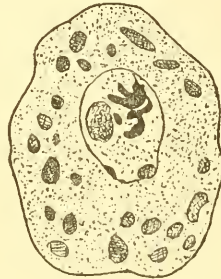
FIG. 2. — Calkins's Fig. 5 reduced one-half.

ever, in ovaries from worms past the breeding season (clitellar region no longer marked), I find, among eggs in various stages of degeneration, some that exactly correspond to Calkins's figure 5 ; the chromatin has for the most part lost its granular structure, forming one or more solid homogeneous masses among the granules, and in the cytoplasm there are large homogeneous masses, which appear to be identical with Calkins's "great yolk plates."

Ovaries from worms without a defined clitellum show various stages of degeneration. In some cases the eggs of one ovary will be entirely degenerated, while those of the other will appear normal ; again, only the older eggs of one or both ovaries will show degeneration ; while in some cases both

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[1] Gary N. Calkins, "Observations on the Yolk-nucleus in the Egg of *Lumbricus*." *Transactions New York Acad. of Sci.*, June, 1895.

ovaries will appear entirely normal.    I am inclined to believe that the condition of the ovary in this respect depends upon the length of time that has elapsed since the ceasing of its functional activity.

## METHOD.

The origin and formation of the polar rings were first traced by various methods, none of which, however, differentiated the substance from all other constituents of the cell.    The fixatives used were chromo-acetic, corrosive sublimate, corrosive acetic, Hermann's fluid, Hermann's fluid followed by Merkel, Merkel's fluid, osmic acid of various strengths, picro-acetic, Parenyi's, Flemming's, etc.    The stains used were various haematoxylins, various carmines, and numerous anilins.    While I was engaged in a systematic effort to find a stain that would differentiate the polar-ring substance from other constituents of the cell, the publication of Calkins's results from his study of *Lumbricus* made it still more important for me to substantiate my results by differential staining.    The use of lithium carmine and Lyon's blue was suggested to me by seeing some beautiful specimens of nerve tissue, prepared (under the direction of Dr. Patten) by Miss Lewis of Harvard University; and I am indebted to Dr. Patten for subsequent advice as to the use of the stains.    Shortly afterwards I found that Korschelt[1] in 1889, had used practically the same method (borax carmine and Lyon's blue) to differentiate the yolk-nucleus (*Dotterkern*) of various insect eggs; and in spite of his beautiful results (clearly differentiating *Dotterkern* from chromatin), I find very few investigators who have repeated his method.[2]

[1] Korschelt, "Beiträge zur Morphologie und Physiologie des Zellkernes. *Zool. Jahrb.*, Bd. IV, Hft. 1, 1889.

[2] Calkins seems to have tried Korschelt's method, and he says of it: "After borax carmine and Lyon's blue the yolk-nucleus and chromatin had the bright red stain of the carmine." In *Allolobophora foetida* the only eggs that do not give a constant reaction by this method are eggs in some stages of degeneration ; for example, the "great yolk plates" (?) of Cut 1 deeply stain with carmine, and persistently retain the red after treatment with Lyon's blue long enough to stain the degenerating cytoplasm deeply.

The method that has proved most satisfactory for eggs of *Allolobophora foetida* is to stain the sections from one to twenty-four hours in lithium carmine, wash in acidulated alcohol for a few seconds, and double stain with a very dilute solution of Lyon's blue. The length of time required for staining depends upon the fixative used. The process must be carefully watched under the microscope; for the lithium carmine in both the spindle fibres and cytoplasmic network may be replaced by the Lyon's blue.[1] If the staining be properly modified to suit the special fixative, all the fixatives tested give results more or less satisfactory; but corrosive sublimate, with or without acetic acid, gives the most brilliant and satisfactory reaction. A more detailed description of the effect of the various fixatives upon the polar ring substance will be given later.

Before describing the figures, I would emphasize the fact that each is an exact representation of the preparation both in form and color, most of the work having been done by an expert draftsman and colorist (Mr. H. Bridgham); not even in shade or tint is there any variation from the original. None of the figures are in the least diagrammatic; they were drawn from sections, the camera lucida being used in all cases. Each egg (taken from a cocoon) was studied under a Zeiss 2mm. immersion lens, and drawn with the aid of an Abbe camera, before it was imbedded for sectioning.[2] The variations in size of eggs of nearly the same stages of development are largely due to unequal shrinkage, dependent upon the fixative used.

Fig. 1 represents a very young oöcyte. In the cytoplasm, in close contact with the nucleus and sharply differentiated from the chromatin, nucleolus, and cytoplasm, is a blue, granular mass, — the so-called yolk-nucleus. This substance

[1] If the oögonial areas are overstained with Lyon's blue, the nuclei appear imbedded in a matrix of blue; the cell boundaries, red cytoplasm, and capillaries being obliterated. If preparations fixed by Hermann's fluid are overstained, the nucleoli as well as the yolk-nucleus will stain blue.

[2] These facts are emphasized in order to prevent a mistake similar to the one made regarding the figures of my preliminary note. The *American Naturalist*, vol. XXIX, January, 1895, speaks of my " diagrammatic figures."

greatly increases as the egg grows, and can be traced (by the sharp contrast of color) step by step during the development, fertilization, and cleavage. It is present at the chief points of activity in the cell: it is in the spindle, it forms the fertilization cone and the archoplasm of both sperm and egg attraction-spheres; and at the pro-nuclear stage a part of it is aggregated at both poles of the egg, forming the structures known as the polar rings. For this blue substance I shall retain the term employed by Boveri in a far more limited sense — the term *archoplasm.*

In no case where the method has been properly applied have I found a cell in the resting stage, — that is, a cell containing nucleus, nucleolus, and cytoplasm, — without being able to recognize at least a trace of the archoplasm, the cell often being so small as to make it impossible to decide whether it belongs to the earlier or to the final generation of oögonia. As growing oöcytes are found close to the stem of the ovary, surrounded by dividing oögonia, the locality of the cell is not a trustworthy indication of the stage to which it belongs.[1]

In Fig. 2 we have an older oöcyte, with the archoplasm increased in proportion to the rest of the cell.

[1] In the spermatogonia of *Salamander mac.,* Meves finds masses of granular substance, which he identifies with the "yolk-nucleus" of other authors and with the attraction-sphere. F. Meves, "Ueber eine Metamorphose der Attractionssphäre in den Spermatogonien von *Salamandra maculosa,*" pp. 143–4. *Arch. f. mik. Anat.,* Bd. 44, Hft. 1, 1894. Through the courtesy of Dr. Watasé, I have been able to apply Korschelt's method to the sperm cells of *Siren,* and I find that these contain a granular substance similar to the archoplasm of *Allolobophora foetida.* Many of them resemble very closely my Fig. 1 (a young oöcyte). These results recall Nussbaum's identification of the *Nebenkern* of a variety of gland cells with the *Dotterkern* of eggs and the *Nebenkern* of the spermatocytes: "Dagegen wird man den Nebenkern der Drüsenzellen wohl mit dem von Wittich entdeckten Dotterkern der Eier, dem durch von la Valette St. George zuerst bekannt gewordenen Nebenkern der Spermatocyten, den von Leydig aus der Epidermis von Pelobates-Larven beschriebenen Bildungen in eine Categorie bringen dürfen." Moritz Nussbaum, "Ueber den Bau und die Thätigkeit der Drüsen." *Arch. f. mik. Anat.,* Bd. 21, 1882, pp. 343–4. Later, Balbiani says: "Le noyau vitellin (Dotterkern) des Araneides est l'homologue du Nebenkern (centrosome de Platner) des cellules seminales et du centrosome des cellules somatiques." E. G. Balbiani, "Centrosome et Dotterkern." *Journ. de l'Anat. et de la Physiol.,* tome XXIX, 1893.

Fig. 3 represents a still larger oöcyte, with a corresponding growth of the archoplasm.

Figs. 4 and 5 represent two types of the distribution of the archoplasm found in the older oöcytes. In some cases the greater part of the substance is aggregated at the periphery of the egg; in others the distribution is more nearly equal throughout the cytoplasm.[1]

Fig. 6 shows a new phase of the archoplasm.[2] Here we not only find it distributed through the cytoplasm, but at points where the membrane of the germinal vesicle has disappeared we see it entering into the germinal vesicle itself.

The presence of the archoplasm in the germinal vesicle reminds one of Korschelt's[3] and Jordan's[4] observations on the entrance of the "yolk-nucleus" into the germinal vesicle, and Lavdowsky's[5] claim to have seen yolk elements within the nucleus. It also recalls the observations of some of the investigators who claim to have seen not only the nucleoli, but also chromatin, *leaving* the germinal vesicle; for this substance in earlier stages has been positively asserted to be chromatin by several authors. In Fig. 6 an attempt has been made to represent the less transparent appearance of the ovarian eggs at the free end of the ovary. A comparison of this with the foregoing figures will show what I have attempted to indicate. With very few exceptions (dependent upon the fixative) this same lack of transparency is shown in all later stages ; *i.e.* in eggs taken from the cocoon.

[1] Fig. 4 recalls the *Diffuse Dotterkern* of Stuhlmann: "Die Reifung des Arthropodeneies nach Beobachtungen an Insekten, Spinnen, Myriopoden und Peripatus." Ber. d. Naturf. Ges. z. Freiburg i. Br., Bd. 1, 1886. *Cf.* His Fig. 137, Taf. VIII.

[2] The lithographer has not sufficiently blended the three masses of archoplasm at the upper left side of the figure. In the original sketch, the masses are more granular in appearance and are connected by less dense areas of the same substance.

[3] Eugen Korschelt, "Beiträge zur Morphologie und Physiologie des Zellkernes." *Zool. Jahrb.*, Bd. IV, Hft. 1, 1889.

[4] Edwin O. Jordan, "The Habits and Development of the Newt." Journal of Morphology, vol. VIII, 1893.

[5] M. Lavdowsky, "Von der Entstehung der chromatischen und achromatischen Substanzen in den tierischen und pflanzlichen Zellen." Anatomische Hefte: Merkel und Bonnet's Anat. und Entwicklungsg., Bd. IV. Hft. 13, 1894.

The study of eggs killed in any fixative containing osmic acid inclines me to believe that this lack of transparency is due, at least in part, to material absorbed from the body-cavity of the worm, and later from the albuminous contents of the cocoon ; for all these eggs contain in greater or less degree the black spots (*Deutoplasm*) represented in Fig. 14, which can be dissolved out with warm xylol or ether, their fatty nature thus being proved.  These, however, are not the only factors ; for the degree of transparency shown by the young ovarian egg is not restored by dissolving away the *Deutoplasm*.

The distribution of the archoplasm in all these eggs is modified by the fixative ; corrosive sublimate or corrosive acetic are especially favorable for its study, as they cause it to aggregate into masses, thus producing the sharpest contrast between the red and the blue.   In chromo-acetic preparations, on the contrary, the distribution of the archoplasm is much more nearly equal throughout the cytoplasm ; but *with all fixatives it is aggregated at the centres of activity of the cell.*

I am inclined to think chromo-acetic the most reliable fixative, for the following reasons :

First, it so fixes the eggs that the subsequent treatment with alcohols produces scarcely perceptible shrinkage ; as a rule, eggs measured before killing and after mounting give almost the same diameter.

Second, after chromo-acetic all the structures of the cell may be constantly and sharply defined : — the cytoplasmic network ; the attraction-sphere, with its centrosome, archoplasm, and rays ; the spindle fibres ; the chromosomes ; the fertilization cone ;  the sperm, and the sperm granules. Structures that are distorted or obliterated by many other fixatives may be constantly and distinctly defined with chromo-acetic.

I am inclined to believe that the apparently granular structure of the archoplasm is largely, if not wholly, due to the fixatives ; certainly the degree of granulation varies with the different fixatives, and at the points of greatest activity of the cell the fusing of the red and blue substances into a structureless homogeneous mass suggests that (at least in some stages) both

substances may be fluid. (The polar rings in the egg of
*Clepsine* consist, according to Whitman, of "a transparent
fluid substance.") The granular appearance of the archoplasm
is most marked at the points of greatest aggregation; that
part aggregated at the poles as polar rings appearing more or
less granular with all fixatives.

Fig. 7 shows a typical example of the presence of the archo-
plasm within the spindle and at its poles[1].

In some cases (possibly caused by the action of the fixative)
the archoplasm is not equally distributed in the spindle, but is
aggregated at its edges and around the chromosomes, leaving
areas entirely free from the substance; again, it is aggregated
in longitudinal lines, which appear as heavy blue fibres among
the red anastomosing spindle fibres. At the poles of the spindle,
we see the red cytoplasmic rays of the attraction-spheres and a
pronounced aggregation of the archoplasm. This egg (Fig. 7)
was killed in Merkel's fluid, which thus far has proved relatively
unfavorable for chromosomes and centrosomes.[2]

Fig. 8 shows a cross-section through the fertilization cone
(the structure which appears to be identical with the "cone of
attraction" of other animal forms).[3] The archoplasm is aggre-
gated around the sperm, and the part in contact with the
sperm appears to be blended with the red cytoplasm, forming
a homogeneous mass, suggesting a fluid condition of both
substances. This figure gives us a typical illustration of the
action of corrosive acetic upon the archoplasm, causing it to
form into masses, as mentioned above.

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[1] This phase of the archoplasm recalls Strasburger's "Kinoplasm"; Flem-
ming's "Zelle." Merkel und Bonnet's Ergebn. der Anat. u. Entwickg., Bd.
III, 1893, pp. 66, 104.

[2] The lithographer has not indicated the faint chromosomes shown in the
original sketch. For the shape of the chromosomes of this spindle see my "Pre-
liminary Note on the Maturation and Fertilization of the Egg of *Allolobophora
foetida.*" Journal of Morphology, vol. IX, p. 94.

[3] For further details as to the cone and the fertilization of this egg, see my
"Preliminary Note on the Maturation and Fertilization of the Egg of *Allolobophora
foetida,*" Journal of Morphology, vol. IX, 1894. The "sperm granules" give
a staining reaction similar to the nucleoli; with aurantia and iron haematoxylin,
the nucleoli of the pronuclei, the nucleoli outside the first maturation spindle, and
the sperm granules, all stain yellow, while the chromosomes and sperm stain blue.

Fig. 9 represents a longitudinal section through one of the fertilization cones, that part of the sperm which is still within the cone being indicated. In this figure we again see the tendency of the archoplasm to accumulate at the centres of activity; it is collected around the entering sperm. *The extent of the cone is dependent upon the length of sperm within the egg*, and the aggregation of the archoplasm (especially after fixatives that destroy radial structure) seems to be the *sole factor* that gives form to the cone. In applying the same method of staining to the spermatozoön at various stages of its development, I have not been able to obtain any reaction indicating the presence of archoplasm; hence it appears that this relatively large mass of archoplasm of the cone has not been brought into the egg by the sperm. The question is here suggested : Is not the archoplasm of the cone identical with the sperm archoplasm of other eggs? Boveri[1] says: "Auf diesem Stadium nun finden wir das Archoplasm als einen dichten kugeligen Hof um das im Centrum des Eies gelegene Spermatozoon (Fig. 10 und 11, Taf. I ; Fig 26, Taf. II). Es stellt sich an den beweisenden Präparaten als eine beträchtliche Ansammlung einer gleichmässig körnigen Substanze dar, die nach aussen ziemlich scharf abgegrenzt ist, während die übrige Zellsubstanz vollkommen homogen erscheint" (p. 65).

Fig. 10. As soon as the first polar body is constricted off we no longer find a fertilization cone ; but we find a sperm attraction-sphere with *blue archoplasm* at the point previously occupied by the blue archoplasm at the apex of the cone. At this stage (Fig. 10) the sperm head is contracted into a relatively short, thick rod, with its attraction-sphere occupying a position nearer the attraction-sphere of the lower pole of the spindle than does the rod itself. The structure of the sperm attraction-sphere is identical with that of the egg attraction-sphere described above (Fig. 7). This figure (Fig. 10) suggests that the archoplasm of both sperm and egg attraction-spheres is furnished by the egg alone. In the case of the sperm attraction-sphere we have evidence only that the middle-piece

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[1] Th. Boveri, "Zellen-Studien." Hft. 2, Jena, 1888.

of the sperm produces the centre of activity, around which the archoplasm (*already present in the egg*) aggregates.[1] *In the egg of Allolobophora foetida this blue archoplasmic mass is so pronounced that there can be no question as to its individuality ; it is as distinct as the cytoplasmic network itself, thus supporting Boveri's assertion of the specific character of archoplasm.*

In this figure (Fig. 10), drawn from preparations fixed in chromo-acetic, we find not only the centrosome but the red anastomosing rays of the attraction-spheres sharply defined.

At the stage represented in Fig. 11 most of the archoplasm (especially in chromo-acetic preparations) is aggregated at the periphery of the egg, only a relatively small amount being present around the very small male and female pronuclei ; but in corrosive acetic preparations the archoplasm is not limited so nearly to the periphery, some masses being present throughout the cytoplasm of the egg. This presence of the polar-ring substance on the periphery of the egg, prior to its aggregation at the poles, supports Vejdovsky's[2] observations on *Rhynchelmis.*

Fig. 12 represents the stage at which both pronuclei are formed, a large part of the archoplasm having aggregated at the two poles of the egg, thus forming the polar rings. In addition to the archoplasm at the poles, a relatively large amount has aggregated around the pronuclei (one of which is shown in the figure); and at the points where the membrane is breaking down the archoplasm is entering into the pronucleus itself. The archoplasm massed around the pronuclei has a different

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[1] In the relatively large spermatozoa of *Amphiuma* (which the kindness of Professor Conklin has enabled me to study), I have obtained a reaction to Lyon's blue both in the middle-piece and in the tail. The red cytoplasm and the blue archoplasm are present in both structures, suggesting that the apparent absence of the archoplasm in the spermatozoön of *Allolobophora foetida* may be due to faulty technique. I think, however, there can be no question that the relatively large amount of archoplasm in the cone and attraction-spheres of the egg of *Allolobophora foetida* is merely an aggregation of the archoplasm *already present in the egg.* This conclusion is in accord with the observations of Wheeler on the egg of *Myzostoma,* in which he finds the archoplasm of the cleavage attraction-spheres furnished by the egg alone. Wm. M. Wheeler, "The Behavior of the Centrosome in the Fertilized Egg of *Myzostoma glabrum Leuckart.*" JOURNAL OF MORPHOLOGY, vol. X, 1895.

[2] F. Vejdovsky, "Entwicklungsgeschichtliche Untersuchungen." Tafs. IV und V, Prag, 1892.

shade of blue from that at the poles. It shows (though in a less degree) the blending of the red and blue described under Fig. 8.

In a somewhat later stage (Fig. 13) the membrane of the pronuclei is entirely gone, the chromatin is in the form of loops, and the archoplasm is present around the loops and in the cytoplasm.

Fig. 14 represents one of the polar rings before staining with Lyon's blue. The egg was killed in Hermann's fluid, and the section was stained for twenty-four hours in lithium carmine. We see here how (in Hermann's preparations) lithium carmine stains the cytoplasm, and does not stain the polar ring substance. This section also represents the black spots referred to above (*Deutoplasm*), which subsequently were completely dissolved away by soaking the sections for twenty-four hours in warm xylol.

In studying the literature on the so-called yolk-nucleus I have found that in many cases the substance follows more or less closely a course of development similar to that of the polar ring substance of this egg. It appears first close to the germinal vesicle, increases in amount as the egg grows, and in some forms becomes distributed in granular masses near the periphery of the egg, while in others it appears as numerous clumps or granules throughout the cytoplasm. In many cases the figures show aggregations of the substance which strongly suggest polar rings. I shall, however, quote only those cases where the author specifically states that the substance is finally aggregated at one or both poles of the egg.[1]

Stuhlmann's[2] results from investigations of a variety of insects furnish the strongest evidence that the *Dotterkern* of these animals and the polar rings of *Allolobophora foetida* are

[1] For the latest historical sketches of the "yolk-nucleus," and for the literature on the subject, see L. F. Henneguy, "Le corps vitellin de Balbiani dans l'oeuf des vertébrès," *Journ. de l'anat. et de la physiol.*, ann. 29, 93 ; and H. Mertens, "Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux," *Archives de Biologie*, tome XIII, 1893.

[2] Franz Stuhlmann, "Die Reifung des Arthropodeneies nach Beobachtungen an Insekten, Spinnen, Myriopoden und Peripatus." *Ber. d. naturf. Ges. z. Freiburg. i. Br.*, Bd. I 1886.

identical structures.    Stuhlmann distinguishes two kinds of *Dotterkern: i.e. " Diffuse Dotterkerne,"* that which is distributed throughout the cytoplasm (*cf.* his Fig. 137 with my Fig. 4), and *" Eigentlicher Dotterkern,"* that which is *aggregated at one of the poles.*    For examples of polar *Dotterkern* see Figs. 164, 165, Taf. IX, and examples on Taf. X.

In a recent paper by J. W. Hubbard[1] on the "yolk-nucleus" in *Cymatogaster,* he clearly *traces the "yolk-nucleus" from the germinal vesicle to one pole of the egg ;* and his figures show an aggregation of these granules fully as pronounced as either of the polar rings of *Allolobophora foetida.*

Leydig's[2] description of the granules (in the egg of *Argulus*), which *aggregate at both poles of the egg,* is strikingly suggestive of the polar rings.    " In der Substanz des Spongioplasmas erscheinen jetzt auch wirkliche Granula oder dunkelrandige Körnchen, *welche sich zu zwei Haufen an beiden Polen des Eies ansammeln"* (p. 300).

I am greatly indebted to Professor Wheeler for generously allowing me to study his *Myzostoma* preparations, and providing me with adult *Myzostoma* to prepare with Korschelt's method.    The study of these preparations has shown that in various tissue cells the cytoplasm can be differentiated into two distinct substances, one reacting to lithium carmine and the other to Lyon's blue.

It gives me pleasure to express my great obligation to Dr. Whitman for most kind and thorough criticism of my work.

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[1] J. W. Hubbard, "The Yolk-nucleus in Cymatogaster aggregatus Gibbons." *Proceed. of the Amer. Philos. Society,* vol. XXXIII, No. 144, 1894.

[2] Franz Leydig, " Beiträge zur Kenntniss des thierischen Eies im unbefruchteten Zustande." *Zool. Jahr., Abth. f. Anat. u. Ontog.,* Bd. III, Hft. 2, 1888.

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## EXPLANATION OF PLATE I.

All figures were drawn from sections; in all but three cases the entire figure was drawn from a single section.

Zeiss. hom. immer., 2 mm., 140 ap.

Abbe Camera.

Figs. 1–5, ocular IV.

Figs. 6–12, ocular II.

Figs. 13–14, ocular IV.

Stains, lithium carmine and Lyon's blue.

Archoplasm (" yolk-nucleus," "archoplasm," polar rings), blue.

Chromatin, nucleolus, and cytoplasm, red.

FIG. 1.  Very young oöcyte, p. 4.

FIG. 2.  Older oöcyte, p. 5.

FIG. 3.  Later stage, p. 6.

FIG. 4.  Archoplasm dispersed in irregular patches throughout the oöcyte, p. 6.

FIG. 5.  Archoplasm peripherally distributed, p. 6.

FIG. 6.  Egg detached from free end of ovary (section through germinal vesicle), p. 6.

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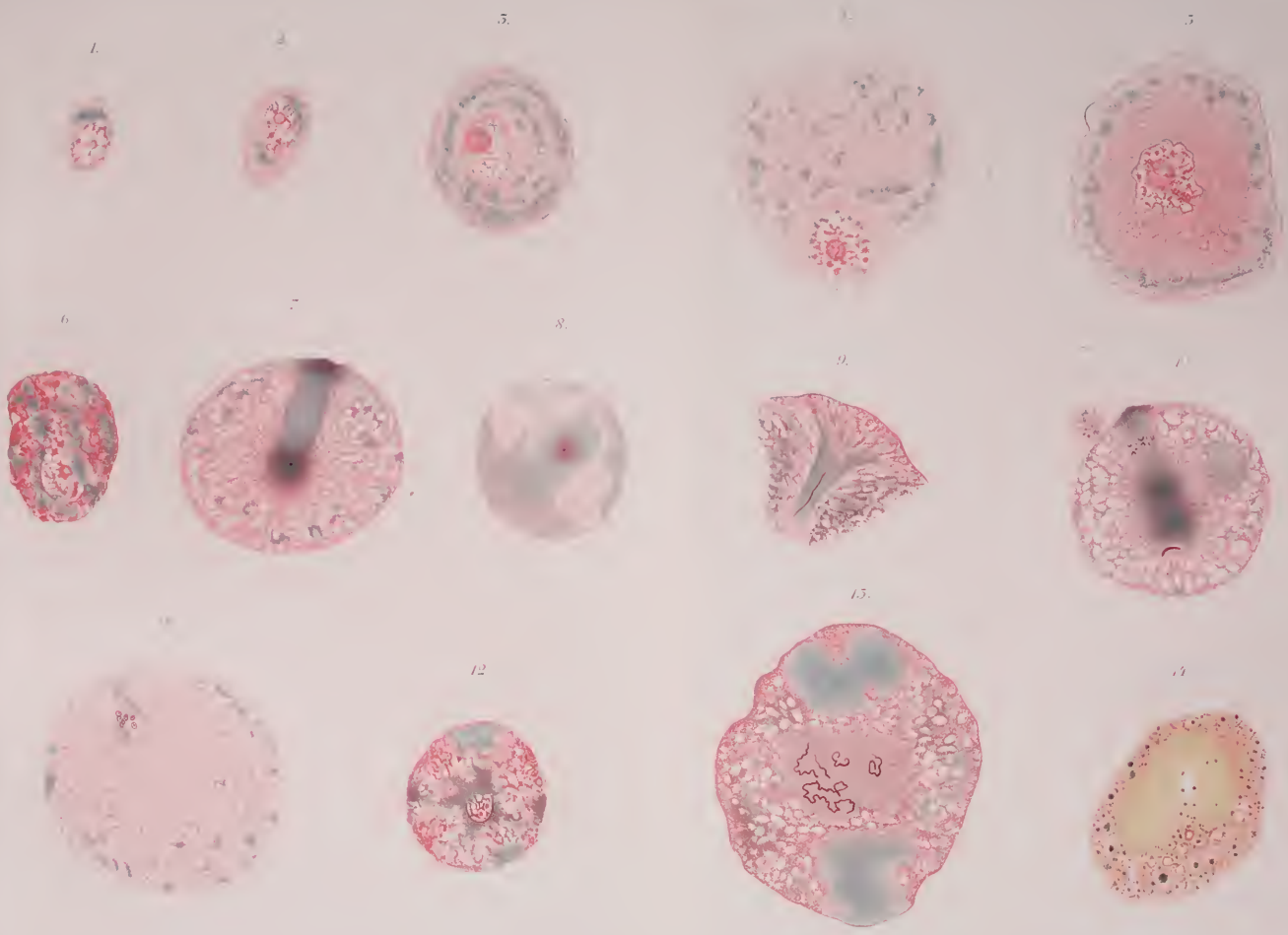
# VARIATIONS IN THE DEVELOPMENT OF
## *LIMULUS POLYPHEMUS.*

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## INTRODUCTION.

MOST of the material for the following paper was obtained during the summer of 1891 at the U. S. Fish Commission Laboratory at Woods Holl. The preparations and drawings were made during the following winter. But since then, from time to time, new material was obtained, till the number and variety of abnormal embryos at my disposal became very great. The principal value of the material lies in the large number of abnormal embryos, and in their range of variation, from nearly normal ones to those so modified as to leave a hardly recognizable being behind. To make this range and diversity of variation obvious, great pains were taken to present as large a number of surface views as possible, made from stained and mounted embryos. In nearly every case the embryos were subsequently sectioned, and used as a guide for the interpretation, or correction, if necessary, of the surface

views.   But the latter usually tell the whole story, so that but few figures of sections have been deemed necessary.

The amount of good material was so great that I have in no case used embryos for illustration if there was the least doubt of the abnormality being a real one, and not one due to post-mortem abrasion, shrinkage, or other causes of like nature.

The present paper is an unexpected by-product of work along other lines.   The time necessary for this digression from a prescribed course was somewhat grudgingly given.   It is partly for that reason, and not because their value for this purpose was unappreciated, that I have not entered into a critical discussion of prevalent theories of heredity and development in the light of these new facts, although I have ventured to make a few suggestions of a theoretical character that naturally arose out of their consideration.

It seemed to me that about everything of value in the way of argument has already been said and resaid on the various phases of epigenesis *versus* evolution.   When the smoke from the volleys of words discharged in the last few years has cleared away somewhat, it will probably be found that the rival disputants are in closer agreement than has been suspected.

But, after all, the most convincing arguments are the plain solid facts.   They are always eloquent, and we cannot have too many.   One who has often had occasion to search the literature on a given subject for definite information must be impressed with the uncertainties that hover thickly around the majority of observations, and which render them practically worthless for constructive purposes.

While it is not claimed that the following descriptions are less open to this criticism than most others, it may be a point in their favor that, almost to the last, the embryos were selected and drawn with great care, merely as an illustration of isolated cases of variation, and without any preconceived ideas as to their meaning or mutual relations.

It was my intention originally to publish a description of the normal development first, as I had abundance of material carefully prepared and studied for this purpose.   The reader would then be better able to appreciate the meaning of the abnormal

embryos.    But in view of the long delay that might attend the publication of the first part, and among other reasons, on account of the interest now centred in variation and abnormal development, it seems advisable not to delay further the publication of the present paper.

But in order to afford some ready means of comparison with normal embryos, prepared by my methods and interpreted on the same basis as the abnormal ones, Pl. I has been introduced, in which are represented the more important stages in the development of normal embryos.

### *Methods of Hardening, Staining, and Clearing the Embryo for Surface Views and for Sections.*

Surface views of opaque embryos are useful for some purposes, certain points being brought out with special clearness shortly after the eggs are put into the hardening fluid.    But in order to make out many important details, it is absolutely essential to stain the egg, and clear in clove oil, balsam, or oil of cedar.    The latter often gave the best pictures and the eggs could be kept longer in this fluid without discoloring the yolk, than in oil of cloves.

To obtain the best surface views, the embryos should be stained and mounted as soon as possible after hardening, or if that is not convenient, preserved in perfectly clean alcohol in glass-stoppered bottles, as the tannin, or other substances in cork stoppers, is dissolved out by the alcohol and discolors the yolk.

Either picro-nitric or undiluted picro-sulphuric acid or Perenyi's fluid may be used for hardening.    The eggs should be immersed in *the cold solution from ten to twenty-four hours*. The yolk is thus made quite hard, and the membranes swell so that they can be easily removed by fine-pointed forceps.    The membranes must always be removed before placing in alcohol; otherwise they shrink, and the embryos are injured or distorted.    Moreover, a white albuminoid substance collects under the membranes, and is precipitated on the embryo when it is put into alcohol, obscuring very much the beauty and clearness of the preparations.

After the eggs are shelled they are rinsed in the hardening fluids and transferred to a large quantity of strong alcohol, say 94%, which is changed frequently the first few days. If not treated in this way, the yolk is likely to swell and crack. Brittleness of the yolk is much diminished by the prolonged treatment with acids.

The most beautiful surface views are obtained by staining the whole egg in borax carmine, or almost any haematoxylin, for a very short time, one-half to one or two minutes, and then washing in acid alcohol. This method gives very sharp and luminous surface contours. It has been used on a great variety of objects, arthropod and vertebrate embryos, etc., and is very useful.

But this method cannot be used if there is any surface cuticle present. The whole egg must in that case be stained throughout, and the color subsequently drawn completely out of the yolk by acid alcohol (10 to 15 drops strong hydrochloric acid to 100 c.c. of 70% alcohol). The decolorizing process may require several days, and the acid alcohol must be changed as often as it is discolored. When this is successfully done, the yolk has the transparent, yellow color it had before staining, but the nuclei are bright red.

If the eggs are to be mounted, after clearing in oil of cloves, they are split in halves with a delicate knife, made by grinding the end of a needle down to a very thin blade.

The halves with the embryos on them are arranged like serial sections, in shallow cells, and fastened in position with a small drop of thick collodion and clove oil. The fixative is hardened by washing in turpentine, and finally the eggs are mounted in balsam.

Each embryo was numbered, drawn, and in most cases finally taken out of the balsam and sectioned.

The embryos were studied with raised condenser and wide open diaphragm, so that they appeared bright red on a clear yellow field.

In such preparations the elevated surfaces and protruding organs appear dark, the depressions light. All the drawings have been outlined with the aid of a camera from such prepara-

tions, *and, with few exceptions, drawn to the same scale, so that the difference in size of embryos of the same age is very evident.*

### Methods of Obtaining Material.

My attention was first drawn to the presence of abnormal embryos, by finding a nest in which about 90% of the eggs were about ready to hatch, while the remaining ones were apparently in very early stages of development. A few of the latter contained double monsters. A number of different nests were then examined, and many were found containing abnormal eggs. The number of abnormal eggs ranged, at a rough estimate, not by actual count, from none to about 10%, or perhaps more.

In order to obtain a greater abundance and variety of abnormal embryos, about 50,000 eggs, from many different nests and in different stages of development, were collected. The sand containing the eggs was placed in shallow dishes and stirred about by strong currents of water till the eggs rose to the surface, when they were poured off into other jars and washed again till perfectly clean. Placed in hatching jars, they sink to the bottom, and are kept constantly but gently agitated by a current of pure sea water. As fast as the young larvae begin to swim about, they are swept out of the top of the jar through the escape pipe.

After from eight to ten weeks most of the larvae have hatched. *Many thousands of the larvae thus obtained have been examined, and no abnormal embryos found among them.*

In the residue left in the jar are many apparently normal and well advanced embryos, some of which hatch from time to time for many weeks or even six months or more after the regular period of hatching has passed. All those that eventually leave the membranes, whether early or late, and swim about freely, are, with very rare exceptions, normal. Among the remaining eggs are found embryos in all stages of degeneration. A very few only are dead and decomposing. The rest appear fresh and healthy, and one would not suspect from their general outward appearance that they were abnormal embryos.

The eggs left in jars that had been running from ten to fourteen weeks were finally treated with hardening fluids, making the shape of the embryos very conspicuous. The whole collection could then be examined under a dissecting microscope, and the most important forms picked out and shelled at once.

As many embryos were already far advanced (six to nine weeks) when placed in the hatching jar, it is evident that the remaining abnormal forms were at least ten to fourteen weeks old, and some may have been even more than twenty, although as seen by the figures, many of them appear to be not more than from ten days to two weeks old. Most of the monstrosities are apparently the result of retrogressive development, rather than of a slow, progressive one. That is, embryos normal in outward appearance may develop up to stage $E$, Pl. I. They then appear to develop less rapidly, or they may remain for a long time practically stationary. But they finally become smaller, and by the fusion and complete atrophy of their various organs, dwindle to some insignificant remnant which is in turn absorbed. Thus a once well-grown embryo may disappear completely, leaving a healthy-looking egg behind, but one which consists of nothing but yolk.

I was able to confirm the above statement by selecting a dozen or more abnormal embryos and keeping them under observation for four or five weeks. It is difficult to make out details on living eggs, but enough could be seen to prove beyond any doubt that in each case the characteristic abnormality, such as asymmetry, fusion, or reduction of appendages, etc., became more and more marked from day to day, until my observations were brought to a close by the complete disappearance of the embryos, due to the increasing opacity of the chorion. On killing and staining these eggs, the embryos could be easily distinguished again, in advanced stages of degeneration.

In other instances I have kept large numbers of the residual eggs in open dishes, for five or six weeks after they were taken from the hatching jars. In such cases, the number of individuals showing extreme median fusion, or atrophy, or general degeneration, seemed to be greater than before. The number

of abnormal embryos seemed to be greatest in the nests taken from sand that was blackened and foul from decaying organic matter (decomposing eggs?) ; but on the other hand, they were often found in abundance in perfectly clean sand close to nests where nearly all the eggs were normal.

It occurred to me that the abnormal eggs were produced by the unusual conditions in a hatching jar, such as the constant movement and the exposure to light, or perhaps to the difference in the temperature or density of the water.

This last summer, therefore, hoping to obtain new classes of variation, about 25,000 eggs were placed in shallow dishes. Some were kept in the dark, others in the direct rays of the sun. The water in some dishes was allowed to evaporate almost to dryness, leaving a thick crust of salt in them. A large quantity of fresh water was then added. Under this treatment, many of the older larvae died, and their bodies were allowed to putrefy in the dishes, so that the water became very foul. At the close of the season, all the eggs that survived these indignities, about two-thirds of them, were brought to Hanover, and kept in shallow covered dishes, exposed during some part of the day to the direct rays of the sun. Up to November, the water was turbid and filled with bacteria. Shortly after that, algae began to grow, and now cover the sides and bottom of the dish with a thick, green scum. In December, it was thought a large number of abnormal embryos might be obtained, *and about 5000 were killed and examined, including trilobite larvae and unhatched eggs, but not a single abnormal larva or embryo was found in the lot !* This fact is not easily explained, because in any case we ought to find a certain number of abnormal forms. But it seemed probable that all those embryos originally abnormal were early exterminated by this drastic treatment, and only the normal ones survived, that is, normal in every respect except their very slow development, and this was probably due in part at least to the increased density of the sea water through evaporation.

## Causes of Variation.

It is assumed that all the embryos were at first normal in exter-
nal appearance, and that the condition in which they were found
was attained by a gradual fusion and atrophy of organs, according
to the methods described under the various headings. But we
have always had in mind the possibility, indeed the strong proba-
bility, that in some cases the normal form was not actually ex-
pressed, but that the embryo from the first appeared in some one
of the various stages of fusion and degeneration. For example,
when a particular appendage is completely or partially invagi-
nated, we have no means of determining whether it was first
normally formed and was subsequently invaginated, or whether
at the moment of its first appearance it gradually assumed the
condition in which we find it. The one condition may be
regarded as the resultant of the simultaneous action of normal
and abnormal factors, the other the resultant of the abnormal
factors acting after the normal ones have found expression.
No number of cases, however great or however varied, can
settle that point. But a complete series of them properly
arranged may be taken to indicate the successive stages a
fully developed normal appendage would assume when subse-
quently invaginated, and the same applies to any of the other
modifications that have been observed.

All variations of the same class are no doubt due to the
action of similar abnormal factors that tend to throw the
normal mechanism out of its beaten track. But while simi-
lar factors will tend to produce similar divergences in the
end, the nature of the preceding forms will depend on the
intensity of their action and on the period in the develop-
ment at which they begin to act. The variations first to
appear are of the greatest import, because the at first
slight divergence becomes continually greater till it leads
to impossible combinations which may necessarily be fatal
to the future organism. Moreover, an organ on its way
toward a position of stability is more readily affected by
external agents than one that has settled down as it were
into that mature and stable condition characteristic of all older

organs.   But there is nothing to indicate with certainty whether the initial cause of variation is due (1) to a variation in the combination, or the quantity, or the quality of the original constructive materials, or (2) to the variation in conditions external to the ovum, or (3) to a combination of both. But as very divergent forms appear among eggs kept under apparently the same conditions, it would seem more than likely that the first set of causes are the real ones.

Variations in the unfertilized ova, and in the spermatozoa or polar globules, at once arise before the mind in their familiar attitude, as factors in some way connected with the phenomena of variation.   And there is the whole infinitely complex Weismannian mechanism, with its endless army of corpuscular brownies, on whose sins of omission or commission we may easily throw the responsibility.

But these corpuscular theories fail to explain anything.   Their agents come or go at the beck and call of him who commands them.   We are in the end left with the sterile formula, that this or that organ is as it is because the necessary corpuscles were there to make it so !

All the variations so far observed can be traced back to variation in the relative rate of growth, specialization, and degeneration in the numerous groups of cells that constitute the embryo.   The problem then is to explain why one group of cells grows faster or slower in one embryo than in another, or why it grows at all.   But the conditions determining differential growth are very complex, and may be different in each particular case.   No simple general statement will suffice. We have passed that stage where it is enough to know that a machine is made of iron and run by electricity.   We must know what electricity is, and the structure and bearing of every pin, screw, and wheel in the whole mechanism.   It is needless to say that we are very far from having any such knowledge even of the simplest bit of living matter.

The facts here presented enable us to catch a glimpse of embryological processes under new conditions and from a different standpoint, and while many of them increase rather than diminish the existing difficulties, they will perhaps, in

some future theories of development, find their proper place and partial explanation.

It seemed inadvisable to add further to this paper by detailed reference to the voluminous literature bearing only indirectly on the facts here set forth.

So little is known about variations in arthropod embryos, and especially arachnids, and the facts we present are so different in character from those already known, that no injustice will be done previous workers along similar lines by not referring in detail to their publications. The only reference to an abnormality in Limulus that I have been able to find is in an article on "Diploteratology, An Essay on Compound Human Monsters," by Geo. J. Fisher, Albany, 1868. A good figure is there given of an adult (?) animal with a double caudal spine and a symmetrically forked abdomen.

### DESCRIPTION OF THE DIFFERENT CLASSES OF VARIATION.

#### I.   INVAGINATION OF APPENDAGES.

This remarkable modification is of comparatively common occurrence in forms which are in other respects more or less abnormal. It is confined, so far as I have observed, to the thoracic appendages, and most commonly affects the middle ones of the series.

It may begin after the appendage is fully formed as a minute, slit-like depression at its distal end, Figs. 10, 11, *th. ap.*[4], *th. ap.*[3]. The slit is always transverse to the long axis of the body, and appears in the stained specimens as a fine line in the middle of a clear band devoid of nuclei. When the invagination is complete, the whole appendage is carried inward, so that in its place is an opening leading into a deep tube with a flattened, conical lumen.

The third or fourth appendages on either or both sides may be invaginated, Figs. 10, 11, or, as in one case — the only one observed — all the thoracic appendages, with the exception of the first and sixth pairs, may be invaginated, the infolding being

greatest in the second pair, and diminishing in depth from that point backwards.

The embryos just described are in other respects nearly normal, the principal deviations being in the abbreviation and atrophy of the abdominal region in Figs. 8 and 10, and the modification of the cephalic lobes in Figs. 10 and 11. But invaginated appendages are frequently found in other types of embryos, as in Figs. 14, 16, 18, 33, 34, 40, 61, 65, 70, 98.

Any appendage, except perhaps those of the first pair, may be invaginated ; the third pair appear to be most frequently affected in this way.

Sections have been cut through several invaginated appendages, and confirm completely the conclusions drawn from surface views. They throw little further light on the subject. Pl. X, Fig. 10, represents a longitudinal, vertical section through the embryo shown in Fig. 10. The only point of further interest here is seen at the inner end of the appendage, where the ectoderm seems to become continuous with the mesoderm, as though there was an inward cell proliferation at that point. If such were the case, a communication, through the hollow appendage, might be established between the exterior on one side, and the mesenteron on the other. A condition would then result like that which obtains in the gill slits of vertebrates.

As to the cause of the invagination, very little can be said further than that it is a local, internal, rather than an external one.

We cannot see how any of the general external conditions, such as the density or the composition of the surrounding medium, could produce such a purely local effect as the invagination of one out of several apparently identical appendages.

There is no evidence whatever that local pressure, such as that which the egg membranes, or the adjacent appendages, might exert, was the cause of the invaginations, for the membranes stand far away from the embryos at this period, and besides, an examination of most any of the figures shows clearly that the position of the invaginated appendages is such that the membranes could not touch them. Such cases as

those shown in Figs. 10, 11, or 65 could not be due to pressure of membranes or of adjacent appendages.

It seems to me, therefore, that the immediate cause is a local, internal one, independent, to the same extent as the normal growths, of external conditions, and having its source in some remote instability of the innermost mechanism.

This class of variations may therefore be called *normal variations*, to distinguish them from those due to the more immediate action of the environment. They must be regarded as necessary incidents of a particular structure and likely to occur in a certain percentage of cases, irrespective of the immediate environment.

In vital processes, what may be at one time or place an incidental and occasional phenomenon, may become elsewhere under other conditions a constantly recurring result. It is therefore clear that in estimating relationships by means of morphological characters, such variations deserve careful consideration. They are as likely to throw light on phyllogenetic problems as ontogeny. The latter indicates the established paths connecting the present with the past ; the study of normal variations shows us possible paths leading out of the present into the future.

These facts, then, are interesting morphologically in two ways :

(1) They may be regarded as forming an indirect confirmation of the view that the lung-books of scorpions and spiders, as claimed by Lankester and Kingsley, are invaginated gill-bearing appendages, modified for breathing air.

(2) I have maintained in a former paper on the " Origin of Vertebrates from Arachnids," that the complete visceral arches of vertebrates are homologous with the appendages of an arachnid-like ancestor, because in Limulus and scorpions, the number of these appendages, their innervation, the position of their important sense organs, the structure of the mesoblastic cavities associated with them, and the nature of the muscles arising from these cavities, resemble as a whole the corresponding structures in vertebrates.

Aside from other considerations, the striking difference between arthropod appendages and the gill arches of verte-

brates renders any relation between them at first sight very improbable.   But the unexpected discovery that in this particular arthropod the appendages are frequently invaginated, leaving in their place a series of slit-like gill openings, such as those shown in Fig. 1, is a potent factor in favor of my view.

If in Fig. 8, the thoracic appendages had been provided with gill leaves like those on the abdominal appendages, we would then have, in place of typical arthropod appendages, a series of respiratory sacs, the cavities of which, by the persistence of embryonic conditions might, after the manner of vertebrate gills, communicate with the alimentary canal, either through its nephridium and somite, or by a secondary opening at the apex, where ectoderm and mesoderm appear to be continuous.   The exact relations in Limulus of the invaginated appendages to the mesoblastic somite, and of the latter to endoderm have not been determined, as in the cases studied the cavity of the somite had disappeared.   But there seems to be no reason to doubt, from what occurs in the normal embryos of Limulus and other arthropods, that something like the condition mentioned above might arise, for each of the assumed conditions is known to occur.

The great difficulties involved in determining what is ectodermic, mesodermic, or endodermic in the vertebrate head, we are just beginning to realize.   The use of the terms is founded on the supposition that the embryological processes in vertebrates present a modification of those assumed to occur in some real or imaginary invertebrate, which is further assumed to be a more or less remote ancestor of the vertebrates.   While the problem is still in this uncertain condition, the fact that these "a priori" methods of interpretation do not harmonize with the suggestions here made cannot be used as an argument against them.

## II. Absence of Appendages.

We shall consider all embryos showing the absence of one or more appendages under the above heading.   In all cases the reduction of an appendage seems to be accompanied by a

degeneration more or less complete of all the other organs on
the same half metamere, but as these organs are less easily seen,
I have confined my statements in the main to the appendages.
The degeneration of an appendage is an indication that degen-
eration has taken place or will take place in the other organs of
that half-metamere, but the reverse is not true, for an appendage
frequently persists long after all associated organs have disap-
peared.  Absence of appendages is one of the most common
abnormalities, and like the invagination of appendages is most
likely to occur in embryos showing other indications of abnor-
mality.  *The three anterior thoracic, first, second, and third, and
the abdominal appendages are most frequently absent.*  The three
posterior pairs of thoracic appendages are found so frequently
after everything else has disappeared, that one might suppose
a nauplius-like larva was a normal feature in the development
of Limulus, but a little consideration will show that such is not
the case.

While the appendages are frequently absent in pairs, leaving
bilaterally symmetrical embryos, this is by no means the rule.[1]

*A. Absence of Abdominal Appendages.* — This is perhaps
the most common of all the modifications observed.  A great
many of the abnormal embryos showed some abbreviation of
the abdomen, accompanied by the reduction or the entire ab-
sence of the appendages.

Fig. 5 shows the condition of the abdomen in normal
embryos at the time all the thoracic appendages have appeared.
The primitive streak is still present as a narrow, longitudinal
furrow, from the floor of which there is an inward proliferation
of cells.

One frequently finds embryos, otherwise normal, in which
the marginal fold, *m.f.*, which represents the margin of the
future thoracic and abdominal shields, sweeps across the
median line just back of the first pair of thoracic appendages.
They are so much like what occurs in Fig. 8, that it was not
necessary to represent them.  In some of these cases, the

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[1] I have unfortunately not tabulated all the cases seen, but I have a strong
impression that degeneration is more likely to affect a half metamere than a
whole one; as though the former were the unit of structure rather than the latter.

abdomen is perhaps merely retarded and may appear later in its normal condition. This is probably the case with Figs. 9, 10, and 15.

*The Telopore.* — In the normal embryos, at a very early stage, the posterior end of the body, or the anal plate, consists of a broad mass of proliferating cells, representing a modification of the posterior of the two primitive cumuli that constitute the beginning of the embryo.

In Fig. 1, there is already formed a primitive streak-like invagination in the anal plate, representing a specialization of this proliferating area. Along this proliferating furrow, ectoderm and inner-layer cells become continuous. Without following out its history in further detail here, it is enough to state that it persists, either as a solid mass of cells or as a furrow, up to stage *C,* after which it gradually disappears. The anus does not appear on the flat abdominal plate till about stage *E,* Fig. 7, long after the primitive streak has disappeared. But these normal conditions are frequently deviated from, giving rise to a great range of variations.

*In many cases when the abdomen is abbreviated or absent, the entire remaining region, where the abdomen should be formed, is invaginated to form a deep depression, varying greatly in size and general appearance.* This depression, or *telopore,* is not a modification of the anus or of the primitive streak, for one or the other of them may, in some cases, be found at the bottom of the depression. It seems to be the result of the active proliferation that is going on in this region during the formation of new segments.

A common form of the telopore is shown in Figs. 9, 10, and 11, another in Figs. 16, 19, 21, 22, 23, 24, 36, 59, 62, 67, 68. In Figs. 20, 21, 23, 51, the whole abdominal region is so deeply depressed that the posterior thoracic and abdominal appendages, when they are developed, are carried into the cavity.

At the bottom of the depression, I have often found in sections the proliferating primitive streak, not markedly different from that in the normal position.

The anus may finally appear at the bottom of the telopore, after the primitive streak has disappeared. In some cases, it

is difficult to distinguish the telopore from the anal invagina-
tion, or in other cases from the primitive streak, so great is
the range and number of modifications that may be observed.

The infolded abdominal region seems to straighten out in
some cases, and in the end give rise to a normally segmented
abdomen. In the majority of cases, however, its presence
indicates a general weakness that leads ultimately to complete
degeneration of the whole embryo. In some cases the defect
persists to very late stages, for trilobite larvae are not uncommon
that are perfectly formed except for the abdomen, which may
show any one of the numerous stages of degeneration.

Whether these defective larvae die, or the abdomen is
restored after successive moults, is not known. I have looked
in vain for traces of aborted abdomens and in fact for any kind
of abnormalities in individuals older than the tribolite stage.

*B. Asymmetry of Abdominal Appendages* has been observed
in a large number of cases. This condition is well shown in
Figs. 29 and 31, where there are three abdominal appendages
on the right but no trace of them on the left. In Fig. 29 the
abdomen is thrown round to the left by the unequal develop-
ment, and a peculiar, hood-like fold of ectoderm covers its
posterior portion.

In Fig. 30, there are four abdominal appendages on the left,
and only two on the right. In Figs. 32, 37, 38, 53, 54, there
is strongly marked asymmetry.

*C. Absence of Thoracic Appendages.* In the normal em-
bryos, the second, third, and fourth thoracic appendages appear
simultaneously, and shortly afterwards in the order named,
the first, fifth, and sixth.

In the abnormal forms, any one thoracic appendage, or almost
any combination of the twelve, may be absent, but, as one might
infer, from what has been said in reference to the invagination
of appendages, it is very difficult to determine in any given
case whether the appendages failed to develop, or whether
their absence is due to degeneration.

In order to obtain some light on this point, a dozen or more
living abnormal embryos were kept under observation from
the 20th of July till the 17th of August. Most of the eggs

either died or became so opaque that after a few days nothing was clearly visible in them. It was, however, clearly established, by means of careful drawings made from time to time on such eggs as were transparent enough to allow one to follow the changes going on within them, that a gradual atrophy of the anterior and posterior extremities took place, while the whole embryo showed a marked decrease in size. These facts were enough to show that progressive degeneration did take place in these embryos, and it is very probable that a similar gradual atrophy would have taken place in most of the cases of abnormal embryos here cited, if they had been allowed to live long enough.

The simplest cases of the absence of thoracic appendages are those where one or more appendages are absent on one side of the body, as in Pl. IV, Fig. 30, where the right second and third are absent. Similar cases are shown in Figs. 13, 14, 29, 30, 32, 33, 34, 35, 36, 37, and others.

These cases are common, but such as that in Fig. 38 are extremely rare, only three similar ones having been seen. In this embryo the whole of the left cephalic lobe, nerve-cord, and mesoderm, — everything, in fact, except what appears to be the left sixth appendage, is absent, while on the right side everything is perfectly normal, except for the slight spiral curvature to the left due to the unequal cell stress.

A very frequent form of abnormality is where there has been *a nearly bilaterally symmetrical reduction of the anterior, or the posterior, end of the embryo, or both.* The cases, in so far as they affect the abdomen, have already been considered. This process carried still farther would affect the reduction of the posterior part of the thorax, but that condition seems to be comparatively rare. There is on the contrary a tendency to leave this region intact, reducing instead the three anterior segments one by one, from before backwards, producing conditions like those in Figs. 16, 18, 24, 25, 26, 27. The small embryos with but three pairs of appendages represent the last stages of the process, and they are so abundant that I at one time supposed they might represent a true *nauplius* stage. It may be that similar embryos gave rise to the statements of

Dohrn and Osborn to that effect. But that they are not
nauplii is obvious because they are mere fragments of embryos,
minus brain, oesophagus, and one or more of the anterior
thoracic segments. One cannot always determine with cer-
tainty just what appendages are preserved in these false
nauplii, but in most cases they appear to be the three posterior
pairs of thoracic appendages.

There are three reasons in favor of this view: (1) In some
cases that clearly belong to this category, the cephalic lobes
and the two or three following segments are quite rudimentary,
showing marked anterior degeneration, while the three pos-
terior appendages are of fair size. In Fig. 25 is a typical case.
Here the cephalic lobes are reduced to a rounded plate of cells
with the remnant of the oesophagus in the centre, and they
are separated from the thorax by a wide space along which no
organs are developed.

(2) In cases where there has been anterior degeneration,
accompanied by the fusion of right and left halves, the last
three pairs of appendages (known to be such by the presence
of the flabellum on the sixth) undergo the least modification or
degeneration, and persist long after every organ in front of them
has disappeared. Figs. 44, 48, also 94, 96, 97, 102–104.

(3) When transverse fission occurs, it divides the embryo
between the third and fourth pairs of thoracic appendages into
two parts; the posterior one usually persists for a long time
after the first part has disappeared, showing that it has the
greatest vitality. Figs. 51, 98, 103.

It is therefore very probable that in Figs. 25, 26, 27, 33, 34,
35, 62, 65, 66 the segments present are the 4th, 5th, and 6th
thoracic. But it must be observed that in Figs. 31 and 32 the
second and third pairs of appendages are much better developed
than the posterior ones. And also in Fig. 27 the rudiments of
the oesophagus and cephalic lobes lie directly in front of the
first pair of appendages. The same position of the cephalic
lobes and oesophagus is observed in Figs. 33, 34, 45, 62, 65,
66, 67. It is thus obvious that entire segments may be omitted
without leaving any apparent break in the series. This is
especially clear in Fig. 27, where three thoracic segments are

certainly absent, because the cephalic lobes and abdominal appendages are in their proper relative positions, and yet only three out of the six thoracic segments are present. It cannot therefore be argued that because in Fig. 62 the oesophagus lies just in front of the first pair of remaining appendages, that they are the original first pair and not the fourth.

Figs. 33, 34, 35, 65, 66 probably represent modifications of this three-legged form in which the appendages have undergone various modifications through invagination, median fusion, and degeneration.

In Fig. 34, the last pair have fused to form a median appendage, and in Fig. 35 there has been such distortion and reduction that it is hard to recognize what remains, but it appears to consist of the three left thoracic appendages and one right. The oesophagus lies at $oe.$, and the telepore at $tp.$, at the bottom of a furrow partly covered by a hood-like fold.

In most of these small three-legged forms, the median ventral surface is deeply depressed and closely surrounded by a thick and high marginal fold.

### III.    Multiplication of Appendages.

Multiplication of definite regions or organs of the body, not including in this category double or triple monsters, is very rare. I have observed but one case. It is interesting and important, since it proves conclusively that a half of a metamere already laid down has the power to multiply independently of adjacent organs. This is shown in Pl. II, Fig. 12, where the right chelicera — which fortunately can be identified here with absolute certainty — has divided twice. The first division apparently gave rise to $a^{1+2}$ and $a^{3+4}$. A subsequent division separated completely $a^3$ from $a^4$. But the third division effected only a partial separation of $a^1$ from $a^2$. The abnormal growth has also modified the right nerve-cord at this point, throwing the head over to the left. None of the neuromeres are very clearly brought out in this preparation, so it is not certain whether there is one for each of the four right chelicerae or not. But there is a prominent enlargement of the

right nerve-cord, *s*, which from its shape and position appears
more intimately related to the third left chelicera than to
any of the others.    What appears to be the neuromere of
the fourth right chelicera is small and triangular, and pushed
to one side by the growth of the neuromere just in front of it.
That this growth affects the whole right half of the segment is
shown by the presence there of two rudimentary lateral eyes, *l.e.*

IV.    FUSION OF RIGHT AND LEFT HALVES OF THE EMBRYO
AND ANTERO–POSTERIOR DEGENERATION.

This remarkable phenomenon has been observed in so many
different stages, that there can be no doubt as to the manner
in which it takes place.   Median fusion and degeneration
begins at the anterior end of the embryo (except in the hour-
glass type), and gradually extends toward the posterior end.
In the typical cases, each organ in one half of the embryo
unites with its fellow of the opposite side to form a median
unpaired organ.   Those nearest the median line unite first,
and then degenerate, and those lateral to them follow in the
order of their position, till the whole of the segment has
disappeared.   The steps in the process are most clearly shown
by the appendages, the dorsal organs, and the nerve-cords.
The other paired organs probably fuse and degenerate in the
same manner, but owing to their indistinctness in surface
views, it is not so easy to follow in detail their successive
modifications.   In this way Λ-shaped embryos are produced,
showing various stages in the progress of the degeneration
from the anterior toward the posterior end.

Toward the close of the process we may find either a median
row of papillae, representing two or three pairs of medianly
fused appendages, Pl. VI, Fig 50, or an exhausted mass of cells
at what was the posterior end of the embryo ; and finally these
useless remnants may in their turn disappear.   The principle
involved in the process is well illustrated by the adjacent Figs.
1 and 2.

In Fig. 1, each square represents a segmental organ of some
kind.   The lower half of the diagram shows how the organs

are produced by the usual method of apical growth, or rather it is more correctly a double rectilinear growth, — a longitudinal one and a lateral one at right angles to it. The longitudinal growth may be represented by the lines parallel with $A$–$p$, and the lateral growth by the lines parallel with $E$–$E$. The relative age of each organ is determined by its position in relation to these two sets of lines, the uppermost $a$ being the oldest and most specialized organ in the body, and the lowest $A$ the youngest. Now it is obvious on examining the diagrams *that the median fusion and antero-posterior degeneration of the organs takes place in the reverse order of their formation, the oldest dying first and the youngest last.* Considering for the present only the small-lettered part of Fig. 1, median fusion and degeneration such as occurs in Limulus will gradually carry the hypothenuses of the shaded triangles toward each other till they meet in the median line, the shaded areas themselves gradually disappearing. The half of the embryo
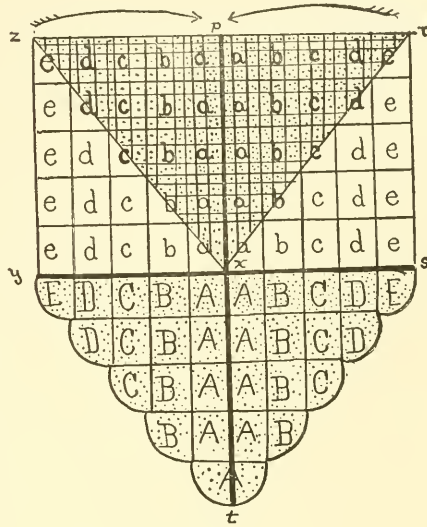
Fig. 1.
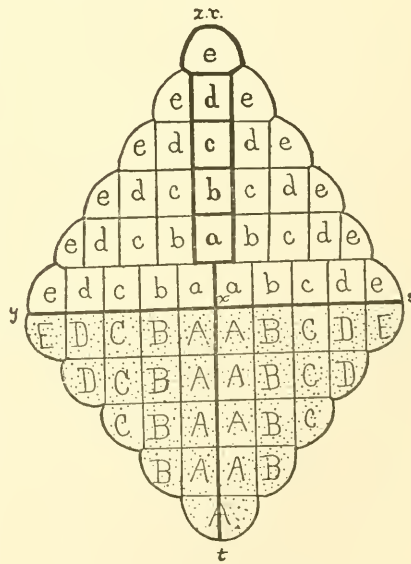
Fig. 2.

Diagrams to illustrate the laws of growth of segmental organs and their disappearance by median fusion.

that dies first, therefore, consists of the two quarters *z.p.x.* and
*r.p.x.*, and with their disappearance the remaining quarters are
thrown toward the median line in such a way that the position
of each organ is shifted a step farther toward the median line
than the corresponding organ just behind it. Furthermore a
row of dissimilar, unpaired organs is formed along the median
line in the same sequence from behind forwards, as from the
median line toward the sides, Fig. 2, *a, b, c, d, e,* and *A, B,
C, D, E.*

The steps in this process are as follows. The most median
organs, *a* and *a* in the anterior row, Fig. 1, fuse with each other
in the median line and then disappear. Their place is imme-
diately occupied by the fused organs that were originally next
to them on the outer side, namely *b* and *b* ; at the same time
*a* and *a* fuse in the second row. In the third step, the fused
organs *b* and *b* of the first row disappear and the fused organs
*c* and *c* take their place ; *b* and *b* take the place of *a + a* in
the second row, and *a* and *a* fuse in the third. The following
steps are of the same nature and are repeated till the condition
like that in the anterior part of Fig. 2 is reached. It may be
continued still farther till all the organs have disappeared, the
last fused organs to disappear being the two most lateral ones
of the first line.[1]

In reality, however, no such condition as the one just
described would ever be completely realized, owing to the
presence at the posterior growing end of the body of groups of
segments in which the organs are arranged in an inverse manner
to that just described, see lower half of Fig. 2. The tendency
will be, therefore, to produce a body in which the arrangement of
the organs is determined by the interaction of growth and
degeneration, one having its greatest activity at the pos-
terior, the other at the anterior, end. The resultant form
will be therefore a more or less elongated rhomboid. The
tendency of all segmented animals to assume such a form,

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[1] It is not claimed that the process of median fusion and degeneration always
follows exactly the steps indicated above, as will be observed on examination of
the surface views of various embryos, but the variations from it are not of such
a nature as to invalidate the general law.

owing to the fusion or reduction of organs at the extremities of the body, and the fullness and completeness of structure shown in the intermediate regions, testify to the universality of this double law of growth and degeneration.

What is the *cause* of this mode of degeneration ?   It seems to be an exaggeration of the forces which have determined the form and manner of growth in normal embryos.

Assume, for example, that the embryo of a segmented animal consists of a double row of independent half-metameres, placed with their oldest ends or *heads* toward each other, and growing laterally like an acrogenous plant.   The series of organs thus formed, extending from the oldest or median end to the youngest, or lateral, extremity, such as the endoderm,[1] mesoderm, neuromere, nephridium, appendage sense-organs, and various somatic structures, will then appear on the half-somite, roughly speaking, in the order of the appearance of such organs throughout the animal series.   And they are analogous to the metameres of a whole animal, or to the succession of morphological units produced in an acrogenous plant.   There is, however, a sharp distinction to be drawn between the arrangement of organs on a half-metamere and the succession of metameres in a segmented animal.

In the latter case we have a succession of homologous parts more or less modified by their position ; in the former, a succession of fundamentally different parts — a logical sequence of morphological structures in accordance with the genesis of physiological specialization.   The dorsal and ventral surfaces are thus forever fixed as parts fundamentally different, and less likely to be confounded through secondary changes than the anterior and posterior extremities of the body.   Broadly speaking, therefore, the ventral surface of a segmented animal is the oldest and most specialized, — the dorsal the youngest and least specialized.

If we had to deal only with lateral growth, — *i.e.* growth at the apex of each half-metamere, and with the formation of new half-metameres at the posterior end, and all this taking place on

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[1] The endodermic and mesodermic portions of a half-metamere are enfolded at an early period, and consequently do not appear to form a part of the series.

a *flat*, instead of a spherical surface, — we should have a succes-
sion of organs like that in the lower half of Figs. 1 and 2.   But
each half-metamere not only grows in length but in width,
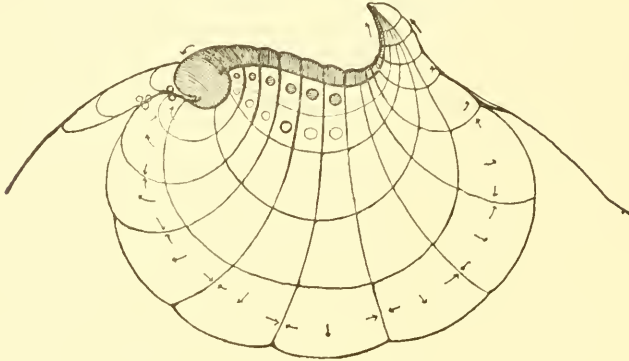


FIG. 3.
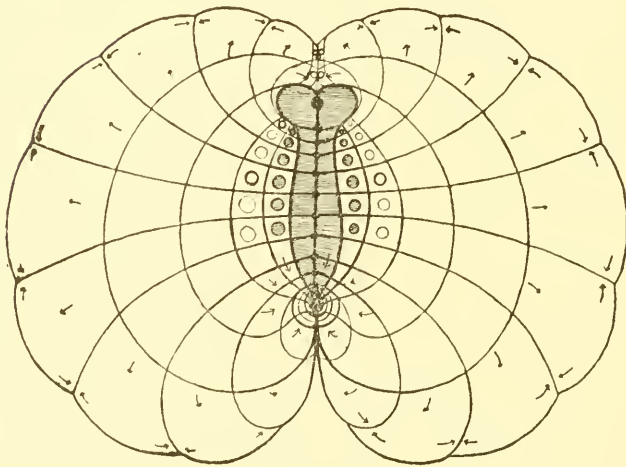


FIG. 4.

Diagrams to illustrate how a double series of half-metameres formed in succession from before
backwards, and growing on a spherical surface, will produce differential growth forces.
These in turn produce median concrescence and degeneration, cervical and caudal flexures,
and the modification of the trunk into regions of different morphological and physiological
potentialities.

and as this is most manifest at the lateral end, they will tend
to be triangular in form.   Moreover, each succeeding metamere
will have to grow under other conditions of cell tension and
available yolk surface than the metamere that preceded it.

The whole tendency will be then to produce a form like that in Fig. 4, where an attempt has been made to illustrate the action of growth forces by lines corresponding with the arrangement of organs. The increase in width of the lateral ends of the metameres will produce a constantly increasing tension which will find relief, and thus favor still further growth, by movements forwards and backwards. The ends of the most anterior metameres will thus be forced together along the median line, causing the median fusion of organs in front of the true anterior extremity of the body. Conditions like these have probably caused the fusion of such organs as the median ocelli and the olfactory organs of Limulus, and in insects the upper lip, which arises, as has been repeatedly shown, from the fusion of originally paired organs lying on the very anterior margin of the cephalic lobes. (See "Eyes of Acilius.")

At the posterior end of the body, concrescence of the mesodermic area is the result, and the true apex of the body is shut off from growth over the surface of the yolk. The new segments formed after this period are therefore forced to grow vertically upward and forwards. Hence the conical, forwardly directed tail seen so constantly in vertebrates and arthropods.

The segments formed at the apex of this conical tail lobe will be produced under conditions very different from those found elsewhere, and we can readily see how these conditions might not only be the direct cause of the diminution in size and fusion of the organs there, but also prohibit the further addition of new segments.

At the head end there is no necessity for such a form, because no new segments are formed there. But there is a gradual thickening of all the organs along the median line, which tends to find relief forwards as well as laterally ; but as the anterior margin of the cephalic lobes is, as it were, shut out from the median line by the ingrowth of the mesodermic area, its only relief is in buckling upward and forwards. Hence the cerebral flexure, and the general S-shaped contour of the whole embryo, Fig. 3.

*Concrescence of the margins of the mesodermic area* occurs in the normal embryos, as shown by the figures in Pl. I.

But there are some very interesting phases of it seen in the embryos that are much reduced in size, and which may be best considered in this connection. The margin of the meso- dermic area is there frequently enlarged so that it becomes very conspicuous in surface views. It serves as a pretty safe index of the grade of development and degeneration, and also to identify the posterior end of the body in embryos reduced to such low terms as those in Pls. VI and VII. This structure is formed by the fusion of the peripheral margin of the mesoblastic somites with the ectoderm and with the yolk cells.[1] A long *primitive, streak-like thickening is thus formed, by the proliferation of which the germ layers are extended laterally in exactly the same way that we are familiar with as occurring at the posterior end of the body.*

There is a great difference in the lateral extension of the segmented portion of the mesoderm. In normal embryos it may reach the very margin of the mesodermic area, while in abnormal cases it may not extend beyond the appendages. But there is universal agreement in normal and abnormal embryos in the union at the periphery of ectoderm, mesoderm, and yolk cells.

*The lateral growth then of a metamere is comparable with the posterior apical growth by which the body of the embryo is increased in length.*

In normal embryos, the lateral margins of the mesoblastic segments, when first formed, are quite indistinct, Fig. 1, but they soon fuse to form the conspicuous marginal thickening just described, Figs. 2–4, *m.a.* The egg being nearly spherical, the anterior and posterior portions of the margin have a shorter distance to go in order to unite with each other in the median line than the middle portions. For this reason and others that we have already discussed, the margins, following the path of least resistance, tend to form anterior and posterior loops, which finally meet in front of and behind the embryo, Pl. I, Fig. 5, forming a rounded, mesodermic area in the centre of which the

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[1] We cannot for lack of space discuss the origin and history of the mesoderm here in the detailed manner that it deserves. We can only point out briefly the more important facts that bear on the subject matter of this paper.

embryo lies.   Its thickened rim, *m. a., usually forms two con-spicuous posteriorly directed loops, which may be found in all stages of concrescence behind the apex of the abdomen.   The sides of the loops concresce like the closing of the arms of a Λ, and a longitudinal post-anal thickening is thus formed, below which lies a great cloud of cells, brought together at that point by the union of the proliferating rim.*

In the abnormal and degenerate forms, the margin of the mesodermic area is usually very distinct, although every out-ward trace of segmentation in the mesoderm, and even the greater part of the embryo itself, may have disappeared.

The margin also shows *in many cases more clearly than in the normal embryos the posterior, median concrescence, and it takes place in such a manner as to bring very forcibly to mind the similar phenomena in vertebrate embryos.*   One of the most striking instances of this exaggeration of the mesodermic margin is shown in Pl. VI, Fig. 63.   Unlike all the rest, this is an opaque embryo shown by reflected light.   The margin of the mesodermic area forms a conspicuous ridge, which is thickened posteriorly.   Where concrescence has taken place, a broad median elevation is produced.

Nothing like *the thickened margin* here described is known to occur in any other arthropod, but a similar concrescence of mesodermic segments probably occurs in all arthropods.

In the embryos very far advanced in degeneration, the margin of the mesodermic area breaks up into isolated, irregular masses containing closely packed nuclei, Pls. VI and VII, Figs. 69, 72, 76, 88, etc.   These masses may lie deeply in the yolk, but they still show very clearly their derivation.   They fre-quently send out pseudopodia-like streamers of nuclei, which fade at the distal ends as though they were gradually dissolving in the yolk.

In Figs. 67 and 72, the margins of the mesodermic area first united a short distance behind the end of the abdomen, leaving a pear-shaped area of yolk, covered by the blastoderm only.

In Figs. 10, 18, 24, 61, 68, the margins have fused over a long distance, leaving along the line of fusion a cloud of yolk and mesoderm cells.   The posterior margin of the fused areas

is usually distinctly indented in such cases, as though the concrescence were proceeding still farther backwards.

Various modifications of the different phases of concrescence are shown in Figs. 14, 16, 18, 20, 24, 43, 49, 59, 61, 62, 63, 65, 67, 68, 70, 71, 72, 79, 80, 82, 83.

The traces of segmentation in the mesodermic area and the very conspicuous concrescing folds are interesting features of Figs. 14 and 20. Observe also the curious knob-like ectodermic thickening at the posterior end of the concresced area in Fig. 16, a not infrequent occurrence. Compare also Pl. III, Fig. 20 with Pl. V, Fig. 49, where the heart is apparently formed for a part of its length.

The whole process of concrescence, as shown by these embryos, reminds one of the concrescence of the so-called margin of the blastopore in vertebrates. The principal difference is that in vertebrates the ectoderm, mesoderm, and endoderm grow over the yolk together, there being no single layered blastoderm covering the whole yolk before the germ layers begin to form, as is the case with Limulus and the arthropods generally. But the presence of this layer of cells can hardly be regarded as a serious objection to a comparison of the processes of concrescence in these two great groups of animals.

In the light of this comparison, the fact that in very rare cases, as shown by Ryder in certain fish eggs, the segmentation of the mesoderm extends to the margin of the concrescing lips of the "blastopore" is very significant. It seems to me most easily explained as a reversion to a condition like that in arthropods.

There are some other interesting suggestions that arise from this comparison. We must not forget that *in Limulus at any rate the growth backwards of this line of concrescence does not increase the body in length. It is merely a part of the haemal surface, thrown back temporarily upon the neural surface by the presence of the yolk. The true increase in length of the axis of the body comes from the proliferation of cells in a primitive streak that lies just in front of where concrescence began.* It is obvious that an application of these principles of growth to vertebrate embryos might clear away some of the difficulties

that have arisen in attempting to explain, first, the increase in length at the posterior end, and second, the relation of the primitive streak to the concresced lips of the "blastopore." In fact, the whole manner of interpreting the formation of germ layers in vertebrates may be advantageously reconsidered from this new standpoint.

I can only touch on these points here, but I shall discuss them more fully elsewhere. The figures are too suggestive to be passed over without comment.

That a half-metamere is to be regarded as the unit of structure of segmented animals is shown, above all (1), as already indicated, by their method of growth and direction of specialization, and (2), by the manner in which half-metameres are omitted or increased or diminished in size, rather than whole ones. On this supposition, certain imperfections in the segments of otherwise typically segmented animals can be explained; such, for example, as *spiral segments*, which may be accounted for as due in part to the imperfect union of twin half-metameres on the dorsal side of the body. Instead of meeting each other squarely, and thus forming a line of equalization of forces, they have grown past each other in opposite directions, and thus each forms a more or less perfect spiral !

*Polarity and embryonic axes* thus appear in a new light. In any segmented animal the embryonic half-metameres group themselves in such a way that they may be represented by axes meeting at a shifting point, as shown in Fig. 5. The line *H. T.* represents the median line and the axis of longitudinal growth, or of *repetition*, and the triangle *ab T*, the form that would result if the individual half-metameres did not mutually modify each other.

*W.x.* and *p.x.* represent the axes of specialization, along which new organs are produced in each half segment. The growth along these axes is at right angles to the median line, and also parallel with it. The tendency to a concentration of organs at the forward end of the embryo, or toward a median fusion and antero-posterior degeneration there, may be explained

as due to the fact that there is greatest tension along those
lines, *H.x.*, and less growth power to resist it, because the
tissues at those points are oldest, and have already exhausted
more of the inherent powers of growth than at *T.*

It may not be venturing too far to claim for this principle still
further applicability ; for example, in explaining the more pro-
found and remote physiological differences between the anterior,
middle, and posterior regions of the body.   Or rather, it would
be better to say that this morphological law is the formal
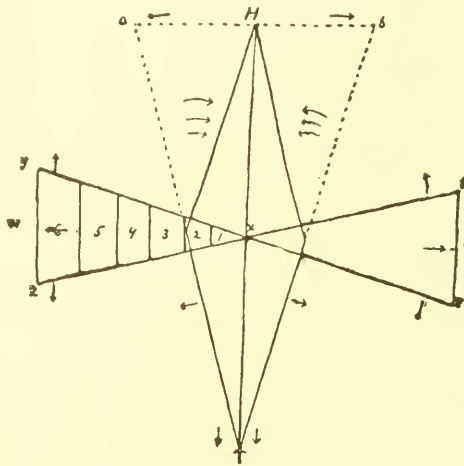


Fig. 5.

expression of the fact that differential growth tension has fixed
the posterior, middle, and anterior regions of the body as the
seats, respectively, of constructive, elaborative, and destructive
physiological processes.   But it will not do to press this
thought too far, certainly not without a precise statement of
the way it is to be applied.

It would also be important to determine whether there is
any relation between these laws of growth and decline and the
different powers of regeneration shown by various regions of
the body, and in this connection we would recall the difference
so manifest in this respect between the head and tail region.
If such were, indeed, the case, there might be some foundation
for the supposition that growth and regeneration are associated

phenomena, antithetical to the associated phenomena of degeneration, specialization, and lack of regenerative power.

We have thus seen how differences in the time and place of growth will in normal embryos produce conditions that cause the fusion and degeneration of organs.

In such cases the fusion and degeneration took place in front of or behind the true extremities of the body. But we see no reason why the same kind of factors should not produce the more extensive median fusion and degeneration seen in the abnormal forms.

This supposition becomes all the more plausible when we consider that the lines of fusion and degeneration are coincident with the lines of greatest stress.

Again, we can see how reversing the conditions that have brought about median fusion and degeneration, *i.e.* diminished lateral cell stress at the anterior end, might permit the formation of double and triple monsters, — as shown by Fig. 7, p. 73.

And finally, if the half-metameres were very much reduced in numbers, the tendency to increase in width at the lateral end would have greater freedom, and more or less ovoid bodies would result, in which the segmentation would be lost sight of in the antero-posterior expansion of the half-metameres. The organs would then tend to be formed along concentric circles, somewhat as in the embryo of a cephalopod.

Having considered what we have regarded as the general principles involved in this class of variations, we will now examine the individual illustrations of the same.

An early stage of fusion is well shown in Pl. V, Fig. 39. The cephalic lobes are constricted, and without character. The chelicerae are absent. The next two appendages are brought closely together, but the space between the appendages of the following pairs becomes greater, till in the last thoracic metamere they are separated from each other by the normal distance.

A tendency in the same direction is seen in Fig. 47, as is shown by the mere trace of cephalic lobes, the absence of

the oesophagus and first two pairs of appendages, and the
drawing together of the appendages of the fourth pair.

In Fig. 46 is a more typical condition.   The cephalic lobes
are entirely absent, and the anterior limbs of the thoracic
margin are converging to form the apex of an inverted V.
The chelicerae have united at $ch.$, and the second pair of
appendages forms a single, long, coiled and slender process, $ap^2$.
The third pair has almost fused, but each appendage still retains
its characteristic shape.

In Fig. 44 the second and third pairs have united, while
every trace of the cephalic lobes and of the chelicerae has
disappeared.   In Fig. 42 about the same change has taken
place, except that the degeneration of fused appendages has
progressed farther backwards, for here both the chelicerae and
second pair of appendages have disappeared.   The fusion of
the appendages of the fourth pair is not quite completed.

Fusion accompanied by degeneration of the two anterior pairs
of appendages is shown in Figs. 43 and 49.   In Fig. 48 fusion
and antero-posterior atrophy have given rise to a form frequently
seen in which little of the embryo but the three posterior
thoracic appendages is left.   In Fig. 41 is a similar embryo in
which the oesophagus is still visible a long distance in front of
the embryo.   It is a pit-like depression merging gradually into
a long cloud of cells lying below the surface, and extending
backward toward the embryo, $d.oe.$   These cells represent
either the degenerating remnant of the anterior portion of the
embryo, or of the oesophagus.

If the process of fusion and degeneration progresses in the
way the various stages just described indicate, we should obtain
embryos in which all the paired organs have been fused and
subsequently absorbed, except the last pair of appendages, or
perhaps the tip of the tail.   This appears to be the case with
the one in Pl. IX, Fig. 106.   We should not forget that
this embryo has been developing as long as the others here
described ; and that it really is in a late stage is shown by its
size, and by the concrescence of the posterior limbs of the
germinal area back of the median appendage or lobe.   There
is no trace whatever of a nervous system, unless the dark area

around the stomodaeum represents the remnants of the cephalic lobes.

What appears to be a still more degenerate condition is shown in Pl. VI, Fig. 69. Here the inner layer cells of the margin of the germinal area are breaking up into dense, irregular masses of degenerating cells, while every trace of the head is absent.

Degeneration of fused organs does not always proceed in that way, as is illustrated by the remarkable embryo shown in Pl. VI, Fig. 50. This is the only instance observed in which three successive metameres show the same degree of fusion. There is no indication as to what appendages are represented in this embryo, probably the last three thoracic, as we have seen that they have greater vitality than any other organs of the body.

Similar conditions are shown in Figs. 52–58, but complicated by partial transverse fission. See Section VI.

*The only exceptions* observed to the law of fusion and degeneration illustrated by the preceding figures — to which many more might have been added — are shown in Pl. V, Figs. 40 and 45. In the first, the cephalic lobes and cheliceral segments have disappeared. The second and sixth pairs of appendages are fused, so that the whole embryo is spindle-shaped. The identity of the appendages is determined by the "dorsal organs," which are very plainly developed. This embryo, then, seems to furnish a case of median fusion and degeneration at both ends. In Fig. 45 the last pair of legs has fused. It probably represents the fifth pair, the sixth having disappeared.

Nearly all of the older multiple embryos show in one or more of the component individuals the typical antero-posterior fusion and degeneration. But a modification of this process occurs there of extreme interest. In such cases the fusion is greatest between the third and fourth thoracic metameres, and diminishes from that point both forward and backward, producing what I have called *hour-glass embryos*. The constriction thus produced may separate the anterior and posterior regions completely. This condition is very beautifully shown

in Pl. VIII, Fig. 98, also in Pl. IX, Figs. 102 and 103. See
also pages 67–71.

The ordinary antero-posterior fusion is well shown in Fig. 94,
where the left-hand embryo consists merely of a tail, the nor-
mal fifth and sixth, and the fused fourth, pairs of thoracic appen-
dages. A mere trace of the anterior part of the embryo is
shown at *oe.*, and the well-developed approximated dorsal
organs are clearly seen at *d.o.* No nervous system or other
organs are visible. In the upper embryo of Fig. 97 fusion and
complete degeneration of the anterior end of the embryo are
shown. The fourth pair has fused and is much reduced, the
fifth is fused and very long and much folded, the sixth pair
is approximated, but not fused. In the opposite embryo the
process has gone on a little farther, resulting in the fusion of
the fifth and sixth pairs. The fourth pair was very small and
partly concealed under the folds of the long and much crumpled
fifth pair. The dorsal organs in the upper embryo are close
together, in the lower they are completely fused, *d.o.*

Still another condition, but similar to that in Fig. 97, is
shown in the right-hand embryo of Fig. 96. There is no mar-
ginal fold here or any dorsal organ. The tail has been thrown
outwards, making a sharp bend in the longitudinal axis, just in
front of the sixth pair of legs. The latter is nearly fused; the
fourth and fifth pairs completely so. I can form no plausible
conjecture as to what the process to the left of the left flabellum
may represent. It is entirely out of place as an appendage, so
far as we may judge from the other cases. It is important to
observe that in Fig. 96, and in both embryos in Fig. 97, the
flabella are larger than usual in embryos of this age, resem-
bling, except in position, normal thoracic appendages. The
problematical appendage of Fig. 96 may be an extra flabellum
belonging to the left fifth thoracic appendage. But no indi-
cation of such an organ has ever been seen in any other
specimen.

In the curiously aborted embryo seen in Fig. 100, the
posterior three or four (?) pairs of thoracic, and two or three
abdominal, appendages are fused along the median line. Here,
all three thoracic appendages show about the same increase in

length and irregularity in shape, which is very unusual, indicating that they fused nearly simultaneously and to an equal degree.

In Fig. 93 the left-hand embryo, at its anterior end, is medianly fused and partially degenerated. The minute pit *oe.* probably represents the remnant of the fused second pair of appendages.

In Fig. 95 fusion and degeneration result in the formation of a slipper-shaped embryo in which nothing is left but a single median appendage and a pit that may represent the last of an appendage, or perhaps the fused dorsal organs.

In Fig. 98 is represented an extremely interesting condition, due in part to transverse constriction or fission. The constriction, followed by fusion, and finally by degeneration, takes place between the third and fourth pairs of appendages, and diminishes from that point in both directions. In Fig. 103, embryo *A*, the same process is carried a little farther, all the anterior appendages being fused to form a row of four median projections which preserve approximately the relative proportions of the three pairs of appendages from which they arose. In the same figure, fusion and degeneration are carried further in embryo *B*, and further yet in embryo *C*, where nothing is left but the fused dorsal organ at *c*, and the last two fused thoracic appendages.

In the triple embryo shown in Fig. 104, all three individuals are in essentially the same condition as embryo *C* of Fig. 103.

In Fig. 102 we have a triple monster in which embryo *A* is nearly normal. *B* has undergone transverse fission, as in Fig. 90, and the anterior half has undergone degeneration till nothing is left but a single median projection representing an imperfectly fused pair of appendages. Embryo *C* is represented by the fused sixth pair of legs and by traces of the abdomen.

It is an interesting fact that in nearly all cases when there has been undoubted antero-posterior fusion and degeneration, the abdomen with its appendages is nearly normal and very well preserved. This is somewhat surprising, as the abdomen is usually much abbreviated and often entirely absent in embryos that show a tendency to abnormality in other respects than in multiple fission or in median fusion.

It should also be observed that in Figs. 102 and 103, much less clearly in Fig. 104, the embryos, beginning with the most perfect embryo, *A*, show increased concrescence and degeneration as we pass in a spiral to embryos *B* and *C.* This interesting fact will be discussed under the head of double and triple monsters.

*Change in Shape of Fused and Degenerating Appendages.*

When two appendages unite, they fuse at the base first, and the fusion extends from that point toward the apex. The resultant appendage is at first much longer and more slender than the unfused ones. It is also usually folded back and forth several times, and otherwise irregular in shape. It subsequently diminishes in length, and finally forms a minute, conical papilla arising from the centre of a saucer-shaped depression. The papilla then disappears, leaving a shallow depression that cannot be readily distinguished from the oesophagus or the fused dorsal organs.

As a general rule the order in which fusion takes place is indicated by the length and coiling of the appendages. When there are several fused appendages visible, they show a gradual diminution in these characters from before backwards, as in Figs. 40, 44, 46, and 102. In rare cases the same characters are presented by all the fused appendages of a series as in Figs. 50 and 100.

V. GENERAL PROGRESSIVE DEGENERATION.

A careful study and comparison of many abnormal embryos of various ages and conditions indicates pretty clearly that most of the abnormalities are due to either a local or a general lack of formative energy. We picture to ourselves two sets of factors at work, the action of one being to increase the quantity and diversification of protoplasm, the other to reduce it to its lowest terms. The action of the first may temporarily prevail, but in the end the second factors are certain to prevent the work of the creative ones.

We assume that there are certain conditions resident in the ovum which, under the action of normal surroundings, guide it through a long series of changes to the expression of that form and mode of action characteristic of what we call the normal organism.

Among the embryos of Limulus, there are some in which there appears to be a very slow discharge of vital processes, producing the retarded or belated forms ; again, nearly perfect individuals are produced within the normal period, but very much reduced in size throughout, suggesting the small but perfect embryos of Amphioxus that have developed from fragments of segmenting ova. There are embryos constituting a third class, in which a particular charge of formative energy was apparently omitted, resulting in the absence of an eye, a leg, a neuromere, or a large, definitely circumscribed area of the embryo, but without visibly affecting the remaining organs. Finally, there is a fourth class, where the embryo seems properly loaded and the various charges properly connected, and it starts off well, following its normal line of flight for a while. But through some inherent defects, the nature of which we cannot even conjecture, progressive development ceases at a point very far from the mark. Then follows a decline, manifested outwardly by a general decrease in size, by fusion and complete atrophy of one organ after the other, till the whole embryo disappears. But as the animal still lives during this decline, and in all outward appearances is sound and healthy, showing even in the last stages the presence of karyokinetic figures, it is obvious that we can only explain this condition by assuming that the death rate among the cells is greater than the birth rate. And as the last survivors are nothing but indifferent, lymphoid cells, we must also assume that the gradual approximation of the death to the birth period cuts off more and more from the period necessary for cell specialization. *The result is a new kind of death for highly organized animals, — one, namely, in which the component cells gradually decrease in number and in specialization till nothing remains of a once complex organism but a few indifferent cells, which in turn themselves disappear by a continuation of the same processes.*

Almost any organ of the body, but more especially the brain, oesophagus, nerve-cord, abdomen, or appendages, may be absent from the start, or may be behind time, or developing normally may quickly disappear, without in any case, so far as could be learned, either affecting neighboring organs or being affected themselves by conditions other than those produced by their own growth.

The variation can be usually traced back to variations in half-metameres. This would seem to indicate that the trunk of the embryo is a group of integral parts arranged in a double series like two rows of segmented animals placed head to head. Each half-metamere seems to be endowed at the outset with a fixed capital of formative material, which when absent in whole or in part, or exhausted, cannot be restored. In addition to this the growth of a half-metamere may be hindered or favored by local mechanical conditions similar to those producing median concrescence and degeneration.

No other supposition, it seems to me, can explain why one leg, for example, out of twelve utterly fails to develop, while the rest go on as usual, although all are equally surrounded by nourishing yolk and by the same medium.

*With these considerations in mind we can understand how a weakening in the developmental forces might be indicated by the four following classes of variation, namely: (1) slowness of development, (2) small size, (3) absence of organs here and there, and (4) gradual reduction of the whole body till it completely disappears.*

Let us now consider these four classes in more detail.

(I) *Almost every one of the embryos we have figured is behind time*, as we have already explained in stating the methods by which the material was obtained, and we have also described how single organs, such as the appendages, disappear or fail to develop, and that the absence of any organ is often followed by the complete degeneration of the rest of the metamere.

(II) One of the most characteristic features of the following embryos *is their small size*, a fact well brought out by comparing the figures on Plates I, VI, and VII, all of which are drawn to the same scale. In these cases the reduction in size

is permanent, and if very marked seems to lead to the final disappearance of the embryo.

(III) *Absence of individual organs.*

*A. Atrophy of the thorax. The most frequent defect in the thorax is the absence of the entire cheliceral segment, a fact of striking significance in connection with its diminutive size throughout the entire group of arachnids.*

Further degeneration may do away, one at a time, with the succeeding thoracic segments in their order from before backwards, but usually leaving two, or more frequently, the three posterior ones, nearly intact. This mode of degeneration may not be preceded by the median fusion previously described. The missing organs are either absent from the start or else degenerate very early. Meantime the cephalic lobes and oesophagus may persist, but in variously modified conditions, as shown in Figs. 26, 27, 33, 34, 35, 62, 67. *These facts show that the anterior part of the thorax is the weakest part and most likely to be absent or to degenerate, and that this weakness gradually diminishes towards the posterior end.*

*B.* When the *cephalic lobes* show partial degeneration, *the reduction seems to take place first along their anterior margins, and to progress backwards independently of anterior degeneration in the thorax.* The law of degeneration of the cephalic lobes as here stated is not so clearly shown as in the case of the thorax, on account of the difficulty of distinguishing the parts. But it seems to hold good in Figs. 18, 20, 21, 22, 27, 47. It is perfectly certain, however, that *the cephalic lobes and oesophagus may be preserved after every trace of one or more segments behind them has disappeared,* as in Fig. 47 and in Fig. 25.

The area where degeneration of the anterior margin of the cephalic lobes has taken place, and which has not been covered by the contracting marginal fold, is nearly always flattened and depressed, as shown in Figs. 10, 11, 16, 20, 27, etc.

*C. In the abdomen, the same law of degeneration seems to hold good, i.e. the most anterior abdominal appendages and neuromeres are the first to degenerate, the posterior ones being the most persistent.* It should be borne in mind that the normal growth of the abdominal appendages is similar to that of the

thoracic. For example, three pairs of thoracic appendages, the second, third, and fourth, appear first, followed by a very small pair in front, the chelicerae, and by two pairs of appendages behind ; the most precocious part is therefore the middle portion. In the abdomen, the second and third pairs first appear, followed by the rudimentary chelaria in front, and by the remaining appendages behind. Compare Figs. 4, 6, and 7.

In the degenerate embryos shown in Figs. 10 and 15, the only abdominal appendages present appear to be the second and third pairs. It is hard to say whether the ones that should develop back of them are merely belated or have degenerated, the general impression at first sight being that there has been a shortening of the abdomen by suppression of its posterior end. However, in more typical cases, the facts seem to support a different conclusion. For example, a large part of the abdomen may be absent, and in its place may be seen either a conspicuous tail-like projection resembling the post-abdomen in young scorpion embryos, or an ingrowth into the yolk, which varies greatly in size and depth in different individuals. If we may use the presence of this depression as an indication of the position of the original posterior end of the abdomen, *it is obvious that it is the anterior metameres that have disappeared, because we usually find this depression or projection, as the case may be, carried forward to a point just back of the thoracic appendages.* Compare Figs. 8, 13, 16, 20, 21, 24, etc.

In Figs. 29, 30, and 32 the same law is illustrated by a different class of cases. Here, also, we see that the principal loss of material is at the anterior end of the right or the left side of the abdomen, not at its posterior end.

*The abdomen, therefore, like an independent embryo, develops its metameres in a sequence similar to that in the thorax. They degenerate from before backwards, and independently of degeneration elsewhere in the embryo.*

*The cases we have just described show that there are three separate points at which backward degeneration may begin, namely, at the anterior end of the cephalic lobes, at the anterior*

*end of the thorax, and at the anterior end of the abdomen. Each of the regions between these points may degenerate independently of the other, and in the same way that the whole embryo sometimes does, suggesting the idea that the embryo consists of a chain of imperfect individuals, such as we see in annelids as the result of imperfect fission.*

A less strongly marked division occurs in Limulus, across the middle of the thorax between the third and fourth segments. It is shown (1) by the marked tendency of the first three segments to degenerate and of the last three to persist, (2) by the transverse fission that occurs there, see following paragraphs (p. 67), and (3) by the presence of an enlarged pair of sense-organs ("dorsal organs") opposite the first of the last three segments, just as the lateral eyes lie opposite the first one of the first three segments.

When the degeneration is incomplete, it may manifest itself by a partial median fusion and a decrease in size of the organs along the lines separating the regions above indicated.

*These cross-lines, where degeneration is most likely to occur in Limulus embryos, are the regions showing the least "vitality"; they correspond to the regions where a reduction in size and a tendency to undergo median concrescence are frequently seen in the adult individuals from various groups of arthropods. In Limulus these lines of weakness are cleavage planes separating regions having different potentialities, and corresponding to regions possessing different morphological characters in many other arthropods. Broadly speaking, the salient morphological characters seen in the various regions of the body of arthropods are similar to those that appear as abnormal variations in Limulus embryos; and, as we have seen, these characters are mainly those due to difference in size, specialization, and median fusion, and these in turn have to be referred back primarily to differences in the power of growth.*

The four regions of the body in Limulus embryos are roughly shown in the adjacent Fig. 6. The lateral constrictions and the transverse lines indicate approximately the position and amount of median fusion and degeneration.

The repetition of three segments in successive regions of

the body is a striking fact, and it should also be observed
that each individual part of the embryo, and especially the
ends, tends to assume the shape of a single or double V,
characteristic of entire embryos
undergoing atrophy by median or
apical fusion and degeneration.

In comparing these variations in
Limulus, Fig. 6, with the normal
conditions in other arthropods, I
will merely bring to mind the fol-
lowing facts, to which others might
be added.

(*a*) In the first region we have to
recall the absence, or median fusion,
or degeneration, of appendages at
the anterior margin of the cephalic
lobes, especially in insect embryos,
and the absence or fusion or de-
generation of the anterior pairs of
ocelli in arachnid embryos (scor-
pions, spiders, Limulus) and the
great development of the antennae
and lateral eyes arising from the
posterior margins, as in insects and
crustacea.[1]



Fig. 6.

Diagram to show lines and regions of
most frequent degeneration. The
lateral constrictions indicate potential
fission planes due to partial median
fusion. The cross-lines indicate the
relative weakness of the different
regions.

(*b*) The reduction and the fre-
quent median fusion of the ap-
pendages just back of the mouth in insects, crustacea and
arachnids, and the much greater size of those at the posterior
end of the thorax. The frequent absence of the chelicerae
in Limulus is to be compared with the small size of these
appendages in the vast majority of arachnids. The manifest
weakness of the first three pairs of thoracic appendages in

[1] The degree of median fusion may therefore be taken as an index of the
original order of serially homologous organs. Applied to vertebrates, this would
indicate that the most anterior sense-organ is the pineal eye, followed by the
olfactory organ and the lateral eyes, just as is the case in Limulus. This
harmonizes with the conclusions that I have reached on anatomical and embryo-
logical grounds of an entirely different nature.

Limulus, as shown by their more frequent absence and median fusion, is comparable with the small size and frequent median fusion of the first three pairs of post-oral appendages in insects and other arthropods.

(*c*) There is at the anterior end of the abdomen a region of condensation, specialization, and degeneration, forming what I have called the *vagus region* of scorpions and Limulus, and which, in a paper on " The Origin of Vertebrates from Arachnids," I showed was probably of wide distribution in the arachnida, including the trilobites and related forms.   In scorpions, it consists of four very much condensed metameres, and in Limulus of two or more, which in both forms are provided with rudimentary appendages, nearly or quite fused.

(*d*) Finally, the very wide distribution of well-developed terminal appendages through all groups of arthropods is a manifestation of the same law of growth.

*In conclusion, therefore, it would appear that the division of the body of arthropods into successive regions composed of several segments is not due primarily to specialization or adaptation, either by or for any particular use, or through disuse, but to some form determining forces that govern growth.   The same factors in all probability produce in a similar way metameric segmentation, transverse fission in annelids, and determine the length of the body in a given individual.*

(IV) *The complete degeneration of the whole embryo* is an obscure process, and probably varies considerably in different individuals.   A continuation of the various local degenerations previously described has, without doubt, gradually produced the misjointed fragments of embryos seen in Pls. VI and VII.   They admirably illustrate Empedoclian fancies.   The embryos themselves give some indication of the various ways in which they have degenerated.   They also indicate that the final stages of degeneration lead to a tolerably uniform condition.

With the disappearance of all the appendages, the embryo may, in the class of cases we shall now consider, be reduced to a mere pit or sac, yet preserving certain features which show

clearly the stage in which the whole embryo would have been, had no degeneration taken place.

Perhaps the best illustration of this is shown in Pl. VII, Fig. 82. The mesodermic area is relatively large, and its posterior margins are thickened and well advanced toward concrescence. There is a very large, projecting tail lobe, like that in Figs. 48, 49, and 94, and *yet of the body proper (which should have all the organs seen in Figs. 5 and 6) there is nothing left but a deep, thick-walled pit, with a triangular opening to the exterior.*

A similar condition is shown in Fig. 83, where the undifferentiated remnant of the body forms a Y-shaped sac with an oval opening at its posterior end.

Figs. 84, 85, 86, 87, and 88 are various modifications of the same condition.

In Fig. 88 the thickened margin of the mesodermic area has broken up into the star-shaped masses of degenerating cells, so frequently seen in the later stages of degeneration.

These embryos *represent what is left after invagination, median fusion, and progressive degeneration have attacked with varying success every part of the body.*

Most of the embryos shown on Pls. V and VI would probably have reached this condition ultimately. In some of these cases one can distinguish here and there an appendage, or some other organ; but the remaining parts may be so distorted or misplaced that it is impossible to distinguish literally as well as figuratively any head or tail to them, as in Figs. 71 and 56, and others which we have not space to figure.

In Fig. 81 the large posterior depression appears to be the remnant of the anal plate, and the three obscure pits in front of it, the last of three fused pairs of invaginated appendages. In Fig. 80 everything has disappeared except the large pit, situated at what appears to have been the anterior end of the embryo.

Whether the huge projection in Fig. 69, Pl. VI, and Fig. 106, Pl. IX, represents a fused and partly invaginated pair of thoracic appendages or a tail-like projection of the anal plate is hard to determine.

*The curious pits and sacs just described themselves finally degenerate into a mere cloud of cells varying in form and appearance,* Fig. 89.    Many embryos of this kind show slight indications of a central depression, probably the last trace of a sac like those just described.

*I have found some eggs among those that had been kept alive for three or four months in which no trace of cells or nuclei was visible in surface views,* yet they appeared perfectly sound and free from decay, even when stained and cleared in clove oil. They are probably eggs that have passed so far beyond the stages of degeneration shown in Fig. 89, that even the last few cells have disappeared.

There is another series of degenerated embryos that are very interesting from their resemblance to the early stages of normal embryos.    They consist of two groups of cells like two primitive cumuli, one corresponding to the head and the other to the tail end of the body.    This condition has already been passed by the embryo shown in Fig. 1.

Whether or no degenerating embryos as a rule pass through this condition with separate *Anlagen* for head and tail is doubtful, nevertheless it is a variety very frequently seen.    It is well shown in Figs. 72–78.

There is no way to determine certainly whether an embryo like that in Fig. 73 has degenerated, or whether it has been kept, by something hindering normal development, in approximately its original condition.    But the great age of the embryo, the evidence in other embryos like this one, of concrescence, and the breaking up of the margin of the circular mesodermic area, bears out the assumption that it has undergone profound degeneration.    The anterior mass of cells and the pit usually seen in its centre probably represent the degenerated cephalic lobes and oesophagus, and the posterior cloud, the anal plate, and telopore.    Both of these discs are in all this class of cases about the same, but the posterior one is usually the larger and thicker.    Sections of the discs show various conditions, from one where there is merely a thick, homogeneous mass of cells, flush with the surface, but sending into the yolk pseudopodia-like masses of degen-

erating cells, to one in which it consists of a thin layer
of cells deeply invaginated in the centre, and from the in-
vaginated part alone arises a cloud of scattered yolk nuclei.
Pl. X, Fig. 73.

Whether the depressions in Figs. 72, 79, and 81 represent
the oral and anal depressions, or the pits left after the degenera-
tion of fused and invaginated appendages, or of one or more
segments, as in Pl. VI, Fig. 61, cannot be determined.

In some cases, as in Figs. 69 and 73, where the outline of
the axis of the embryo may be faintly distinguished, there is
no cephalic cloud of cells visible.

*It would thus appear that degeneration may carry old embryos
back to a state resembling that seen in very young embryos, i.e.
one where it consists of a cluster of proliferating cells at either
end.*

*These facts seem to indicate that the body of the embryo is
not a single organic unit, such as it would be if it were an
elongated gastrula with fused lips, but rather one of a double
origin.*

We may regard the head *Anlage*, from which arise the
cephalic lobes and oesophagus, as representing the remnants
of a trochosphere, and the posterior *Anlage* as the primitive
trunk which arises from the trochosphere as a bud-like out-
growth.   I have already shown elsewhere,[1] that the origin of
the stomodaeal nerves in Limulus supports such a view.


### VI.  Fission.

All the cases of fission that I have seen in Limulus affected
embryos in which the thoracic appendages were well developed,
or if they were not present it was evident that their absence was
due to degeneration.

The multiple embryos must necessarily, from the methods of
obtaining them, have been well advanced ; and any abnormality
in the early stages is easily overlooked among the hundreds of
eggs that one must examine in order to find them.   Neverthe-
less it seems probable that fission does not usually begin till a

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[1] Morphology and Physiology of the Brain and Sense-Organs of Limulus.

comparatively late period. This is certainly the case with the embryos shown in Figs. 90 and 91, the type most frequently seen.

In the formation of multiple embryos, we may distinguish two kinds of fission:

(1) Transverse fission, dividing the embryo into anterior and posterior portions, the plane of fission being usually between the third and fourth thoracic appendages.

(2) Longitudinal fission, beginning at the anterior end. This is the most common form, and one presenting a great number of modifications through degeneration. *The process is essentially different from that of transverse fission, for the latter is the result of a local transverse concrescence and degeneration, while longitudinal fission consists in the formation of two new halves of an embryo along the median line of one already existing. The formation of the new halves begins at the anterior end and extends gradually backwards, one new half being a mirror image of the other. The old halves are thus thrust apart, and with the newly formed halves make new embryos.*[1]

*This process may be repeated a second time in one of the new embryos, thus producing three embryos, tail to tail, consisting of the two original halves plus four new ones.*

### A. Transverse Fission.

There are more or less clear indications of this form of fission in a great many embryos, but it is rarely that it is very clearly marked. The fact that the plane of fission occurs at a definite point is very remarkable, and indicates a break in the morphological continuity of the embryo of considerable theoretical interest.

In normal embryos, neither the order of development of the appendages, nor their size or shape, gives any indication of this cleavage line. The great development of the lateral, segmental

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[1] Indications of longitudinal fission, beginning at the posterior end and extending forwards, are very rare. No *bona fide* case has been observed, and I doubt whether it ever occurs in Limulus, the cases in which it appears to exist being perhaps better explained as malformations of the posterior end of the body, rather than as the beginning of true fission.

sense-organ, *d.o.*, is the first thing to suggest a break in the apparently homogeneous series of thoracic metameres.

But we frequently see in embryos which in most respects do not depart from the normal type, quite constant differences in the arrangement of the thoracic appendages, which may be regarded as indications of the cleavage plane in question. In Pl. III, Fig. 23, *the potential fissure plane is indicated by an increased distance separating the third and fourth pairs of appendages.* This would be of little importance perhaps taken alone, but such cases are frequently seen, so that it probably has the significance attached to it.

Another class of cases illustrating the same thing consists of embryos in which the *three anterior pairs of appendages have a markedly different direction of growth* from the posterior ones, suggesting the characteristic difference in appearance between the mandibles and maxillae of an insect embryo and the thoracic appendages. These cases are also comparatively common, typical cases being shown in Pl. III, Figs. 19 and 22.

None of these cases would attract attention if it were not for the fact that the constriction about to be described occurs between these two sets of thoracic metameres.

A most beautiful example of transverse fission, and one that throws a good deal of light on the ones about to be considered, is shown in Pl. IV, Fig. 29. This case is, however, complicated by the partial degeneration of the left half of the thorax, and the complete absence of the left half of the abdomen. The constriction is due to the presence of a transverse line of degeneration having its greatest intensity along the fourth segment.

In Pl. VI, Fig. 51, is an obvious constriction between the third and fourth thoracic metameres. When the constriction is more conspicuous, it has evidently been preceded by fusion of the right and left halves, the degree of fusion diminishing from the point of greatest constriction toward the anterior and the posterior end. This is beautifully shown in the lower embryo of Pl. VIII, Fig. 98. As every pair of appendages is present in this case, there is no question about the exact point of constriction.

A similar case is shown in the single embryo in Fig. 52.   A comparison of this embryo with the preceding leaves little doubt that the constriction has taken place between the third and fourth segments.   The cephalic lobes are much degenerated, and the chelicerae are absent.   The modification of the remaining appendages is easily determined from the lettering.

In Figs. 54 and 55 are two other illustrations of transverse fission accompanied by degeneration.   In Fig. 55 the anterior portion of the thorax, consisting of three pairs of appendages, but without cephalic lobes, oesophagus, or neuromeres, is widely separated from the posterior portion, which consists solely of a conical projection representing either an enlarged caudal lobe, or else the last pair of fused, thoracic appendages.   One or two small pits in front of it indicate, probably, where the other posterior thoracic appendages have disappeared.   In Fig. 54 is a curious modification not easily explained.   It appears to be due to a transverse constriction separating the thorax, the only part that is left, into two portions.   The two pairs of appendages of the anterior part have fused to form a median row, the fusion being indicated by the great length of the two twisted median appendages.   The appendages were apparently absent on the right side of the posterior portion, so that it is spirally twisted.

In Fig. 53 an earlier stage of the same process is shown. There has been a partial constriction between the third and fourth pairs of thoracic appendages.   In the anterior portion the third pair have fused, the second are still widely separated, while the first pair and most of the cephalic lobes are absent.   In the posterior portion the fourth pair are fused, and the right fifth and sixth appendages, including their neuromeres, are absent. The fifth and sixth appendages of the left side are very large, and owing to the unequal bilateral development, thrown spirally toward the right.   The distortion of the axial line is also shown by the position of the telopore, *t.p.*

Still other embryos where median fusion has played a conspicuous part, but which still show evidences of transverse fission, are shown in Figs. 56, 57, and 58.   In Fig. 58 the anterior projection probably represents the remnant of the fused

anterior thoracic appendages ; and the posterior infolding, the invaginated remnants of the posterior thoracic appendages. It is not improbable that Figs. 74 to 78, Pl. VII, represent still further degeneration of this kind.

We cannot be certain whether a common form of embryo such as that in Figs. 48 and 94 is due to gradual antero-posterior fusion and degeneration up to the fourth thoracic appendages, or whether there has been previous fission at that point, followed by the degeneration of the anterior portion. But in either case it shows a distinct difference in the vitality of the two parts, which confirms our view of their morphological difference.

In Fig. 103 we have the most remarkable example of constriction, followed by antero-posterior degeneration, that has been met with. The least modified embryo, *A*, is a further modification of the condition seen in the lower embryo in Fig. 98. All the organs in the anterior portion have fused leaving a median row of three long appendages, and fairly developed cephalic lobes and oesophagus.

In embryo *B* the whole anterior portion has degenerated, while the closely approximated dorsal organs appear like a pair of eyes in front of what is left of the posterior part of the thorax.

In embryo *C* the same process is carried still further, the dorsal organs now forming a median pit in front of the remainder of the thorax, analogous to the median eyes of the cephalic lobes.

This condition has been reached by all three of the embryos shown in Fig. 104.

The persistence of the posterior part of the thorax, following transverse fission, is also well shown in the very advanced embryos in Figs. 96, 97, and 100. The point where transverse constriction has occurred, and the degree of antero-posterior degeneration are about the same in each.

That in cases like those just referred to there may be actual separation of the anterior part of the thorax from the posterior is shown by Fig. 102, in embryo *B*, where in the two widely separated portions of the thorax there is nothing left in the anterior one except an imperfectly fused pair of appendages.

*In conclusion, therefore, it may be stated that transverse fission occurs in the region of the fourth thoracic segment, dividing the body into two parts, which show different morphological characters and different degrees of vitality. The separation of the two parts is the result of a constriction brought about by the successive median fusion and degeneration of the organs lying along that segment, the most median, and therefore the oldest and most specialized, fusing and degenerating first, and the others follow-ing in the order of their position on the segment. There is a gradual diminution of the effects of degeneration both in front of and behind this line. A similar line of degeneration is seen in the region of the cheliceral segment, just back of the cephalic lobes, and in the region of the first abdominal or cheliceral seg-ment,* Fig. 6. Further evidence of degeneration at these points is seen in normal adult animals, in the small size of the organs on these segments, and in their tendency to unite in the median line. In the abnormal embryos it is shown by an exaggeration of these conditions, resulting very frequently in the absence of entire segments.

*The inherent tendency to diminution of growth along such lines is what has, in all probability, led to the division of the body of arthropods into successive regions, such as cephalic lobes, pro-thorax, thorax, abdomen, post-abdomen, etc.*

*B.* Median Longitudinal Fission; Double and Triple Embryos.

This method of forming multiple embryos is comparatively common. It often begins at a late period, after the full number of normal appendages and metameres is formed.

The first steps in the process have not been observed, but a study of Fig. 90, where fission has not progressed very far, shows what the initiatory processes must have been like. There can be no doubt that we have actual fission here, and not fusion of two originally independent embryos. It is also clearly shown by such cases as that in Fig. 98, where the left half of the larger embryo is defective, owing to the lack of the formative material necessary to produce an entire new half; and especially by the fact that in all these cases the embryos

*match each other exactly, and always in the same way, which could hardly be the case if two separate embryos had united with each other through accidental contact.*

In Figs. 90 and 91, then, if we have fission instead of fusion, one half of each new embryo must come, not from the fission of already existing organs, *but by the formation of two new halves.* Where does this new material come from, and by what processes of growth are the new halves formed?

In answer to the first question we may say at once that there is not the slightest evidence of the existence of any formative material in the shape of proliferating cells along the median line where the new parts are forming. The old halves are, to all appearance, separate from the new. As they are already specialized, and sharply circumscribed, there seems no way open to explain the origin of the new half of a segment by lateral budding, or by regeneration, or by growth, from the corresponding old one. We might suppose, perhaps, that the new neuromeres in Figs. 90 and 91 come from a kind of regeneration of the old one, *but that could not possibly be the case with any of the new organs lateral to the neuromere,* such as the appendages, sense-organs, and margin of the mesodermic area.

We are left, then, entirely to conjecture as to the origin and causation of the new growth. We shall return to this point later.

The new halves are formed, however, in a very definite manner, which we shall now proceed to explain. *In brief, they appear in exactly the reverse order of that by which the old ones disappear by median fusion!*

### 1. Double Embryos.

In the formation of double embryos, two new halves belonging ultimately to separate embryos, are produced, each half being the mirror image of the other, to which it is united along its *lateral* margin. The new halves first appear at the anterior end of the median line of the old embryo, probably between the anterior median margins of the cephalic

lobes. They form a triangular body which grows backwards at the apex, and laterally in either direction, along lines parallel with the base of the triangle. The two old halves are thus wedged apart till, with the complete formation of the new halves, the embryos form a straight line, tail to tail. Fig. 92.
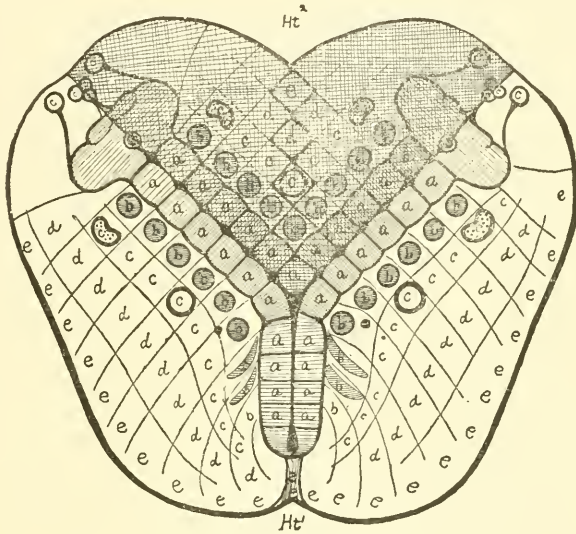


FIG. 7.

Diagram to illustrate the law of formation of new halves in double embryos.
The new halves are shaded.

*Each new organ of a metamere appears first as a single organ common to both embryos, and having a normal position for each,* Fig. 7. Additional organs are formed in the same way, in the order of their arrangement on the metamere. For example, the organ nearest the median line is formed first; this then divides into two, and the one lateral to it appears between them as a single organ common to both embryos; this divides, and the next one appears in the same place, till all the organs of a given metamere are formed. The same process takes place in the next posterior metamere, but it is always one step behind that in the metamere in front of it.

The result is that in an embryo that has nearly completed its division, as in the diagram (Fig. 7), we find a row of

median, unpaired organs, which in their serial arrangement
follow the same order as in the metamere itself, namely, *a*, *b*,
*c*, *d*, *e*.

It is thus obvious that the rate and direction of growth is
such that each new half tends to form a right-angled triangle,
the apex of which coincides with the posterior end of the
embryo, the altitude with the mid-ventral line, and the base
with the width of the oldest half-metamere.

We thus see a gradually increasing series of new organs
appear along the altitude of the triangle, or the old mid-ventral
line.   They attain their full size and perfection of form for
that stage ; then each divides into two (the one a mirror image
of the other), which move away from the median line, and in
their former place appears a new unpaired set, composed of
the organs that normally lie lateral to the ones just formed.

The successive ·eruption of new series of organs along this
median line, and the manner in which they divide and move
away from it to right and left, is so entirely different from
what we have been accustomed to see that it is very impressive.
This effect is not diminished on further reflection.

Examination of the diagrammatic figure illustrating an
incomplete double embryo shows that each metamere has a
lateral growth similar to that which occurs at the posterior end
of segmented animals. *In posterior, apical growth a number
of like parts, or segments, increasing in age and in differentia-
tion toward the anterior end, is produced.   In the lateral growth
of a half-metamere a series of unlike parts is produced.   But
while there is a similar method of growth in both cases, there is
in the second case a greater increase of specialization in passing
from the growing point toward the part first formed.*

If we carry the process seen in Pl. VIII, Fig. 90, back to its
beginning, we are led to conclude that fission began by the for-
mation of new organs in the median line at the very anterior
end of the body, that is, in the indentation separating the right
and left semicircular lobes of the brain.   The first organs to
appear then must have been the cephalic lobes.   But each half
of a cephalic lobe consists of at least two parts, a lateral one,
the optic ganglion, and a median one, the semicircular lobe and

the cerebral hemisphere. It is not probable, therefore, that a common median cephalic lobe developed in the same way as a common appendage, for the optic ganglion of one side would have to take the place of the cerebral hemisphere of the other, or *vice versa*. The different parts are probably formed as independent organs in a sequence from the median line laterally, as is the case with the different organs on a metamere. That is, a common semicircular lobe is first formed, then the cerebral hemisphere, then the optic ganglia, and finally the lateral eyes.

In the earliest stage of a double embryo observed, Fig. 90, we see how the characteristic median row of unpaired organs is forming. The fourth neuromere and the third appendage are unpaired, but the tips of the second appendage are just visible as two minute papillae, at the summit of a great bilobed projection.

A comparison of this appendage with the third median pair in Fig. 91 shows that *each new median appendage divides first at the apex, the separation gradually extending toward the base. This, it will be observed, is the exact reverse of what occurs when the appendages of the right and left sides unite to form a single median one.* Compare Figs. 42, 43, 48, and 49.

In the median line, at the anterior end of the double embryo shown in Fig. 90, is a small depression in a dark mass of cells. The pit probably represents the common *Anlage* of the dorso-ventral muscles, which are seen to the right and left of each head, reaching the surface ectoderm near the apex of the optic ganglion.

In Fig. 91 the separation of the two heads has, by the wedge-like ingrowth of the two new halves, been carried down to the fifth thoracic metamere.

There is perhaps an actively growing point at the apex of the V, which gradually works backward, thrusting the old halves apart. But there is no indication whatever in surface views of such a proliferation, for each new part has the appearance of being as complete in every detail as the corresponding organs in the old halves.

My sections of double embryos were not perfect enough to be

of much assistance. I doubt, however, whether the most perfect
sections would indicate any materially different condition from
that seen in the surface views. It is somewhat surprising that
the tension necessary to push the old halves over the yolk does
not produce in them any other distortion than a gentle curvature
to right and left. It shows how truly each part assumes its char-
acteristic forms, dominated by its inherent structure rather than
by the mechanical stress of adjacent organs. For example, in
swinging the head of either embryo to the right or left, the
movement may coincide on one side with certain lines of
growth, but be directly opposed to them on the other. This
may be seen in the diagram, Fig. 7, where it is obvious
that the left side of the right-hand embryo is being swept
along in the direction of its own lateral growth, and it must
receive some additional impulse with which to overcome the
resistance to it. But on the right side of the same embryo
the movement is against the line of lateral growth. The
internal stress at the lateral $C's$ is very different from that
at corresponding points in a single embryo, and very different
from what it is at the median $C$, and yet the resulting organs
under these diverse conditions are the same !

The reason these varying mechanical conditions have so
little effect on the form of the organs is probably because they
are so transitory. They differ essentially from the permanent
and gradually increasing stresses that produce concrescence
and degeneration.

In Fig. 92 the separation into two embryos began at an
earlier period and is carried much farther than in the ones just
considered. The head of each embryo has been swept over
an arc of 90°. Further movement in that direction is pre-
vented by the interference of the lower margins (as the figure
stands) of the old mesodermic area. It is probable that the
tension produced by this interference would about equal the
devaricating force in the new halves, as soon as the two
embryos formed a straight line tail to tail. If this condition is
reached at an early period, the same forces, *i.e.* the tendency
of the posterior margin of the mesodermic area to concresce
behind each embryo, will ultimately force them apart. But

if the embryo is well developed before division takes place, the head ends will meet each other on the side of the egg opposite to the tails and will thus tend to prevent their further separation.

In Fig. 93 separation of the two embryos has taken place, as shown by the arrows, in a manner similar to that in Fig. 92. But before the new sixth thoracic appendage and those of the abdomen were produced, median fusion and antero-posterior degeneration took place in the left-hand embryo in the manner so frequently observed in single embryos. See Pl. V. The cephalic lobes have disappeared, and the first three thoracic metameres have fused in the median line, leaving nothing but a minute pit to represent the second pair of appendages, and a small, median papilla to represent the third and fourth.

In Fig. 94 the same process is carried further. In the way the egg now stands, the common axis of the two embryos was originally nearly vertical, as in Fig. 95. The lower one then moved upwards past the left side of the other one to its present position. It now occupies the free surface of the yolk between the dorsal margins of the right-hand embryo. Median fusion and antero-posterior degeneration then followed, whether before or after separation cannot be determined, reducing the left-hand embryo to the posterior part of the thorax and the abdomen. The latter has a very conspicuous tail lobe similar to that in Pl. V, Fig. 48.

It is seen on comparing Figs. 90, 92, and 98, that one of the hearts and tail lobes must have belonged to the original embryo; the others must be entirely new. For example, in Fig. 92 the heart and tail lobe just above $y$ will be formed by the concrescence of the right and left margins of the old embryo, while the heart appearing at $x$ will be entirely new. The two sides are so much alike in Fig. 97 that we cannot tell which is the old half and which the new. Now if the tail ends of these embryos should grow past each other, as in Figs. 94 and 96, then one embryo would carry off, according as it passed to the right or left of the other, either a new tail and a new heart, or the old ones. If we knew whether the heart of the original embryo in Fig. 97 was to the right or the left, as the figure now

stands, we could tell which was the new tail and which the old. This can be done in Fig. 96, for it is obvious that both halves of the tail lobe of embryo *A*, probably as far up as the last left thoracic appendage, are those of the original embryo. Embryo *B*, however, has carried off an entirely new tail lobe. The result is that the right half of embryo *A* will consist of the right half of the original embryo, and all its left half will be a new formation, except the abdominal part. In embryo *B*, its left half is that of the old embryo, except the posterior end, which is new, and was probably produced by the backward regeneration of that half of the body. Its right side is entirely new. A similar condition must prevail in Fig. 94, except that embryo *B* has here carried off the *old* abdominal lobe, and *A* the new one.

These conditions are best seen in the diagrams, Figs. 8 and 9, where the shaded portions represent the old parts, and the light ones the new. In Fig. 9, embryo *B* pushes past the left side of *A* and carries off a new heart and tail lobe, and the posterior portion of the abdomen on the left side.

In the well-balanced condition seen in Fig. 97, it is unlikely that further changes in the relative positions of the two embryos would take place. But if they should be forced to grow past each other, a different proportional combination of old and new elements would be produced from that in Fig. 96, and the cause of this would be due, in part at least, to the different periods in the formation of the new halves at which the embryos separated from each other.

In Fig. 95 the two embryos, arising by longitudinal fission as in the preceding ones, are still united tail to tail, but degeneration of the lower one has progressed so far as to reduce it to a slipper-shaped thickening with a depression in its centre, from which arises a papilla, probably representing the last trace of the fused sixth pair of thoracic appendages. A dark-rimmed depression in front of this probably represents the remnants of another pair.

In Fig. 98, division has produced two nearly complete embryos, the new half of the abdomen of each embryo being absent. The lower embryo has already begun to degenerate,

and presents a very good case of transverse fission, accom-
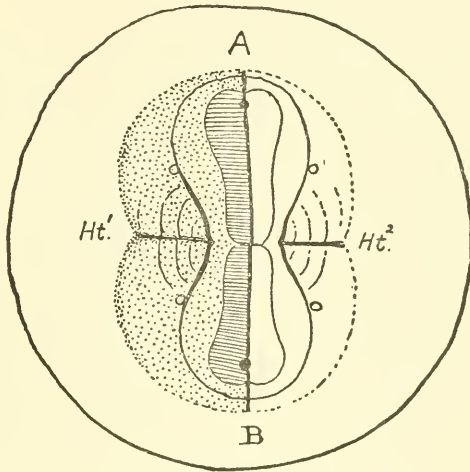


Fig. 8.

Diagram to illustrate the probable proportional composition of double embryos out of
old and new parts.
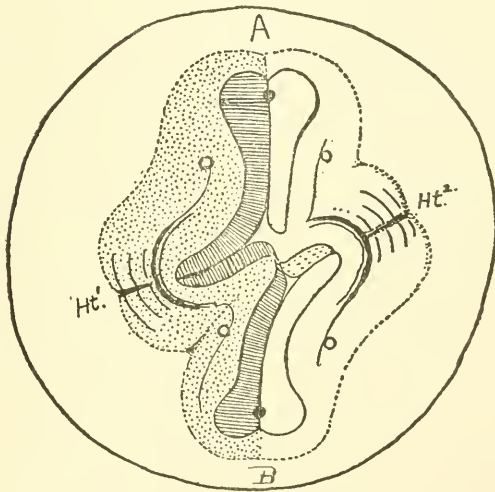


Fig. 9.

Diagram to illustrate the relation of old and new parts in the abdomen of separated double embryos.

panied by median fusion.   There is a marked "weakness"
of the new half of the upper embryo, shown by the absence of

the second, and the small size of the invaginated fourth, appendage. There is no indication of segmentation lateral to these appendages, and the left half of the neuromere opposite the fourth appendage is apparently absent. The weakness of this half is further shown by a gentle curvature of the head toward the left, although at this stage in other double embryos it is curved to the right.

In Fig. 97 is a very late stage (about the trilobite stage) of a double embryo. The two embryos are tail to tail in a straight line, and so symmetrically developed that there is no indication whatever of the direction in which the two embryos separated from the primitive median plane. Median fusion and antero-posterior degeneration of each embryo has progressed as far as the fourth thoracic segment. As indicated by the large segmental sense-organ and the appendages, fusion and degeneration have progressed farther in the lower embryo than in the upper one. Interesting features of these two embryos are the two perfect hearts and tails, extending laterally at right angles to the continuous nerve-cords, and the large flabellae, which look like a separate set of appendages.

In Fig. 96 is what appears to be a case of division of a different nature from those just described ; but a careful examination will show, I think, that it is after all the same. We can easily and satisfactorily explain its condition by assuming that longitudinal division gave rise to two embryos in a straight line tail to tail, and that they separated and pushed past each other in opposite directions. The anterior end of the right hand embryo is turned vertically downward and has undergone median fusion and degeneration. This resulted in the fusion of the fourth and fifth pairs of thoracic appendages, leaving the sixth pair and the abdomen in a nearly normal condition. The abdomen, as indicated by the dotted line, has finally been thrown sharply to the right by the growth of the left side of the thorax of the larger embryo. The result is an embryo much like that in Fig. 97, only its longitudinal axis is bent at right angles, the anterior portion of what is left being in its primitive position, parallel with the axis of the larger embryo.

In Fig. 100 is a much older embryo, with the remains of a second rudimentary one attached to its right side. Separation of the two embryos probably took place in the direction indicated by the arrows, — the left-hand embryo undergoing median fusion and antero-posterior degeneration. The remaining appendages are so twisted and obscure that their identity could not be certainly determined. They appear to represent the fused third, fourth, and fifth pairs of thoracic appendages arranged in single line. The sixth pair are fused at the base, leaving the ends free. The first two pairs of abdominal appendages have also fused, something that rarely occurs with them, to form two median, tongue-like projections, each of which is bent almost at right angles.

On examination from the dorsal side, Fig. 101, the outlines of the two embryos are distinctly seen. The median dark streak in the smaller embryo probably represents the remnants of the oesophagus, or perhaps the heart. The large semilunar band of cells consists of the *fibre cells* that I have described elsewhere, and represents the concresced margins of the mesodermic area. It should lie on the anterior dorsal surface of the thorax, but is here thrown forward towards the ventral surface.

The only instance observed in which there seems to be a deviation from the method of forming double embryos, just described, is shown in Fig. 99. This embryo was accidentally destroyed before a finished drawing of it was made, but it had been completely outlined and carefully studied. There is no question therefore about the correctness of the details of structure, as far as they are given.

At first sight the right-hand embryo appears to have arisen by longitudinal regeneration from the left side of the larger one. Aside from the fact that no indication of such a process has been seen elsewhere, it is difficult to imagine in detail the method by which it was brought about. It is not necessary to discuss these possibilities, as long as we can reduce this type to the usual one by assuming that the form of degeneration frequently seen in single embryos, has affected one of the embryos almost coincident with its separation from the other.

For example, if fission progressed in Fig. 91 down to the
abdominal region, leaving the newly formed abdominal ap-
pendages as unpaired organs, just as the fourth appendage now
is ; and if the anterior part of the right-hand embryo under-
went median fusion and antero-posterior degeneration up to
the fifth thoracic metamere, we would then have a condition
like that in Fig. 99. I see little reason to doubt that the
embryo in question was formed approximately in that way.


### 2. TRIPLE EMBRYOS.

Illustrations of triple embryos are seen in Figs. 102 to 104.
The steps by which they were produced were probably as
follows : It is assumed that in the beginning there was a
single, normal embryo, and that it gave rise, by longitudinal
fission, in the manner already described, to two embryos, each
one composed of a new half and an old one, Fig. 7. The
right-hand embryo then divides in the same way as the first,
by the formation of two new halves, Fig. 10. The result
is that the halves of the original embryo are now separated
from each other by an angle of about 240°. The second
embryo, $B$, is an entirely new formation, but embryo $A$ con-
sists of the original right half plus a new left half, and embryo $C$
consists of the old left half plus a new right half.

The original line of concrescence of the posterior margins of
the mesodermic areas, $Ht.^1$ (along which the heart is formed),
remains unchanged, except in its position on the yolk, just as
the original line of concrescence becomes the lower one in the
double embryo. The other two heart lines, $Ht.^2$ and $Ht.^3$, are
*entirely new formations.*

If we may speak of the new halves as separate genera-
tions, their relations to each other in a triple embryo are
as follows : In embryo $A$ the body consists on the right
side of the right half of a mother, and on the other of the
left half of a daughter. Embryo $B$ is composed on the right
of the right half of a daughter, and on the left of the left
half of a granddaughter. Embryo $C$ is composed on the left
side of the left half of a mother, and on the right side of the

right half of a granddaughter.   The line of concrescence of
the heart lines will have at $Ht.^2$ on one side the right half of
one daughter, and on the other the left half of another daugh-
ter.   These two halves cannot be said to belong to the
same daughter without assuming some preformation jugglery
by which the molecules of one half were shifted to the
wrong side of the other.   At $Ht.^3$ there is on one side of
the concrescence line the margin of the right mesodermic
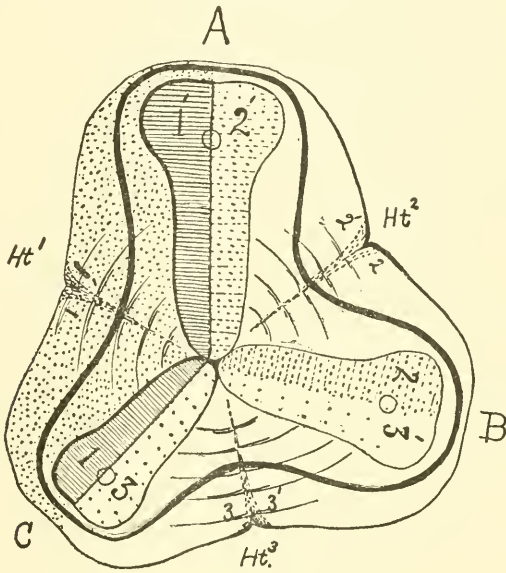


Fig. 10.

Diagram of a triple embryo, to show the relation of the old and new parts.   Original halves,
1 + 1'; second generation, 2 + 2'; third generation, 3 + 3'.

area of a granddaughter, and on the other the margin of an-
other granddaughter.   At $Ht.^1$ the concrescence line has, on
either side, in their original relations, the right and left mar-
gins of the mesodermic area of the mother.   We may there-
fore call the hearts, or other organs developed along these three
lines, respectively the mother, daughter, and granddaughter
hearts, etc.

In explaining the condition of the triple embryos in Figs.
102 to 104, we shall assume that the original embryo divided

lengthwise in the manner already described, producing *A* and *BC,* and that *BC* divided, giving rise to *B* and *C.* In Fig. 102 *A* remains practically normal ; *B* has undergone median fusion and transverse fission across the line of the fourth segment. The abdomen and last two thoracic appendages are practically normal, while the anterior part of the thorax and cephalic lobes have disappeared, except one pair of fused appendages.

Passing around to embryo *C,* we find median fusion and degeneration have obliterated everything but the abdomen and the last pair of fused thoracic appendages.

In Fig. 103, *A* has undergone median fusion and degeneration, forming a pretty good example of an hour-glass embryo. The same process has affected *B,* obliterating entirely the cephalic lobes and anterior portion of the thorax. The dorsal organs, however, are not quite fused in the median line. But this has taken place in *C,* and in other respects the degeneration is carried farther than in *B.* In both these triple embryos, then, *the path of increasing degeneration is that of a spiral from A to C.* [1]

In Fig. 104 all three embryos are reduced so nearly to the same level that it is hard to determine which is the most degenerate. They are reduced to the last two fused thoracic appendages and a remnant of the abdomen. Embryo *C* has the smallest appendages and may be taken to be the most degenerate. But between *A* and *B* there is so little distinction that one cannot determine whether the line of degeneration follows a right or a left handed spiral. We shall return to this point later.

*Discussion of Observations on Defective and Exuberant Embryos.* — There is little hope in the present condition of our knowledge of finding anything like a satisfactory explanation of the phenomena of either defective or exuberant embryos, because the solution of the problem is bound up in that of vitality. While the futility of seeking final explanations of vital phenomena is fully recognized, we have ventured, supported by the facts on variation here described, to approach some of the outposts of the subject. These facts are numerous and in some

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[1] Possibly, from *C* to *A,* as more recent evidence indicates.

instances novel, and it will probably be admitted that they are somewhat conducive to speculation.   I shall make them alone the basis of the argument which follows.   There is an obvious advantage in treating the subject in this way, for the chances of misconception due to confounding unlike results or conditions with one another are thereby reduced to a minimum.

We may assume that the three embryos of triple monsters are endowed at the outset with equal potentialities and that it is merely a question of time that will bring them all to the same condition of degeneration.   But one embryo must be older than the other two, both of which are of the same age. If the age of the embryo, that is the time that has elapsed since it became an independent embryo, determines the amount of degeneration, then $C$, which is the most degenerate, ought to be the oldest ; and $B$ and $C$, being of the same age, ought to show the same degree of degeneration.   But as this is not the case we must assume that some other factor than the time each has had to degenerate determines the degree of degeneration. We may also dismiss as possible factors the environment, for as we have seen that was the same for all classes, yet, in spite of that, defective, exuberant, multiple, and normal embryos were produced ; and also that when the conditions of development were made excessively abnormal, no abnormal embryos were to be found.[1]

In discussing the phenomena of multiple embryos as well as the other variations described, we must bear in mind the following facts, which although apparently contradictory in some cases, must nevertheless be made to harmonize and be mutually confirmatory before any approach to an explanation is possible. These facts are :

(1) Whatever variations are here considered are probably due primarily to structural variations resident in the ovum, and not to differences in the environment.

[1] It is obvious that we should find at least as many abnormalities under the abnormal condition as under the normal, especially if the primary cause of the variation is to be sought in the eggs themselves.   The absence of abnormalities under the former condition is probably due to the fact that under the prolonged drastic treatment to which they were subjected, only the normal healthy ones survived.

(2) There is a great difference in the growth period under apparently the same conditions.

(3) There is a great difference in the size of different embryos, some being much larger than the normal and others smaller.

(4) Certain organs or regions of the body may be entirely absent and are not subsequently restored.

(5) When organs once formed disappear in certain regions, it is usually by median fusion and degeneration in the reverse order of their age and specialization.

(6) Multiple embryos are due to the formation of new parts, which appear in the reverse order of that in which old organs disappear by median fusion and antero-posterior degeneration.

(7) Multiple embryos thus formed quickly disappear again by median fusion and antero-posterior degeneration.

(8) Individuals of triple embryos recently formed differ greatly in size and in the amount of degeneration.

(9) In old triple embryos the individuals are more nearly alike.

Taking up each set of facts, except the first, it would seem from a consideration of the variations of the second class that every individual and every part of it has a definite *rate* and *range* of growth, a rise and a decline like a time clock that has been set to go a definite period at a definite rate. The time for the whole embryo may be reduced apparently to almost any fraction of the normal one, as in those that die a natural death before reaching stage *C* ; or individual organs may die and disappear by median fusion and degeneration before the other organs have completed their development ; and defective parts either remain defective, or dwindle and disappear in the midst of plenty, side by side with healthy flourishing organs. Embryos six or eight months old are found in the same stage, to all appearances, as normal ones only six or eight weeks old. Both these kinds of variation are apparently best explained by assuming a primary variation in the quantity or quality of growth material, or of both. Either variation may express itself as a variation in the intensity of the "growth force" that may be measured in terms of the range of development, that is, the number of intermediate stages produced, or

in terms of the rate.   The original variation may be local or general, but in either case, the deficiency, if there be one, is not restored by nutrition, or by drafts on a general supply.

(3 and 4).  Variation in the size of separate organs or of the whole embryo, or the entire absence of organs, seems to be due to a variation in the amount of formative material of that particular kind out of which the variable organs are formed.

The variation in the amount of formative material expresses itself in these cases in a variation in the size of the parts, not in their rate or range of development.   The formative material when diminished or absent does not appear to be restored or replenished, because regeneration of defective or absent parts does not take place, although the organs that are present find plenty of material with which to continue their own growth.

The thing then that is lacking in this case seems to have a definite location and is not distributed throughout the embryo. The deficiency is due apparently to the absence of some kind of formative material, and not merely to a diminution of its formative powers or to a variation in the quality.

(5).  Organs once formed frequently disappear by median fusion and degeneration, *in the reverse order of their age and specialization*.   In other words, on a given segment the right and left organs telescope into each other at the median line, and disappear one after the other in the order of their position and original formation.   This mode of degeneration must be due to some inherent structural conditions, and not alone to mechanical stress or tension, because it always occurs in the same way at various but well-determined places, where the mechanical stresses due to growth of the surrounding parts must be quite different.   We may assume that the reason the median end of a half segment disappears first is *because it is most specialized or most highly developed, and therefore most likely to feel the effects of diminished vitality and increased tension*.   The other parts follow in order for the same reason, but the reason the latter move bodily toward the median line is because a path of least resistance is constantly reëstablished there by the degeneration of the organs nearest that point.

(6). Multiple embryos are produced by the formation of new parts which appear in a definite sequence and in a definite place, and in a manner the reverse of that by which old organs disappear by median fusion and degeneration.

This result may be attributed either to the presence of an excess of formative material, or to forced drafts on the reserve brought about by some unknown internal conditions: If the former supposition were correct, there is no obvious reason why the additional parts should not be permanent acquisitions. We cannot assume that such an arrangement of parts as we see in multiple embryos is necessarily fatal to their coördination, because the large numbers of double embryos of all kinds that have reached advanced stages of development, as is well known, would testify to the contrary. On the other hand, our next fact (7), that multiple embryos almost immediately degenerate by median fusion and antero-posterior degeneration, shows, it seems to me, that *there is no production of new "formative material," but a misdirection of that already existing. There is in some way the dividing up of the sum total of formative material so that it crystallizes out along two or three lines instead of one.*

The fact that these new centres start out all right but soon begin to degenerate in the same way that poorly endowed single embryos do, shows that there was not enough formative material to go round ; that multiplying the formative centres simply cuts off specialization and longevity at the other end.

This supposition is still further supported by the next two facts, namely (8) :

The individuals of triple embryos recently formed differ greatly in size and in the amount of degeneration, and (9) In old triple embryos the individuals are more nearly alike. Before we consider these two points further, let us assume for a moment that in the formation of multiple embryos there has been, figuratively speaking, a forced growth producing material faster than it can be differentiated, and that the point of greatest differentiation tension is at the oldest point, where the most growth and specialization had already taken place.

This point, as we have already shown, is the median part of the most anterior end of the body. The tension there would be most likely to be relieved by the production of a new organ at that point, exactly duplicating in size and differentiation the one side of which it was formed. If the differentiation tension, if we may use the term, is still too great, it will be relieved by the production of more new organs at the next point of greatest differentiation, namely, at a point lateral to the two newly formed organs, and on the median side of the corresponding organ in the next posterior segment. The process might go on till two entirely new halves were produced in that way, or it might stop at any time that the extra tension was relieved.

This supposition may in a measure account for the remarkable fact that the newly formed organs do not pass through the various stages of development the other organs did, but assume at once whatever characters the old organs may have at that time. For example, the tip of the new unpaired appendage in Figs. 90 and 91 is just as perfect in form and character for that age as the other appendages on the old halves, and for every successive part of it that appears, it is the same. It looks as though the perfect appendage had been previously concealed in the yolk, and gradually rose to the surface point first, till completely exposed.

If two entirely separate embryos are produced by this forcing process, the growth force, if it is an exhaustible quantity, will be irretrievably subdivided between two embryos. Each will have half the energy of the parent, provided there has been an equal distribution between the new and old halves. But on the formation of a triple embryo, the undivided one will have twice the energy of either of the other two.

On the other hand, if there is at first no recuperation of embryo $B$ from $A$, or $C$ from $B$, the latter must contain the least amount, and $C$ less than $A$, *provided* there is recuperation of the weaker half of each embryo from the stronger during the process of division. This is clearly in accordance with their degrees of degeneration, as shown in Fig. 102. But if we suppose that there is subsequently a slow diffusion of formative

energy throughout the three embryos, so that each one gets an
equal share, there will be a tendency to bring all three embryos
down to the same grade of degeneration.    We may thus explain
the diminished difference between the three embryos in Fig. 103
and those in 102; and finally in Fig. 104, where the three embryos
have evidently been formed a long time, all three are reduced
to nearly the same condition.    At first sight these conclusions
seem to be in direct opposition to the fact that in single
asymmetrical embryos *there is no indication whatever that the
stronger half possesses any power by virtue* of which missing
parts on the opposite side of the median line can be restored.
If one embryo can produce two new halves by drawing on
its bank account, as in the formation of double and triple
embryos, why do not defective single embryos restore an
absent half or quarter?    We can only say that in such
cases the right half of the embryo, for example, was absent
because the formative material for that part was absent
from the start.    Theoretically there is no reason why the
lost part should not be restored by a forced draft on the
corresponding organs of the whole side.    *But there is no
particular reason for assuming that forced growth would be
likely to occur in an embryo that was already defective in
growth material.*

On the other hand, it is a point to be borne in mind that in
all the defective embryos shown in Pl. IV very few show a
defect at the anterior end of the body, unless, as in Fig. 38,
the whole left half is absent, or in Fig. 37, which is probably
a double embryo.    The parts most frequently absent are the
posterior ones, or ones across the middle of the thorax.    *That
is, they are the parts least likely to be restored from the opposite
side, provided their restoration took place in the same order that
the organs of new halves are formed in double and triple
embryos.*

According to Hertwig and others, the stimulus of a modified
environment alone is sufficient to call forth the new embryo.    But
as in the cases we are considering the environment was nearly the
same for all, it can only mean that out of many thousands of eggs
*the few that produced double or triple monsters were different at*

*the outset, and for that reason responded in a different way to the same environment.*   Again, if it is the environment alone that produced the excessive growth manifested in the formation of two or three embryos out of one, we should expect that under these favorable conditions the new embryos would be large and vigorous, but as a matter of fact they are not, for no sooner are they once formed than they begin to degenerate, and finally all of them may be reduced to mere remnants.

Hertwig's criticism of Weismann's explanation of polymorphism in ants and bees, as well as of multiple embryos, is, it seems to me, a valid one.   For, as he points out, we have no right to assume that the embryo is provided with several sets of germ plasm destined to develop, under proper stimuli, into new embryos or organs, unless the necessary stimuli are likely to occur in nature.   And it might also be added that this condition could not have been fixed by natural selection, if its realization brought death with it.

On the other hand, the assumption that Hertwig makes, that the stimulus of a new environment is alone sufficient to produce new formative material, is not a necessary one.   Indeed, the facts seem to me to point to an opposite conclusion.

The factors producing forced growth of this kind, it seems fair to assume, are the reverse of those that cause median concrescence, because the sequence of events is nearly the reverse.   That is, excessive tension causes organs to fuse and disappear along the median line.   Is it not probable then that abnormal reduction of this tension would facilitate the formation of new organs there, which will then reverse the tension conditions and cause the organs to disappear ?

The formation of multiple embryos may be the result of some inherent defect in the ovum, and the stimulus of a modified environment may exaggerate these defects, and increase the percentage of multiple embryos ; but there is no evidence to show among the higher, segmented animals, at least, that double or triple embryos may be produced by the sole action of any definite environment.

The fact that double and triple monsters appear under abnormal conditions, is not a valid argument against the mosaic

theory, or, at least, not in its widest sense, but only against the rigid limitations placed on it by Weismann and others.

It is well known that an embryo may, under stress of new environment, divide and produce two or more new ones ; and perfectly formed embryos are produced from fragments of segmenting eggs, but these embryos have *suffered either a diminution in size, or in vitality, or both*. No one, so far as I know, has succeeded in raising a fraction of the original ovum into a mature individual showing the same longevity and vitality as those raised from whole ova. The same is true of double or triple embryos produced by excessive growth.

If we assume with Weismann that the material for two or more embryos is present in each egg that is capable of producing multiple embryos, and that the stimulus of the environment has called them all into activity, it will be difficult to explain how it happens that the newly formed parts disappear so soon after formation, and also— a difficulty that, so far as I know, has not been foreseen before — *why the new material should be in isolated halves of different individuals instead of in one or more entirely new individuals distinct from the old*, or why these halves should develop and unite with the old ones in the way they do.

It is probable that the same method of forming the new parts in multiple embryos of Limulus is followed in other animals, only it has not been recognized there, because the embryological processes are more obscure.

It should also be observed in this connection that there is no evidence that the new organs come from some indifferent reserved cells. In addition to the fact that, under very favorable circumstances, no trace of such cells can be seen, it would be difficult to explain why they should always first manifest themselves at the point of greatest specialization, that is, at the very anterior median line, and grow backwards instead of forwards. We should, on the contrary, naturally look for them at the posterior end, and expect that they would produce a new embryo there in the usual manner.

It seems to me that we must assume that in all normal ova there is a definite quality and quantity of formative material

that will under normal conditions produce at the end of a certain time an animal of a given form, capable of performing a number of activities. The capital each ovum starts with determines the result, if the conditions are normal. A change in environment may retard or accelerate the mechanism ; it may throw certain parts into new tracks ; it may influence the distribution of formative material as a whole, but it cannot, within the period of one generation, change the nature of the formative material.

However the machine may vary, we recognize it as the same machine, — incomplete perhaps, but if so, we recognize the vacant places. In very rare cases (only one observed) a Limulus embryo may have more than twelve thoracic appendages, but the extra appendage is exactly like one already existing. In no case is there found a new organ or part different in kind from those already existing, and in no case is an organ out of place in reference to others. The chelicerae always come back of the cephalic lobes ; and a flabellum, if it is present at all, always occurs on the outer margin of the sixth pair of appendages. We can only attribute the original absence of an organ, or of any part of the embryo, and the subsequent failure to reproduce that organ, to the original absence or diversion into other channels of the material out of which that organ was to have been formed.

We can explain the formation of double and triple embryos, not on the assumption that the original formative material has been increased, but that it has been divided and diverted into separate channels, and the consequent diminution in the quantity available for the new embryos has been one of the causes of their subsequent degeneration.

There are in normal embryos inherent lines of weakness along which there is certain to be diminished growth. They mark off the different regions of the body from each other; and when through the action of the environment or through congenital conditions there is a diminution of vitality, it is shown by the diminished size of these regions and by a tendency to fuse along the median line.

Increased vitality is shown by increased size, persistency of form, and by the production of new organs in the reverse way of that in which they disappear. If the increase is due to the original presence of an increased amount of formative material in the ovum, the increase in size, vitality, and number of organs will be a permanent characteristic of the individual ; but if it is due to the accelerating action of the environment, there will be a corresponding loss at some other time or place. We may further conclude that :

(1) In multiple embryos of Limulus the new organs are formed by forced drafts on the old material.

(2) That the new halves are weaker in formative power than the old.

(3) That equality is established by the interchange of material from the stronger to the weaker halves.

(4) That at first the sum total of formative energy in both halves of embryo $C$ is less than in $B$, and in $B$ than in $A$.

(5) That equality is finally established between all these embryos by interchange of material, so that in the end all three are reduced to the same grade of degeneration.

(6) In defective single embryos, the absent parts are absent because their specific formative material was absent. Theoretically the absent organs might be restored by forced growth of the other side. But there is no reason to expect forced growth will occur in an embryo already defective in formative material. If it does occur, it will more likely be at the anterior end, and of course the restoration will not be detected. If it does occur in that way, it explains why unilateral defects are more frequently seen at the posterior than at the anterior end.

## VII. Degeneration and Death of Limulus Embryos.

The causes of degeneration and death of the embryos described in the preceding sections are due to abnormal limitations in either the power of division, specialization, or longevity of the cells, or to various combinations of the same.

The structure of a fully developed adult animal, it seems to me, must depend on the relation that exists between (1) the rate of production of new cells, (2) the rate and the amount of specialization of these cells, (3) the longevity of the completely specialized cells, and (4) the death rate of the cells, or their rate of decline toward a simpler, less specialized condition. The interrelations of these factors must be extremely complex and to a certain extent independent of each other ; for reproduction, specialization, and decay may, apparently, take place simultaneously in any part of any organ, or in the entire organism, in almost any conceivable proportion.

An essential feature of organic death in the higher animals is the cessation of normal activity in many different organs, because other organs, by accident, or by inherent conditions, cease to perform some particular work, perhaps insignificant in itself, upon which all the others depend.

The nerve cells, for example, may be performing faithfully and well their particular work, and yet may perish for lack of proper nutrition, or owing to the presence of poisonous substances that should have been eliminated from the body. We do not know how long they might have continued to act under favorable conditions.

Every living thing has a more or less definite size, form, life period, and kind of activity. It is apparently assumed by some writers that these manifestations are not merely predetermined by the organization of the ovum, acting under the guidance of a changing environment, but that there is an actual deposit of formed material corresponding in some way to every one of the almost infinitely numerous parts of the future organism. That there are definite potentialities in every ovum cannot be denied, but these potentialities must not be confounded with what actually exists as specific matter, and which forms the actual physical basis of the ovum at a given time.

The future organism is the resultant of the action on an initial, specifically constructed mass of forces outside of and independent of the mass (1) of such forces acting on the newly formed material added to the old, and (2) on the continued interaction of these masses and forces on one another, under

constantly changing conditions brought about by these inter-
actions. To affirm that the resultants of such complex inter-
actions can be in any sense preformed is grossly inaccurate
and misleading.

To use the term "preformation" to designate in any way
the remote antecedents of a bit of living protoplasm is like
asserting that the germs of a banner cloud are preformed in
the south wind, or that the germs of a watch are preformed
in the iron and coal used in making it.

Embryological processes are not to be interpreted merely
as necessary preliminaries to a higher condition. There is no
beginning or end. Each phase and part of a living thing
should be treated as it actually is, and be accorded its full
value as a perfected, completed thing, — not as it is going to
be, ignoring the present to catch some imaginary glimpses of
the future.

In the higher animals, death comes to the organism in most
cases, it would seem, as a result of the increasingly complex
interrelation of cells. Non-living compounds accumulate in
the tissues with age and destroy their elasticity or permeability;
the calibre of conducting tubes is diminished and they fail to
furnish the necessary supplies, or excessive demands lead to
rupture, lack of coördination between volume and surface ex-
posure, excess of capillary friction accompanying increased
size of organs, etc. Such slight defects may place insuperable
barriers to further growth and specialization. The same may
be true of the individual cells themselves. We need not
assume that they cease their activities at the end of a certain
period because a certain amount of inherited energy has been
liberated, as in the uncoiling of a watch-spring, but because
they cannot clean themselves or adjust the necessary repairs to
the new conditions. Environment, within and without, winds
up and liberates the springs, not the ancestors. As long as the
environment does this, the vital mechanism will continue to go
till stopped by the products of its own activity, and the mechani-
cal impossibility of adjusting old parts to new requirements.

The more complex the relation of parts and the more per-
fect their mutual adaptation, the more sure and complete will

be the collapse of the whole, when the working of one part is interfered with. Each organism by itself is a world of individual cells where heredity, use, disuse, and struggle for existence are the determining factors of form and function, just as in the larger world organisms as a whole are the resultants of these factors. In both cases, sudden and great changes in the environment result in a rapid collapse or death, owing to the impossibility of the cells on the one hand, or the organism on the other, adapting themselves to the new conditions ; and the result is death of the individual organism in one case, or extermination of the species in the other. If the change is a slow one and one calling for less specialization, in fewer directions, degeneration, or reversion to a simpler plan of structure, may follow till perhaps something resembling the original starting-point has been reached.

*It is an exactly analagous process to this that occurs in some of the degenerating embryos of Limulus. It is a new kind of death for an individual organism, or, at least, not like the one with which we are familiar.* The embryo, a community of thousands of different kinds of cells, does not die like a nation swept by a pestilence, or like the starving inmates of a helpless vessel, but like a flourishing community in the midst of plenty, where some of the infinite niceties of adjustment are such as insensibly to reduce the birth rate below the death rate, to reduce the complex interrelation of individuals, until after many generations the last survivor, reduced to the lowest terms, disappears.

The point we wish especially to emphasize is that in the degenerating embryos of Limulus, cell reproduction, cell specialization, and cell decay are progressing side by side in every part of the body. Karyokinetic figures and the fragments of decaying nuclei are found side by side. Whether the animal develops or degenerates depends on the relative intensity of these three factors. The embryos dwindle in size because the death rate of the cells is greater than the birth rate. The embryo loses nerve centres, sense-organs, and appendages because the amount of specialization of individual

cells is cut down more and more, till only the simplest kinds
remain, or because the new ones die before they become
specialized. The lack of specialization may affect different
parts unequally, as when the surface details of structure fail
to appear on the otherwise well-developed appendages, as in
Fig. 21, or the nerve-cords, the appendages, or sense-organs
are lacking.

As we might expect, the process is never exactly the
same, but it invariably tends to carry the organism back, in
the main, over the old lines of progressive development, till
it reaches its primitive condition, namely, a small community
of untrained, insubordinate individuals, which in turn die
one by one, till the death of the last survivor exterminates
the race.

This may be called the true natural death of an organism,— all
others are more or less catastrophic, due to the increasing lack
of coördination and adjustment to the new conditions produced
by its own growth and specialization. A natural death like this
is only possible where the degree of specialization has been com-
paratively small, and where every cell may receive the stimulus
and material necessary to the continuance of its activities and
the discharge of its waste or noxious products.

If this be true, then there is no such distinction to be made
as "mortal and immortal" protoplasm. All protoplasm is "im-
mortal," in the same sense that chemical compounds, or mix-
tures of the same will continue to be formed, manifest their
specific properties, and disappear so long as the proper environ-
ment is maintained.

Cessation of vital activity, then, is due solely to inadequate
environment, whether we are dealing with highly organized
individuals, or with bacteria, amoebae, or human ganglion cells,
or with any part of these.

The growth of the smallest protoplasmic part of a cell differs
from the growth of a crystal in the fact that, having grown, the
conditions of growth and persistency are more materially
altered in the former than in the later case.

The metabolic changes that take place in and around the cell
are to a certain extent processes of filtration. The vital pro-

cesses cease, not because some hidden spring within needs to be renewed, or because, like a spent rocket, it needs to be recharged, but because the still perfect mechanism is choked with dust and weighed down with uneliminatable material. Before the final collapse some virgin fragment of the original material escapes to begin the same process anew.

We need not assume that this new material, or germ, is essentially different from the living parts of the old machine. We certainly need not look on it as a wonderful piece of pyrotechnics, with countless preëxisting fuses, percussion caps, and hidden chambers, adjusted to discharge themselves at the right instant and in the proper sequence — a something which needs but the spark and afflatus of proper environment to start on its heedless career with infinite splutterings and explosions, to cease only when it becomes an empty shell!

There is in our opinion nothing to be gained by such a view of developmental processes. Nevertheless, in discussing such problems the use of figurative language cannot be avoided, for nothing more than a vague conception can be formed of the infinitely complex mechanism at work within the embryos even of the very lowly organized animals. To follow its normal course of action or development to the end, there must be in the ovum infinite niceties in the qualitative and quantitative adjustment of the new parts to the old ; the chemical time-locks, that are to fix or release, must be set with exactness to the time and place, and the whole mechanism made adjustable to a rather wide variation in its surroundings. This being the case, who will venture to say that the entire absence of the third thoracic appendage was due to the fact that a certain particle, destined to grow into that particular appendage, was accidentally omitted from the chromatin of the segmentation nucleus?

Is it necessary to suppose because the third left thoracic appendage in Limulus was invaginated and its mate projected freely from the surface, that the former was derived from a peculiarly constructed molecule, or biophor, or whatever you please to call it, that was bound to develop into an invaginated appendage and not one projecting in the normal way? As well assume that a river inherits two kinds of sand grains, one of

such a peculiar structure that they give rise to sink holes, and the other to sand banks !

The facts of variation are of value to the morphologist as well as to the biological metaphysician.   If, for example, invaginated appendages are formed, and are formed frequently, it shows that in that direction is, in its broadest sense, a path of low resistance likely to be followed again and again.   The same is true of the deviations affecting the margin of the mesodermic area, and in fact any of the deviations that occur often and in a definite way.

In the inevitable shifting of internal relations that occur in all living organisms, this path is as likely to become a permanent path of least resistance as the other is to remain as it is.   In other words, the normal and abnormal exchange places.

In conclusion, there seems to me no evidence in the variations here described to support the theories which attempt to explain heredity by assumptions that leave no room for the idea that the ovum is an _organism_ — theories which make the ovum a mere receptacle to hold job lots of ancestral organs, to be shuffled together and dealt out again during segmentation by some archoplasmatic prestidigitator!   It makes little difference whether the germules, plastidules, biophores, or whatever we may choose to call these corpuscular " brownies," come from immediate producers of the ovum, or from ancestors ten thousand generations back ; they are things, it seems to me, which exist only as mere names that help to bring before the mind a few of the factors in an extremely complex process.

## EXPLANATION OF PLATE II.

Nearly all the figures were outlined with a camera and drawn to the same scale. They were made from mounted and cleared specimens, viewed with a raised condenser and wide open diaphragm. In this way they appear bright red on a clear yellow field; the elevations showing dark, the depressions light.

The embryos *A* to *E* represent normal stages introduced here for comparison with the abnormal ones. Figs. 1 and 2 belong to an earlier series, where a different method of designating the stages has been adopted.

FIG. 1, × 50.  Surface view of a normal embryo, stained and cleared in oil of cedar. The cephalic lobes form a clearly outlined, semicircular plate of ectoderm, thickened on each side. There is no trace of an oesophagus, but very faint indications of the cheliceral segment may be seen on the posterior margin of the lobes. The next three thoracic metameres are fully formed. In reflected light they appear, in surface views of opaque preparations, as gentle undulations of the surface, forming a series of ridges and valleys. The lateral ends of the low ridges are bent backwards and unite with each other near the margin of the mesodermic area. When the eggs are cleared in oil, the mesodermic segments are seen beneath, and about coextensive with the surface ridges. The fifth segment is just separating off from the anal plate. Along the median line of the latter is a slit-like invagination to form the *telopore*, from which a sheet of inner layer cells extends laterally and forwards, while beneath the invagination a line of cells extends into the yolk.

FIG. 2, × 50.  Surface view of an embryo about 10 days old. The drawing was made from studies of the opaque embryo in alcohol, and from studies of the same embryo cleared in clove oil and stained in borax carmine. The sixth segment has appeared on the anterior margin of the anal plate. The lateral ends of the four preceding mesodermic somites have become confluent, and the mesodermic area thus formed is provided with a distinct thickening along its lateral margins, *m. a.*, forming what I have called the "thickened rim of the mesodermic area."

The cheliceral segment is now quite distinct, and a faint, dark band is seen in front of the cheliceral segment connecting the two sides of the cephalic lobes.

FIG. 3, × 55.  Surface view of embryo about twelve days old, stained in borax carmine and cleared in oil of cedar.

All six thoracic segments are now formed. The sixth, as was the case at a corresponding age with each of the preceding segments except the chelicerae, is very plainly marked by its dark color, and by the sharp furrows that lie on either side of it.

The second, third, and fourth pairs of appendages have appeared, and on either side of them are faint spots, that are perhaps the beginnings of the sense-organs, seen in this region more clearly at a later stage.

FIG. 4, × 50.  Surface view of a normal embryo 14 days old, stained in borax carmine and cleared in oil of cedar. The three pairs of appendages are more conspicuously developed, the oesophagus has appeared at the anterior margin of the cephalic lobes, and the brain and ventral nerve-cords are now easily distinguished by the characteristic mottlings, due to the presence of many minute pits, precisely similar to those described and figured by me in scorpions. The appearance is due to the presence in the nerve-cord and cephalic lobes of numerous independent, bud-like thickenings of the ectoderm, which in histological speciali-

zation and in general appearance resemble sense-organs. The suggestion that the appearance is due to a folding to increase the surface, or that they represent "neuroblasts" (Wheeler) is inadequate, and both suggestions are based on misapprehension of the facts. We shall treat of them more fully elsewhere.

The rim of the mesodermic area is considerably enlarged, and is growing towards the median line back of the anal plate.

The telopore in this particular individual has disappeared, but the median streak of inner layer cells, derived from it, is still visible.

Fig. 5, × 60. Surface view of a normal embryo 18 (?) days old. All the thoracic appendages except the first pair are now elongated processes having the characteristic shape of thoracic appendages. The sixth pair are still transversely elongated, and resemble in form the early stages in the development of the abdominal appendages. The most striking feature of this stage is, however, the series of large, shallow depressions on the outer side of the marginal fold, *m.f.* It is only in exceptional cases that the depressions are visible. They are most readily seen by reflected light in shelled ova, shortly after treatment with the hardening reagent, picro-nitric acid. The second and third, and the fifth and sixth, quickly disappear, leaving no trace behind. The first is a little smaller than the rest and lies a little in front of the chelicerae, but appears to belong to that segment. It develops into the lateral eyes. The fourth, *d.o.*, lies exactly opposite the fourth thoracic appendage, and growing rapidly, gives rise to the conspicuous organ, undoubtedly of a sensory nature, found in nearly the same position as late as the time of casting the first larval shell. The lateral eyes gradually move backwards till they lie on the dorsal side of, and posterior to, the so-called dorsal organ, *d.o.* The rim of the mesodermic area is decidedly thickened, and consists entirely of the remarkable cells containing a coiled fibre. Along the whole extent of this thickening, the inner and outer layers of cells are continuous ; elsewhere, except along the median line, they are sharply separated. Sections show clearly that the inner layer cells along the entire length of the thickening are receiving extensive additions from an inward proliferation of the overlying ectoderm. The posterior margin of the mesodermic area is drawn out into two backwardly directed lobes, which frequently show traces of segmentation comparable with that seen in the early stages of the older somites (Fig. 1).

Fig. 6, × 41. Surface view of entire ovum about 21 days old. Picro-nitric acid, borax carmine, balsam. The stained embryo is shown as a transparent object.

The operculum and first pair of gills are well developed, but the chelaria are as yet barely visible. The cephalic lobes have become specialized into the semi-circular lobes, *s.l.*, and the optic ganglia of the lateral eyes, *o.g.* ; the remaining portions constitute the brain proper, *br.* The ectodermic thickening to form the corneagen of the median eyes is shown at *m.e.* The openings of the tubular invaginations of the median eyes and their nerves are shown at *p.e.t.* The fused distal ends of these tubes will form the median eye vesicle ; the remaining distal third of the tubes is converted directly into the corresponding portion of the median eye nerve. The proximal portion of the nerves is formed by the separation of the nerve fibres from the peripheral surface of the tubes, leaving a collapsed epithelial tube behind, which persists for a long time after hatching as a functionless remnant. The separation of nerve fibres from the proximal ends of the eye tubes takes place before these parts of the tubes fuse with each other, so that the

distal end of the median eye nerve is unpaired, while the proximal end is paired. The roots of the $\lambda$-shaped nerve terminate in the semicircular lobes.

The lateral eyes have moved backward, away from their original position to the dorsal surface opposite the second and third thoracic appendages, *i.e.* The "dorsal organ" has retained its original position on the fourth segment. The dorso-ventral muscles, dividing the yolk into segments, and passing between the primary liver lobes, have appeared.

FIG. 7, × 40. Surface view of a normal embryo about four weeks old. Picronitric acid, borax carmine, clove oil. The point that will interest us here is the further specialization of the cephalic lobes. The median eye tubes are still unfused, and open by separate pores, *p.e.t.* The proximal portion of the median eye nerves is seen at *p.m.n.*, connecting the median eye tubes, *m.e.t.*, with the semicircular lobes, *s.l.* The latter, which are formed by invagination of the anterior margin of the cephalic lobes, have grown inwards and backwards, so that they now lie on the dorsal surface of the same. They are seen through that part of the brain which lies over them, and which will soon give rise to the cerebral hemispheres.

The olfactory organs, *o.l.*, are now visible for the first time as oval ectodermic thickenings on the anterior margin of the cephalic lobes, at the point where the future cerebral hemispheres, the optic ganglion, and the semicircular lobes meet. The coxal sense-organs are visible as elongated transverse ridges at the median margin of the base of the appendages. From these thickenings arise the enormous ganglia of the pedal nerves and the coxal sense-organs. See my paper on the "Brain and Sense-Organs of Limulus."

Pl. II
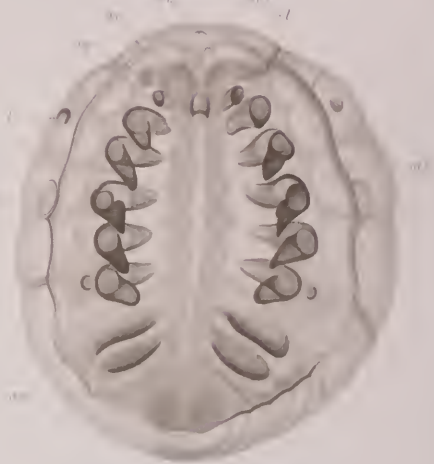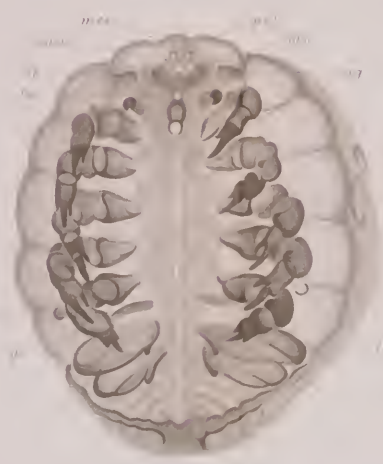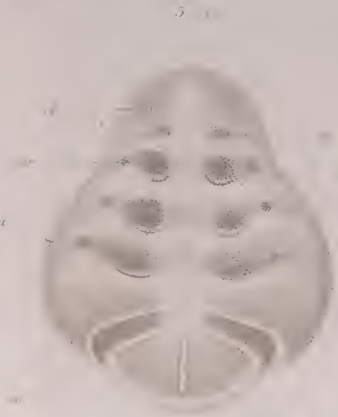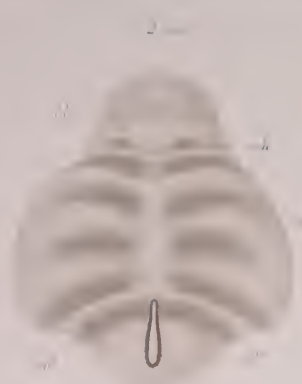
### EXPLANATION OF PLATE III.

FIG. 8, × 60. This is a well-developed and nearly full-sized embryo of stage *C–D*.

It is remarkable on account of the invagination of all the thoracic appendages except the first and last pairs. The first pair are small for this stage, and hardly recognizable even in sections. The last pair are well developed, and project freely from the surface in the normal way.

The invagination of the remaining thoracic appendages is greatest on the left side. The series of low oval elevations represents the uninvaginated bases of the appendages, and the transverse slits at their summits, *w.ap.*, the openings leading into deep cavities formed by the invaginations of the outer two-thirds of the same. The dark ring around the slits represents the optical sections of the ectoderm nuclei that form the wall of the invagination. Sections of these appendages show that they do not differ materially from those shown in Pl. XI, Fig. 1.

The abdominal plate is very short and devoid of appendages. A small, deep pit is present in it, which in sections resembles the proctodaeum. Comparison with stages *C* and *D* indicates that the embryo is too young to be normally provided with a proctodaeum. It probably represents the last stages of the *telopore.* The cephalic lobes are abnormal in shape and structure. They contain two central depressed areas of thickened ectoderm, appearing as light round spots in surface views, *br.iv*, and in section, Pl. XI, Fig. 8. The optic ganglia, *op.g.*, are partly concealed by a broad fold of ectoderm, whose free edge is directed diagonally backward, *g.f.*, Pl. XI, Figs. 8 and 8². This fold does not extend across the median line in front of the oesophagus.

The lateral eyes are conspicuous, but the sense-organs opposite the third thoracic appendage are not visible in surface views, although they can easily be detected in sections.

FIG. 9, × 60. This embryo is between stages *C* and *D*. The anterior end of the body is narrowed, and there is a slight lateral constriction opposite the fourth pair of appendages. The latter are completely invaginated into the yolk, leaving two slit-like openings on the surface of the embryo. The boundaries of the inner ends of the invaginated appendages are not sharply defined, and in the yolk around them is a halo of degenerating nuclei apparently formed by the disintegration of its walls. The thoracic portion of the marginal fold is very faint, except at the posterior end. The whole embryo lies flush with the surrounding surfaces, instead of being deeply depressed as in Figs. 8, 10, 12.

The cephalic lobes are clearly outlined. A series of transverse sections (Pl. XI, Fig. 9) shows that the dark area bounded by the lines *ec.* and *gf.* is covered by an amnion-like, ectodermic fold (although the middle layer is very obscure), extending backward and medianly over the cephalic lobes. Over the light inclosed area, *br.*, there is no superficial ectoderm, and the very light spots, *br.iv.*, are the same pit-like depressions of the brain seen in Figs. 8–12. The ectoderm over these depressions is covered with a layer of vertically striated, cuticular substance, looking like a layer of cilia, and similar to that seen over the surface of most developing sense-organs.

FIG. 10, × 60.   A large embryo, a little older than stage *C–D*.   All the thoracic appendages are large.   The third pair are completely invaginated, and project their whole length into the yolk.   The pockets thus formed open to the exterior by two sharply circumscribed transverse slits, *p.*

The apex of the fourth appendage of the left side, *ap.*, is invaginated, and the base is expanded and folded as though vertically compressed.   A section of the third pair is shown in Pl. XI, Fig. 10$^1$.   The ectoderm is sharply defined except at the tip, *x*, where the arrangement of the nuclei indicates a proliferation of ectoderm cells into the yolk.   A similar condition has been observed in other embryos. The cephalic lobes are modified in appearance by the extensive overgrowth of an amnion-like, ganglionic fold along their anterior margin.   Longitudinal sections in the planes indicated by the lines 1, 2, and 3 are shown in Pl. XI, Figs. 10$^1$, 10$^2$, and 10$^3$.   Abnormal depressions in the region of the future cerebral hemispheres are seen at *br.iv.*, in Fig. 10, and in sections in Fig. 10$^2$.

Near the median line the brain is covered with a layer of ectoderm cells, Fig. 10$^3$, not developed in the lateral areas, Figs. 10$^1$ and 10$^2$.

The lateral eyes have moved back to a point opposite the second pair of appendages.   The large thoracic sense-organs are very feebly developed, if not entirely absent.

There is a conspicuous mass of cells lying in the yolk back of the tail lobe, *p.a.c.*   It is formed by the concrescence of the thickened rim of the mesodermic area.

FIG. 11, × 60.   This embryo is similar in general appearance to that shown in Fig. 10.   The thoracic appendages are, however, longer, and there are four pairs of abdominal appendages present.   The distal half of the third thoracic appendage on the left side is invaginated, *th.ap.*   The fourth and fifth appendages are directed forwards instead of backwards.   The brain-pits, *br.iv.*, are here smaller than in Fig. 10, and pushed farther forward beneath the ganglionic fold.   The whole brain and optic ganglia, *op.g.*, are depressed below the level of the ventral cord and the margin of the ganglionic fold.   The ganglionic invagination, *g.iv.*, is about the same as in Fig. 10.   The light area, which indicates the mouth of this slit-like depression, gradually runs into the rounded brain-pits on the median side, *br.iv.*   The ganglionic fold is continued as a thickened band, without infolding, across the median anterior border of the cephalic lobes.

The lateral eyes are unusually conspicuous, and lie very nearly on a line with the chelicerae.

A large peripheral vesicle, *pr.ve.*, extends inwards almost to the centre of the egg.   It is a conical cavity in the yolk, lined with a thick layer of cells, derived from an abnormal growth of the thickened margin of the mesodermic area.   A well-marked depression is present in the abdominal plate that in surface views appears to mark the beginning of the proctodaeal invagination.

FIG. 12, × 60, stage *C.D.*

The thoracic appendages have the character of those seen in the early phases of stage *D*, while the abdomen has the characters seen in stage *C*.   The apex of the third left leg is reduced in size and deeply invaginated.

The cephalic lobes, which have run together to form a broad, unpaired thickening, are thrown to the left by a remarkable growth of the right half of the cheliceral segment.   The original right chelicera has apparently divided, producing appendages $a^{3–4}$ and $a^{1–2}$.   A second division followed, producing $a^3$ and $a^4$,

and a third has imperfectly divided $a^{1-2}$. The resemblance of the extra append-ages to the normal chelicerae, and the fact that all the remaining appendages are in their proper positions, remove all doubts as to their identity.

It should be observed that the fourth chelicera, if this explanation is correct, is the oldest, as indeed its size and isolation suggest. This condition, however, is the reverse of what usually obtains in segmented animals. There is no distinct segmentation of the right cord corresponding to the extra appendages. But if present it could not be easily seen, as the segmentation of the nerve-cord in other parts of the embryo is very indistinct. But there is a triangular swelling of the nerve-cord, $S$, which widens forwards, and seems to include the first three append-ages of the right side. A small, triangular thickening, posterior and lateral to the one just described, lies opposite the fourth chelicera.

The lateral eye of the left side is normal. But on the right are two dark areas which probably represent two right lateral eyes. It would thus appear that the right half of the cheliceral metamere has given rise by division to four more or less complete half-metameres.

FIG. 13, × 60. A large embryo in stage $C$.

The first two thoracic appendages are absent on the right, causing a spiral curvature of the head toward that side. The anal plate forms a conspicuous oval protuberance, suggesting the early stages in the formation of the tail in insects and scorpions.

The most singular feature, and one not seen in any other abnormal form, was the fact that the cephalic lobes formed a continuous thickened mass of ectoderm, without distinction into right and left halves. In the median line was an enor-mous wedge-shaped ectodermic thickening, reinforced by an underlying layer of mesoderm, extending backward as far as the third thoracic metamere. This median thickening seems to be, in part, an exaggeration of a median post-oesophageal proliferation frequently seen in normal embryos of this age. On the right side of the cephalic lobes mesoderm and ectoderm are indistinctly separated from each other, and the former consisted of a mass of the characteristic, degen-erating nuclei. In place of the first two appendages on the right, are irregular, flattened masses of loose cells containing degenerating nuclei. Nearly all the mesoderm of the right side, and especially the great masses in the appendages, showed the same kind of degenerating nuclei. None were seen on the left side.

A large area in the yolk, beneath the posterior, thoracic region, and nearly the whole surface of the embryo, especially on the right side, was strewn with innumerable, intensely red dots, that look like bacteria. But these dots are also similar in size and color to the chromatin granules in the yolk nuclei, and also to those in the nuclei of certain ectoderm cells. After a careful study of them it seems probable that the red dots will turn out to be bacteria, but we must not lose sight of the possibility that they may be chromatic granules, liberated by the rupture of degenerating nuclei. The granules appear to lead an independent existence, at least for a limited period.

FIG. 14, × 60. A small embryo in stage $C.D.$

The right chelicera is absent, and the last two thoracic appendages on the right side are completely invaginated. There is a difference in direction of the first three and the last three pairs of thoracic appendages, similar to that in Fig. 22.

The abdominal plate forms an elongated, thick-walled lobe, the posterior half of which projects freely from the surface of the egg, a not infrequent abnormality.

The cephalic lobes are well defined, and in shape and in the numerous small, pit-like depressions of the surface suggest the early stages in the cephalic lobes of scorpions and spiders.

The thoracic portion of the marginal fold is very faint except at the anterior and posterior ends.

The posterior margins of the mesodermic area form conspicuous, triangular bands, approaching each other toward the median line. Just beneath them in the yolk are many degenerating nuclei, especially numerous just beneath the posterior median margin, where they form two elongated, parallel cords of yolk nuclei.

FIG. 15, × 60. This is a remarkably large and well-developed embryo in stage *D*. It represents a type very frequently seen. It is quite normal except in its size, in the abbreviation of the abdominal plate, and in the absence of the invagination of the cephalic lobes. The whole embryo is deeply imbedded in the yolk, and with a very prominent marginal fold. There is a series of rounded ectodermic thickenings on the outer side of the base of each of the first four thoracic appendages, resembling the flabellum of the sixth pair, but being in reality the ventral ends of the dorso-ventral muscles.

Pl III

## EXPLANATION OF PLATE IV.

FIGS. 16, 17, (18), 19, 20, 21, (22), 23, 24, 26, 27 represent very common types of about stage *C*. They are often laterally compressed, as well as diminished in length by the antero-posterior compression of the head region, and by the absence of the abdomen. Except in Fig. 19, the marginal fold, *g.f.*, is very conspicuous, extending nearly around the embryo. All the parts included in this fold, *i.e.* the nervous system and appendages, are deeply depressed below the surface of the ovum. In Fig. 19, there is a difference in direction between the appendages of the anterior and posterior thoracic regions, and a wide space between the third and fourth pairs, in Fig. 23. In Fig. 16, the second and third pairs of existing appendages are partly invaginated. There is no invagination of the abdominal plate in Figs. 17 and 19, but a very conspicuous one in Figs. 16, 20, 21, and 23. The full number of thoracic appendages are present in Figs. 17, 19, 20, 22, and 23. In Fig. 16, two metameres are absent, probably the first two, although there is no positive evidence in this case as to which ones. In all the figures the cephalic lobes, and the cheliceral segment when present, are much more deeply depressed than the rest of the nervous system. This is a very common feature in this type of embryo, and is especially well shown in Fig. 16, where there is a very abrupt descent from the level of the ventral nerve-cord to that of the cephalic lobes. Compare also Figs. 62–67, etc.

Two extreme modifications of the ganglionic folds of the cephalic lobes are to be seen. One is shown in Fig. 19, where the cephalic lobes are nearly flat and naked, or as in this embryo, covered by a very thin single layer of cells, *g.f.*, advancing posteriorly and medianly over the lobes and adhering very closely to their outer surface. The other type is seen in Fig. 21, where there is a deep invagination on either side of the cephalic lobes, overarched by a very prominent, amnion-like ganglionic fold. In Figs. 17 and 23, only a small part of them is so concealed, while in Fig. 16 they are entirely exposed. In Pl. IV, Fig. 34, the whole cephalic lobes are covered by this fold.

In nearly all of these figures, the lateral eyes are very well developed for this stage, and are easily seen in surface views about opposite the chelicerae, or if the chelicerae are absent (as in Fig. 16) about opposite the position the chelicerae would have occupied had they been present. On the other hand, the so-called "dorsal organs" are either entirely absent, or so faintly developed that they cannot be detected in sections or surface views. They are shown in Fig. 22 only.

In these compressed embryos, the rim of the mesodermic area is generally very thick and distinct. In Fig. 20, the outlines of the posterior mesoblastic somite are visible up to the margin, and the manner in which they grow toward the median line, and unite there to form the post-anal cloud of cells, is clearly shown.

In most embryos of this type, the outlines of the somites are not preserved, but the posterior portion of the thickened rim of the mesodermic area, and the part that has concresced in the posterior median line are all the more distinct. One of these forms, seen as an opaque object and illustrating the extreme development of this character, is shown in Pl. VI, Fig. 63. An important point to be observed here is that the rim of the mesodermic area is so abnormally large, that it appears as a white and prominent ridge, resembling in shape and position the so-called concrescing margin of the blastopore in sharks and reptiles.

The prominent mesoblastic rims of this type of embryo are subject to local vesicular enlargements, which are usually filled with a clear fluid and lined with several layers of rounded cells derived from the mesoderm. The round cells present all stages of degeneration.

The individual characteristics of these embryos are as follows:

FIG. 16, × 60, sectioned. Two thoracic metameres are absent, probably the first two. Last three pairs more or less invaginated at apex. Embryo somewhat elevated as a whole, but depressed in centre, and surrounded by a high, thick, marginal fold. Abdomen absent. Cephalic lobes, sharply depressed below level of rest of nervous system, forming a steep descent between the first two appendages. Two deep oval invaginations on the lateral margins of the cephalic lobes.

There is a slight depression at the summit of the elevation between the first two thoracic appendages, from which arises by inward proliferation, a great mass of degenerating cells, which give this area its dark color. The region of most active proliferation seems to extend a short distance along the median line, the crowd of cells thus produced mingling anteriorly with those about the oesophagus, and diminishing posteriorly till they disappear about opposite the anterior margin of the second pair of appendages.

The mesodermic area is extensive anteriorly, but without a conspicuous rim, which was too remote from the body of embryo to represent in the figure. Two broad masses of mesoderm radiate from the head of embryo to the rim of the mesodermic area. Posteriorly the mesodermic rim is well marked and notched in the median line, where there is a small ectodermic elevation, *p.a.c.*, continuous with a great mass of underlying yolk cells. The latter are also continuous with the great cloud of cells arising from the deep oval invagination, *t.p.*, in the anal plate. There is a small marginal vesicle on the right side, *m.v.* No trace of segmentation in the mesodermic area.

FIG. 17, × 60, sectioned. Embryo short and rather broad. Abdomen absent. Marked difference in size of the first, the following three, and the last two, pairs of appendages. Cephalic lobes broad and disproportionately large. Large oval depressions, *br. iv.*, on sides of lobes partly covered by an overhanging ganglionic fold. Marginal fold conspicuous posteriorly, where it extends across the median line, forming a very prominent spindle-shaped enlargement. No marked concrescence of the posterior margin of the mesodermic area.

FIG. 18, × 60, sectioned. The body of this embryo lies well below the surface and is much compressed laterally, so that the nerve-cord forms a single, median, roof-like ridge, not adequately represented in surface views. The cephalic lobes are absent, but there is a small median invagination, probably representing the oesophagus.

There is a very large tail lobe, projecting upward and forward, and entirely separated from the body except at its posterior end. Its interior is filled with yolk. On either side are seen the median portions of two great marginal vesicles, *m.v.* The outer margins of the vesicles coincide with the peripheral margin of the mesodermic area. In the depressions beneath the legs and the tail lobe, on the nerve-cord and elsewhere, the surface of the embryo was covered with clusters of the intensely red dots that look so much like bacteria.

Beneath the base of the tail lobe is a conical invagination best seen in sections, directed diagonally backward into the yolk. From its posterior end, streamers of closely packed cells extend into the yolk. Around these streamers are many free nuclei resembling yolk cells.

Back of the tail lobe is the usual ectodermic thickening, *p.a.c.*, and beneath it is the usual post-anal cloud of mesoderm, formed by the concrescence of the margin of the mesodermic areas.

FIG. 19, × 60, not sectioned.    Embryo not depressed.    Thoracic appendages show a common, but abnormal mode of growth.    Abdomen absent.    Cephalic lobes slightly convex, with very thin ectodermic fold, probably consisting of a single layer of cells growing over their lateral portions.    Marginal fold knotted and conspicuous posteriorly, but not continuous across the median line.    No invagination of anal plate.    Mouth very long and narrow.    Mesodermic area, comparatively small, circular, and with conspicuous rim, *m.a.*, especially at sides.    No conspicuous mass of cells due to concrescence of mesodermic rim back of anal plate.

FIG. 20, × 60, sectioned.    Embryo short and narrow and deeply depressed. Marginal fold sharply defined.    Cephalic lobes shortened and partly concealed by a broad overhanging ganglionic fold.

Abdominal plate without appendages and sharply infolded to form a very deep oval invagination, from the walls of which arises a cloud of degenerating yolk cells.

The mesodermic area is extensive.    It rim is well defined, and posteriorly shows very beautifully its mode of concrescing.    In this case the outlines of the posterior, mesoblastic segments are very clearly shown.

FIG. 21, × 60, sectioned.    Chelicerae and abdomen absent.    Large open depression on margin of cephalic lobes, bounded on the sides by a prominent, overhanging lip.    Mouth very large, with rostrum-like projection in front.    Body of embryo depressed and surrounded by prominent, knotted, marginal fold, which is discontinuous posteriorly.

A deep and broad invagination of the anal plate has carried the last pair of thoracic appendages inwards till they project from its sides.    A conspicuous elevation back of anal plate, *p.a.c.*, due to the concrescence of the posterior margin of the mesodermic area.    The lateral and anterior portions of the rim are not represented.

FIG. 22, × 60.    A very common form of embryo in stage *D*.    Not sectioned. Differs from the normal in its shortened, compact shape, and in being deeply sunken in the yolk.    It was outlined to show a rather frequent modification of the direction in which the thoracic appendages grow, the anterior pairs pointing backwards and laterally and the posterior ones forward and inwards.    It is interesting because it occurs frequently, but especially because it is between the third and fourth thoracic metameres that transverse division sometimes takes place.    The difference in the disposition of the appendages recalls a very similar one that obtains between the mouth parts and the walking appendages of insect embryos.

FIG. 23, × 60, not sectioned.    Embryo narrow and depressed.    Appendages normal except in the separation of third and fourth pairs, a rather frequent occurrence, that seems to have some connection with the transverse fission that often occurs here, and with the difference in direction between the appendages in front of and behind this space.    Two pairs of abdominal appendages present, but they are carried inwards by the invagination of the anal plate, so that they project from the sides of the cavity.    Marginal fold prominent, especially posteriorly, and continuous anteriorly with a well-defined fold overhanging the invagination on the sides of the cephalic lobes.    Mesodermic area not conspicuous or well defined.

Fig. 24, × 60, sectioned.    In this embryo the cephalic lobes and first two tho-
racic segments have disappeared.    The marginal folds have closed in front of the
third pair of appendages, and the nerve-cord terminates abruptly just back of
them.    The abdomen is absent, and the marginal folds are contracted so as to
extend across the median line just behind the sixth pair of thoracic appendages.
In the median line, the fold is interrupted by a deep, thick-walled invagination
with an oblong lumen.

The invagination dips deeply into the yolk, and from its thick walls arise
numerous nuclei which are seen scattered about in the neighboring yolk.    The
yolk nuclei are most numerous back of the invagination.    An ectodermic thick-
ening forms the broad, dark, post-anal band seen in surface views, *p.a.c.*    At its
posterior end, the band becomes continuous with the thickened rim of the meso-
blastic area.    The post-anal cloud of yolk cells is formed in part by cells that
have migrated from the walls of the invagination, and in part by those arising
from the thickened mesodermic rims, which have concresced along that line.

A large marginal vesicle, *m.v.*, is seen in the right anterior margin of the
mesodermic area.

Fig. 25, × 60, sectioned.    A remarkable embryo in stage *C*, in which the
cephalic lobes are reduced to a flat circular plate, slightly depressed, so that it is
surrounded on all sides by a vertical wall.    Near the centre of the disc, which is
separated by a considerable distance from the remainder of the embryo, is a small
pit representing the oesophagus, and in the yolk below the disc is a great, irregular
mass of cells, with numerous pseudopodia-like streamers of cells, extending still
deeper into the yolk.

The body of the embryo, which consists of three appendage-bearing segments,
is bent into the yolk at the posterior end.    Back of this abbreviated trunk is a
broad depression bounded on either side by steep walls, which gradually shallow
posteriorly to the surface of the ovum.

Fig. 26, × 60, sectioned.    A very compact embryo of stage *C* and *D*.    All the
ectodermic layers are very thick, and the mesodermic area is constricted so that
its peripheral margin lies close around the body of the embryo.    The greater part
of the mesoderm forms two thick bands on either side of the body, close to the
median line.

The cephalic lobes are completely covered by a hood-like fold extending back
almost to the second pair of thoracic appendages.    At the bottom of the cephalic
cavity is a small pit, the histological character of whose walls indicates that it is
the oesophagus.

Surface of embryo covered with bacteria.

Fig. 27, × 60, sectioned.    Very much shortened embryo in stage *C*.

The rudimentary cephalic lobes are covered by a backwardly directed fold, and
only three pairs of appendages, probably the fourth, fifth, and sixth, are represented.
What looked in surface views like an anus was present, but its nature could not
be determined in sections.    Such embryos as those in Figs. 26 and 27 are rather
common, and possibly they were seen by Dohrn and Osborn, and gave rise to the
statement that Limulus passes through a nauplius stage.

Fig. 28, × 33, not sectioned.    An embryo in stage *C*.

It consists of an abdominal plate and four metameres, probably representing
the last three thoracic and first abdominal ones.    The appendages are separated
by a very wide space, over which the neuromeres (?) and mesoblastic somites
extend as long, narrow bands.

Pl. IV

## EXPLANATION OF PLATE V.

Fig. 29, × 60. Embryo in about stage *E*

The most striking features are : (1) a diffuse atrophy of the left side, resulting in the complete disappearance of the left abdominal appendages and neuromeres, and the reduction in size and absence of surface details in the left thoracic appendages; and (2) *the hour-glass form* due to a diffuse, transverse atrophy of both halves along the fourth thoracic segment. When examined more carefully, it is seen that the three anterior thoracic appendages on the right side are nearly normal ; the second and third being perhaps a little stouter than usual, but still showing all the characteristic details of this stage. The fourth right appendage is reduced to a broad, low elevation, probably representing the basal joints of the same, the rest of the appendage being reduced to a very small, medianly directed protuberance springing from an oval depression. The next appendage is larger than the fourth, but smaller and less perfect than the sixth ; and that is smaller and less perfect than it should be. The last two look as though made of wax that had been warmed on the surface, thus melting off sharp angles and other details.

The right side of the abdomen is apparently perfect.

On the left half of the thorax the chelicera is thrown outwards, but is otherwise normal; but the second and third appendages are strikingly smooth and rounded on the tips, as though the surface details had been melted off. The fourth is a minute papilla, and the fifth and sixth are small, rounded, unjointed elevations.

The whole left half of the abdomen is absent, except a trace of the marginal fold.

The cephalic lobes are somewhat distorted, and the semicircular lobe is apparently interrupted in the median line.

The median ocellus and the nerves extending to it are very distinct.

The ventral nerve-cord is not clearly divisible into right and left halves. Back of the third pair of thoracic appendages, it narrows, and finally the right half only extends beyond the rudimentary fourth pair of appendages into the abdomen. In another specimen similar to this, it is very clear that the right half of the cord only is represented on the posterior part of the thorax and abdomen.

The marginal fold is deeply constricted opposite the fourth thoracic segment. The right lateral eye and "dorsal organ" lie beyond the limits of the figure, thus preserving their normal position in reference to the body of the embryo, but not in reference to the marginal fold. The left eye and dorsal organ could not be seen, and were probably much reduced, if not entirely absent.

Fig. 30, × 60, not sectioned. Embryo with two thoracic appendages and the corresponding neuromeres of the left side absent. The missing appendages appear to be the second and third, as the next three correspond to the last three of the opposite side. It should be observed that the second and third appendages of the right side are unusually large, as is the case with the appendages on the corresponding segments in Figs. 31 and 32. There appeared to be four abdominal appendages on the left and only two on the right. There is a very large peripheral vesicle on the right side, *p.v.*

Fig. 31, × 60, not sectioned. Embryo with the last three thoracic appendages on the left side absent. The left half of the abdomen is also absent. The

remaining half, which is provided with two distinct appendages, is thrown sharply to the left, so that its long axis is at right angles to the rest of the body.

All the thoracic appendages are present on the right; but the second and third are very large, and the fifth and sixth correspondingly small. On the left, three appendages are absent, probably the last three of the series, as the most anterior one seems to represent the chelicera.

Fig. 32, × 60, not sectioned. On the left side the second and third thoracic appendages, as in the preceding figures, are unusually large, and the last three thoracic appendages are abnormally small. On the right the chelicera is absent, and the last three appendages, of which one is entirely absent, are very small. The right half of the abdomen is absent, but on the left three appendages and their corresponding neuromeres are present. What is left of the abdomen is thrown to the right, and its end covered with a large hood-like overgrowth of the marginal fold.

Fig. 33, × 60. Small embryo in stage *C*.

Cephalic lobes are very small and partly concealed by the ganglionic fold, which nearly reaches to the mouth. Three well-formed appendages, fourth, fifth, and sixth (?), are present on the left. On the right the corresponding appendages are invaginated, forming three shallow pits, *i.a.* The abdomen and the anterior part of the thorax are absent.

The outline of the mesodermic area is visible, showing its thickened rim, *ma.* Its concresced posterior portions form the usual post-anal cloud of cells, *p.a.c.*

Fig. 34, × 60, not sectioned. A much reduced embryo in stage *C*.

The head is turned toward the left, although that side is better developed than the right. The cephalic lobes are covered by a distinct, amnion-like fold, that extends back to the first remaining appendage. Through the fold may be seen the reduced oesophagus. Two appendages are present on the left, and corresponding to them on the right, a shallow pit and small papilla. Back of these appendages is a large unpaired one, probably formed by the fusion of the appendages of the sixth pair. Back of this is a deep furrow, flanked on either side by a rounded elevation.

On either side, just within the limits of the mesodermic area, are two large marginal vesicles.

Fig. 35, × 60, not sectioned. A much distorted and abbreviated embryo.

The cephalic lobes are broad, and contain a good-sized oesophagus. The remainder of the embryo is bent sharply to the right. This is due to the presence of three distinct appendages on the left side, and only one (the second?) on the right. The tail end of the embryo is invaginated, and covered by a small, overgrowing fold. The margin of the mesodermic area is clearly defined, and plainly shows the notch, due to concrescence of its posterior margin. At the anterior end of the embryo is a dark band of yolk cells (?) extending forward to the anterior border of the mesodermic area.

Fig. 36, × 42. Embryo in somewhat older stage than Fig. 29.

The whole right half is practically normal. The most characteristic feature of the embryo is the absence of most of the left half of the thorax; although the left half of the cephalic lobes, and of the abdomen, is nearly normal.

The whole embryo is somewhat shortened. The appendages of the right side, and the right ganglionic fold, extends backwards over the outer surface of the

brain and optic ganglion farther than usual.   Otherwise the right side appears to
be normal.

The left sixth thoracic appendage is a small, three-jointed organ, showing
distinctly the characteristic flabellum near its lateral margin.   The fifth is a mere
papilla, and the third and fourth are entirely absent.   The second is relatively
large, and seems to be partially invaginated at the tip.   The left chelicera is
absent.   In the left half of the abdomen the appendages are partly fused, forming
a great three-lobed mass.

It is, therefore, plain that while the left half of the thorax is greatly reduced, as
a whole, it shows in addition a *diffuse transverse atrophy with its line of greatest
intensity between the third and fourth appendages, and diminishing gradually in
front and back of this line.*   We thus have a case of *hour-glass atrophy, confined
to the left half of the body.*   (Compare Fig. 29.)

The nervous system is not sharply outlined.   The parts of the cephalic lobes
are run together, and a great dark fold extends diagonally backwards over the
right half.   The left is much smaller, and has been carried backward by the
contraction of the left side.   The oesophagus and mouth are small and inconspic-
uous.   It is not clear from surface views whether a part of the left nerve-cord is
absent or not.

The lateral eyes and dorsal organs were in their normal positions on the right
side, but could not be seen on the left.

The marginal fold is normal on the right.   On the left it disappears near the
optic ganglion.   There is a large, laterally directed fold opposite the left sixth
appendage that consists, in part at least, of the marginal fold.   In the abdomen the
left marginal fold is normal, and it meets the fold just described at a sharp angle,
but does not appear to be continuous with it.   Between the two folds is a very
deep triangular depression.   This condition of the marginal fold is the usual one
when there has been atrophy of the anterior quarter of the same side.

In the anal region the marginal fold is greatly thickened, forming a conspicuous
U-shaped boundary to the posterior part of a deep depression.

FIG. 37, × 40.   This embryo is, in part, in an advanced stage of develop-
ment, corresponding to that seen in embryos about ready to hatch.   There are
only a very few markedly abnormal embryos found in this late stage.

The right side is nearly perfect and normal.   The ganglionic fold over the
right half of the brain is large, as in the preceding figure.   The right nerve-
cord, which was very well developed and plainly outlined, is bent sharply at
the junction of the thorax and abdomen.   This bend, as seen in the drawing,
is due, to a small extent only, to the foreshortening produced by looking down
on the upturned abdomen.   Almost the entire left half of the cephalic lobes
are absent, but the left nerve-cord, as far as the beginning of the abdomen,
is present in its normal condition.    All the left thoracic appendages have
disappeared completely, leaving no trace behind except in the third segment,
where there is a shallow depression, seen edgewise in the figure, with a small papilla
projecting from its centre.   This papilla probably represents the last trace of the
third thoracic appendage.   The left nerve-cord seems to terminate abruptly at
the posterior end of the abdomen, without uniting with its abdominal part.   The
anterior end of the latter turns off sharply to the left, toward a large, dark,
conical elevation.   To the left of it is a short, conical projection, with its apex
directed forwards.

The embryo is probably in a state of incomplete longitudinal fission, or a double embryo, similar to those in Pl. IX, Fig. 98. The two problematical appendages, *x* and *y*, would then represent the medianly fused, posterior thoracic appendages of a second embryo, whose axis is in the line *B*. The two embryos possess an abdominal nerve-cord in common. The abdominal appendages on the left side of embryo *B* are absent (its right abdominal half was not formed at all according to this supposition), and in embryo *A* the thoracic appendages failed to develop after the longitudinal fission of the embryo.

This explanation, requires us to assume nothing more than what we know takes place in the double embryos described in plates IX and X. If it is correct, this embryo, although resembling that in Fig. 36, owes its present condition to a totally different sequence of events.

FIG. 38, × 33, unsectioned. A well-advanced embryo, the right half of which is complete and perfectly normal, except in its slight curvature to the left. Of the left half nothing remains but a dark band of inner-layer cells and a small posterior appendage situated in a rather deep depression. The long axis of the mouth is rotated nearly 45°, so that the apex of the rostrum is thrown toward the left.

## EXPLANATION OF PLATE VI.

FIGS. 39–49 illustrate the more important phases in the formation of the inverted V-shaped embryos. These embryos are formed by the fusion in the median line of the corresponding right and left organs of each metamere. The organs nearest the median line are the first to unite, forming in that way an unpaired organ, having the characteristic features of each member of the pair. The unpaired organ thus formed then decreases in size, and finally disappears. In its place the organs next to it, on the same metamere, unite, and in turn degenerate ; and so on till the whole metamere has disappeared. The process seems to begin in every case in the most anterior metameres ; and in the most typical cases, as soon as the first unpaired organ, formed in, say, the first thoracic metamere, has disappeared, the same organ is found unpaired in the second metamere ; and by the time that has disappeared the unpaired condition of that organ obtains in the next following metamere, and so on, till every paired organ has become median and unpaired, and then disappeared. In the last phase of the process, if realized in full, there would be nothing left of the embryo but a single unpaired organ, situated at what was the posterior end of the body, and formed by the median fusion of the most laterally situated paired organ of the last metamere.

Such a condition has not been observed, the nearest approach to it being an embryo of which nothing remained but the mesodermic area and a posterior unpaired process, representing either the last thoracic appendage or the tail lobe.

In very rare cases one of the posterior pair of appendages may fuse in the median line, while there is no indication of fusion in front of that point. But in such cases there is no evidence of a progressive median fusion and degeneration extending toward the anterior end.

There is another exception to the median fusion and progressive anteroposterior degeneration seen in the hour-glass embryos shown in Pl. IV.

FIG. 39, × 60, not sectioned. This embryo is instructive, as it is apparently in the early stages of median fusion. The cephalic lobes are reduced to a thin, circular disc, showing no trace of separate optic ganglia and cerebral hemispheres. The oesophagus is a shallow pit in the centre of the disc, and the cheliceral segment has disappeared entirely. The appendages of the fifth and sixth thoracic segments are nearly normal in position, but in passing forward from this point the appendages decrease in size and approach more and more the median line, till in the second segment they have nearly united with each other. The nerve-cords terminate back of either the third or the fourth pair of appendages, but as this embryo was not sectioned that could not be determined with certainty.

The end of the left third appendage is invaginated. The abdomen is normal except for the rather prominent anal plate.

FIG. 40, × 60. This is a very unusual form, and the only one of its kind observed. Median fusion has taken place at both ends. The dorsal organs are very conspicuous, and as they always lie opposite the fourth pair of appendages we can see that the following changes have taken place :

The cephalic lobes, oesophagus, and first thoracic neuromere have disappeared. The appendages of the second pair have fused completely, and those of the third pair have approached each other, preparatory to the same change. The fourth pair is nearly normal. The fifth appendage on the right is invaginated for half its

length. The appendages of the sixth pair are short and thick, and have fused with each other except at their tips. Just in front of them is a large, deep pit, *i.v.* I do not know of any explanation of the presence of such an invagination at this point, unless it may be regarded as an invaginated tail lobe or a telopore, carried forward to its present position before the fusion of the last pair of thoracic appendages.

Every trace of the abdomen is absent, something that is very unusual, for it is a noteworthy fact that in this class of embryos the abdomen usually remains nearly normal, however profound may be the changes that have affected the anterior part of the embryo.

Fig. 41, × 60, sectioned. In this embryo the cephalic lobes and first two thoracic segments have disappeared, apparently after median fusion. The primitive position of the cephalic lobes is indicated by a conical mass of degenerating cells projecting into the yolk, and seen in profile on the edge of the egg. From the apex of the mass, an irregular train of the same kind of cells, lying deep in the yolk, extends backwards to the surface at the anterior end of the embryo. These cells probably represent the last remnant of the degenerating oesophagus and anterior portion of the embryo.

The right appendage of the third thoracic segment is absent, and the fifth pair is invaginated. Three depressions along the median line are indicated by white areas.

All the parts of the embryo appear very dark, owing to the unusual thickness of the cell layers, and the large amount of staining fluid the cells have absorbed.

Fig. 42, × 60, sectioned. This is a rather common form. The cephalic lobes, oesophagus, and first two thoracic metameres are absent. The appendages of the first pair are fused nearly to their tips, those of the fourth pair are fused at the base only. The two nerve-cords fuse with each other just in front of the fifth pair of appendages, and the single median cord thus produced terminates abruptly just back of the base of the fourth pair. Within the fused bases of the appendages of the fourth segment is a large mass of cells that looks like the remnant of the neuromere of this segment, but forced to assume a spherical form by the fusion of the appendages. It is connected posteriorly with the rest of the nerve-cord by a narrow chain of cells. In front of the fused appendage is merely a thin layer of ectoderm and mesoderm, every trace of the nerve-cord being absent.

Immediately in front of the fused appendages of the third thoracic segment is a short, flattened tube, directed diagonally forward into the yolk. It is surrounded by a thin layer of mesoderm, and opens outwards by a transverse opening between the marginal fold and the anterior edge of the unpaired appendage. It has all the appearance of an oesophagus, and probably is one, but of course it is entirely out of place here.

There is a slight asymmetry of the abdomen, due to the absence of one appendage on the left side, but otherwise the embryo back of the fifth pair of thoracic appendages is quite normal.

Fig. 43, × 60. This embryo is similar to that in Fig. 49. The original position of the cephalic lobes is indicated by a shallow, saucer-shaped, ectodermic thickening, *c.l.* In the figure, owing to the curvature of the surface of the ovum, it is seen edgewise, and only the thickened posterior rim is shown. It is connected with the remaining part of the embryo by a broad train of *yolk cells,* lying close to the surface. They appear in the figure as a broad, faint band, *y.c.* The third

pair of appendages are fused at their bases, and back of them terminates the ventral nerve-cord.

The abdomen is smooth and flat, showing no trace of appendages.

The limits of the mesodermic area are clearly defined by the usual thickened rim.  The mesodermic area back of the tail lobe is darker, and its posterior margin, owing to incomplete concrescence, is deeply notched.

FIG. 44, × 60.  In this embryo the cephalic lobes and the cheliceral segment have disappeared, leaving some distance in front of the embryo a large irregular patch of cells, continuous right and left with the mesodermic rim.  In the figure it is seen in profile on the edge of the egg, *c.l.*

The appendages of the second post-oral segment have fused with each other, and a similar fusion of appendages has taken place in the third segment.  The smaller size of the anterior median appendages indicates that it was formed previous to that of the following segment, and has already undergone some degeneration.

The marginal fold, *m.f.*, extends *anteriorly* between the fused appendages of the second and those of the third segment.  It usually appears to contract with the atrophy of the anterior end of the body, so that it closely encircles all the remaining appendages.  If that really occurred here, the fold must have in some way passed around the second pair of appendages, a process difficult to explain.  The only alternative is to suppose that a new fold was formed back of the second pair of appendages, and that the old one has disappeared or was never formed.

The nerve-cord terminates as a blunt, unpaired process, a little in front of the fourth pair of appendages.  The posterior part of the thorax and the abdomen are normal, except that the apex of the latter projects freely away from the ovum, and the whole abdomen hangs over a great blister-like vesicle filled with fluid and enclosed between the blastoderm above and a thickening of the mesoderm below. There is nothing in the preparation to suggest that this condition is due to shrinkage, etc.

The outlines of two similar vesicles (or marginal vesicles) are seen in the mesodermic area on either side of the head region, *m.v.*

FIG. 45, × 60, not sectioned.  This embryo shows a slight reduction of the cephalic lobes and of the third and fourth thoracic appendages of the right side. In place of the fifth and sixth appendages and the abdomen, is a large median conical protuberance.  Whether the latter represents the fused fifth and sixth appendages or the tail lobe could not be determined.

FIG. 46, × 60, not sectioned.  The cephalic lobes have disappeared, or at least together with the oesophagus are reduced to an invaginated, conical layer of cells, constituting the dark mass at the apex of the V-shaped marginal fold, *d.c.l.*

The chelicerae have fused to form a short median process, which lies in a depression that in front is partly overarched by a semicircular fold of the ectoderm.

The appendages of the second pair have fused to form a very long, slender, corkscrew-like filament.  It is attached to the embryo just back of the apex of the fused chelicerae.  The appendages of the third pair are fused at their bases and somewhat diminished in size.  The nerve-cord terminates abruptly just back of them.

The parts of the embryo lying back of the third thoracic segment are practically normal.

FIG. 47, × 60, not sectioned. The first and second thoracic segments are absent. The cephalic lobes, however, persist as a faint disc of cells without character. This case differs somewhat from the preceding ones, in that the effects of degeneration do not increase gradually from before backward, for degeneration has been greater in the first two post-oral segments than in the cephalic lobes.

The median space between the appendages of the remaining pairs gradually increases toward the posterior end.

A still further difference between this embryo and the preceding ones is seen in the fusion across the median line of the abdominal appendages, the last appendage being the smaller one.

FIG. 48, × 60, sectioned. In this embryo, the sixth pair of thoracic appendages are identified by the well-developed flabella. The long median appendage is formed by the fusion of the fourth pair of the thoracic appendages, the unfused tips of the two original appendages being still visible. The first three pairs of appendages and the entire cephalic lobes have disappeared. The nerve-cord is normal and well developed from the root of the tail lobe to the base of the unpaired appendage of the fourth segment. The abdomen is small, but possesses two pairs of normal appendages. There is a very conspicuous tail lobe, the size and shape of which suggest the idea that with the diminution in size of the embryo, the marginal fold became too large, and the excess accumulated at the posterior end to form the tail lobe. *Back of the tail lobes is a small conical projection, also visible in the sections, having all the appearance of an unpaired thoracic appendage.* The presence of an appendage in this place is very remarkable, and I am unable to offer any explanation for its occurrence there.

The body of the embryo forms the floor of a deep depression bounded by the marginal fold. The embryo stained very deeply, as it is composed of dense tissue containing a large amount of chromatin. Anteriorly, the rim of the mesodermic area, which was not visible, or perhaps overlooked in the surface views, is easily seen in the sections, apparently preserving its normal size and extension for this stage. In the sections that pass a long distance in front of the present anterior end of the embryo, the mesodermic rims are seen as widely separated as in the normal embryo of this stage. In the line midway between them, instead of the nerve-cords and appendages, there is merely a thin, undifferentiated layer of ectoderm with a few scattering mesoderm cells beneath it.

FIG. 49, × 60, sectioned. The cephalic lobes and first two thoracic segments are absent. No trace whatever of these organs is to be seen in sections, although there is a dark area, due to an accumulation of loose cells, where the anterior end of the embryo should be.

The marginal folds, *m.f.*, are distinct and well developed, and extend across the median line just in front of the second pair of thoracic appendages. The latter are fused with each other at their bases, and just back of them the nerve-cord teminates abruptly in a blunt, unpaired process.

The abdomen is very well developed, and terminates in a broad tail lobe that projects upwards and forwards, thus overarching the posterior part of the abdomen.

Such a prominent tail lobe, although occasionally seen, is not a common form of abnormality It is suggestive of the prominent tail fold in insects and crustacea, but in this peculiar case recalls that seen in scorpion embryos.
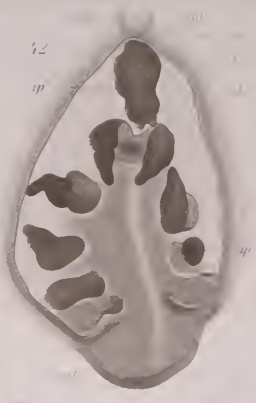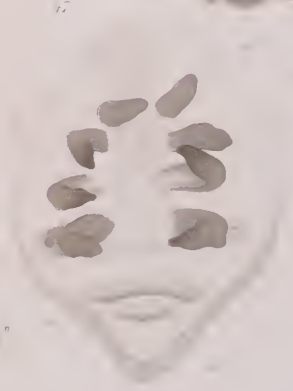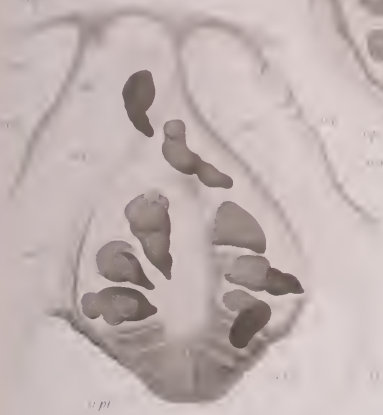
## EXPLANATION OF PLATE VII.

FIG. 50, × 60.   This embryo evidently represents an extreme case of median fusion, but differs from those on the preceding plate in that there are no indications of that V-shaped arrangement of paired organs usually seen when a progressive antero-posterior degeneration has followed the median fusion.   The cephalic lobes, oesophagus, and nervous system are entirely absent.   Nothing remains of the body but an oblong elevation of thickened ectoderm with three median appendages arranged along its summit.   Behind the third median appendage was a partly invaginated plug of cells, $t.p.$, that may be the remnant of the telopore. Besides this there was nothing to indicate to what segment these fused appendages belonged, or which was the anterior and which the posterior end of the body.   The mesodermic area was slightly raised and was pentagonal in outline, with a thickened rim.

FIG. 51, × 60.   A large embryo in stage $C$ showing a distinct transverse constriction between the third and fourth thoracic segments.

The anterior part of the embryo is normal, except in the absence of the chelicerae.

The posterior portion is infolded between the appendages of the sixth thoracic segment to form a deep, circular cavity.   The sixth pair of legs, identified by the flabella on their outer margin, have been carried inwards by the infolding, till they project toward each other from its lateral walls.

FIG. 52, × 60, sectioned.   An hour-glass embryo in stage $C$.

The cephalic lobes are deeply depressed, and nearly covered by two lateral folds.   The chelicerae are absent, the next two appendages are brought closely together, and the third pair completely fused to form a thick median appendage, $ap^3$, extending backwards.

These three appendages project from a circular depression, bounded on all sides by a thickened margin, which anteriorly forms the folds overlapping the cephalic lobes.

The fourth pair of appendages are also fused, $ap^4$, and the fifth are either absent, or fused and invaginated to form the pit just back of the base of the preceding pair, $ap^5$.

The sixth pair are small and devoid of flabella, but are nearly normal in shape and position.

The posterior part of the thorax and a part of the abdomen are nearly surrounded by a marginal fold, well developed posteriorly, but not extending across the median line in front of the fused appendages of the fourth segment.

FIG. 53, × 60, sectioned.   A remarkable embryo in stage $C$ that has undergone extensive reduction and fusion.   As nearly as one can determine by a study of surface views and cross-sections, the following changes have taken place.   The cephalic lobes, oesophagus, and chelicerae have disappeared, leaving hardly any recognizable traces behind.   The second thoracic appendage on the left is nearly normal, that on the right, a low, irregular papilla.   Between the two, in sections, traces of a double nerve-cord may be seen.   Back of this point, the nerve-cords fuse and finally disappear as such, near the large, irregular, median protuberance that probably represents the fused appendages of the third segment, $ap^3$.

Following the fused appendages is a rather broad expanse, covered by a thin (single?) layer of cells.

The next thickening, $ap^4$, is elongated transversely, and probably represents the fused appendages of the fourth segment.

The next two appendages of the left side are of full size and normal, but owing to the absence of the corresponding opposite appendages and nerve-cord they have been twisted spirally toward the right.

The abdomen is absent. At what represents the posterior end of the embryo is a deep, tubular invagination, $t.p.$, with thick walls, from which have arisen innumerable cells that form a dense cloud in the surrounding yolk. The embryo is therefore to be regarded as an hour-glass embryo, still further modified by the absence of the right posterior half of the thorax. It should be compared with the following one.

The surface of the embryo is covered with bacteria.

Fig. 54, × 60, sectioned. This remarkable embryo in stage $D–E$ belongs to the hour-glass type, but it is modified by the median fusion of the anterior end, and by the absence of the right half of the posterior end of the thorax. It seems to be the result of still further progress along the lines followed by the embryo shown in Fig, 53.

In such cases as this it is very difficult to determine just what changes have taken place, especially as regards the amount of nerve tissue, if any, that is left, and the segments to which the remaining appendages belong.

As near as can be determined, the changes have been as follows : The cephalic lobes are absent, and in their place is a broad, triangular, ectodermic plate, beneath which is an unusually large number of yolk cells.

The marginal fold has contracted anteriorly to form a thick rim around an oval depression. At the anterior end of this depression is seen in surface views a dark spot, which is the optical section of a very long oesophagus-like tube extending vertically into the yolk, about as far as the adjacent appendage is long. The tube is not closed at its inner end, and its walls are folded longitudinally, as in a true oesophagus.

Back of this tube is a long irregular appendage arising at first directly upward from the bottom of the depression, and then bending over to the side, so as to lie flat on the surface of the egg. Back of this is another appendage; it is bent double, and in such a way that its distal end points toward the median line, toward a point a little in advance of its proximal end.

The lateral growth of these two appendages at first seemed to me to indicate that the whole right half of this portion of the embryo had disappeared, and that the left half had rotated on its own longitudinal axis 180° toward the right. But after a careful examination of the sections, I failed to find any traces of either nerve-cord ; and, on the whole, the sections seemed to indicate that each appendage had been formed by the fusion of a pair, and, as is so frequently the case in such embryos, the nerve-cord had entirely disappeared.

The posterior end of the embryo, which is separated from the anterior one by thin, characterless layers of ectoderm and mesoderm, consists of a deep, thick-walled and irregular sac, from the posterior wall of which arise two long-pointed appendages, extending upward and forward. There are some parts of the wall of the sac that look histologically like parts of a nerve-cord, but the whole layer is so folded and crowded together that nothing in regard to this point could be

determined with certainty. There is, however, little doubt in my mind that the two appendages in question are the fifth and sixth thoracic appendages of the left side. The sixth has been moved in the plane of the paper 45° toward the embryo's right side. The cause of the rotation is to be sought, as in Fig. 53, in the absence of the posterior right half of the thorax.

The mesodermic area is much contracted, and in place of the continuous thickened rim are isolated masses of closely packed nuclei, some of which lie quite deeply in the yolk; others are continuous with the surface mesoderm. The masses are irregular in shape, but are usually provided with radiating streamers containing many nuclei.

FIG. 55, × 60, sectioned. This is also an *hour-glass embryo*, but one in which the two parts are completely separated. The cephalic lobes are absent. The second pair (chelicerae?) of thoracic appendages have fused in the median line. The next pair are widely separated, and following them is a second pair of fused appendages. Between the unfused appendages is a well-developed double nerve-cord, which gradually narrows at either end to an unpaired cord. One end lies just back of the anterior median appendage and the other in front of the posterior one.

Back of these four appendages is an area devoid of external features. In sections it is seen to be composed of a slightly thickened homogeneous layer of ectoderm with a similar underlying layer of mesoderm. At the posterior end of this area is a deep, tubular invagination or telopore, *t.p.*, directed vertically into the yolk, and surrounded by the usual cloud of migrating cells. In front of the telopore are two small depressions, *i.a.*, that may represent invaginated appendages.

Projecting forwards over the telopore is a broad conical process. It probably represents a tail lobe such as is seen in Pl. VI, Figs. 48 and 49.

Back of this lobe is a broad convex area, composed of slightly thickened ectoderm and mesoderm, and evidently formed by the concrescence of the posterior margins of the mesodermic area. The rest of the mesodermic area is roughly A-shaped, the two backwardly directed lobes showing notches produced by partial concrescence.

FIG. 56, × 30, not sectioned. This embryo is in about stage *C*.

The remnants of the right and left sides have undergone complete median fusion. There is now nothing left but an anterior, oesophagus-like tube, *oe.*, opening beneath a backwardly directed fold, and two median appendages. The posterior one is the larger, and projects backwards a short distance over the floor of the depression in which both lie. This depression is roughly boat-shaped, but wider and considerably deeper at the posterior end.

The nervous system is entirely absent, or, at any rate, the ectoderm over the bottom of the furrow is not thicker than, or in any way distinguishable from, that covering other parts of the embryo. The mesoderm is much thickened under what may be considered the body of the embryo, as shown by the dark rim in the figure. But a thin layer of mesoderm extends much farther than this, and along its periphery may be seen, in surface views, irregular star-shaped masses of cells, deeply imbedded in the yolk, but probably derived from the disintegrated, thickened margin of the mesodermic area.

As seen in sections, the tissues appear to be perfectly normal and healthy.

There is an indication of a transverse constriction between the two appendages. As similar constrictions occur most frequently between the third and fourth

segments, it is possible that the appendages on each side of this constriction belong to the third and fourth segments.

Fig. 57, × 60, not sectioned. This is an embryo in stage *C* that has undergone partial median fusion, accompanied by transverse constriction. It could not be determined which was the anterior end of the embryo. It has been placed in its present position on account of the hood-like fold at what is now the anterior end, as it resembles a fold that sometimes covers the cephalic lobes of other embryos. At the anterior end of the embryo are two pairs of appendages nearly fused. Back of them is a thin layer of undifferentiated ectoderm with a thick mass of underlying mesoderm, the latter causing the dark color of the preparation. There is a longitudinal fold of ectoderm, probably the displaced marginal fold, on the left side of this area, and in about the middle of the same is a conical invagination, *i.a.*

At the posterior end, the fold extends around a second depression, from which arises a large protuberance, evidently formed by the fusion of a pair of appendages. There is no trace of a nervous system in this embryo, or any distinguishable lateral boundaries to the mesodermic area.

Fig. 58, × 60, sectioned. Here we have a thick, oval mesodermic area, from whose inner surface several pseudopodia-like bands of nuclei extend vertically into the yolk. There are no appendages or nerve-cords, but there is an axial, ectodermic thickening, which, at what we may call the anterior end, forms a large pyramidal elevation, containing a large number of degenerating nuclei. This elevation probably represents one or more pairs of fused appendages. At the posterior end, the axial thickening gradually culminates in a tub-shaped elevation with a slit-like depression on its upper surface. At the bottom of the slit is a small circular depression. Underlying the whole is a great mass of mesoderm cells.

Fig. 59, × 60, sectioned. In this embryo the cephalic lobes are hardly distinguishable. There are three small appendages on the right and four on the left side. The identification of the appendages can be approximately determined by the presence on the right of the dorsal organ. There is a great depression across the posterior thoracic and abdominal region, and below its thickened ectodermic floor, and apparently arising from it, are a great many free-yolk cells.

The mesodermic area is nearly circular. Along the whole extent of its thickened margin arises a cloud of isolated cells that extends deeply into the yolk.

The concrescing posterior margins of the mesodermic areas are very beautifully shown. The median limbs, *c.m.a*, are the most conspicuous in surface views. Sections show that this is due to the presence of a ridge-like thickening of the ectoderm, continuous with a compact band of underlying mesoderm.

On the peripheral margin of the mesodermic area the ectoderm is thin, and the underlying mesoderm is composed of isolated, lymphoid cells. The dark spot on the right, where the third appendage should be, is due in part to a small protuberance there, but in the main to the presence, *below the surface, of an oval sac with clear-cut walls*. It has the appearance of an enlarged mesoblastic somite.

Fig. 60, × 60, sectioned. This is probably a very old embryo, for there are thick layers of chiten over the tips of the remaining appendages, such as is only seen in embryos ready to hatch. It at first seemed probable that the small depression, *tp.*, was the remnant of the cephalic lobes. But on sectioning the embryo, the thickenings at what is now the upper end seemed to be, without much

doubt, the brain and optic ganglia. The deviations from the normal, then, in this embryo are as follows: The whole right optic ganglion and a part of the right cerebral hemisphere are absent. On the left side the cephalic lobe is perfect, and one can distinguish in sections the minute pit that develops into the lateral eye, *l.e.* The chelicerae are probably absent, the two pairs of appendages now present belonging to the second and third thoracic segments. The appendages of the fourth segment have fused in the median line, and the marginal fold has closed in behind them. From this point in the marginal fold a faint cloud of yolk cells extends backward a considerable distance to a small thick-walled depression in the ectoderm, *tp.* From the posterior wall of the shallow depression arises a minute papilla, which may be the remnant of the tail lobe. (Compare Figs. 27, 43, and 64.) It seems too far back to represent the fused appendages of the last thoracic segment. The anterior part of the depression is still further invaginated to form a short, conical pocket, directed diagonally forward into the yolk. The inner end of the pocket is continuous with a great mass of cells which seem to be wandering into the yolk there to degenerate and disappear. It would appear, therefore, that transverse fission has taken place, and that contrary to every other case that has come under my notice, except, perhaps, Fig. 45, the subsequent fusion and degeneration have been greatest in the posterior half of the thorax.

Fig. 61, × 60, sectioned. The mesoderm and ectoderm are much thickened and concentrated along the axial portion of the embryo. There is no distinction into head, thorax, and abdomen. The body of the embryo forms a deep, elongated furrow, constricted laterally to form a chain of four or five clearly marked dilatations, each one of which represents a metamere. At the bottom of two of the dilatations are shallow, slit-like infoldings that represent the remnants of invaginated thoracic appendages, *ia.* In sections there is no recognizable remnant of the nerve-cords to be seen.

The mesodermic area is sharply defined on its periphery, where there is the usual thickened rim, but it contains very few mesoderm cells, except beneath the axial portion, where they form a very thick, compact layer ten to fifteen cells deep. It is this deposit of cells which forms the dark ring seen in surface views.

This embryo is also remarkable for the fact that the surface ectoderm of the entire mesodermic area is covered with a very thick deposit of a soft, vitreous exudation, resembling chiten. Over the axial furrow it has accumulated in great botroidal masses, among which in each section may be seen four or five rounded, amoeboid cells that look like those seen in the peripheral vesicles. There is no indication as to where these isolated cells, which are separated by considerable distance from the embryo, come from, or what their function may be. Their occurrence at this place suggests a rudimentary amnion and serosa, but otherwise there is nothing to indicate that they develop in the manner characteristic of these organs.

Fig. 62, × 60, not sectioned. Embryo with three pairs of appendages, perhaps the fourth to sixth pairs. The cephalic lobes are deeply depressed, and separated from the thorax by a sharp, transverse fold. The rim of the mesodermic area is very distinct, and shows clearly the posterior concrescing margins, and the post-anal cloud of yolk cells between, *p.a.c.*

The abdomen is deeply depressed, forming a cavity with nearly vertical walls surrounding a triangular opening.

FIG. 63, × 50, not sectioned.   This embryo is shown as an opaque object by reflected light.   It was drawn shortly after it was killed in picronitric acid, and while still in the fluid.

The embryo is small, but has six pairs of thoracic appendages and well-developed cephalic lobes.   It shows in a very striking manner a conspicuous ridge extending completely around the embryo.   It is broadened and thickened posteriorly, and is continuous, with a thick, broad elevation extending forwards to the posterior portion of the body.   This ridge is the very well-developed marginal thickening of the mesodermic area, such as has been shown in many of the preceding figures.   The post-anal band is formed by the concrescence of the posterior margin of the mesodermic area.   When seen in this way, as an opaque object, its resemblance to the blastodisc of a vertebrate embryo is obvious (shark, lizard, or chick).

FIG. 64, × 60, not sectioned.   A peculiar embryo without appendages, but showing three pairs of distinct, hollow, mesoblastic somites.   In shape it resembles the embryos of scorpions or spiders more than those of Limulus.

FIG. 65, × 60, sectioned.   A small, abbreviated embryo in stage *C*.   The rudimentary cephalic lobes are depressed and rotated to the embryo's right.   Their lateral margins are still further invaginated to form two oval depressions, nearly concealed by distinct folds that have grown over them from the sides, *b.iv.* The oesophagus is comparatively large, and the slit-like opening is also rotated toward the left.

The chelicerae are probably absent.   On the left are the fourth (?) and sixth (?) appendages, but the fifth is invaginated.   On the right the fourth (?) and fifth (?) are invaginated, and the sixth (?) persists.

A telopore is present behind the last pair of appendages.   Back of the embryo is seen a long, faint band of yolk cells, *p.a.c.*, formed by the concrescence of the margin of the mesodermic area.

FIG. 66, × 60, not sectioned.   A small embryo in stage *C*.

The cephalic lobes are not distinguishable as such, but the oesophagus is distinct.   There are three appendages (fourth, fifth, sixth[?]) on the left, and two on the right.   In place of the posterior part of the thorax is a broad depression.

FIG. 67, × 60, not sectioned.   A narrow embryo, depressed along the median line, and surrounded by high and sharp marginal folds.   The cephalic lobes are depressed below the level of the thoracic nerve-cord.   The second(?) and third(?) pairs of thoracic appendages are present with one more appendage on the right.   In the abdominal region is a deep, pyramidal depression, directed nearly vertically downwards.

The dark area along the marginal fold of the embryo is produced by unusually thick layers of mesoderm.   The margin of the mesodermic area is not clearly defined; it lies, in part, at least, beyond the limits of the figure.   On the left side of the embryo, in the thickened mesodermic margin, is a large, marginal vesicle. The two dark areas back of the abdomen are formed by longitudinal, ridge-like thickenings of the ectoderm and mesoderm.   Between the two ridges is a depressed area covered by a thin layer of mesoderm and ectoderm.   It appears, therefore, that the posterior median margins of the mesodermic area have not completely united, except at their very posterior ends.   In front of this point is a rather large rhomboidal space not covered by the mesodermic area.

FIG. 68, × 60, sectioned. In this embryo the cephalic lobes and cheliceral segment have disappeared, except for a cloud of degenerating cells in the underlying yolk. These cells lie where the cephalic lobes ought to be, and perhaps they represent the remnants of these organs ; but, on the other hand, they have more the histological character of loose mesoderm cells, and are produced, in part, at least, by the concrescence of the thickened anterior margins of the mesodermic area ; they thus resemble the post-anal band formed by the concrescence of the posterior margins of the mesodermic area. This anterior band of cells gradually becomes more dense posteriorly, till it finally merges into a flattened, oesophagus-like invagination, which opens just over the anterior margin of the fused second pair of appendages, *oe*.

A remarkable feature of this embryo is the curious invagination extending along the whole left margin of the mesodermic area, *i.m.a.* This very narrow-mouthed infolding converts the mesodermic rim into a thin shelf of cells, projecting deeply into the yolk. In most sections the cavity of the invagination is very plain, and it is then seen to be lined with a continuation of the thin surface cuticula. (See sections, Pl. XI, Fig. 68.)

Along this furrow, and in the margin of the aborted cephalic lobes, the surface of the ovum is covered with masses of minute, intensely red dots that look very much like bacteria. The dots are also found just below the surface, and then it often appears as if some of the nuclei had ruptured, allowing a swarm of chromatin granules to escape.

The second pair of appendages have fused, forming a broad, biloped plate back of which the nerve-cord terminates. The fifth and sixth appendages of the left side are absent. The abdomen is absent ; in its place is a very deep, thick-walled depression. The marginal fold is very thick posteriorly, and terminates abruptly in a foot-shaped enlargement that dips down into the depression, and forms a part of its lateral wall. The posterior end of the right marginal fold is weak, and is not connected with the fold on the opposite side.

The dark area back of the abdomen, *p.a.c.*, represent a great cloud of cells lying in the yolk, and formed by the concrescence of the posterior portions of the mesodermic rim.

FIG. 69, × 60. Embryo with nearly circular mesodermic area. The nerve-cord appendages and cephalic lobes have disappeared.

At the anterior end is a mass of mesoderm with a conical cloud of yolk cells beneath, so faint as to be hardly visible in surface views. From this point the longitudinal, ecto-mesodermic band, gradually increases in distinctness toward the posterior end, where the two layers that compose it are sharply separated. The outer layer is here raised to form the median protuberance, which probably represents an enlarged tail lobe. The outline of the mesodermic area is clearly defined, and along its lateral and posterior margin are numerous irregular masses of cells that extend deeply into the yolk.

The tissues appear healthy and normal, but there is no trace of organs other than the median appendage.

Pl VII

64. *d.cl* 63.

65. *b.io* *n* 64.

66.

67. 68.

Pl. VII
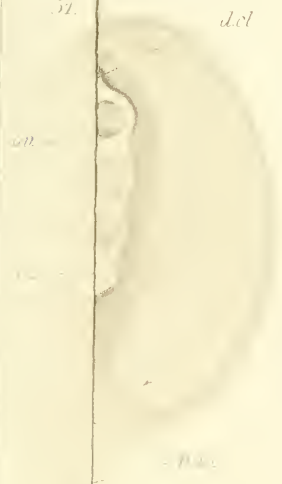
### EXPLANATION OF PLATE VIII.

FIG. 70, × 60, not sectioned.    A very much reduced embryo probably in
stage *C*.   There are faint indications of the nerve-cord and cephalic lobes and
oesophagus.   Two appendages are present on the right ; on the left the corre-
sponding appendages are invaginated.

Back of the embryo is a long band of inner layer cells formed by the concresced
margin of the mesodermic area.   The length of this band affords some indication
of the advanced stage of development of the embryo, and the amount of reduction
it has undergone.

The mesodermic area is nearly circular and very large, only the posterior por-
tion being shown in the figure.   Its extent may be approximately determined by
continuing the curved lines, *c.m.a.*

FIG. 71, × 60, sectioned.    This embryo is so flattened and distorted that its
parts are hard to identify.   Beginning at the anterior end of the embryo and
passing backward, we find first a deep depression, at the bottom of which is a
well-defined nerve-cord.    From the right wall of the depression arises a large
appendage, $ap^1$, that projects over and conceals the nerve-cord.   Its mate on the
opposite side is absent.

Back of the depression, the surface of the embryo rises to nearly the level of
the ovum, and here is a nearly normal neuromere, *n.m.*, with an invaginated
appendage on either side of it, $ap^2$.   The ectoderm then thins out considerably,
but thickens again to form an indistinct nerve-cord, $n.m^2$, with a large invaginated
appendage on the left, $ap^3$.   Following this are two swellings that may represent
appendages ; and finally this decidedly mixed-up collection of organs terminates
in a thickened ectodermic disc, which appears to represent the anal plate.    In
both sections and surface views indications of the thickened mesodermic margin
are seen on the side.   Posteriorly the margin is very broad, and, owing to
concrescence, deeply notched.

This embryo is in the last stages of degeneration that show recognizable organs.
In the following figures, even these rudiments disappear, and nothing remains
but simple layers of mesoderm and ectoderm, complicated by more or less irregular
foldings.

In the following figures, 72–78, so far as one may judge from what little
remains of the embryos, there has been median fusion, and antero-posterior
degeneration.   The embryos were probably laid down throughout their whole
extent, and then underwent a gradual median fusion and degeneration, *confined
principally, however, to the axial organs, and to the region midway between the two
extremities*.   In none of this series is there any recognizable trace of nerve-cord,
appendages, or other axial organs ; although the peripheral mesoderm retains in
many cases very nearly its normal character.   It is assumed in each case that the
present condition was preceded by one having nerve-cords, appendages, cephalic
lobes, and sense-organs, and that these have gradually faded away, leaving only
the mesodermic area in a recognizable condition.   Within this area are two (sel-
dom but one) great masses of mesodermic (?) or yolk (?) cells, either with or
without an overlying ectodermic invagination.   They represent the remnants of
the head and tail.

It is very interesting to note that these two cell masses resemble in position and general appearance the two proliferating areas, "primitive cumuli," which at a very early period give rise to the head and trunk of normal embryos.

No trace of primitive cumuli is visible in normal embryos after stage *C*, and as these embryos are certainly as far advanced as that stage, they either must have retained to this late period a very early embryonic character, or else the processes of degeneration have carried them back a second time to their original condition.   In either case, the facts seem to emphasize the morphological importance of these two proliferating centres.

There is no very reliable indication as to what is the head or tail *Anlage*, except perhaps in Fig. 73.   The embryos have been oriented in each case with the smaller *Anlage* at the anterior end, and arranged and numbered as far as possible in accordance with the degree of degeneration.

It is obvious that such a plan of arrangement cannot be followed with any accuracy, since each embryo has its own way of degenerating, begins to degenerate at different stages of development, and may have been defective at the outset. What finally happens to these degenerated embryos cannot be determined, since further degeneration of either head or tail *Anlage* would make it impossible to determine whether it belonged to this series or the next.   But I see no reason to doubt that degeneration goes on till even the head and tail *Anlagen* are absorbed and disappear completely.

While Fig. 73 is the first one of the series here represented, numerous intermediate stages between it and normal embryos of stages *C* and *D* were found.

Just how far normal development may proceed before this general degeneration begins could not be determined.   I do not recollect seeing any embryo undergoing this phase of degeneration, which in whole or in any part had reached a higher stage of development than that characteristic of stage *D*.   But it must be borne in mind that all these embryos had been developing or degenerating in the same jars with normal eggs, for from fifteen to eighteen weeks.   The normal ones passed through their whole development and escaped from the jars as trilobite larvae, in but little more than half this time.

FIG. 72, × 60, sectioned.   In this embryo, every trace of cephalic lobes, oesophagus, nerve-cords and appendages has disappeared.   The mesodermic area is very large and irregular.   Anteriorly the margin of the same is pretty close to the axis of the embryo.   It there consists of numerous star-shaped masses of cells, densely crowded together, *m.a.*   The posterior margins are much thickened to form two great diverging arms, which are united at their distal extremities, but are separated medially by a triangular area devoid of mesoderm cells (compare Fig. 67).   These thickened arms unquestionably represent the posterior margins of the mesodermic area which has not completely concresced along the median line.   It is important to recognize this, because it shows approximately to what a late stage of development this embryo belongs.   Such embryos as this and those that follow on this plate, cannot for a moment be considered as belated normal embryos, in early stages of development.   Their great age (two or three months), the concrescence of the mesodermic margins, the histological character of the tissues, and a comparison with the early stages of normal embryos, is sufficient to prove this beyond any question.

A thin layer of ectoderm covers the greater part of the mesodermic area, but it is thickened and elevated in the axial region, over what probably represents the

body of the embryo. The elevation forms a broad, figure-8-shaped ridge (marginal folds?), with a slit-like depression in about the centre of each loop. There is a thickened layer of mesoderm beneath the whole of this axial portion.

Beneath the anterior end is a very large solid mass of mesoderm cells, *c.m.s.*, from which pseudopodia-like streamers of cells radiate deeply into the yolk.

FIG. 73, × 60, sectioned. In this embryo the tissues are beautifully clear, and sharply differentiated, resembling histologically the late stages in the formation of the blastoderm of normal embryos. The ectoderm, instead of containing flattened cells with little protoplasm, is composed of high columnar cells with nuclei at their outer ends, their inner ends being filled with masses of yolk granules. The whole layer is sharply separated from the underlying yolk and mesoderm.

There is no cluster of yolk cells at the anterior end. The dark median band consists of columnar ectoderm cells, like those described above, but higher, and below them is a clear band of mesoderm. Both layers are entirely undifferentiated.

At what seems to be the posterior end, is a deep, oblong depression with two infoldings of its anterior wall, like steps leading down into it. Numerous degenerating nuclei arise from the inner surface of the infolded layer, and lie scattered about in the neighboring yolk. The mesodermic area is circular, with a faint thickening of its anterior and lateral margins. There is no trace of a posterior concrescence.

FIG. 74, × 60, sectioned. Embryo similar to the preceding. It consists of two disc-shaped ectodermic thickenings, connected by a longitudinal band of the same nature. Beneath the thickened ectoderm are corresponding mesodermic thickenings, but especially enlarged at either end.

There is a shallow depression in the centre of each ectodermic disc. In both Figs. 74 and 76 the ectoderm of the posterior thickening shows a higher grade of histological differentiation than those of the anterior one.

The peripheral outline of the mesodermic area was not visible.

FIG. 75, × 60, sectioned. An embryo with a circular, mesodermic area, and a "blastopore"-like invagination at either end.

The invaginations are similar to those already described, except that comparatively few yolk cells surround the invaginations. The outer surface of the invagination is covered with a thick layer of bacteria (?). The ectoderm is a uniformly thin layer of cells, showing no trace whatever of a median thickening connecting the two invaginations. The mesoderm is remarkable in that it is of nearly uniform thickness throughout its whole extent, except at the margin where it is decidedly thickened, *m.a.*

*Most of the marginal mesoderm is composed of the characteristic, striated cells seen in stage D, and later.*

FIG. 76, × 60, sectioned. This embryo consists of a circular, mesodermic area containing scattered, star-shaped masses of cells, either extending from the mesoderm into the underlying yolk, or lying freely in it. There is.a deep, circular, ectodermic invagination at the anterior end with a thick layer of mesoderm surrounding it, and a similar, but larger, deeper, and more irregular cavity, at the posterior end. The ectoderm lining this invagination is considerably thicker than that of the anterior one, and the thickening extends along the surface for some distance back of the infolding, *p.a.c.* This thickening is probably the result of the concrescence of the posterior margins of the mesodermic area. There are

many degenerating nuclei in the ectoderm and mesoderm of the posterior thickening, but comparatively few in the anterior one.

There is a gently elevated, thickened band of ectoderm, connecting the two invaginations, but it does not show any histological differentiation, and the slightly thickened layer of mesoderm underlying it is not segmented or otherwise specialized.

FIG. 77, × 60, sectioned.    In this embryo everything has disappeared except two masses of cells, one at either end of what was the body of the embryo. There is a small cluster of mesodermic cells at the anterior end, and a similar one at the posterior extremity.    Over the latter is a thickened ectodermic area with a rather deep, vertical invagination in its centre.    There is no indication of any ectodermic or mesodermic thickening connecting these two masses of cells.

The outlines of the mesodermic area are not well defined.

FIG. 78, × 60, sectioned.    Embryo with well-defined, circular, mesodermic area.    There is an axial band of thickened ectoderm, slightly enlarged at either end, and beneath it is a broader band of mesoderm, which becomes enlarged at either end to form two great irregular masses of cells, *c.d.*.    The latter project deeply into the yolk, and from them radiate pseudopodia-like processes, composed of dense masses of cells.    Over the centre of these masses, ectoderm and mesoderm are continuous, as though the inner cells arose by proliferation from the outer ones.    The outer layers at these two points, however, contain no karyokinetic figures, but instead, numerous degenerating nuclei.

The ectoderm over the posterior mesodermic enlargement is composed of a very thick layer of columnar cells, but shows no noteworthy histological differentiation.

No bacteria (?) were visible on the surface of the ovum, and the tissues appeared healthy and normal.

FIGS. 79–89.    In this series, the processes of degeneration have followed somewhat different lines than in the one just described (Figs. 72–78).    But we do not assume that there is any sharp distinction between the two series.

We have here in some instances undoubted evidence that the embryos in question represent mere remnants of highly developed individuals, or to put it more accurately, some of these embryos may consist of a single recognizable organ only, which may be in the condition seen in normal embryos of stage $D$, while every other organ characteristic of this stage has either failed to develop or has completely degenerated.

This is especially true of the embryo shown in Fig. 82, where the large tail lobe and the concrescing mesodermic margins suggest the conditions seen in stage $A$ and $D$ (see Figs. 5 and 6), while the remainder of the body of the embryo is reduced to a mere sac, invaginated into the yolk.

In the cases that seem to lead up to this condition, the body of the embryo is usually pinched and narrowed and much depressed, with high, conspicuous, marginal folds that approximate each other toward the median line, Fig. 70. The appendages may or may not be present, but if present, they are usually invaginated.

Practically all conditions, from normal embryos, and ones like those in Figs. 61, 62, and 67, to those shown in Figs. 81 to 89, have been observed.

From the first condition towards the last there is a gradual reduction of sense-organs, nervous system and appendages, till nothing is left of the embryo, but a

more or less clearly defined mesodermic area, with a central thick-walled sac, representing the remnant of the axial portion of the body. This sac may be a single one, regular in outline, or it may be more or less irregular, as though composed of two or more sacs arranged one behind the other along the median line (Fig. 81).

These sacs may be of a different nature in each case. In Fig. 61, the dilatations of the common furrow represent single metameres, from which the appendages and nerve-cords have nearly disappeared. In Figs. 72 and 79, each sac probably represents the remnants of several segments. These embryos may belong to the hour-glass series, where there is a transverse constriction between the third and fourth thoracic segments dividing the embryo into two distinct parts. Subsequent degeneration of the appendages and other organs would reduce each part to a separate sac.

When the sac is comparatively large, and communicates with the exterior by a small opening only, as in Figs. 82, 83, 84, 85, and 86, a study of the sections through the sac reveals an unexpected complexity of structure, the walls being thickened or variously curved, so as to suggest that the cephalic lobes, with their various ganglia, and the anterior part of the nerve-cord, still persisted, while the appendages had disappeared. I have not been able to satisfy myself that such was really the case, for the various parts are thrown so much out of position, that it is impossible to identify them. But the whole histological character of the thickened walls of these sacs resembles nerve tissue more than anything else. For this reason, and owing also to the shape of the sacs, cross-sections of them resemble cross-sections of embryonic vertebrate brains. This is true of Figs. 83 and 84, especially the latter, where the tubular outgrowths from the anterior of the sac recall the similar outgrowths to form the lateral eyes in vertebrates.

These sacs themselves degenerate. Their walls collapse and lose their character as distinct cell layers, till there is left merely a central mass of cells in a much contracted mesodermic area. All distinction between these two parts, *i.e.* ectoderm and mesoderm, is gradually obliterated, and what then remains seems to disappear completely by absorption. It would be a very difficult matter to prove that this complete absorption really does occur, for one cannot follow any single individual through the process, but there is no difficulty in finding among these degenerating eggs a few apparently healthy ones in which there is no trace of nuclei or cell clusters to be found. There is also no difficulty in finding ova, showing various stages, from embryos composed of a barely recognizable cluster of cells, to ones like that in Fig. 89. These ova without embryos may be three or four months old, and if they had failed to develop in the first place and died, it is difficult to understand how they could be preserved so long without putrefaction.

In all these embryos, 79–89, as well as those in the preceding series, the tissues at first sight appear perfectly normal and healthy. They stain well, and one may find some nuclei in karyokinesis. But in one part or another may be seen, among the perfectly normal ones, numerous nuclei, in which the chromatin is crowded at one or both poles, leaving the centre perfectly clear. In those apparently just entering on the process, the chromatin stains very deeply, as in nuclei undergoing karyokinesis, but in the later phases the color becomes less intense. The nuclear wall disappears, and finally nothing is visible but a few faint, isolated chromatin granules, which in turn disappear also. Immense masses of these nuclei are sometimes seen at the anterior and

posterior ends of the degenerating embryos. They are also seen along the longitudinal, median line, in the nerve-cords, and in the appendages.

I have described and figured just such nuclei as these in the ectoderm about the eyes of Acilius, and have seen them in the degenerating masses of endoderm cells, or yolk nuclei, at the anterior and the posterior ends of healthy and normal scorpion, Limulus, and insect embryos.

Similar nuclei are found, and they undergo the same changes, in the degenerating amnion and serosa of insects, where these membranes, after forming a "dorsal organ," are invaginated into the yolk.

In none of these cases are the degenerating nuclei ingested by more vigorous amoeboid cells; on the contrary they disintegrate and then fade out of sight, probably by forming solutions that diffuse gradually throughout the yolk or other tissues.

FIG. 79, × 60, sectioned. The mesodermic area is ovoid. There is a well-marked marginal thickening; also a post-anal one, *p.a.c.*, showing where concrescence has taken place. Along the axis of the embryo the thickened ectoderm is elevated and contains two distinct, elongated invaginations. At the posterior end of the second one is a small and still deeper invagination. The lateral walls of the second invagination are constricted at a point that probably marks the line of separation between two adjacent segments; compare Figs. 61 and 81. The invaginated ectoderm, here, as in the preceding figures, probably represents the remnant of the whole axial ectoderm of normal embryos, *i.e.* cephalic lobes, nerve-cords and appendages, as far laterally as the marginal fold.

This embryo may represent the last stages of an hour-glass embryo, the anterior sac being the remnant of the cephalic lobes and first three pairs of appendages, and the posterior sac the remnants of the last three thoracic appendages and the abdomen. Compare Figs. 52, 53, 54, 55, and 57.

FIG. 80, × 60, sectioned. This is similar to the preceding, only the concrescence of the posterior margins of the mesodermic area is much more clearly shown, *p.a.c.* There is hardly any trace of the axial portion of the embryo, other than an elongated, ectodermic thickening with a large diamond-shaped depression extending along it. In surface views, there are no indications of nerve-cords or appendages, but in sections there are slight differentiations of the thick wall of the furrow, that may represent the last traces of the nerve-cords and appendages.

FIG. 81, × 60, sectioned. Over a heart-shaped, mesodermic area lies a thin layer of ectoderm, elevated considerably and thickened along the median line. Along the summit of the ridge is a series of depressions increasing in size from before backwards. The third depression is so deep as to be almost tubular. The last is broad and shallow, but has a rather deep depression in its centre. Each depression probably represents a segment from which nerve-cord, appendages, etc., have disappeared. The ectoderm has the appearance of old specialized tissue, although one cannot identify any particular kind of tissue or organ in it.

FIG. 82, × 60, sectioned. This extremely instructive specimen shows clearly the real stage of development, and the nature of the changes this class of embryos has undergone. The mesodermic area is kidney-shaped, and precisely defined by a thickened margin. The latter forms two distinct lobes that are manifestly growing toward each other in order to concresce along the posterior median line. On examination of the normal embryos it will be seen that this phase of concrescence is not due till stage *C–D*. The abdomen of this embryo

has apparently persisted as a large, conical lobe, projecting almost vertically upwards from the surface of the yolk. It is evidently the same kind of a lobe seen in Figs. 13, 48, and 49. The body of the embryo, as in the three preceding figures, is reduced to a mere sac. The opening of the sac is triangular, and almost as wide as the sac is broad. A little to one side, on the floor at the anterior end, is a small, tubular invagination, oesophagus (?), directed vertically downwards.

FIG. 83, × 60, sectioned. This is another important embryo. The mesodermic area is relatively small, and not very sharply defined anteriorly, owing to the small number of mesoderm cells contained in it. Posteriorly, and this is a point of value in determining the poles of the embryo, the mesodermic margin is decidedly thickened, and shows very clearly the effects of concrescence, *p.a.c.* The axial ectoderm shows the usual thickening, and is invaginated to form a complicated, thick-walled sac, opening by an oval aperture. The sac projects forwards beneath the ectoderm, and at its anterior end expands into two transverse tubes. If the nerve-cord and the cephalic lobes are still present, although they cannot be recognized as such, it is obvious that the floor and sides of the sac will consist of these organs, while the lateral eyes would be situated somewhere near the end of the lateral diverticula. This condition is what normally obtains in vertebrates, and is of obvious significance in view of the other resemblances which we have pointed out elsewhere, between the normal brain and eyes of Limulus and those of vertebrates. Such embryos are extremely rare, and may have no morphological value. But the possible significance of such variations should not be overlooked.

Beneath the aperture of the sac, the floor of the same suddenly slopes downward, and at the bottom of this depression is still another smaller and tubular one that projects downward and forward into the yolk. The character of the tissues, which seem to be perfectly normal, and further structural details, are shown in the three transverse sections (Pl. X, Figs. $83^{1,2,3}$).

FIG. 84, × 60, sectioned. Of the mesodermic area, which is nearly circular, only the posterior portion is represented. The whole area consists of a thick layer of rounded, and often isolated, lymphoid cells, lying in a space between the yolk and a thin layer of ectoderm. There is a prominent marginal thickening extending completely around the mesodermic area. In the centre of the latter the ectoderm is thickened and deeply invaginated to form a roughly cubical cavity, partly closed over in front by a backwardly directed lip, or fold, and behind by a similar one directed forwards.

The thick walls of this cavity probably represent that part of the embryo from which the nerve-cords and appendages are developed, the whole complex mass being reduced to a continuous, undifferentiated layer.

From the anterior wall of the cavity projects a short, tubular outgrowth that looks very much like an embryonic stomodaeum, the resemblance being increased by the presence of *a pair of muscle strands*, which extend laterally from the tip of the tube to the surface ectoderm. The presence of this specialized tissue offers a sharp contrast to the very early embryonic character of the rest of the embryo.

This fact supports my view, if further evidence were necessary, that these are very old embryos whose organs have been reduced, with this exception, to the simplest embryonic tissues.

FIG. 85, × 60, sectioned. The mesodermic area here is small, circular, and poorly defined. The margin, however, at the anterior border is greatly enlarged

to form a marginal vesicle, *m.v.* In the centre is a kite-shaped sac opening to the surface by an elongated figure-8-shaped opening, *c.* The anterior part of the floor of the sac protrudes upwards and forwards (cephalic lobes[?]), and from this inclined wall projects forwards a short tube or stomodaeum(?), *b.* Both structures are seen in sections in Pl. X, Fig. 85$^{1,2}$. The sac extends backwards some distance beyond the aperture, *c,* as a cylindrical tube which ends blindly deep in the yolk.

There is a shield-shaped thickening of the ectoderm, lying over and a little to the right of the sac. It is represented by the shaded area, *d.* A section through this thickening and the posterior end of the sac is shown in Fig. 85$^2$.

FIG. 86, × 60. This embryo is similar to the preceding one. Unfortunately, it was lost or injured before it could be sectioned. In the centre of the mesodermic area is a thick-walled sac, which communicates with the exterior by a diamond-shaped opening. The anterior end of the sac is very broad and spacious, and the posterior part a deep, nearly closed furrow terminating in a tube that dips vertically into the yolk. The relation of these parts, so far as they could be determined in surface views, is shown in the accompanying median, longitudinal, optical section. Fig. 86, *B.*

FIG. 87, × 60, not sectioned. The mesodermic area appears to be nearly circular, but its outline is very indistinct. In its centre is a conical, thick-walled projection, with an opening at the summit leading into a large, nearly spherical sac. Specimens resembling this one in all but unimportant details are comparatively common. They are evidently further modifications of the conditions seen in the five preceding figures. This specimen was not sectioned, as it did not appear to present any noteworthy features.

FIG. 88, × 60, sectioned. In this case a sac like that seen in the preceding figures is beginning to break up. The mesodermic area is distorted, and on the left ill defined; on the right the rim of the mesodermic area shows the character-istic, star-shaped masses of cells that are spreading out into the yolk, preparatory to their final dissolution.

In sections of other specimens, apparently in a similar condition to this one, the walls of the sac have lost their sharp outlines, as though they were gradually falling apart; and the cells composing them have the character of closely packed, lymphoid cells, rather than that of the columnar ones seen in the preceding figures. Still one would hardly suspect, from a mere inspection of the sections, that these specimens were anything else than very early stages of normal embryos.

FIG. 89, × 60, sectioned. In this remarkable specimen we have a good example of the last stages in the degeneration of the embryo. In all the other cases I have studied, where the thick walls of the sac had broken up into a form-less mass of cells, the latter were usually rounded, lymphoid ones, showing no further differentiation. But in this instance the entire mass, representing all that is left of the embryo, is composed of those peculiar cells, containing coiled filaments that are only found at a late embryonic period in the thickened margin of the embryonic area. I have already referred to these cells in one of my earlier papers ("Origin of Vertebrates from Arachnids," p. 375), and at that time supposed they were purely embryonic, and gave rise in some instances to the future muscle cells. Since then, however, I have found them as free, amoeboid cells, in great numbers in the tissues of the adult animals. They differ somewhat in histological characters from the embryonic ones of the mesodermic margin, but there can be no question whatever about their identity. In the adult these cells are very

abundant all through the tissues about the median and lateral eyes and the olfactory organs, and I see no reason to doubt that they will be found as abundantly elsewhere. Kingsley failed to confirm the existence of these cells in the embryo, which is somewhat surprising, for in stage $D$ they form as conspicuous an object in sections and surface views as any other organ of the body.

I expect to describe in more detail the history of these cells in another paper. I refer to them here to make clear the remarkable fact that in these degenerate embryos there is almost nothing left but a mass of these highly specialized and peculiar cells. The only thing remaining besides them are the ordinary yolk cells and the blastoderm, and even this disappears, as such, over the mass of cells under consideration. It is as though a mammalian embryo should gradually degenerate into a mass of cells consisting solely of osteoblasts, or muscle cells, or any other specialized form of tissue. However, it must not be assumed that other kinds of cells are during degeneration converted into the fibre cells. The latter merely persist as such after the others have disappeared.

On careful study of one of the sections we see the fibre cells usually in ill-defined groups, with intermediate areas containing some nuclei, unquestionably in karyokinesis. Other nuclei, however, seem to have made preparations for division, but instead of doing so they break into numerous deeply stained globules, which become disseminated through the yolk, and growing fainter and fainter, finally disappear.

A portion of a section through the embryo is shown in Pl. X, Fig. 89. Below the disc is a clear area containing a few yolk globules, and many degenerating nuclei and cells, apparently derived from the mass of cells above.

Pl VIII

## EXPLANATION OF PLATE IX.

In this plate, double monsters are shown in various stages of formation.  In all the cases I have seen in Limulus, double embryos are formed by fission, or more correctly by the gradual intercallation, beginning at the anterior end, of two new halves between the old.  If there are five paired organs on each segment, and *a* is the most median one, and *e* the most lateral, then *a* will be the first new organ to appear, and it will appear in the median line of the first segment, as an unpaired organ, having the same appearance as each member of the paired organ.  It divides, and in its place in the same segment will be found an unpaired organ like organ *b*.  But at the same time a new, unpaired organ, like *a*, will be formed in the median line of segment number two.  At the next division, organ *a* will be produced in the median line of the third segment, *b* in the second, and *c* in the first; organs *a* and *b* being now completely formed in pairs in the first segment, and organs *b* in the second.  This process goes on till two complete new halves are wedged in between the old, and two new individuals are produced, each individual consisting of an old and a new half.  The old halves were produced by normal apical growth from behind forward ; the new half was also produced by apical growth, but from before backwards.  Each new half is a mirror image of the other, and at the same time the mirror image of the old half next to it ; but the new halves are united by their lateral margins instead of the median ones.

The very first steps in this process have not been seen.  Hence the manner in which the new halves of the cephalic lobes and the oesophagus are produced, can only be inferred from the manner in which new organs are formed in the post-oral segments.  The process of forming two new organs by the division of the unpaired ones, takes place in exactly the reverse order that unpaired organs are formed by the fusion of paired ones.

Fig. 90, $\times$ 33.  The entering wedge formed by the newly produced organs has reached the fifth thoracic segment.  At the end of the wedge is an unpaired neuromere ; in the next in front of it are two new halves of a neuromere and an unpaired appendage.  In the next segment in front of that are two complete and newly formed chelicerae.

Fig. 91, $\times$ 33.  Here the process has progressed still further.  The division of the new appendage in the third segment is almost completed, the separation extending from the tip nearly to the base.  In the fourth segment the new appendage is perfect, but shows no trace of division.

Fig. 92, $\times$ 30.  The growth of the new halves is practically completed, forming two distinct embryos, which have, however, an abdomen in common.  The new halves lie on the upper side, as the egg is placed in the figure, the old ones being below.  Each of the old halves has been rotated on its tail end 90°, one to the right, the other to the left.  They have been forced apart in this way, at first by the wedge-like ingrowth of the new nerve-cords.  But as the organs lateral to the two nerve-cords develop, they push the previously formed parts still farther right and left.  This goes on till the new embryos form a straight line tail to tail.  Further rotation of the old halves will be stopped by the interference of the lower margins of the mesodermic areas at *y*.

FIG. 93, × 33. The process seen in the preceding figure has evidently begun here. But before the two new halves were completely formed, the left-hand embryo began to concresce along its own median line. The cephalic lobes and first two segments have disappeared in that way, exactly as in single embryos (see Pl. IV). The sixth pair of appendages has not been formed in the new halves.

FIG. 94, × 33. Here the process seen in the preceding figure has been carried further. Fission probably occurred at first, as in Fig. 92; degeneration of one embryo then followed, as in Fig. 93; and finally the two embryos separated, moving tail first in opposite directions to the position they now occupy. The right-hand embryo is apparently normal; the left-hand one has been reduced by antero-posterior fusion and degeneration, till the last two pairs only of thoracic appendages remain. The fourth pair have united in the median line. The "dorsal organs," *d.o.*, are still separate and very distinct. Between them is a small mass of cells, the remnant probably of an unpaired appendage. This embryo, with its five legs and large tail lobe, is very similar to that in Pl. V, Fig. 48.

FIG. 95, × 33. This is a double embryo which, at one stage of its development, was probably like that in Fig. 92. The normal upper embryo still occupies its former position tail to tail with the lower one, which median concrescence and antero-posterior degeneration has reduced to a small, median appendage, projecting from an oval depression. The abdomen is a narrow oblong thickening, and at the opposite extremity of the body is a median depression that may represent the fused dorsal organs (compare Fig. 97), or the remnants of an oesophagus.

On either side of this much-reduced embryo is an irregular, dark band, formed by the concrescence of the margins of the mesodermic areas of the two embryos, and probably representing two rudimentary hearts.

FIG. 96, × 33. This is a much older embryo than any of the preceding. It has passed successively through approximately the same stages seen in Figs. 92–94. The exact sequence of events, of course, cannot be determined; but we can understand the position of appendages in the right-hand embryo, by assuming that after a tail-to-tail condition was reached median fusion and antero-posterior degeneration of the lower embryo followed, somewhat as in Fig. 93. Then the tail of the lower embryo pushed past the tail of the upper, going to the right of it instead of to the left, as in Fig. 94. But meantime the margin of the thorax of the larger embryo had grown so large that it had practically preëmpted the territory in that region; the tail of the smaller embryo was thus forced to bend to the right, through an angle of about 90°. The curvature thus produced of the axis of the smaller embryo is shown by the arrow *x*, and by the dotted line which runs along the median line from the fourth thoracic appendage, *ap* $^4$, to the abdomen. The latter is perfectly normal and well developed. The cephalic lobes and first three neuromeres have disappeared, through median fusion and antero-posterior degeneration. The appendages of the fourth and fifth segments have fused along the curved median line, and are very much reduced in size. The sixth pair, *ap* $^6$, are still quite large, and fused only at their base. The flabella, *fl.*, are very large, and each is separated by a relatively wide space from the base of its respective appendage.

Just under the end of the left sixth appendage is a small projection that cannot be accounted for, as its position is such that it cannot be brought into line with any of the other appendages. It may be the left flabellum of embryo *A*.

Fɪɢ. 97, × 33. This embryo is in an older stage than the preceding one. It is an almost perfectly symmetrical double embryo, tail to tail as in Fig. 92. Median fusion and antero-posterior degeneration have affected both embryos, but the lower one more than the upper. In the latter, everything as far as the fourth segment has disappeared. The dorsal organs are very large, and are supplied on one margin with considerable black pigment, a condition that has not been seen in the normal embryos. They have approached the median line, but have not fused with each other. The appendages of that segment, *i.e.* fourth, have fused, and the single appendage thus produced is reduced to a small conical projection, *ap* 4. The fifth pair have also fused, forming a long zigzag appendage. The sixth pair have fused at the base to form a large oval vesicle, from the summit of which project the separate ends of the reduced appendages. The chelaria and abdominal appendages are normal.

In the lower embryo, *B*, the "dorsal organs" on the fourth segment have fused. Everything anterior to that has disappeared, but the appendages of the fifth and sixth segments remain as elongated, crumpled, unpaired organs.

The nerve-cords extend without interruption or modification from one animal to the other. There are two perfectly normal hearts and two abdominal lobes, both organs being shared in common by the two embryos.

Fɪɢ. 98, × 33. This is a very interesting case, as it shows clearly three entirely separate phenomena, *i.e.* (1) longitudinal division ; (2) median concrescence ; (3) transverse fission. The two latter conditions have been seen in the single embryos previously described. The two embryos now form almost a straight line. The abdomen of the original embryo is intact, and would have become, in all probability, the abdomen of embryo *A*. A remarkable fact is the obvious "weakness" of the new half of embryo *A*, as compared with its old, right side. This is shown by the absence of its second and fourth appendages, and the fourth neuromere. In place of the fourth appendage is a minute pore that may be the invaginated remnant of the same. Embryo *B* is a beautiful example of the hour-glass type, the constriction occurring in the usual place, between the third and fourth segments. The appendages of the third and fourth pairs are fused in the median line. In front of the third pair, and back of the fourth, is a gradual diminution of the concrescence. The large size of the chelicerae in embryo *B* is surprising, considering the otherwise reduced condition of the embryo.

Fɪɢ. 99, × 35. This is a very rare form, and the only one of the kind I have seen. It at first sight seems to belong to a different class from the preceding, and to have been produced in a different manner. However, it is easily explained by assuming that during fission, like that in Fig. 91, median fusion and antero-posterior degeneration destroyed the anterior part of embryo *B* as fast as it was formed. Two new nerve-cords extended to the tip of the abdomen, and a row of unpaired appendages, extending from the fourth abdominal to the fifth thoracic segment, have been formed.

Pl. IX
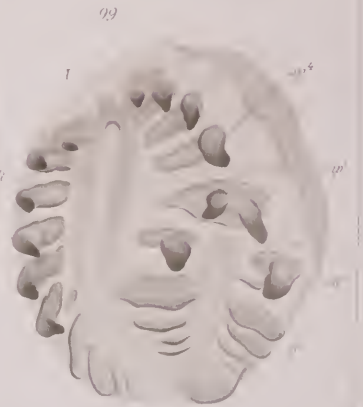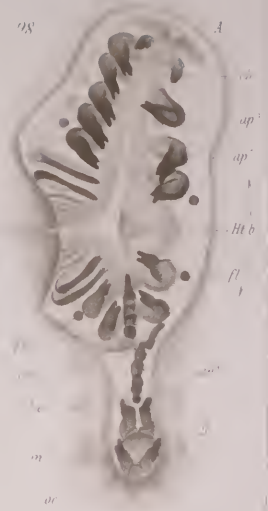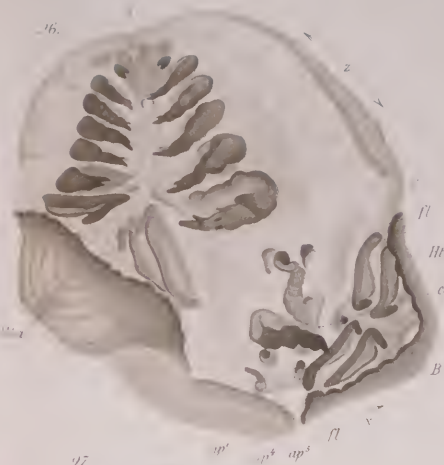
## EXPLANATION OF PLATE X.

FIG. 100, × 25. This is one of the oldest double monsters seen. It should be compared with Figs. 96 and 98. The development of the new halves had apparently not separated the old ones much more than in Fig. 98. The main embryo has the abdomen of the original embryo.

In the smaller embryo are two unpaired, tongue-like, abdominal appendages. The appendages of the sixth segment are fused at their base, and in front of them is a row of three long, crumpled filaments, representing the medially fused appendages of the third, fourth, and fifth segments. All the segments in front of the third have disappeared.

FIG. 101, × 30. Same embryo as in the preceding figure, seen from above. The dorsal surface of the thorax of the smaller embryo is spread out on that of the larger. Below the dark mass of cells that represent the remnants of the thickened margin of the mesodermic area is an elongated cloud of cells, probably the remnant of the oesophagus, *oe*.

There is a well-developed heart in the abdominal region of the larger embryo, but none in that of the smaller one.

FIG. 102, × 30. There are three embryos on this egg. Embryo *A* is normal and perfect in everything except the abdomen. *B* has undergone median fusion and degeneration, and transverse fission. The cephalic lobes and first four segments have disappeared, except two incompletely fused appendages. The abdomen and the posterior part of the thorax persists. The latter is bounded in front of the fifth pair of appendages by a great fold that extends completely across the median line. The nerve-cord in this posterior remnant of an embryo forms a conspicuous, unpaired ridge.

Embryo *C* has undergone such fusion and antero-posterior degeneration that nothing remains but the fused appendages of the sixth segment, and a rudimentary abdomen.

It is probable that the original embryo divided lengthwise, giving rise to *A* and *BC*, and the latter then divided, giving rise to *B* and *C*.

FIG. 103, × 30. In this triple embryo the individuals, *A*, *B*, and *C*, were probably produced in the same way as in the preceding. As all the embryos could not be seen at once, each embryo was drawn separately with the aid of a camera, and finally all three united and represented as though spread out on a flat surface.

Embryo *A* has undergone median fusion and transverse fission. The fused appendages of the first four segments are arranged in a single row; it is very rarely that one sees as many unpaired appendages as this.

The cephalic lobes are narrowed, and covered by a hood-like fold of ectoderm, through which one sees the oesophagus.

The marginal fold has grown across the median line in front of the fourth pair of appendages, as in the extreme forms of hour-glass embryos. In front of this fold, and near the median line, are the dorsal organs.

The fifth pair of appendages have not fused entirely. Embryo *B* has degenerated completely in front of the fused fifth pair of appendages, with the exception of the dorsal organs, which have almost reached the median line.

The sixth pair of appendages have nearly fused at the base, but are distinct at the tips.

In embryo *C* the same kind of fusion and degeneration as in embryo *B* has occurred, but it has progressed still farther, for the dorsal organs have fused, and also the six pairs of appendages.   At the central ends of all the embryos are paired and unpaired ridges, representing abdominal appendages.   The dorso-ventral muscles are well developed, and may be seen radiating from the triangular, marginal fold to all three embryos.

FIG. 104, × 33.   This extraordinary embryo is a triple one like the preceding. By median fusion and antero-posterior degeneration, each embryo is reduced to a condition like that in embryo *C*, Fig. 103.   The dorsal organs and the fifth and sixth thoracic appendages have fused in the median line.   Everything anterior to the fourth or fifth segment has disappeared.   There is a dark, central area, composed of thickened layers of mesoderm and ectoderm, that represents the anal plates of all three embryos.   A series of two or three concentric ridges encircle it, representing the abdominal segments.   Between embryos *A* and *B*, and *A* and *C*, are seen the concrescing margins of the mesodermic areas, divided into distinct mesoblastic segments.   Nothing of the kind can be seen between embryos *B* and *C*.

The three following embryos belong on the preceding plate, but could not conveniently be placed there.

FIG. 105, × 60, not sectioned.   A very flat embryo with inconspicuous, marginal folds.   The right chelicera is absent.   The embryo is remarkable for the large size of the mouth, and especially for the series of organs arranged along the median line behind the mouth, *a–d*.   The first two are transverse depressions, and in the yolk beneath the second depression, as though arising from it, is a collection of cells.   The third depression is similar to the second, but with a thick lip, projecting forwards from its posterior border.   The fourth is a conical, ectodermic elevation.   The abdominal plate is thrown toward the right.   The embryo conveys the impression (more strongly than appears in the figure) that it is provided with a series of five mouth-like openings, arranged along the median line.

FIG. 106, × 60, sectioned.   This embryo is in an advanced stage of development, but extensive degeneration has taken place, leaving but little of the original embryo behind.   The cephalic lobes are represented by a dark patch of cells, with a still darker portion, the remnant of the oesophagus, in its centre.   In sections, it appears as a slightly thickened layer of ectoderm, with a much thicker mass of mesoderm beneath it.   The remainder of the embryo, except the terminal lobe, consists of a single layer of flattened ectoderm cells, with a thin underlying layer of mesoderm.   There is no indication of a nervous system, or other ectodermic structures.   The margins of the mesodermic area, especially on the side, are thickened, *m.a.*, to form a conspicuous band, composed of several layers of cells.   At the posterior end of the body of the embryo is a median, conical projection, probably representing the tip of the abdomen, but possibly a fused pair of thoracic appendages.   The appendage rises out of a depression, the anterior wall of which is thrown forward into a shallow pocket.

Back of this papilla, the margins of the mesodermic area have concresced in the typical manner for stage *D*, and in the yolk beneath the concresced margins is an oval cloud of degenerating cells, *p.a.c.*   The condition of the posterior end of the embryo shows clearly, in spite of its apparently simple condition, the advanced stage of development it has reached.   While degeneration seems to be

going on rapidly and extensively in this embryo, there are numerous karyokinetic figures in both the ectoderm and mesoderm at the posterior end of the body.

FIG. 107, × 60. A very small, circular embryo, with a bare trace of cephalic lobes and oesophagus, the latter appearing as a white spot with faint bands on either side, which probably represent the anterior extensions of the nerve-cords.

Two pairs of appendages are present (second and third thoracic ?) and back of them are traces of other appendages. The abdominal invagination is small, but quite deep.

Pl X

The number opposite each figure corresponds to the number of the embryo that was sectioned. The exponent indicates the position and number of the section, and agrees with the number opposite the dotted section line, *s*, in the surface views.

The sections are not intended to show histological details, but merely as a help in the interpretation of the surface views.

FIG. 8 [1], [2], × 200. Two longitudinal sections of the brain, optic ganglion, and invaginated appendage.

FIG. 9 [3], [5], [7]. Cross-sections of the cephalic lobes, to show the nature of the fold that grows over the brain.

FIG. 10 [1], [2], [3]. Longitudinal sections showing the ganglionic fold over the optic ganglion, and the brain pits.

FIG. 10 [11], × 200. Longitudinal, vertical section of an invaginated appendage. Same section as 10 [1], but shows a part farther back, and more deeply invaginated.

FIG. 11 [1], × 400. Section of a marginal vesicle, showing fatty degeneration of the mesoderm cells.

FIG. 20 [1], [2], [3], [4], × 100. Cross-sections, showing absence of nervous system at the anterior end, and the medianly fused appendages.

FIG. 68 [1], [2], × 100. Cross-sections, showing invaginated, marginal thickening. *A*. Same more highly magnified, to show the bacteria-like dots on the surface.

FIG. 73 [1], [2], × 100. Section of a late stage of a degenerate embryo, showing absence of organs and histological specialization.

FIG. 77 [1], [2], × 100. Section of embryo similar to that in Fig. 66. It shows a remarkably large, horseshoe-shaped body cavity, possibly formed by the fusion of several somites. The cells lining the floor of the cavity are flattened and mingled with fibres, so it has the character of connective tissue seen in very old stages. The somatic mesoderm is on the contrary composed of lymphoid cells, which are here and there wedged in between the inner ends of the columnar, ectodermic ones, as though they arose from the latter by inward proliferation.

On each side of the cavity is a thickened cord of mesoderm cells, which seems to represent the thickened rim of the mesodermic area. Such a relatively enormous body cavity (?) has not been seen in any other embryos.

FIG. 83 [1], [2], [3], × 100. Cross-sections of an invaginated embryo, showing absence of recognizable nerve-cords and appendages.

FIG. 84, × 100. Cross-section of an invaginated embryo, showing the last traces of the nerve-cords, appendages, and eyes.

FIG. 85 [1], [2], × 100. Cross-sections of invaginated embryo, showing the relation of the foldings.

FIG. 89. Section of a very old embryo, reduced to a formless mass of cells. Of the latter, some are multiplying by karyokinesis, others degenerating; some are lymphoid, others finally are the very remarkable amoeboid cells, containing coiled fibres. These cells are found in great numbers in the marginal thickenings of the mesodermic area of very old embryos, and are also found in great numbers in the adult. *B*. Two fibre cells enlarged, showing the fibre lengthwise and in optical cross-section.

*Pl. XI*
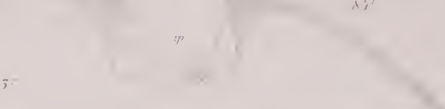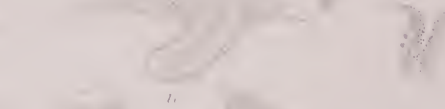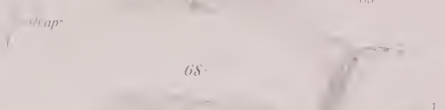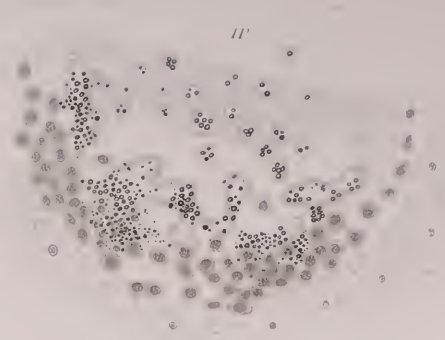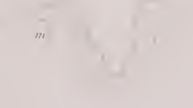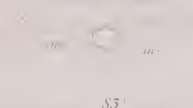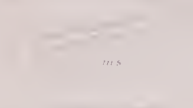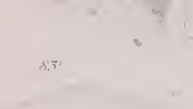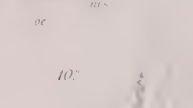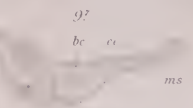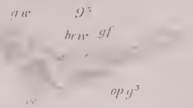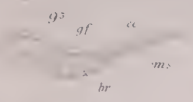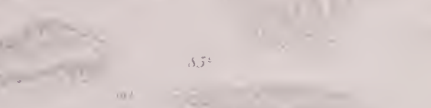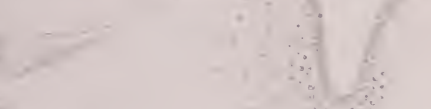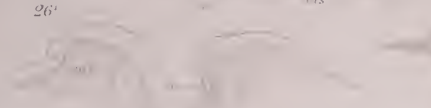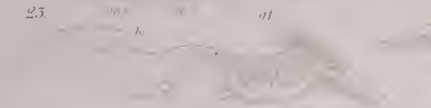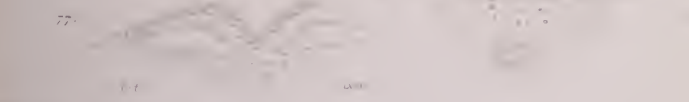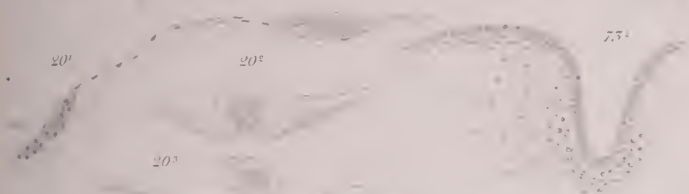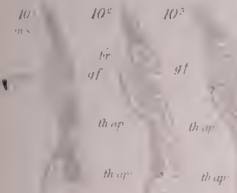
# BUDDING IN COMPOUND ASCIDIANS, BASED ON STUDIES ON GOODSIRIA AND PEROPHORA.

W. E. RITTER.

## CONTENTS.

## INTRODUCTION.

THE material that has served as the basis of the paper here presented was collected by myself at various points on the California Coast during the years 1892 and 1893. A considerable part of the investigation has been carried on under

circumstances that have placed me under obligations to several persons and institutions. These I would here acknowledge, and that most gratefully : first of all to Mr. Alexander Agassiz, through whose generosity it was that I was enabled to occupy a table at the Zoölogical Station at Naples for some months during the fall and winter of 1894 ; next, to Prof. Dohrn and all those associated with him in making the Naples Station the realized ideal of what such an institution should be ; and last, but by no means least, to Prof. F. E. Schulze, who so kindly and generously placed the excellent facilities of the Zoölogical Institution of the University of Berlin at my service for some months.

## *A.* GOODSIRIA DURA NOV. SP.

### 1. MATERIAL. TECHNIQUE.

My specimens were all collected by myself at Santa Barbara, California, during a brief visit there in the last days of December, 1892.

They were all found upon the beach where they had been thrown by the waves; I saw no colonies in their original positions. As they were found in abundance and in a perfectly fresh condition, I conclude the species must be plentiful at this point, and that it lives on the sea bottom not far from the shore. This latter supposition is likewise supported by the fact that most of the colonies are attached either to shore-inhabiting seaweeds, or to *Styela rubra*, an Ascidian very common at Santa Barbara on the piles of the wharf, and on the rocks in shallow water. It is almost certain that the dredge, when brought into use here, will bring it up from its natural abode in quantities. On account of its obvious abundance at this point, I have been somewhat surprised at not finding it elsewhere on our coast; but there is little doubt that further work with dredge and tackle will bring it to light at other places.

As the only killing reagents with which I was provided on my visit to Santa Barbara were picro-sulphuric mixture and

alcohol, I was limited to these for the preservation of my specimens. Luckily, however, the former proved to be a favorable medium for the purpose. Some of my material has proved to be excellently well preserved, better than any I have been able to get of some other Ascidians by using a large variety of reagents. Some specimens preserved in absolute alcohol were found to be valuable as collateral material.

No opportunity has been afforded me for studying living specimens with any detail, but they are much too dense and opaque to permit of being very satisfactorily studied in this condition. Likewise, since the species belongs to that category of Ascidians in which the mantle clings closely to the test, it is impossible to free the zooids from the colony in preserved specimens so as to study them whole with much satisfaction. One is consequently obliged to depend on the study of small colonies, or small pieces of colonies cleared in oil; on dissections; and on thin sections. The first mentioned method is particularly valuable.

Of the numerous stains employed I have found Paul Mayer's Hæmalum, and Grenacher's Alum Carmine to give the most satisfactory results.

## 2. Description of the Species.

As we are dealing with a new form, it will be necessary to describe it ; and this is the more incumbent because it belongs to a group of Ascidians not very well known to science.

As mentioned in my preliminary paper ('94), it belongs to the *Polystyelidae,* a family founded by Herdman ('86). The author has given a good historical résumé of our knowledge of the group, and I may consequently touch this phase of the subject lightly. The six genera of which the family is at present composed were, with one exception, all established between 1843 and the time of Herdman's description of the Challenger collection in 1886. This one exception was added by the author himself. They were described by different writers and assigned by them to different larger groups already known ; Carus ('43), for example, regarding the genus described by him

as being allied to *Clavelina;* while Giard ('74) considered the
two founded by him as related to the *Cynthiidae.* Most of
the authors believed the particular forms which they studied
were true compound Ascidians, though none of them were
able to produce the crucial test of this, *viz.,* evidence of the
occurrence of multiplication by gemmation. This evidence
remained wanting till the publication of my recent pre-
liminary.

By personally studying as many of the known species as
were accessible to him, and by critical examination of the
descriptions and figures given by other writers, Herdman has
amply justified his uniting the genera into a single new family.
He has also contributed many valuable observations on the
species described by him.

Since the work of this author appeared, there has been, so
far as I know, but one other contribution to our knowledge of
the group. This consists in the addition by Gottschaldt ('94)
of a new species, *Goodsiria borealis.* To this, further reference
will have to be made because of its close resemblance to the
species now under consideration.

The following is the diagnosis of the new species as my
present knowledge enables me to give it :

*General Character of the Colonies.* Predominating form flat
and encrusting. Occurs most frequently on various sea-weeds,
and on *Styela rubra,* and often so completely covers these that
the outlines of the colonies are determined by those of the
particular substrata. But in addition to the flat and encrusting
condition, colonies not infrequently occur with fleshy knobs
composed of a large mass of test material containing zooids in
the entire surface layer but none in the centre.

Colonies from 1cm. to 5 or 6 cm. in diameter, apparently
tending to expand equally in all directions when permitted to
do so by form of substratum.

Color in living state, dull red ; light brown in preserved
specimens.

*Zooids.* Fully imbedded in the test, not projecting from sur-
face of colony ; not arranged in systems, no common atrial
orifices ; for the most part evenly distributed in surface layer

of test and rather close together. Size of zooids, length 3 to 5 mm., width 2 to 3 mm.

*Test.* Rather dense, opaque, cells small and not numerous, no bladder cells, slightly fibrillated. Vessels numerous, much branched and anastamosing, terminating, particularly in margins of colony, in many large pear-shaped ampullae; mostly occupying a deeper position in test than the zooids.

*Musculature.* Not highly developed, fibres not arranged in distinct bands.

*Branchial Apparatus.* Position of branchial and atrial orifices vary with the character of the colony; when the colony is thin and encrusting they are both placed more dorsally, *i.e.* opposite the endostyle; when the colony is fleshy and massive, the orifices are more anterior and nearer together. No distinct siphons; orifices not lobed, at least not discoverably so in preserved specimens; in some specimens orifices obscurely quadrilateral.

Branchial tentacles simple, usually twenty long and strong ones, and about same number of smaller ones alternating with them. Atrial tentacles present, about twenty in number, much smaller than the branchial. Branchial sac without folds; internal longitudinal vessels rather prominent, 5 on each side, the two dorsal ones on each side nearer together than the others. Small intermediate transverse vessels frequently present. About 12 series of stigmata; 5 or 6 stigmata between each two longitudinal vessels excepting between the two on each side which are closer together, where there are only two or three. Dorsal lamina a plane membrane tending to roll up.

*Endocarps.* Present in the form of large globular structures attached to the parietal wall of the peribranchial sac, from which they project prominently into the peribranchial space.

*Digestive Tract.* Situated on left side of branchial sac, distinctly divided into oesophagus, stomach, and intestine. Oesophagus nearly as broad as intestine, and approaching the stomach in length. Stomach somewhat pear-shaped, about 8 deep folds extending lengthwise of the organ, parallel with one another, not converging toward the point of entrance of the oesophagus. Course of intestine, first ventralward from

stomach for a short distance, then changing by a rather wide curve to a nearly dorsal direction which is followed for a distance about equal to the combined length of oesophagus, stomach, and ventrally directed limb of intestine. Anus directed somewhat anteriorly, nearly as broad as any part of intestine, provided with a thick lip. A peculiar thickened band in the wall of the intestine, beginning in the loop, extends in an oblique direction half way to the anus. Lacteal system consists of a prominent coecum projecting from the stomach near its pyloric orifice, into one side of which opens a much smaller tube, the common stem of the greatly branched intestinal part of the system.

*Sexual Organs.* Both ovaries and testes in the form of "polycarps" attached to the mantle on each side of the endostyle, and projecting into the peribranchial chamber. These few in number and small in size so far as known. Uncertain whether the same zooids produce both ova and sperm or not.

*Hypophysis.* A simple duct, no branched glandular part, the mouth a simple elliptical opening.

*Ganglion.* Situated ventrally to the hypophyseal duct, consisting of the usual outer layer of multi- and uni-polar ganglion cells, and an inner cell-less core of nerve fibres.

*Budding.* Pallial, *i.e.* from the parietal wall of the peribranchial sac.

I have hesitated much in deciding in which of the two genera, *Goodsiria* or *Synstyela*, this species ought to be placed. The only well-marked distinction between them appears to be in the character of the colony, this being designated as massive in the first, and thin and encrusting in the second. Certainly the latter characterization applies to the greater number of colonies of *G. dura* which I have seen, but at the same time it does not apply to all in all their parts. It will surely be allowed by all zoölogists familiar with the compound Ascidians that the form of the colony alone is a rather frail peg on which to hang a genus. It appears to me that the difference between a pedunculated and a massive colony is at least as great as that between a massive and an incrusting one. If the latter difference is worthy of being used to separate species into genera,

the former ought to be likewise; but two of the Challenger species of *Goodsiria* described by Herdman, *viz.*, *G. pedunculata* and *G. placenta*, are decidedly pedunculated, while this charracter is as markedly absent from the other known species. As we shall see presently, if the structure of the *individual zooids* and not the *form of the colonies* is made the basis of comparison, the closest ally to our present species is certainly found in the genus *Goodsiria*, instead of in the genus *Synstyela*.

I have consequently reached the conclusion that while, so far as morphological agreements and disagreements are concerned, my species might be placed with about equal propriety in either genus, since *Goodsiria* is the older of the two (it having been founded by Cunningham in 1872, while *Synstyela* was founded by Giard in 1874) it is more entitled to receive the new comer.

The specific name *dura* I have chosen as applying to the firmness of the colonies due to the density of the testicular matter.

There are three other known species to which this one is closely allied, and with which, consequently it must be compared in some detail in order that its differentiating characters may be brought out.

These are: *Goodsiria coccinea*, Cunningham, *G. borealis*, Gottschaldt, and *Synstyela incrustans*, Herdman. With the first mentioned species it agrees not only in the numerous points of structure common to all species of the family, but also in the form and size of the zooids, points of considerable determinative value for species in this group; but most important of all, *in the absence of folds* in the branchial sac. So exceptional is this condition among the representatives of the family that its occurrence in two species rather closely related in many other particulars may be regarded as evidence of their very close relationship. But that these two forms are specifically distinct there can be no doubt. The differentiating characters are the following. The colonies are much larger and much more distinctly massive in *G. coccinea* than in *G. dura*. According to Herdman ('86), on whose description of the former I base my comparison, the colonies of this species

sometimes reach a length of 46 cm., while I have never seen a colony of *G. dura* more than 10 cm. in length.

The branchial and atrial apertures of *coccinea* are conspicuous and are irregularly four-lobed; in *dura* they are not conspicuous, and in preserved specimens show no trace of lobes. In *G. coccinea* the "meshes" of the branchial sac, *i.e.* the areas of the sac between the internal longitudinal vessels, contain eight stigmata. In *G. dura* the number is not the same in all the meshes, but at the most is less than eight; at the least it is three. But probably the best distinction between the two species is in the character of the stomach. In *G. coccinea* this organ, as described by Herdman, is globular in form, and concerning its folds he says: "There are usually six well-marked folds upon the right side of the stomach. A transverse section shows in addition a single large fold, which projects far into the centre, nearly dividing it into two cavities." Reference to my Figs. 4 and 6, Pl. XII, shows at once that the stomach of *G. dura* is quite different from this. In the first place it is not globular. It is rather schizaster-shaped, if I may be permitted to suppose that the form of this echinoid is any more familiar than that of the stomach which I am comparing with it; but I can think of no other object which it so closely resembles in form as it does some species of this genus. In the *G. dura* stomach there is no one fold that exceeds the others in the extent to which it projects into the chamber.

Another distinction between the two species, that would appear to be constant, consists in the presence in *G. coccinea* of a vessel which, "enclosed in a prolongation of the mantle, leaves the posterior end of each Ascidiozooid and runs for a longer or shorter distance through the test before ending in a dilated bulb." No such vessel occurs in *G. dura*.

Although it is evident from Gottschaldt's description of *G. borealis* that this species and *G. dura* are closely related, it is unfortunately impossible to make as exact a comparison between them as is desirable, owing to the incompleteness of the author's description at one or two critical points. In the form of the colony they appear to agree more closely than either agrees with *G. coccinea*, for the figure representing a

colony of *G. borealis*, shows it to be quite "flat and encrusting," though the author speaks of it as being fleshy. The points in the structure of *G. borealis* which preclude the placing of our California form in that species are these: The four-lobed branchial and atrial orifices; the eight series of stigmata (*G. dura* has twelve); and the presence of folds in the branchial sac. It is stated by Gottschaldt that "zarte Muskelfibrillen" occur in the test; but almost certainly this is an error, the "Fibrillen" which he supposes to be muscle fibres, being similar to those found in the test of many other Ascidians, but which are in all probability correctly considered as not contractile, but mere filamentous differentiations from the matrix of the test. The author also says that the "liver gland" is well developed; but he has in all probability mistaken the lacteal system for a liver, since nothing corresponding to what is generally understood to be the liver in many simple Ascidians is present in at least three closely related species which I have examined with special reference to this point. These species are *Goodsiria coccinea, G. dura*, and an undescribed Australian species of *Chorizocormus* kindly furnished me by Prof. Herdman.

I have regarded *Synstyela incrustans* and *Goodsiria dura* as closely related, more on account of resemblances in the form and character of the entire colonies than from similarities in the structure of the zooids. In the former particular, they undoubtedly agree rather closely, but in the latter they are, on the whole, as previously remarked, less closely alike than are *G. coccinea* and *G. dura*. The colonies of *Synstyela incrustans* are said by Herdman to reach a length of 20 cm. in some cases, while it will be remembered that no colony of *G. dura* has been found more than half that size. The individual zooids are also much larger in the former than in the latter species, their length being as great as 8 mm. in the one, and never more than 5 mm. in the other.

The branchial and atrial apertures of *S. incrustans* are described as "conspicuous, but not distinctly lobed." We have seen that in *G. dura* they are not conspicuous, nor are the lobes recognizable at all in preserved specimens, so that in

this particular the last named species agrees about as well and about as little with *G. coccinea* as with *S. incrustans*. But it is when we regard the branchial sacs of the two species now being compared that we find their most important differences. The sac of *Synstyela incrustans* has a rudimentary fold on each side, while it will be remembered that no folds are present in *G. dura*. Unfortunately, Herdman does not tell us the number of internal longitudinal vessels in the sac of either *G. coccinea* or *S. incrustans;* but he has shown eight in a figure of a portion of the sac of the latter, so we are certain that the number here exceeds the number in *G. dura* by *at least three;* of course the excess may be greater, since it is not certain that the eight shown in the figure referred to is the entire number present; in fact it is more likely not to be, since in making such preparations of the sacs as that from which this figure was drawn one does not usually obtain intact the entire width of one side.

In *S. incrustans* there are intermediate vessels, "normally three in number," crossing the meshes of the branchial sac. In *G. dura* I have never seen more than one of these, and frequently this one is absent.

There also appears to be a difference between the two species in the arrangement of the polycarps. In the former they are figured by Herdman as scattered promiscuously over a portion, at least, of the wall of the peribranchial cavity, while in the latter, so far as I have found them present, they are confined to a single row on each side of the endostyle. But great weight cannot be attached to this distinction, since the sexual organs are too poorly developed in all my specimens of *G. dura* to enable one to decide what their arrangement might be in a better-developed condition.

### 3. The Zooids in the Colony.

This subject is so intimately connected with that of bud-development that it must be treated somewhat more fully than have been the other points of adult structure. I cannot discover any constant arrangement of the Ascidiozooids in the

colonies.  Fig. 1, Pl. XII, represents a colony, natural size, growing on a laminaria leaf.   As here seen, the zooids are, as compared with many compound Ascidians, rather distant from one another.   They are very regularly distributed, there being no suggestion of systems.   Neither have the zooids any constant relation either to the colony as a whole or to one another, with reference to their antero-posterior axes ; though in general where the colonies are narrow and elongated the longer axes of the zooids correspond to the longer axes of the colonies. The arrangement of the adult zooids is of course determined by the relations which the developing buds hold to their parents, and in this there appears to be no constancy beyond the fact that the buds are generally confined to the borders of the colonies.   I say generally this is the case ; but it is not without exception, for in several instances I have found young blastozooids so situated that older ones intervened between them and the edge of the colony.   (Fig. 2, Pl. XII.)

The question of the relation of the buds to the older zooids is important because it obviously involves the questions whether all the zooids of a colony are capable of producing buds, and at what age in the life of the zooids their buds are produced. And this last question leads back again to the fundamental one of whether or not the cells that initiate the bud-development are derived as unmodified or embryonal cells from the parent, the grandparent, and so on to the sexually produced embryo that was the common ancestor of the colony.   What I have to say on this point will be better reserved until I speak in detail of the *Anlage* of the bud, my purpose here being to describe merely the form and composition of the colony as a whole.   In a few instances (Fig. 2, buds *a* and *b*), for example, there is a suggestion that at the borders of the colonies the younger buds occupy positions alternating with the next older ones, but a little in advance of them toward the edge of the colonies.   If, however, such a law of arrangement exists, it prevails less frequently than do the exceptions to it.

To Metchnikoff's ('69) denial that buds are produced by the vessels in Botryllus, Giard ('72) raises the objection that if this

were true it would be impossible to explain "la production d'etoiles multiples et distantes dans le cormus d'un Botryllien." The remoteness of the young buds from any older zooids in *Goodsiria* has likewise frequently proved a stumbling-block to me in seeing how they could in such cases have been produced in the usual way, *i.e.* from the wall of the peribranchial sac. But I have given much attention to the point, and am quite convinced that in reality this is their only source. Herdman ('86) expresses the opinion that the ampullae of the vessels will be found to give origin to the buds, but such is apparently not the case here any more than it is in *Botryllus*.

The frequent remoteness of the buds from their parents must be due to their having grown away from the latter before they become fully severed from them. From the firmness of the test and the character of the young buds I can hardly believe that they have any power of independent migration throughout the test.

This conjecture, that the remoteness of the buds from their parents is due to the growth of the bud before it becomes severed, harmonizes with the view that pallial budding as it takes place in *Botryllus* and *Goodsiria* is not of necessity fundamentally different from the stolonic method of budding. The comparison between these two methods becomes still more interesting from the discovery that in *Perophora* the buds are connected with the septum of the stolon by their peribranchial instead of their branchial sacs. But I shall discuss this point further after having described in detail the development of the bud in each species.

There is certainly no septum in the vessels of the test, (Figs. 3, 5, 7, etc., Pl. XII, *ec.ves.*); and if buds were to be produced in connection with them, it would have to be by a method essentially different from any of the generally recognized types of gemmation among Tunicates. Since the blood of the zooids passes freely into the testicular vessels, and since the young sexual cells float in the blood, it is not beyond the range of possibility that these cells may pass into the ampullae of the vessels, and there form themselves into the "inner vesicle," the all important precursor of the blastozooid.

It is an interesting fact that Herdman ('86, p. 90 *et seq.*) has observed a process in *Colella pedunculata* which would appear to be a realization of that here imagined. As the author himself says, his observations are rather fragmentary, and consequently his account is much less full than we might wish it to be.

That the buds arise in this anomalous manner in this genus, he, however, seems to be convinced, and his description and figures undoubtedly furnish good ground for his conviction. It is certainly very much to be hoped that opportunity will before long be afforded some zoölogist to study the subject more fully. In a few instances I have found a massing of cells within the ampullae that is strongly suggestive of the process described by Herdman. Minute examination of these aggregates has, however, failed to furnish any evidence that they produce buds. Two such cases are shown in Fig. 7, Pl. XII. They are quite conspicuous when cleared in oil and examined with a low magnification.

The vessels generally occupy a deeper position in the test than do the zooids, so that the test surrounding the zooids is less thickly penetrated with them than are its deeper portions. This is shown in Fig. 5, Pl. XII.

It has been mentioned above that the buds become fully severed from the parent zooids at an early stage in development. This fact raises the question of the extent to which the zooids in this species are independent of one another. It is certain that many of them are in connection with the vessels of the test for at least a portion of their lives, and are consequently in vital connection with one another. But this connection is almost certainly secondary, and, I believe, not essential to the development of the zooids.

As the young buds become larger, they press more and more closely against the vessels that were in contact with them, or nearly so, at the beginning, and by this pressure a fusion of the vessel wall with the outer or ectodermal wall of the bud is produced, and then later a perforation of the fused walls occurs, and the lumen of the vessel is thus brought into communication with the body space of the zooid.

Figs. 37 and 38, Pl. XV, taken from two different zooids, represent the points at which the vessels open into the body space of the zooids. I would call particular attention to Fig. 37, since this illustrates a much more common appearance than that shown in Fig. 38. The projections, *ec.ves'*, into the cavity I understand to be due to the vessel's having pushed itself therein, either by its own growth or by its passive resistance to the expansion of the developing zooid. In many cases it appears that the wall of the zooid has been pushed in to a considerable extent by the vessel ; and by an interfolding of the vessel wall and the zooid wall, the character of the connection has become quite complicated. In some instances these projections extend entirely across the body space to the wall of the peribranchial sac.

I said above that it is *almost certain* that these communications are secondary. This qualification was made because one might ask if the vessels do not sprout out from each of the blastozooids, as it would seem they must have done from the sexually produced ancestor of the colony.

That this process never occurs I cannot positively affirm, but I have no evidence that it does, and two considerations incline me to the belief that it does not. In the first place, the peculiar character of the connection, as described and shown in Fig. 37, seems to me to speak against such a process ; and in the second place, one would suppose that if it takes place at all it would do so at a rather early stage in the development of the zooid, before the ectodermal cells in the region from which the vessels would have to be produced had undergone modification. But I have searched in vain for cases of such formation.

By carefully examining all the sections of numerous buds at various stages in development, one can determine that there are no connections whatever between them and the vessels. This is the fact that makes me believe that the connection of the buds to the vessels is not essential to the development and life of the zooids.

### 4. Development of the Undifferentiated Bud.

The earliest stage which I have observed in the development of a bud is represented by Fig. 11, Pl. XII. Here the bud *Anlage* is clearly marked, *bd.a.*, both by the character of the cells and by the slight evagination already seen. But as yet the ecto-derm shows no indication of having been affected by the process which has begun in the wall of the peribranchial sac beneath it. It is true its cells are higher immediately over the evagination than they are at any point more ventralward; but they are not higher than they are from this point upward toward the dorsal side of the zooid, as the figure shows. That is to say, the ectoderm cells are not different over the bud from what they are in a corresponding position of a zooid in which no bud is developing.

The figure shows approximately the stage of development of the parent zooid. The endostyle, *end.*, is not yet fully differen-tiated, histologically, and the thickened places, *st.a.*, in the wall of the peribranchial sac opposite the bud show where branchial stigmata are going to form. The string of cells, *cn.c.*, near the bud, is a fragment of one of the "endocarps" that has been broken and displaced somewhat in the section cutting. It consequently has no significance.

To the important question of whether the bud *Anlage* arises from a "budding zone" on the peribranchial wall in which the cells are "embryonal," and are endowed from their very origin with peculiar bud-producing powers, my observations do not enable me to give a wholly satisfactory answer. However, certain facts are suggestive in this connection. I have never found anything that appears like a "budding zone" similar to that described by Oka, for example, in Botryllus, and I find that the walls of the peribranchial sacs in the developing bud are, after the very first stages of their growth, very thin through-out, the cells being considerably flattened. Their development is, of necessity, accompanied by cell division, but I am unable to discover that this is more noticeable in one region than in another; or that the cells have a different appearance or distribution in one locality from what they have in another.

One of two conclusions seems to be inevitable from the facts observed : *Either there are no budding zones containing specially endowed cells ; or a large majority of zooids are incapable of producing buds.*

It is true that a comparatively small number of zooids with buds actually in connection with them have been found ; but I suppose this to be due generally to the early stage at which the buds are severed from their parents, rather than to the incapacity of the zooids to produce buds. But I would by no means assert that all zooids are capable of asexual reproduction. Later stages in the development of buds, though before severance from their parents, are shown in Figs. 9 and 10, Pl. XII. and Figs. 12–15, Pl. XIII. Fig. 9, Pl. XII, represents a zooid with its bud, seen as a transparent object, but not sufficiently cleared to render distinctly visible the several organs and layers. On the other hand, Fig. 10 was drawn from a specimen well cleared in cedar oil, and consequently so transparent as to make the optical section at the level represented almost as distinct in its various parts as an actual section in the same plane would be. These figures explain themselves sufficiently. The position of the buds far forward on the zooids will be noticed in all these cases. This appears to be their usual position. A transverse section of a bud of a stage about corresponding to that shown in Fig. 10 is presented in Fig. 14. This represents the tenth section from the tip of the bud. The thickness and irregularity of the inner vesicle are here conspicuous. The sections of the same series near the parent zooid show the inner vesicle to be here considerably thinner than it is in the section figured. The difference in thickness in the two regions is, I suppose, due to the fact that growth is taking place chiefly toward the end of the bud. The thinner, basal part is where, a little later, the endoderm will become disorganized in course of the cutting off of the bud from the parent.

The question may arise here if the unequal thickness of the endoderm at various points, as shown in Fig. 10, is not an indication that the differentiation of the organs has begun at this stage.

This does not seem to be the case. At any rate, as we shall presently see, at a still more advanced stage, *i.e.* after the bud has become fully severed, the wall of the inner vesicle is, in some buds at least, still entirely undifferentiated.

Fig. 15, Pl. XIII, shows a section of a bud that is very nearly severed from its parent; in fact, the severance is practically complete, there being a mere neck of scattered cells, *v.c.*, marking the former connection between bud and parent. It is here seen that the ectoderm of the zooid is not fully closed together at the point where the bud was cut away. Here the inner vesicle is wholly undifferentiated, it presenting in every section the same appearance as that shown in the one figured; and it should be said that buds occur in which every trace of the connecting strand has disappeared, but in which the inner vesicle still retains its simple, unmodified condition. However, it does not appear to remain long in this state.

In describing the further growth of the bud it will be best to follow the course of development of each organ separately, but before beginning the description I wish to call attention to the interesting fact that *in different buds the order of appearance and stages in development of the several organs are subject to considerable variation with reference to one another.* I will point out specific instances of this as I proceed with the description.

## 5. Development of the Organs.

### a. *The Branchial and Peribranchial Sacs.*

I have been unable to make my observations on entire buds and on sections agree exactly regarding the initial step in the formation of these structures. By examining whole buds the impression is gained that the process is begun by the growth of two folds which will ultimately form partitions separating the primitive simple vesicle into three portions — two lateral ones, the peribranchial sacs, and a middle one, the branchial sac. These folds are seen at *p.f.*, Fig. 17, Pl. XIII, in which their formation is already advanced to a considerable extent; but in Fig. 16 the slight angles marked by the same

letters are the very beginnings of the folds. In neither of these figures, nor in fact in any of the numerous similar ones that might be given, does it appear as if the peribranchial sacs begin as *evaginations from the primitive* vesicle. It does not appear here as if the peribranchial sacs are initiated by the active growth of the portions of the primitive vesicle walls which are to enter into them, but, as already said, by the growth of the partitioning folds. On the other hand, sections of a very early stage in the development of the sacs show that in one instance, at least, there takes place an active growth of the *Anlage* of the sacs themselves, resulting in *well-defined evaginations*. (See Figs. 20 and 21, *br.s.a.*, Pl. XIII.) These figures are drawn from sections of a bud presenting an earlier stage in the development of the sacs than that represented by Fig. 16, and so it might be assumed that the sacs of the latter individual had their beginnings in such evaginations as those shown in Figs. 20 and 21, even though all indications of them are by this stage completely lost.[1]

But if I am right in supposing the angles *p.f.* in Fig. 16 are the beginnings of the folds, it is not quite clear how such a condition as that presented by this bud would be related to one in which the well-defined evaginations of Figs. 20 and 21 were present. The only way of harmonizing the two conditions, so far as I can see, is to suppose that the sacs begin with the evaginations, and that these grow rapidly in width, but very little in depth, till they extend over nearly the whole of the sides of the primitive vesicle. Posteriorly they extend ultimately so far as to be separated only by the angle in which the intestine (*int.*, Fig. 16) develops, and anteriorly the angles are almost obliterated, but the sacs do not approach each other so closely as behind, but remain separated by the thickened area (*end.*, Figs. 16 and 17), which will give rise to the endostyle. Thus we should have the condition presented by Fig. 16 as a more advanced stage of development derived directly from the earlier distinctly evaginated stage. And the fact that the hypophyseal duct and intestine are both begun in the bud

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[1] I have since seen in a whole bud an evagination from the inner vesicle, at least on one side, that in all probability corresponds to *br.s.a.* in Figs. 20 and 21.

represented by Fig. 16, while neither is yet indicated in the bud with the peribranchial evaginations, also suggests that the former is older than the latter. But the discrepancy between the two conditions may be explained in another way; it may be due to individual variation; in any case, however, it cannot be a matter of fundamental importance.

The initial steps in the formation of the peribranchial sacs accomplished, the remainder of their development is followed without difficulty. The sickle-shaped partitioning folds, *p.f.*, Fig. 17, extend their arms, one on the dorsal, the other on the ventral side of the inner vesicle, farther and farther backward. But of the two arms of each fold, the ventral one takes by far the more important part in effecting the ultimate complete separation of the peribranchial from the branchial sacs.

The process is clearly illustrated by Figs. 16–19, Pl. XIII, representing dorsal views of whole buds examined as transparent objects; and by Figs. 22–24, Pl. XIII, and Figs. 26–29, Pl. XIV, inclusive. The series of transverse sections represented by Figs. 22–24 are from a bud in a stage of development slightly more advanced than that shown in Fig. 17, Pl. XIII. Fig. 22 is the most anterior of the three sections drawn, and, as will be seen, passes through the point at which the branchial siphon, *br.sip.*, is being formed. Fig. 23 is fourteen sections farther back. It shows both the dorsal and ventral arms of the sickle-shaped folds, *d.f.* and *v.f.*, those of the right side (left side of the figure) very nearly meeting each other, and so making the separation of the right branchial sac at this point nearly complete. Fig. 24 represents a section farther back, and shows at this position merely a trace of the folds, the three sacs of the anterior region being here merged into one common cavity representing the unmodified remnant of the primitive inner vesicle. Figs. 26–29, Pl. XIV, represent similar sections of a still older bud. That shown by Fig. 26 is most anterior, and passes through the opening of the hypophyseal duct into the branchial sac, *hy.m.*, and hence is slightly farther back than the section shown in Fig. 22 of the preceding series. Fig. 28 represents a section sixteen sections farther back. It is seen that the dorsal folds take no part in the formation of

the peribranchial sacs in this region. Eight sections farther back, Fig. 29, the ventral folds no longer appear, and we have again an unpartitioned portion of the primitive vesicle.

From the figures of the whole buds, and also from those of the series of sections, it will be seen that the folds do not extend back parallel to each other, neither do they occupy a vertical position. They converge both posteriorly and ventrally, so that the middle or branchial sac is made cone-shaped, the apex of the cone being directed backwards. This becomes still more distinct at a later stage in development (see Figs. 18 and 19, Pl. XIII). An inclination toward the left side of the posterior extremity of the cone is now noticeable, and this becomes more pronounced at a later time (compare Figures mentioned). In some buds it is much more prominent than in others.

We may now pass to the practically completed condition of the branchial apparatus. A dorsal view of an entire bud in such a stage is shown in Fig. 19. The only changes that will take place between this and the fully adult condition will be some unimportant ones, so far as our present purpose is concerned, in the relations of some of the parts to one another, and a great increase in size of all the parts. From the preceding stage the most important changes have perhaps been those taking place in the posterior region of the animal.

Recurrence to Figs. 23 and 24, Pl. XIII, will recall the fact that at this stage the three sacs opened widely into a common chamber in the posterior part of the bud. In the stage we are now considering such is no longer the case. The two ventral folds by growing up to and fusing with the dorsal wall of the primitive vesicle for some distance, and then by fusing with *each other* but *not* with the *dorsal wall*, for the rest of the way back to their extremities have effected the complete separation of the branchial sac from the peribranchial sacs. These relations will be easily understood by comparing Fig. 24 of the preceding stage with Fig. 28 of the present one, and then Figs. 30 and 31, Pl. XIV, with Fig. 28. Figs. 30 and 31 are from a bud considerably more advanced than the one represented by Figs. 26–29. Fig. 31 shows at *br.s.* the posterior tip of the

branchial sac. One or two sections farther back this cavity disappears, and to the very last is wholly shut off from the large single surrounding cavity marked *at.* The section shown in Fig. 31 is thirteen sections farther back than the one shown in Fig. 30.

It thus appears that while the peribranchial sacs become wholly separated from the branchial sac, they do not become separated from each other, but remain in very wide communication, this communication occurring both behind the posterior extremity of the branchial sac and over its dorsal part. (Compare Fig. 31.) It is this common cavity into which the two peribranchial sacs open, and which may be regarded as an unmodified remnant of the primitive inner vesicle that has been called the cloaca ; but it is important to recognize that in this species, as in many others, it is neither morphologically nor physiologically wholly distinct from the peribranchial sacs. By this time numerous branchial stigmata have formed, particularly in the anterior portion of the branchial sac. The peripharyngeal band and the branchial tentacles are already partly developed, and the dorsal lamina, *d.l.*, Fig. 30, has become quite a conspicuous object on the sections, though it has not yet become rolled over at its edge as it always appears in the adult (Fig. 6, Pl. XII). The endostyle is also distinctly marked out, though it is not histologically fully differentiated, (*end.*, Fig. 30). Likewise the internal longitudinal bars (*i.l.b.*, 1, *i.l.b.*, 2, etc., Fig. 6) have begun to form.

The formation of the branchial and atrial openings seems deserving of a little special attention. The development is initiated by an evagination from the inner sac, for each orifice (Fig. 35, *at.sip.*, Pl. XIV). A little later an invagination forms immediately over this in the ectoderm ; the two meet, fuse, the fused wall becomes broken down, and by gradual but later-accomplished perforation of the overlying test, communication is established between the outside world and the respective sacs (Fig. 36, Pl. XV). The fact to which I wish to call attention appears in the course of later development. It is illustrated by Fig. 36, drawn from a section through a branchial siphon in which the opening is completed so far as the cellular

walls taking part in its formation are concerned. The over-lying test is not yet perforated, however. The great growth of the epithelium lining the newly formed siphon, resulting in its remarkable thickness, and the wide, circular double folds, *s.cc.*, *s.f.*, and *s.f.*[1], is the fact to which I refer. Study of various stages in this growth shows that all the thickened part of the epithelium is derived from the ectodermal invagination, and that the formation of the folds is due to growth of this ectodermal epithelium. The secondary fold, *s.f.*[1], represents the evagination from the inner layer, it remaining passive, while the other portion grows rapidly, and produces, as the figure shows, a well-marked valve. As development goes on the whole siphon expands, and this fold or valve becomes narrower and narrower, and finally in the adult it is wholly obliterated, at least in the normal uncontracted condition of the animal. The formation of this fold is probably to permit of the protrusion of the siphon above the general level of the colony in its fully developed state. As shown by this figure, at this stage, *i.e.* previous to the perforation of the test, there is no such protrusion. This explanation hardly accounts, however, for the great height of the entire lining epithelium during these stages of development.

A very different explanation suggests itself, though one too imaginative to be deserving of more than a mere mention. One might conjecture that as incident to the abbreviated development of the Ascidian blastozooid as compared with the embryonic development, the earliest, paired stage in the origin of the atrial opening, so well known in the larval history of many Ascidians, had been entirely lost in the development of the bud, and that the peculiar conditions above described rep-resent the stage in the formation of that opening immediately after the fusion of the two primitive paired embryonic orifices. Van Beneden et Julin ('84), pp. 625–627, to whom we are indebted for the facts in the embryonal development above referred to, quite insist on the importance of distinguish-ing the cloaca proper from the peribranchial cavities. They point out that in *Phallusia scabroïdes* the cloaca is *wholly* lined by an ingrowth of ectoderm from the dorsal side

of the larva between the two atrial orifices ; while, on the other hand, "les cavités péribranchiales sont . . . delimitées en dehors *seulement* par l'épiblaste," and even this partial ectodermal lining arises independently, both in time and position, of that which lines the cloaca.

If there is any value in the suggestion here made that the ectodermal fold, *s.f.*, Fig. 36, is comparable to the true cloaca of the above-quoted authors, it would not be lessened by the rejection of their distinction between cloaca and peribranchial cavity in so far as this distinction rests upon their view that a portion of the peribranchial epithelium is of endodermal origin.

But, as already said, I do not regard the suggestion, with the only facts that I now have to base it on, as deserving more than a mere mention.

### b.  *The Digestive Tract and its Appendages.*

The *Anlage* of the intestine is established at a very early stage in the development of the bud.  It appears at a point on the primitive inner vesicle that is ventral, posterior, and slightly to the left.

In the earliest condition seen it is a mere short, simple projection growing from the wall of the vesicle, situated in a notch, or indentation of its side in the region mentioned. Fig. 16, Pl. XIII, representing a dorsal view of a whole bud, illustrates the above statements, but it must be borne in mind that the bud is seen as a transparent object, and that the hypophyseal and intestinal *Anlagen* would not, as represented, be seen at the same level.  The notch above mentioned is clearly seen in this figure as far as the posterior side of the vesicle is concerned ; but sections from similar buds enable one to see that it is quite as well marked on the ventral side as it here appears on the posterior side.  Concerning this notch, or rather the parts of the vesicle adjacent to it, I shall speak further in dealing with the development of the heart ; I shall, therefore, leave the subject for the present after mentioning that at *no time in the life of the zooid does the digestive tract*

*extend so far posteriorly as do the backward extensions of the
vesicle.*

During its early stages of development the intestine projects
downward and to the left from the vesicle to which it is
attached, so as to lie for the greater part in the wide body
space that surrounds the vesicle (Fig. 29, *int.*, Pl. XIV).   As
development advances, however, it becomes pushed into the
vesicle, so that ultimately it lies wholly within it, *i.e.* within
the atrium in the adult, and by carrying the wall before it as it
enters the vesicle, it comes ultimately to be wholly enveloped
by a thin layer of epithelium, which is attached through a thin
double-layered mesentery to the epithelial lining of the atrium.
Figs. 29, 30, 32–34, Pl. XIV, illustrate these statements.
Fig. 29 is from a section that passes through the oesophagus,
*oe.*, and at the same time cuts the intestine, *int.*, in its widest
part at this stage.

At this time the intestine lies, as mentioned above, in a
notch in the ventral side of the vesicle, but projects largely
into the body space.   It has as yet grown very little in length,
but its lip is already directed forward where it appears in the
fourth section in front of the one here figured.   The distal
part is considerably smaller in diameter than the proximal, the
stomach and intestine proper being thus distinguished from
each other even at this early period.

The organ grows both forward and outward with advancing
development, and very soon begins to take on the curved form
so characteristic of the Ascidian digestive tract.   The curva-
ture is produced by the distal end becoming directed at first
outward and dorsalward, and then a little later backward.   The
plane of the loop stands at first at an angle of about 45° to the
horizontal plane.   Owing to the backward direction of the tip,
a section tangent to the loop first appears in a series cut from
before backward ; and then a few sections farther back the
loop is wholly passed and a double section of the organ is
made.   This condition is shown in Fig. 32, *rcs.* being the
rectum, and *st.* the stomach.   The long pouch-like appendage
from the stomach, *l. coc.*, is the beginning of the lacteal coecum
and duct.   It is noteworthy that at this stage it is almost as large

as the intestine proper. By this time the pushing of the organ into the vesicle, as mentioned above, has advanced considerably (Fig. 32). With further growth the rectum becomes directed inward toward the branchial sac, as well as backward and dorsalward, so that, in the nearly adult state, transverse sections of the animal, which pass through the anal opening, already formed at a considerably earlier time, cut the rectum lengthwise for some distance, at the same time that they pass transversely through the stomach (Fig. 6, *an.*, *rec.*, and *st.*, Pl. XII). It will be observed in this figure how fully the whole digestive tract has now come to lie in the atrium and left peribranchial sac. The rectum reaches across the chamber, and comes in contact with the wall of the branchial sac, and actually forms a secondary attachment to it, so that a rectal mesentery is produced (Fig. 34, *mes.rec.*, Pl. XIV). In the section this mesentery does not appear, but it does in sections a little farther back, none of which, however, would illustrate some other points as well as does this one ; consequently, I have chosen this for the figure, and marked the position of the mesentery diagrammatically. The original mesentery (Figs. 33 and 34, *mes. gas.*, Pl. XIV) is in the adult attached to the parietal portion of the atrial epithelium well out on its side, *i.e.* quite remote from the median ventral line, and extends across as a rather narrow band to the digestive tract in the region of the stomach and lacteal coecum (Fig. 34). The differentiation of the lacteal system into the coecum, the duct, and the ramifying portions, as we have seen them to exist in the adult animal, has been fully accomplished by the time the stage we are now considering is reached. The same is true of the formation of the longitudinal folds of the stomach, and the peculiarly modified longitudinal band in the wall of the intestine.

A secondary attachment of the rectum to the wall of the peribranchial sac is said by Hjort ('93), p. 596, to take place also in *Botryllus*.

c.  *The Pericardium and Heart.*

The origin of the common *Anlage* of these two structures
has been the subject of almost as much diversity of statement
for different groups of Tunicates as has been the origin of the
nerve ganglion.    It is, therefore, particularly satisfactory to
find that in this species its method of origin is so clear as
to leave no doubt about what it is.

At the time of writing my preliminary communication I had
not succeeded in finding the earliest stage in the development
of the structure ; further search, however, since then has borne
the desired fruit.

It arises from the postero-ventral wall of the inner vesicle,
but does not become fully severed until the ventral folds which
play the major part in separating the branchial from the peri-
branchial sacs have extended nearly back to the intestinal
*Anlage*.    These folds, consequently, enable us to locate more
precisely the point of its origin.

Figs. 39–41, Pl. XV, are from a series of transverse sections
of a bud in a stage of development considerably younger than
that shown in Fig. 18, Pl. XIII.    Fig. 39 represents the tenth
section in front of the one from which Fig. 40, passing through
the pericardial *Anlage*, *pc.a.*, is drawn.    The ventrally directed
pouch-like fold, *r.pb.s'.*, seen in Fig. 39, is a portion of the
right peribranchial sac, and it is at the posterior extremity of
this that the pericardial *Anlage* originates.    As shown most
clearly in Fig. 41, it is an evagination from the inner vesicle,
but, as just stated, is at the point reached at this stage by the
posterior extremity of the right peribranchial sac.   *r.v.f.*, in
Figs. 40 and 41, indicates the right ventral partitioning fold.
The *Anlage* of the pericardium appears to become separated
from the vesicle at its anterior end first, then later at its
posterior end.    A later stage, slightly after its complete separ-
ation, but while it is still a simple small vesicle, is shown in
Fig. 42, *pc.v.*

According to Oka ('92), p. 535, the pericardium in Botryllus
arises as " eine solide Wucherung des inneren Blattes der

ursprünglichen Knospe ; aber da die betreffende Stelle gerade im Winkel zwischen der mittleren Blase und der Anlage des linkern Peribranchialsackes liegt, ist es schwer zu entscheiden, ob sie speciell der ersteren oder der letzteren entstammt."

Hjort also ('93), p. 602, found the pericardium of the *Botryllus* bud to appear first as a solid cell mass ; but concerning the origin of this mass, whether from the "endoderm" or from mesoderm cells he was not able to decide.

Pizon ('93), p. 44, on the other hand, describes it as arising in this genus as a small diverticulum, and neither from the branchial nor the peribranchial sacs, but from the primitive vesicle.

But whether it begins as a solid cell mass or as a diverticulum signifies very little ; and of almost as little significance is the question whether it arises from the primitive vesicle, from the branchial sac, or from the peribranchial sac, since its point of origin is at this stage of development an indifferent point as regards the three structures mentioned. It is not at all impossible that absolute exactness in observation would find it to arise from the branchial sac in some individuals, from the peribranchial sac in others, and from the primitive vesicle in others. Indeed, the observed facts in relation to variations in the time and position of appearance of different organs make such a conjecture rather probable.

The only fundamental question raised is as to the possibility of a mesodermal instead of an endodermal origin. This question, and also the question of the epicardium, I shall discuss after having presented my observations on the development of the organ in *Perophora*. The formation of the heart by an invagination of the pericardial vesicle is here so entirely similar to what is well known in many other Ascidians that a detailed description of the process would be superfluous. A mere reference to Figs. 29 and 30, Pl. XIV, in which two stages in the development are incidentally shown, will suffice.

### d.  *The Hypophysis and Ganglion.*

  *a  The Ganglio-hypophyseal Duct.* — The hypophysis always arises very early, in some buds its origin being the first interruption of the simple spherical form of the primitive inner vesicle.   This is the case, for example, in the bud a section of which is represented in Fig. 43, Pl. XV.   Its method of origin can be very well seen by examining with a low magnifying power a whole bud from its dorsal side, the bud having been first cleared in oil.   A drawing of such a bud is shown in Fig. 16, Pl. XIII, where *hy.a.* is the *Anlage* of the organ now under consideration.   By observing it under a constantly changing focus, one finds that it is a groove-like evagination from the dorsal wall of the inner vesicle.   A transverse section of a bud in the same stage of development, and cutting the evagination, is presented in Fig. 43.   The groove is very simple and is quite uniform throughout its length.

  The same section is shown at *bd.*, in Fig. 5, Pl. XII, where the clearly seen orientation of the bud in the colony, and the position of the evagination on the dorsal side of the bud, leave no room for doubt that the evagination is the beginning of the hypophysis.   The *Anlage* remains in this groove-like condition only a short time ; for in a stage of development of the bud only a little more advanced than that shown in the two figures to which attention has just been directed, it appears as a tube wholly separated from the vesicle, except at its anterior end, where its lumen communicates with the cavity of the vesicle. This communication remains throughout the life of the animal as the mouth of the hypophysis.   As the tube becomes constricted off from the wall of the vesicle, it terminates posteriorly as a simple blind pouch, and does not at any stage communicate with either of the peribranchial sacs, as is agreed by Oka, Hjort, and Pizon to be the case in Botryllus.   Hjort ('95) also shows such a communication in *Glossophorum sabulosum.*   But as remarked by Hjort and Bonnevie ('95), p. 392, this communication can have very little significance.   They base this remark on the fact that it does not take place in the buds of *Distaplia magnilarva,* studied by them, while it does in the

buds of genera no farther removed from this genus than are *Botryllus* and *Polyclinum.* I agree that the fact has very little significance, but would point to a different reason for so regarding it. The posterior communication is always transient and without physiological importance. The hypophyseal duct forms from the dorsal wall of the inner vesicle at an early stage of development of the bud, before the limits of the peribranchial sacs are sharply established ; and since their limits on the dorsum of the vesicle are always very close to the posterior extremity of the duct, a slight variation in the relative position of the point at which the duct terminates, and the course of the partitioning folds of the peribranchial sacs ; or of the time of closure of the duct opening ; or the formation of the folds, would determine whether the posterior opening of the duct should be into the primitive inner vesicle, the branchial sac, or one or the other of the peribranchial sacs, or into the atrium. I shall show presently that there is a brief period in the history of the duct in *Goodsiria* when it also has both an anterior and a posterior opening. The posterior opening closes, however, at a stage so early in the development of the peribranchial sacs, that we can only regard it as pertaining to the primitive vesicle itself.

As regards the later history of the duct as such, little more need be said. In the nearly adult state its dorsal wall, Fig. 50, Pl. XV, is exceedingly thin, it being but one layer of cells thick, and the cells of this one layer are considerably flattened, and their nuclei are far apart. The ventral wall, on the contrary, remains, at least in the oldest stages of which I have made sections, several cells thick. Attention may here be called to the entire absence of the hypophyseal gland.

The formation of the duct as I have here described it differs in some unimportant particulars from its formation in *Botryllus*, where, according to Hjort and Pizon, it is not at its beginning a groove, but an evagination of about equal diameters transversely and longitudinally. This evagination then grows out into a forwardly directed pouch, the blind tip of which fuses later with the wall of the branchial sac, and its lumen gains communication with this sac by a perforation at the

point of fusion. The permanent hypophysis mouth is therefore in this genus secondary, and not primary as in *Goodsiria.* The groove-like *Anlage* of the duct in the latter genus is apparently very similar to that which Kowalevsky ('74a), p. 450, has described in *Didemnium styliferum*, the bud-development of which resembles that of *Goodsiria* in some other respects.

β *The Ganglion.*—The three papers of Oka, Hjort, and Pizon, on budding in *Botryllus*, reference to which has already been made, were written so nearly simultaneously that neither author was acquainted with the result of the others' work while prosecuting his own. *No two of these investigators agree concerning the origin of the ganglion*, though each was duly impressed with the theoretical importance of the question, and was also familiar with the discordance in the previous results obtained by several investigators who had studied the blastogenesis in various other Ascidians. This fact testifies convincingly to the difficulties involved in the subject. To these difficulties I too can bear witness, and while doing so may be permitted to say that I have striven hard to search out every fact that might bear on the question, and to draw my conclusions uninfluenced by any bias in favor of one or another of the several prevailing views. Four different origins have been found for the ganglion in the buds of different compound Ascidians by different students. 1. It has been derived directly from the central nervous system of the parent zooid. 2. It has been derived from the free so-called mesoderm cells of the body space. 3. It has been derived from the ectodermic, or outer vesicle of the bud. 4. It has been derived from the inner primitive vesicle, or, more precisely, but what is the same thing, from the hypophyseal duct. The first was advanced by Pizon ('93), for *Botryllus*, evidently suggested by the method of origin of the ganglion in the buds of salpa. But as the author does not pretend to have proven such a process to take place here, and as the facts on which he bases his belief are exceedingly meagre, this may be dismissed without further remark.

The suggestion of a mesodermal origin was, so far as I know, first made by Seeliger in his studies on *Clavelina.* It

was, however, based on indirect and theoretical grounds, viz. :
on the facts that in its earliest stages the constituent cells
much resemble those of the mesoderm by which it is sur-
rounded ; that no other source for them was observed which
appeared more probable ; and that in the embryonal develop-
ment a portion of the dorsal nerve undergoes dissolution, its
cells becoming transformed into the free mesoderm cells. It
was conjectured that these latter might reassemble again to
form the ganglion of the bud. But this view has, I believe, been
given up by the author. The belief in a common mesodermal
origin of hypophysis and ganglion, as described by Seeliger
('89), in the buds of *Pyrosoma*, appears to rest on a much
securer foundation. Very recently Lefevre ('95) has main-
tained such an origin for the ganglion in *Perophora*.

In *Goodsiria*, the very early stage at which the ganglion is
found to be fully separated from the hypophyseal duct, together
with the conditions which I have observed in *Perophora*, have
induced me to look for evidence of its mesodermal origin here.
The close resemblance of its cells in its early stages to certain
of the surrounding cells in the body space, *i.e.* mesenchyme cells,
and the difficulty of observing its origin from any other source,
are the only facts that lend any countenance to such a view —
facts certainly of little weight, particularly when opposed to
direct evidence of a contrary kind. What I shall have to
say on this point when treating the gangleo-hypophyseal
development in *Perophora* will be of greater moment.

The ectodermal origin of the ganglion in the bud has
been asserted by Van Beneden et Julin ('87), for *Clavelina ;*
by Oka ('92), for *Botryllus ;* by Salensky ('92), for *Pyrosoma ;*
and by Brooks ('93), for *Salpa*. Of these authors' works
the most important for us in the present connection is
that of Oka. This is most important because of the obvi-
ously close relation between *Botryllus* and *Goodsiria ;* because
of the positiveness of the author ; and because the work
is very recent, and consequently was done by methods in
common use at the present time, and in the light of pre-
vailing theoretical views. It must, therefore, be examined
critically. The author first describes and figures an irregular

accumulation of cells in the blood space between the already
formed hypophyseal tube and the walls of the branchial
and peribranchial sacs. This becomes more compact and
regular in form; in short, it develops into the ganglion.
Concerning the origin of the cells composing the mass, the
author's essential words are : " Was die Herkunft dieser Zellen
betrifft, so entstammen sie der Körperwand und zwar gehen
sie durch Proliferation des dorsalen, oberhalb des hypophysären
Rohres gelegnen Epithels hervor (Fig. 33). *Dieser Vorgang
. . . kann nur in günstigen Fällen beobachtet werden*, denn die
Zellen verlieren sehr rasch den Zusammenhang mit den Epithel-
zellen, von denen sie sich abspalten, und wandern einzeln oder
gruppenweise rechts und links um das Rohr herum und sammeln
sich an der unteren Seite desselben " (p. 540). The italics are
mine, employed because these are to me the most significant
words of the description. No one who has had experience in
studying such a process as the author here so briefly describes
(for the quotation contains all he has to say on the point) will
be convinced by the evidence which he has produced that he
has not fallen into error. Only a single figure illustrates his
statements, and while in this two cells are shown which
undoubtedly appear as though they might be in the act of
leaving the ectoderm to enter the blood space, still in the
absence of further illustration, or more precise statement, this
instance cannot appeal to one as being necessarily more than a
deceptive appearance.

The inadequate support produced by Oka for his view,
together with the fact that he is opposed by all other observers
(Della Valle, Hjort, and Pizon, who have studied the point in
*Botryllus*), and likewise by my own results in *Goodsiria*, compel
me to reject his conclusions.

Concerning the ectodermal origin of the ganglion in *Clavelina*,
it is worth while to point out that Von Beneden et Julin have
not given the subject special attention in their paper. Under
the topic, "Development du coeur etc." they incidentally
mention the ganglion several times, and it is shown in numerous
figures ; in most of them in close contact with the ectoderm,
but also in *most quite distinct from it*. However, figures

representing sections of the ganglion well toward its posterior
end show it apparently in organic connection with the ecto-
derm. Their most pronounced statement concerning its origin
which I can find is the following, occurring on page 311:
" Dans les dernières coupes il n'est pas possible de voir la
limite entre l'ébauche neurale et l'épiderme ; elle paraît être
un simple épaississement de l'épiblaste (Fig. 10, 11, et 12)."
The authors' conclusions certainly need confirmation before
they are entitled to unqualified acceptance.

The ectodermal origin of the ganglion asserted by Salensky
and Brooks for *Pyrosoma* and *Salpa*, respectively, appears to
be well supported in both cases, though it does not stand
unchallenged, Seeliger ('89) claiming a mesodermal origin for
it in both these genera. I have had no opportunity for personal
observations on the point, and consequently shall express no
opinion upon it further than this, that to my thinking even
*Pyrosoma* is sufficiently remote in its relationship to the com-
pound Ascidians to make possible an ectodermal origin of the
ganglion in its ascidiozooids, while the same organ arises from
the endoderm in the buds of the compound Ascidians. Seeing,
as we do, the central nervous system arising from the *ectoderm*
in the *embryozooids*, and from the "*endoderm*" in the *blasto-
zooids* of the same species, its origin from either of these
sources, or even from the mesoderm, in *Pyrosoma*, or still more
in *Salpa*, ought not to cause great astonishment. That, how-
ever, there are such radical differences within the same genus
as results indicate cannot be accepted till more evidence is at
hand than we yet have.

The last-mentioned source of the ganglion, *i.e.* from the
inner or " endodermic " vesicle, was asserted by several inves-
tigators whose observations were made a number of years ago,
before the exacter methods of section-making had come into
use in morphology, and before morphology had gone so greatly
under bondage to the germ-layer theory as it has done more
recently. And so it happened, as is not unfrequently the case
in science, that the greater intellectual freedom enjoyed by
the earlier workers more than offsets their cruder technique,
and they were enabled to reach conclusions more nearly true

than are those reached by some later students under conditions reversed as to technique and intellectual freedom.

Among the earlier works to which reference is above made those of Giard ('72), and Kowalevsky ('74a and '74b), are particularly to be mentioned.

The results of my study of both *Goodsiria* and *Perophora* agree essentially with this last view ; but since in neither genus were they reached without special perplexities, I must present the facts with considerable detail. In *Goodsiria* the formation of the ganglion is by no means a complicated process, but the difficulty in finding just how it occurs is due to the apparent quickness with which it becomes fully separated from its source, which is, I may say in a word, the ventral wall of the hypophyseal duct. At the time of writing my preliminary paper I was still in some doubt about its source, but more careful study since leaves, as I hope the following will show to the satisfaction of every reader, no room for question. Fig. 49, Pl. XVI, presents a transverse section of the duct in the earliest stage that I have found after its complete separation from the primitive inner vesicle. That the stage is one *very soon* after the separation takes place is certain from the state of development of the other organs of the bud as compared with the stage in which the duct is still merely a groove-like evagination. The peribranchial sacs are very slightly developed, the dorsal partitioning fold of the right side being barely indicated in this position, while the left does not appear at all in the section. Yet it is seen that the ganglion, *gl.*, though very small, is already distinctly separated both from the wall of the primitive vesicle beneath it, and from the duct above it. The condition here shown, and even a slightly earlier one, but with the separation still complete, occurs not infrequently ; but the critical stage, the one between this and that with the duct in the groove condition, escaped me for a long time, and made it seem quite probable that the cells entering into the ganglion were derived from some other source than the duct or primitive vesicle, *i.e.* either from the ectoderm or from the surrounding mesenchyme cells.

In only three or four buds have I found the decisive stages, but two of these, those from which Figs. 44–47, Pl. XV,

and Fig. 48, Pl. XVI, are drawn, are particularly to the point, and these I have consequently selected to describe.   Figs. 45–47 represent transverse sections, in order from before backward, of a bud slightly less advanced than the one in nearly longitudinal section shown in Fig. 48.   The sections are considerably oblique, so that the one, Fig. 44, passing through the mouth of the duct makes it appear to be inclined to one side.   The ganglion is not touched by this section, my purpose in figuring it being to show that the hypophysis mouth exists at this time, though very small.   This section, with the following one, not reproduced, leaves no doubt on the point. Figs. 45 and 46 are from sections a little farther back.   They need very little explanation.   They show the thickened ventral wall of the duct, which is (particularly in Fig. 46, six sections farther back toward the place where the duct is still unsevered from the vesicle) not distinguishably separated from the wall of the vesicle.   In this thickened region, Fig. 46, cell division is more frequently seen than in other parts of the tissues. Fig. 47, one section farther back, shows that the duct still communicates by a wide opening with the primitive vesicle. Three more sections take us beyond the duct entirely, so it is obvious that the communication shown in Fig. 47 is at the posterior part of the duct.   If now we turn to the longitudinal sections, one of which is shown in Fig. 48, Pl. XVI, we find that the lumen of the duct no longer communicates with the vesicle posteriorly, but that there exists a trace, *r.c.o.*, of the opening by which this communication was earlier effected. But the most important fact to be learned from this section is that anteriorly the ganglion, *gl.*, has now become separated both from the ventral wall of the duct and from the wall of the vesicle ; but that posteriorly, where the duct has not yet fully severed its connection with the vesicle, the ganglion is still confluent with the large cell mass that forms at the same time the ventral wall of the duct and the dorsal wall of the vesicle.

We are thus led to recognize that *simultaneously with the closing off from the inner vesicle from before backward of the hypophyseal duct, the ganglion becomes differentiated in the same*

*order from the cell mass that forms the last connection between the duct and the vesicle.*

### e. *The Genital System.*

The results obtained from the study on this system are far from complete. It is very imperfectly developed in all my specimens — so much so, in fact, that I cannot regard as final any of the observations made. As already said, my material was all procured in one locality, and at the same time, *viz.*, December 30 — midwinter. It is not at all impossible that when opportunity is afforded to examine specimens taken from other places, and particularly at other seasons of the year, the sexual organs will be found to tell a different story from that intimated by the fragmentary facts presented by the colonies so far studied.

The character of the sexual organs in the adult, already described, and the manner of origin of the sexual cells in the *Botryllus* bud, lead us to expect that here also we shall find the youngest ova and sperm cells floating free in the body space, having been brought hither by the blood directly from the parent zooid. That such is the case seems quite certain from the facts observed. In several instances I have found ova in the blood (Fig. 52, Pl. XVI), but it is noteworthy that in all these cases they were in *buds far advanced in development.* Although I have given particular attention to the point I *have not yet succeeded in finding an undoubted case of sexual cells in a very young bud.* This seems the more remarkable when it is remembered that the bud becomes fully severed from the parent at a very early stage in its development. But the young buds examined are far too few to justify the negative conclusion that recognizable ova never enter them before their complete severance from the mother zooids. Nevertheless the facts observed are of a kind and sufficiently numerous to make very interesting the several alternative questions which they raise. Direct observation shows that in some colonies in which some of the blastozooids contain ova and are consequently presumably capable of sexual reproduction, certain other zooids are developed without having received recognizable ova directly

from their parents. Do these latter zooids remain throughout their lives incapable of sexual reproduction; or do they receive their ova from the blood vessels with which, as previously shown, the zooids become secondarily connected; or have some of the cells contained in the blood the capability of being transformed into sexual cells; or finally, are the sexual cells really brought into the bud from the parent, which again received them in the same way from its parent, and so on till their ultimate origin was the sexually produced common ancestor of the entire colony? The last is, I suppose, the alternative that would appear most probable to the great majority of embryologists, since it is the one most in conformity with prevailing theoretical views concerning the origin of the reproductive elements. It is also in keeping with the early appearance of the sex cells in the *Salpa* stolon, as made known by Kowalensky, Salensky, Brooks, Seeliger, and others; also with what occurs in the budding of *Pyrosoma*.

But most of all it is supported by the conditions presented by the buds of *Botryllus*. The large ova in the young buds of this species, known since the time of Savigny ('16), have by many writers been supposed not to appear in any of the buds before the third or fourth generation. Pizon ('93), has, however, shown that they really originate in the sexually produced embryo, and migrate, as he expresses it, through the first generations of buds, leaving these to develop into sterile zooids, only to become permanently fixed as ovaries in later generations.

The fact that I have not been able to distinguish sexual cells among the blood cells in the young buds of *Goodsiria* may not be regarded as proof that they do not exist there. They may be in so early a stage of differentiation that, with the methods of preservation and staining employed, their distinguishing characters were not brought out, as they might have been by some other treatment.

It is true that I have occasionally found large polynuclear cells (Fig. 55, Pl. XVI) that have seemed to me to be possibly the forerunner of the cell aggregates. These I have again conjectured to be a stage in the formation of the polycarps.

But I have been unable to get decisive evidence of such a
relation between these elements ; and the structure of the
youngest undoubted female polycarps found does not strengthen
the conjecture, for in these the ova are in different stages of
development, but even the smallest show the characteristics of
ova.　Fig. 51, Pl. XV, represents such a polycarp.　It has not
yet advanced sufficiently to have pushed its way from the body
space into the peribranchial chamber.　Certain clusters of a
few cells larger, more deeply stained, and more granular than
the other blood cells, Fig. 54, Pl. XVI, I suppose to be the
male sexual cells ; but this too is not beyond question.

As has been pointed out in describing the species, the poly-
carps have a rather regular position on the ventral side of the
zooids, on each side of the endostyle and not far from it.　In
other words, they occupy undoubted determinate positions.
With the sex cells at first apparently wholly subject to the
movements of the blood, one would much like to know how it
comes about that their final points of attachment, where they
develop into the polycarps, are always nearly the same.　That
they are in the ventral part of the zooids suggests that the
greater weight of the cells, while still swimming in the blood,
prevents their being carried into the higher parts to become
attached ; but that indicates nothing as to why they should
become arranged so regularly on each side of the endostyle.
Unless we may assume determinants for these particular cells
of a considerably higher grade of intelligence than is generally
attributed to these convenient phantoms, it would seem that
there must be some physical or chemical condition in the
membrane in the region where the polycarps are situated that
attracts to it the sex cells from the blood when they happen
to be carried near it.

With the young sex cells entirely subject to the movements
of the blood, and as a consequence liable to be carried to
various parts of the colony, — into one zooid or another, — it is
interesting to notice how completely, from the sexual point of
view, at least, the *colony*, and not the *ascidiozooids composing it*,
is the individual.

*B.* PEROPHORA ANNECTENS, RITTER.

1. INTRODUCTORY. MATERIAL. TECHNIQUE.

I have described this species in detail in a former paper ('94), and consequently am relieved from doing so here. One point only concerning it I must speak of briefly. In the description referred to, I have said in substance that the species includes at one extreme colonies in which the zooids are quite as distinct from one another as they are in *P. Listeri*, for example, they being nearer together, merely, on the stolons; and at the other extreme colonies in which the zooids "are as completely enveloped by a common test as they are in *Botryllus* or *Goodsiria.*"

I wish here to reaffirm and emphasize this statement in its fullness. I have several times reëxamined my material with a view to finding some constant structural difference that would enable me to separate specifically the one extreme from the other; but each effort has resulted in strengthening the conviction that such a separation is impossible. It may be worth while to add that I have reëxamined the case since having had opportunity to study several other species of *Perophora*, new and old, *P. Listeri* included. Last summer I had the pleasure of submitting my specimens to the experienced judgment of Prof. Herdman, and he agrees with me fully that they are all one species. I reassert, then, *that we have here within the limits of the variability of a single species a complete transition from the Simple or Social Ascidians (depending on what author's system of classification be adopted) to the Compound Ascidians;* or in other words, a complete transition within a single species between two groups that have been considered as distinct families, Bronn, Gegenbaur; or as distinct suborders, Herdman; or even as distinct orders, Haeckel, Claus. For a summary of the various systems of classification that have been proposed from time to time, see Seeliger ('94). Lahille ('90) has proposed a wholly new classification and nomenclature of the groups above genera, in which the simple or compound conditions are entirely ignored as differentiating characters for

any groups higher than species. To some extent this radical departure receives support from *Perophora annectens.* But I fully agree with Herdman and Seeliger in thinking that the system adopted by Lahille, at any rate as regards his families and suborders, comes no nearer being a natural one than the other systems which it is intended to supplant. But it would be outside the purpose of the present paper to enter upon a general discussion of tunicate classification.

In view of the apparent hesitation of some students of the Tunicata, with whom I have had personal conversations, to accept my conclusion that *P. annectens* really does include such widely varying forms, I had thought to present in the present publication a more detailed and fully illustrated description of all the transitional forms than is contained in my former paper. However, further reflection has made it seem to me that such a discussion will belong more appropriately to a comprehensive treatment of all our Pacific Coast species of the genus. Such a treatment will be contained in a monograph of the California Tunicata which is now in course of preparation by myself and one of my students, Mr. F. W. Bancroft.

As stated in my preliminary, my results on the budding of *Perophora* have nearly all been obtained from the study of *P. annectens.* From the abundance of this species in Monterey Bay, Cal., and from the compactness of the colonies, it is very easy to get unlimited material, and whenever I have collected it, the buds in all stages of development have been present in great numbers. *P. Listeri*, on the other hand, at least in the Bay of Naples, I found to present almost insuperable difficulties in the way of procuring buds in sufficient quantities to enable me to get the necessary stages, and those in such numbers as to make a satisfactory study of their development. Both the zooids and the stolons are very delicate, and they cling so closely to the stones on which alone I found them that the young buds are removed with very great difficulty. I succeeded, however, in securing specimens enough to enable me to make sure that there is no difference of moment between the bud-development in the two species.

For the killing and preservation of material I have used the various methods that have been recommended by the numerous investigators who have been occupied in recent years with Ascidian morphology and development, but in spite of the variety of treatment I have not been able to get preparations of this genus quite as satisfactory as those obtained from *Goodsiria*. On the whole, as in *Goodsiria*, the specimens fixed by the weaker solution of the picro-sulphuric mixture of Kleinenberg has given the best results; but the glacial acetic acid method and the chromic-acetic acid solution have also been found useful.

Several zoölogists have studied the budding of *Perophora*. Giard ('72), Kowalevsky ('74), Van Beneden et Julin ('87), and Pizon ('93), have published their observations on the subject, but of these by far the most important work is that of Kowalevsky. It was this investigator who first described in detail the entire development of the blastozooids, and his results were so nearly complete that the points which he left in doubt, with perhaps a single exception, remained in that condition up to the present time, nothwithstanding the studies that have since been bestowed upon the subject. The points to which I refer are the origin of the ganglion, the heart, the sexual organs, and the precise relation of the zooids to the stolon; or more exactly, to the cloison, or septum of the stolon. The first, second, and fourth of these, as the sequel will show, have been rather obscured than clarified by the more recent works. Concerning the third it appears that the results of Van Beneden et Julin are more exact than those of Kowalevsky; but of this I am unable to judge from personal observations. In none of the specimens studied by me have the genital organs been sufficiently developed to permit a satisfactory study of them.

Because of Kowalevsky's paper a redescription of the general development of the buds would be quite superfluous. Excepting the genital system, I shall confine myself, therefore, to the points mentioned above as having been left doubtful by him.

## 2. Relation of the Bud to the Stolon.

The point to be made in this connection may be briefly
stated by quoting what I have already said in my preliminary
paper: "When the differentiation of the 'endoderm' into the
branchial and two peribranchial sacs takes place, it does so in
such a way that the developing blastozooid is connected with
the double-walled partition of the stolon, not by the *branchial
sac*, as has been hitherto supposed, but by the left *peribranchial
sac*."

Figs. 57–64, and 65, 67, and 70, Pl. XVI, will suffice to
illustrate the facts. Of these Figs. 67 and 68 are from the
youngest stage. At this time the inner vesicle is still almost
a perfect sphere, no differentiation of organs having yet begun
excepting that a mere trace, *pc.a.*, of the heart is present.
The connection of the inner vesicle to the stolonic septum,
*cl'n.*, does not show in his section, but it does in the third
section of the series preceding this. The bud at this time
does not stand out at a right angle to the stolon, but is
inclined somewhat toward its tip. The series passes from the
posterior, or point of attachment of the bud, toward its ante-
rior, *i.e.* toward the tip of the stolon. It is for this reason
that we find the section of the stolonic septum in several sec-
tions before we come to its place of attachment to the inner
vesicle.

The process by which the vesicle becomes divided up into
the branchial and two peribranchial chambers differs so little
from the same process in *Goodsiria* and various other Ascidian
buds, that I have thought it would be sufficient to pass over
the various stages excepting those that pertain especially to
the condition about to be described. It is, however, necessary
to refer to the account given by Lefevre of the first stages in
the differentiation of the vesicle. He states that in *P. viridis*
the inner vesicle rotates to the right through 90° during the
initial steps in the formation of the peribranchial sacs. Accord-
ing to him the heart *Anlage*, the first organ to appear, is located
at the beginning 90° farther to the dorsal and left of the
vesicle than the position that it ultimately occupies in the

adult zooid. The rotation is not so great in *P. annectens*, as may be determined by considering Fig. 67. The heart *Anlage* is certainly somewhat higher than it will be at a later time, as its position with reference to the stolonic septum, in this section shows. I have seen no specimens in which the *Anlage* was at a higher point, and I think that this particular individual furnishes evidence that no appreciable rotation could have taken place in the earlier stages of development. This evidence consists in the uniform thickness of the wall of the vesicle in all its parts, thus indicating that there has been no considerable inequality of growth, to which Lefevre probably rightly attributes the rotation ; and to the fact that at this time the connection of the septum to the vesicle is not yet thrust off to one side as it later comes to be (Fig. 70) when the rotation is complete. It is undoubtedly to this rotation that the attachment of the septum to the left peribranchial sac is due.

The greater or less extent of the rotation in various species probably has no particular significance beyond the general significance that attaches to variations in development.

From this early stage we may now pass to the examination of one much farther advanced. Such a one is shown by the series of Figs. 57–64, all from the same bud. The sections pass from the anterior toward the posterior of the animal, and are seen on their anterior surfaces, thus causing an apparent reversal of right and left. In Fig. 57 the peribranchial sacs are entirely distinct from the branchial sacs, and this is the condition for a considerably greater portion of the anterior part of the bud. Fig. 58 shows the point at which the three cavities become confluent. From here the ventral partitioning folds, *v.f.*, rapidly fall away ; compare Fig. 59, seven sections farther back. A few sections farther back (Fig. 60) they have almost wholly disappeared, and the three cavities have become practically one. This section, with those going before it, shows clearly, however, that the bay, *l.pb.s.*, is the posterior tip of the left peribranchial sac. But this is the point at which attachment to the stolonic septum, *cl'n.*, occurs. It will be seen that the ventral side of what will later be the branchial sac is far below this, it being marked by the already forming endostyle, *end.*

Series of figures similar to these, representing both younger and older stages, might be multiplied indefinitely were it necessary ; but the main facts, *viz.*, those concerning the relation of the zooid to the stolonic septum, are so clear that no further illustration is required.   And this is the more so because Lefevre ('95) fully confirms my results in this particular.   In Fig. 65 I have shown a single instance from many that might be given of the same relations in a bud of *Perophora Listeri*.

The connection between the septum and the peribranchial sac becomes entirely lost at a stage only a little later than that represented in the series 57–64.   Lefevre states that the severance does not occur until a somewhat later time in *P. viridis*.   This difference in the two species may be correlated with the fact that the zooids of the *P. annectens* colony are closely bound together by the test, and hence depend less on the stolon for maintaining their colonial character than do those of *P. viridis*.   It is certain that in some cases, at least in *P. annectens*, the buds become wholly severed from the stolon, the ectodermal as well as the endodermal connection being cut away.   I have shown an instance of this in Fig. 66.   This is the section of a complete series in which the stolon, *sto.*, with its septum approaches most nearly to the wall of the zooid; yet there is a distinct absence of connection between them.   I am unable to say with what frequency this complete separation takes place, but so far as I have been able to determine it appears that it is rather exceptional.   Certain it is that the stolons of a colony are always in communication with many of the zooids because the blood is always in motion in the living colonies, and there is, of course, no other propelling power than the hearts of the zooids.

I have stated that the connection is always to the *left peribranchial sac*.   This has been true for every undoubted instance observed, but it does not seem in the least improbable that exceptions might be found were one to examine a sufficiently large number of cases ; though that it is well-nigh the invariable rule is certain from the large number of instances of its occurrence that have been observed.

That Kowalevsky failed to make out the precise relations between the bud and the septum of the stolon is not surprising, since he studied whole buds almost exclusively ; at least he could have had no complete series of sections. The septum is very delicate, and is entirely surrounded by tissues denser than itself, and for these reasons it is almost, if not wholly, impossible to trace the actual condition of things in the living bud. But the failure of Van Beneden et Julin, and of Pizon, I cannot account for so easily, since these authors made use of thin sections in their studies. It seems probable that they either missed the stages in which the facts are most easily seen, or that their series of sections were imperfect. Under other circumstances it appears hardly possible that they would have overlooked the facts, patent as they are.

It would appear that Van Beneden et Julin, having carefully followed through its entire development the bud of *Clavelina*, and having studied the bud of *Perophora* sufficiently to see that in most particulars it agreed with *Clavelina*, thought themselves safe in assuming the same agreement would hold in all points. So many common characters do the two genera present, not only in adult structure but also in development, both of embryozooids and blastozooids, that such an assumption might very naturally seem warranted. The case furnishes only another illustration of the dangers to which morphologists are subject when they assume certain things to be true of one animal because they are known to be so for another closely related one. The fatality lies most frequently, I suspect, in the supposed, and not real, close relationship between the animals.

These authors practically, though unconsciously, say that their observations on this particular point are defective for *Perophora*. They say (p. 307), "nous n'avons pas pu nous édifier complètement sur l'histoire du coeur et du système nerveux," etc. Now, as will appear from my account of the development of these organs, it would have been well-nigh impossible for them to fail of a clear understanding of the development and relations of structures mentioned without likewise missing the true relations of the bud to the stolonic septum.

That they did suppose the same conditions to hold for both genera is evident, although they say so rather indirectly. Thus, in summing up their results on the development of the heart " et de ses dependances chez la Claveline," they say, p. 317, "Chez la Claveline, comme chez la Pérophore, la vesicule interne du burgeon," etc. ; and then follows a brief restatement of the relations between the primitive inner vesicle, the branchial and peribranchial sacs, the epicardium, the pericardium and heart, and the cloison, as they have been described in detail for *Clavelina*. In this connection I shall merely say that the description which they give for *Clavelina does not apply in a single essential particular*, excepting as to the formation of the heart from the pericardium, to what actually takes place in *Perophora*. In describing the development of the heart I shall point out in detail the difference between the two.

Pizon's paper is entitled "Histoire de la blastogénèse chez les Botryllides," and the author does not claim to have made so exhaustive a study of the numerous other genera which received his attention as he has of *Botryllus* and *Botrylloids*. As regards *Perophora* he seems to have examined it just enough to make himself feel safe in adopting the errors on this point, at least, into which Van Beneden et Julin had fallen. Thus, he says, p. 105, "Disons d'abord que chez des bourgeons *d'Amaroncium proliferum*, de *Circinalium concrescens* (Giard), de *Perophora Listeri*, de *Clavelina Rissoane*, je suis arrivé identiquement aux mêmes résultats que Kowalevsky (*Am. proliferum*) et que Van Beneden et Julin (*Clavelina Rissoana*) à propos de l'origine du tube epicardique." Then follows a brief description of the epicardium and its relation to the branchial sac and pericardium, which is the same as that already referred to as having been given by Van Beneden et Julin. But this writer's statements on this point I shall also have to consider further after having described the development of the heart.

### 3. Pericardium and Heart.

It appears to be the rule that the pericardium is the very first organ to be founded in the *Perophora* bud. At least, I have found a structure present in several buds at a time when the primitive inner vesicle is still wholly unmodified, which I suppose to be the *Anlage* of this organ. One of these is represented in Figs. 67 and 68, Pl. XVI.

My reason for thinking this to be the beginning of the pericardium is this : the sections of this bud are cut from the posterior toward the anterior end of the future zooid, consequently they are seen in their natural position as regards right and left. This, as I have pointed out a few pages earlier, makes this structure in the position, the slight lateral rotation of the vesicle to the right having been performed, that the heart occupies in the adult bud. The only question that could be raised against this identification would rest on the possibility, first, of error concerning the anterior and posterior ends of the bud ; and second, that in this instance we have an exception to the rule that the septum is connected with the left peribranchial sac. As regards the first, the possibility of error is remote because another older bud in the immediate vicinity is clearly cut from behind forward, and the two are so close together that a reversal of their anterior ends is hardly possible. As regards the second, nothing can be said beyond what was asserted in discussing the relation of the bud to the stolon ; *viz.*, that in the large number of buds observed, no exception to the rule has been found. If this is an exception, it is the only one hit upon.

We may then regard it as certain that the cell mass under consideration is the *Anlage* of the pericardium. The origin of it is a difficult matter to decide. The numerous and widely separated groups of Tunicates in which the pericardium has been satisfactorily shown to arise from the endoderm is a circumstance that in itself makes its similar origin here probable ; and my observations on the whole point in the same direction, though I have been unable to remove from my mind some traces of doubt on the subject. As shown in Fig. 68,

there seems to be in the section here represented a passing of cells from the wall of the vesicle into the *Anlage, pc.a.,* but the evidence of such a process is more convincing in this section than in either of the other three sections of the series in which the *Anlage* appears ; and even here, as the figure shows, the line of separation between the wall and *Anlage* is distinct in places.[1] At the same time the cells of the *Anlage* are some-what more deeply stained than are the immediately adjacent ones in the wall of the vesicle, a fact which I have attempted to bring out in the figure by making the former slightly darker than the latter. Again, as seen by this figure, the cells of the *Anlage* are not closely massed together as one would suppose they would be had they been derived from the vesicle at the single point where, as shown by the figure, they are passing from the latter into the former.

These two last-mentioned conditions raise some doubt about the origin of the *Anlage* from the vesicle, and at the same time they, together with the close resemblance of the cells compos-ing it to some of the surrounding mesenchyme cells, suggest these latter as being its source. I have not seen an earlier stage than this in the formation of the *Anlage*, but have found about the same and slightly older stages not infrequently, and in all cases the conditions presented are very similar. In Figs. 69 and 71, Pl. XVI, are represented sections from different buds both considerably more advanced in development than that just described, though of the two the one shown by Fig. 71 is somewhat older. Here the cavity of the pericardium is already established, though it is quite small, particularly in the younger bud. The portion of the wall of the organ in contact with the wall of the inner vesicle is thicker than else-where, and the two appear to be in organic connection ; but any one who has had experience in determining whether two cell masses in contact with each other are really organically connected or not, knows well how extremely easy it is to be deceived. I have carefully examined with an oil-immer-

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[1] The interruption of the line separating the *Anlage* from the wall is at a point toward which the index line *pc.a.,* Fig. 68, points. The lithographer has failed to accurately reproduce my drawing here.

sion lens all the sections of these two, and numerous other
cases, and believe that the connection is real and not ap-
parent ; and since at a later stage the separation certainly
becomes complete, the connection cannot be supposed to be
secondary. I therefore conclude *that the pericardium originates
from the wall of the primitive inner vesicle.*

At the same time, however, I must expressly state what is
already obvious from the description, that the conclusion rests
upon a *preponderance of evidence;* there is certainly some
evidence against it, and that is indicative of a mesenchymal
origin of the cells.

(Since the above account of the origin of the heart was writ-
ten, Lefevre's short paper has reached me, in which he affirms
that the mesenchyme is the source of the heart. I have
reëxamined my preparations with much care, and must say
that, although, as my words above indicate, Lefevre's state-
ments found my mind in a condition of equilibrium, almost, on
the point, I am still of the opinion that my conclusion is cor-
rect, at least for *P. annectens.* In fact, my more recent study
has discovered some additional evidence in support of my
earlier conclusion. For example, Fig. 69 represents a section
in which, *at the focus here shown,* as seen under the oil-immer-
sion lens, it is certain that no interrupting line is present, and
at $\beta$ is one cell, at least, that is undoubtedly about to divide,
though I do not stake much on this fact. A circumstance
connected with another section of this same series is I think
quite indicative that the *Anlage* is in organic connection with
the vesicle. It is this. The outer thin wall of the pericardial
vesicle has been, in the section referred to, by some means
artificially broken away from the rest of the vesicle ; yet the
thickened side next the endodermic vesicle is still as indistin-
guishably confluent with the latter as in the section figured.

It seems to me quite likely that a force considerable enough
to break this wall would have moved the whole pericardial
vesicle from its contact with the endoderm were its relation
merely a contact.

I have found no sections in which *at some focuses* I cannot
see the separating line to be uninterrupted ; but, very distinctly

in many sections, and less distinctly in many others, I am sure that the line is interrupted.

And, as already said, the fusion can hardly be regarded as secondary, because at a little later time the pericardium is clearly wholly distinct from the "endoderm." Now what is the explanation of the facts that have left traces of doubt in my mind, and have led Lefevre to believe that the cells under consideration come from the mesenchyme ? I believe it to be this : *That the mesenchyme cells themselves are being constantly produced from various parts of the endodermic vesicle for a considerable time during the early stages in the development of the bud.*

My evidence for this is not as conclusive as we would wish it to be, but at the same time I have observed a considerable number of cases in which I believe cells are being set loose from the "endoderm," and are passing into the blood. Figs. 77 and 78, Pl. XVII, represent cases of this kind, *b.c.'*, being the cells referred to. These are both remote from the position at which either the ganglion or heart will form.

And, on theoretical grounds, is not such an origin of the mesenchyme cells highly probable ? Certainly new ones must be forming rapidly somewhere, for the newly developing zooids must make large demands on them for their blood and muscles, both of which undoubtedly come immediately from the mesenchyme. To suppose that all the muscle, blood, and mesenchyme cells of a colony have been derived directly from the mesenchyme cells of the embryozooid does, it seems to me, rather more violence to general developmental principles than to suppose that each new bud is capable of producing such cells for itself. And what part of the bud is more likely to do this than the "endoderm" ? If my belief on this point is correct, then it is not at all impossible that the heart, or even the ganglion, may be formed, at least in part, from mesenchyme cells, for the mesenchyme cells would themselves have the same source, and when first severed from the "endoderm" would be of the same character as the cells which certainly produce the heart and ganglion in the buds of some other species. It would be merely a question of what position on the primitive inner vesicle the cells should be given off. And

in this connection it is significant that the resemblance of the *Anlage* cells to the mesenchyme cells does not apply to *all* the mesenchyme, or blood cells by any means. As a matter of fact, it is to a comparatively small number of these latter that the former have such a resemblance.)

A detailed account of the development of the heart from the pericardial vesicle is just as little necessary here as in the case of *Goodsiria*, where it was stated that such a description would be superfluous, so entirely does the process correspond with what has been many times described for other Tunicates. Fig. 61, *ht.*, Pl. XVI, incidentally shows the heart beginning to form by an invagination of the large pericardial vesicle.

The matter of chief interest in connection with the development of the heart in the buds of *Perophora* remains to be considered. I refer to its *direct* origin from the primitive inner vesicle, and its consequent independence of the stolonic septum. The facts in relation to the subject were partly presented, as will be recognized, in describing the manner of attachment of the blastozooid to the septum; and the whole question might have been very properly discussed there. But, since it has been more commonly treated in connection with the heart by other authors, it seemed best to adopt the same plan in this instance. It has already been pointed out that Van Beneden et Julin supposed *Clavelina* and *Perophora* to agree in this respect, as they do in many others. In order to make it clear that they do not, it will be necessary to point out what, according to these authors, are the conditions in *Clavelina*. An understanding of them in all their details can hardly be reached, however, without aid of the numerous figures by which the authors have illustrated the subject. These are all the figures of plate XI; figures 1 to 7, plate XV; and figures 3ᵃ, 3ᵇ, 3ᶜ, 3ᵈ, and 3ᵉ of plate XVI of their well-known memoir ('87).

The parts essential to an adequate understanding of the conditions described by them are : the primitive inner vesicle, the branchial sac, the "tube épicardique," the "sac épicardique," and the "cloison stoloniale."

To make the subject clear, and at the same time to do so as

briefly as possible, I will quote their words in part, and in part give my own description condensed from theirs. On page 317, previously quoted in part, they say "Chez le Claveline, comme chez la Pérophore, la vésicule interne du bourgeon résulte de l'écartement des deux lames cellulaires adjacentes de la cloison stoloniale. La vésicule, allongée dans le sens de l'axe du bourgeon se continue en arrière dans la cavité virtuelle de la cloison stoloniale. *Cette vésicule se divise transversalement en une portion terminale et une portion basilaire. La portion terminale de la vésicule donne naissance au sac branchial et au tube épicardique. . . . La portion basilaire engendre le sac péricardique. . . . Puis, par une sorte d'étranglement progressif, les deux portions de la vésicule interne primitive se séparent l'une de l'autre.*" The italics are mine, and are inserted to call attention to the points most important for the present purpose. One might understand from the first part of the quotation that the "portion terminale" is fully separated from the "portion basilaire" before any farther differentiation; but such is evidently not the meaning, since the lines omitted between the last and next to the last parts of the quotation give the character of the connection between them and the relation of the whole to the developing heart. I should perhaps have included in the first part of the quotation another line or two which state that the "portion terminale" gives rise also to the peribranchial sacs and the intestine.

On pages 315, 316 the following is much to the point. "Il résulte de l'examen de la série des coupes successives faites á travers le bourgeon partiellement représenté pl. XVI, figure 3ª à 3ᵉ, que *le péricarde, l'épicarde,* et *le sac branchial* sont des parties, incomplètement séparées l'une de l'autre de la vésicule interne du bourgeon. . . . Cependant, le sac péricardique a commencé à se séparer de l'épicarde, et l'étude de ce bourgeon nous permet de nous faire une idée très exacte de la manière dont s'accomplit cette séparation." Then follows a description of the way in which the separation takes place, and still further on the statement: "Plus tard ces communications cessent d'exister et dès lors la vésicule interne primitive du bourgeon s'est subdivisée en deux parties distinctes : *sac branchial* et

*épicarde,* en avant *péricarde* et *cloison stoloniale* en arrière."
And on another page they show that the cavity of the *peri-
cardium is directly continuous into the virtual lumen of the
cloison,* and that this is the condition retained in the adult
zooids.

The essential facts here set forth, summed up in the fewest
words possible, are these : The epicardium is derived from the
branchial sac, or more precisely from the part of the primitive
inner vesicle that later forms the branchial sac. The peri-
cardium is derived from the epicardium. The epicardium
remains in connection with the branchial sac, but becomes
fully separated from the pericardium. The pericardium re-
mains in connection with the cloison.

That the epicardium is a well-defined structure in *Clavelina,*
is obvious from the description and figures of it by the authors.
Thus it communicates with the branchial sac by two openings
called by them "orifice épicardique," these being in reality
two short tubes passing between the branchial sac and the
"tube épicardique" proper, which is a single wide cavity
terminating posteriorly in two "cul-de-sacs épicardiques."

That the course of things in *Perophora* is very different from
this is clearly seen by an examination of the series of Figs. 60,
61, and 62, Pl. XVI. The most striking difference is in the fact
that in *Perophora* the pericardium *neither at its origin, nor at
any later time, has any connection whatever with the cloison of the
stolon;* see particularly Fig. 70, Pl. XVI, from another some-
what younger bud. From these it is seen that it arises directly
from the primitive inner vesicle on its *right* side, consequently
remote from the point of attachment of the cloison to the
inner vesicle, which point is on its *left* side, corresponding in
this stage of development to the posterior extremity of the
left peribranchial sac. This difference implies the further one
*that there is no epicardium in Perophora,* at any rate holding
such a relation to the pericardium as this structure does in
*Clavelina.* It may be asked if the portion of the primitive
vesicle from which the pericardium is derived may not be
regarded as representing the epicardium of *Clavelina.* It
certainly does hold the same relation to the inner vesicle ; *i.e.*

it is at its posterior middle part, so that later it becomes
a posteriorly extended portion of the branchial sac; compare
Figs. 62, 63, and 64, in which *br.s'.* indicates the portion of
the vesicle referred to. But it must be noticed that this is
nothing more than the somewhat narrowed rear end of the
branchial sac leading to the oesophagus (*oe.*, Fig. 62), and ex-
tends back only a very short distance behind its opening.
Fig. 64 represents a section only three sections, $7\frac{1}{2}\,\mu$
thick, farther back than the one shown in Fig. 62, passing
through the opening of the oesophagus; and in this it will
be noticed that the cavity *br.s'.* does not appear, though the
stomach does. This is practically the condition found in the
adult zooids. However, the fact that this coecum, as it may
be called, is the vestibule to the oesophagus need not, in view
of its relations to the branchial sac and pericardium, stand
seriously in the way of regarding it as representing the
epicardium. But to make it correspond with this structure
in *Clavelina*, its relation to the septum would require such
a radical transformation of things as makes it very difficult to
believe that the two structures are genetically related to each
other. To shift the connection of the septum from the *peri-
branchial sac* on the *left side* of the body, to the *pericardium* on
the *right side*, would be a rather serious matter, it seems to me.

Having thus shown a radical difference between *Clavelina*
and *Perophora* in the relations of the pericardium to the stolonic
septum, and of the septum to the branchial apparatus, I leave
the subject for the present to resume it again later on from a
more general point of view.

## 4. THE HYPOPHYSIS AND GANGLION.

It was pointed out in my preliminary communication that
while in both *Goodsiria* and *Perophora* the investigation of
this subject encounters special difficulties, it fortunately hap-
pens that the difficulty is not at the same point in the two
genera.

In *Goodsiria*, as we have seen, the origin of the *common
Anlage* of duct and ganglion from the *inner vesicle* is observed

with the greatest ease and certainty; the difficulty is found in ascertaining the *source of the ganglion*. On the other hand, as we shall presently see, in *Perophora* the origin of the *ganglion from the common Anlage* is seen with perfect distinctness, while the *source of this common Anlage* is ascertained with considerable difficulty.

It is a suggestive fact that in *Perophora* the difficulty encountered in making out the origin of the ganglio-hypophyseal *Anlage* is precisely the same as that which we have already seen in the way of ascertaining the source of the pericardium. In *Goodsiria* it will be remembered that we found both hypophyseal duct and pericardium to arise as well-defined evaginations from the primitive vesicle. In *Perophora*, on the contrary, we have already seen the pericardium arise, almost certainly from the inner vesicle, but, instead of by an evagination, by a cell egression so difficult to observe that a trace of doubt might be entertained as to whether the cells have in reality come from the vesicle or from the surrounding blood, or mesenchyme cells. We shall now see the ganglio-hypophyseal *Anlage* to arise in the same way as the pericardium, and hence in the same way its source is not as absolutely beyond question as might be desired. In both genera it appears as though the influences which have determined the character of the development of one of these structures have also determined that of the other.

Since in *Perophora* the cloudy point is not the origin of the ganglion alone, as in *Goodsiria*, but of the common *Anlage* of duct and ganglion, my reference to the four different alleged sources of the ganglion, made when treating the development of that organ in the latter species, should be recalled here and considered in connection with the origin of the ganglio-hypophyseal *Anlage*.

The supposition that it originates directly from the nerve ganglion of the parent zooid would, in this instance, be so obviously unjustifiable that Pizon himself would hardly venture to make it.

In my preliminary I have declared it to be "absolutely certain" that the *Anlage* does not arise from the ectoderm.

This declaration was made in the face of a full appreciation of the general danger there is in unqualified affirmations that certain developmental processes do *not* take place; but in this instance I believe such positiveness is justified. I must give my reasons for believing so with particular care and detail, because of the slight uncertainty of my conclusion that the inner vesicle is the source of the *Anlage*, and furthermore because of the supposition by Van Beneden et Julin ('87) that the ganglion originates from the ectoderm in the *Clavelina* bud.

It is in the *character of the ectoderm* and the *relation of the Anlage to it* that my conviction finds its justification. The ectoderm is composed of a single layer of cuboid cells, so large, regularly placed, and distinctly set off from one another that they are quite diagrammatic in their clearness in most preparations. The nuclei are round, and generally sharply contrasted with the cell-body by their distinct membrane and their less deeply stained ground substance. They are as a rule situated somewhat nearer the inner side of the cells.

The inner surfaces of the cells are remarkably even and clear cut, and the layer of protoplasm forming them appears to be denser than the rest of the cell-body; at least, it is generally stained more deeply (Figs. 68, Pl. XVI, and 72 and 74, Pl. XVII). From this character of the individual cells the inner surface of the layer which they compose appears in sections as a very sharp line; and as this line would have to be broken were cells to enter the body space from the layer, either by cell division, or by migration, I cannot believe the process could escape all the search I have devoted to the point. Further than this the distinctness of the cells from those adjacent to them in the body space and those composing the *Anlage* is evidence to the same end. Their nuclei are in general smaller; but the most important difference is in their behavior toward reagents. By some methods of treatment, most markedly apparent perhaps in some specimens preserved in Perenyi's fluid and stained with Kleinenberg's Haematoxylin, the protoplasm of the ectoderm cells, after having been decolorized, shows a dark dirty greenish tint that is entirely characteristic of them, not only

as compared with the blood and *Anlage* cells, but also as compared with any other cells whatever of the animal.

And with almost all the methods of preparation used the ectoderm cells stain considerably more deeply, *particularly on their inner sides*, than do the other cells with which we are concerned. In some instances where the blood cells are particularly numerous between the ectoderm and the inner vesicle, it is wholly impossible to decide whether the "mesenchyme" cells are being given off from the inner vesicle or not, so much do they resemble the cells of the latter, and so imperfect is the separating line between them. But in these cases there is *not the least possibility of deception about the distinctness of the body-space cells from the ectoderm.* The difference in staining and the clear boundary line, as described above, preclude it. Now of course, a critical reader, particularly if he be inclined to be skeptical, might reply that for a particular instance it may be true that the case against the ectoderm is as clear as here presented, and that in this instance no cells are being given off from it ; but that this is very far from proving *that at some other stage in development*, or in *some other region* this process does not take place. I fully appreciate the weight of this rejoinder, but in this instance think it wholly overbalanced. As to the second part of it, I would say that the descriptions and figures all apply to the ectoderm on the dorsal side of the bud immediately over the *Anlage*, or, for stages before this has appeared, over the region where it will appear, consequently in the region where we should expect the nervous system to arise, and the region where, according to Oka and Van Beneden et Julin, in *Botryllus* and *Clavelina* it does arise. It is also the region in which more than elsewhere the character of the cells is such that they might most easily be supposed capable of giving origin to new cells. As already shown, the cells are here cuboid in form and have round nuclei and a considerable quantity of cell protoplasm. In all other regions, on the contrary, the cells are flattened, as are their nuclei also, and their protoplasm is relatively less in quantity. The first part of the objection is more weighty than the second, but even this must, I believe, yield to the facts. We shall allow that it

is not sufficient to prove that the ectoderm is *not giving off cells* into the body space *at the time when the Anlage is being formed*. It must also be shown that the process does not take place at *any earlier time*. But when it is considered that the *Anlage* is formed at a very early stage in the life of the bud, it will be seen not to be a matter of great difficulty to examine a complete series of stages from the very inception of the bud up to the time when the *Anlage* is fully formed. This I have done repeatedly, that is, on numerous series of sections, and the description of the ectoderm already given applies as well to one stage as to another. To emphasize this fact I would again call attention to Figs. 68 and 74, *ec.*, the first from a bud in which the *Anlage* has not yet appeared, the second from one in which it is forming. I thus hope to have successfully assumed the risk of *positively denying that the nerve ganglion comes from the ectoderm in the blastozooids of Perophora*, even though I cannot be so positive as to what its source really is. Concerning the difficulties in the way of deciding whether the inner vesicle or the "mesenchyme" cells are the real source of the *Anlage*, I need only refer the reader to what has already been said about the difficulties involving the study of the origin of the pericardium. The well-nigh universal distinctness of the line of separation between the *Anlage* and the wall, the lack of compactness of the *Anlage* in its early stages, and the close resemblance of its cells to many of the surrounding blood cells, here, in the same degree as there, suggest the latter as being the source of the *Anlage*. Fig. 72, Pl. XVII, affords a typical illustration of this. It is drawn from the section in which, if in any one of the series, the separating line is interrupted by cells passing from the wall to the *Anlage*. At the middle point of the separating line there seems to be such an interruption.

By far the most convincing evidence that I have of the origin of the *Anlage* from the vesicle consists of the occurrence in a single specimen of what is almost certainly *an imperfect evagination by which it is formed*. Fig. 74, Pl. XVII, represents the section in which this is most clearly seen. There is no doubt that the separating line is interrupted here at the point

*gl.ev.*, which I believe to be the evagination; and there is also no doubt that the cells of the *Anlage* are continuous with those of the vesicle wall. While it is true that, as shown by the figure, the evagination is not clearly defined either as to its cellular wall or as to its cavity, I am unable to see any valid objection against regarding it as such; the chief difficulty, so far as I see, lies in the fact that the instance is an isolated one. It is impossible that such a condition can be general, since, as already said, I have seen it in a single bud only among the large number examined of practically the same developmental stage. It may be asked if we have not here a rather late stage of development in which the mouth of the hypophysis is already formed; and if the opening here figured does not represent it. But when the entire series of sections is examined and compared with those from younger and older buds, there is really no ground for question on this point. The hypophysis mouth is not formed till a considerably later time, and it is always a well-defined opening, but smaller than this. It is my strong belief that originally the *ganglio-hypophyseal Anlage arose from the inner vesicle by an evagination in Perophora*, just as it does in *Goodsiria*, *Botryllus*, *Glossophorum*, and *Distaplia*. For some unaccountable reason the process has undergone secondary modification, till in most cases, in *P. annectens* at least, the evagination has been wholly replaced by a cell proliferation. Occasionally, however, the earlier evaginate process is reverted to; and such a case is presented in the individual shown in Fig. 74. The individual variation here seems to be somewhat similar to that which occurs in the gastrulation of *Aurelia flavidula*, where it has been recently shown, Miss Hyde ('94), that in some instances the endoderm is formed by a regular invagination, while in others it is formed, in part at least, by delamination and by inwandering of the blastosphere cells.

The completion of the hypophyseal duct and the differentiation of the ganglion from its dorsal wall takes place relatively later here than in *Goodsiria*. It will be remembered that in the latter species the duct is well formed, and the ganglion entirely separated from it at a stage when the peribranchial sacs are but just begun and the endostyle is still scarcely

indicated. In *Perophora*, on the other hand, the ganglion is not fully separted from the duct till the peribranchial sacs are well developed, and the stigmata have begun to form. Likewise the endostyle is well advanced to its final form.

The first differentiation that occurs in the solid, irregular cell mass, that at first constitutes the *Anlage*, consists in the modeling of this into a quite regular cylinder, in which there soon appears a lumen. The wall of the tube thus formed is several cells thick, and is of about equal thickness on all sides throughout its length. Before the lumen is wholly completed a fusion between the walls of the anterior end of the tube and of the branchial sac takes place, the fused area becomes perforated, and thus the hypophysis mouth is produced. The formation of the ganglion begins by a rapid proliferation of cells in the dorsal wall of the duct. Nearly the entire length of the duct participates in the process, although in the nearly adult condition the ganglion does not extend entirely to either end of the duct. Fig. 76 represents a transverse section of a duct at an early stage in the development of the ganglion, and Fig. 75 a longitudinal section of a much later stage — a stage, in fact, when the ganglion is almost completely separated from the duct.

The intervening stages I have not thought it necessary to figure. They occur in many of my sections, however, and the whole process is very clear, and easily observed. The separation takes place considerably earlier in some buds than in others, and in a majority of cases it is completed before a stage so late as that shown by Fig. 75.

I may, perhaps, here refer again to the fact that the ganglion in this species develops on the dorsal side of the duct, while it develops on its ventral side in *Goodsiria*.

It is unnecessary to follow the development further. In a short time the ganglion reaches a diameter considerably exceeding that of the duct, and it acquires the characteristic mantle of ganglion cells.

A single developmental point has been omitted that ought, perhaps, to be mentioned. In a few individuals I have noticed a distinct thickening in the *ventral wall* of the tube. Where

this has occurred most conspicuously it has been before the appearance of the ganglionic thickening in the dorsal wall. It is possible that this may be an embryonic trace of the glandular portion of the hypophysis that is so well developed in some Ascidians. There is no particular evidence for this, but the fact that this structure is known to undergo degeneration in some Ascidians gives the suggestion some probability.

In my description of *Perophora annectens* ('94) I have called attention to the fact that the posterior ends of the ganglion and duct are not situated in the median plane of the body, but are considerably to one side. This condition is assumed early in the development of the organs, and is almost always quite pronounced, but I do not see that it has any particular significance.

### *C.* GENERAL CONCLUSIONS AND REFLECTIONS.

### 1. APPLICATION OF GENERAL DEVELOPMENTAL PRINCIPLES TO AN INTERPRETATION OF THE FACTS.

It being now, as I believe, fully established that the nervous system of the Ascidian bud has a different origin from that of the embryo, we must seek for an explanation for so anomalous a fact,—for the *processes* of evolution are of more importance from a scientific point of view than are their particular *products*.

The explanation, which I think to be the true one, has already been pointed out, first by Seeliger ('85), and more recently by Hjort ('95). The latter, in particular, has dwelt quite fully upon the point. It is, however, a matter of such importance that I believe it deserves to be emphasized still further. This is an instance where nature herself has performed an experiment in modifying the ordinary course of development. The revolutionary and comparatively crude, but still, in considerable degree, successful efforts of experimental embryologists to artificially divert cells that would normally become ectoderm into entoderm, or structures ordinarily of endodermic origin, have been deservedly held to be of the highest moment for solving the fundamental problems of animal development. Cer-

tainly, then, if cases can be found where nature, with her conservative and infinitely delicate methods, has entered upon the same general line of experimentation, and has carried her efforts through to complete success, such cases cannot be too carefully studied.

Let us observe with attention the state of things in the Tunicate bud. The ectoderm is part and parcel of the ectoderm of the parent (this is strictly true in forms like *Goodsiria* and *Botryllus*, where no stolon is present ; and is also essentially true when the budding is stolonic, since here the ectoderm of the stolon is only a prolongation of that of the parent). This is equivalent to saying that the ectoderm of the bud is not an *embryonic structure* at all. It is, on the contrary, a *differentiated organ*. Its function in the parent is to secrete the cellulose test, and in the bud from the very earliest stage it has the same function. The specialization of this secretory function must be deep-seated, for, as Hjort has pointed out, the cellulose character of the test necessitates this. Furthermore, not only is the specialization deep-seated, but also there must be a great and constant activity of the cells ; for not only is the test considerable in quantity, but it must be perpetually produced through the whole life of the zooids to replace the continual waste that is taking place from the external surface. One not infrequently finds great quantities of diatomes embedded in the surface layers of test, and it is well known that many species of animals, particularly small Crustacea, work their way into the test of Ascidians and there lead a semi-parasitic life. Even where no foreign organisms were present, I have often observed the surface test in various species to be eroded and ragged.

The ectoderm, then, has a well-established physiological function to perform in the bud from its very earliest stage of development. How is it with the endoderm ? It is scarcely possible to see how a structure could be more favorably situated for retaining, so far as its functional relation to the organism as a whole is concerned, an undifferentiated character than is the " endoderm " in the early bud. Not only is it wholly protected from contact with the external world, it being enclosed in the ectodermic vesicle, but it has nothing to do in the preparation

of its own food, for it is entirely bathed in the maternal blood. It is relieved from all offices except to assimilate already digested food, and to grow.

Why should not the production of structures which in the embryonic development belongs to the ectoderm be here transferred to the endoderm? And so it is. This conclusion is the more justified when it is considered how different are the conditions under which the two layers develop in the embryo. Here the neural canal is formed while yet the ectoderm is strictly an embryonic structure, and before the endoderm (particularly in the compound Ascidians, *e.g. Amaroecium*, Maurice et Schulgin, '84) is differentiated from the richly laden yolk cells which ultimately give it origin.

We have here an instance where physiological requirements have run counter to the course in which, through heredity alone, development would proceed ; and the former have proved more powerful. To my mind the chief difficulty in such a case is not that developmental conditions can so profoundly modify the usual course of things, but that by such different courses precisely the same result should be reached. In *Perophora*, for example, no one has detected any difference between the adult embryozooid and the adult blastozooid. The same is true of *Clavelina*, and this case is more important, perhaps, because the embryology of this genus has been very thoroughly studied up to the practically adult state.

It appears certain that heredity has here an *ultimate aim*, as we may call it, and that it is able to reach this even though it be thrown considerably off its regular course by special functional requirements ; in other words, that heredity is prospective.

In this connection one may refer to the fact that in some compound Ascidians, *e.g. Botryllus*, there is no such thing as an adult embryozooid, and the suggestion may be made that the much abbreviated life of the embryozooid in such instances is in some way correlated with the profound modification undergone in the development of the blastozooid as compared with the embryozooid.

I have already pointed out on another occasion ('95a) that at least one other instance of anomalous bud origin may receive

a physiological explanation somewhat similar to that above supported as the cause of the course of things in the Ascidian bud. I refer to the ectodermal origin of the bud in *Rathkea octopunctata*, as recently described by Chun ('95). The buds of this medusa develop on the manubrium, and on that portion of it in which the endoderm cells, as is clearly shown by the author's description and figures, are highly modified for the digestive function. On the other hand, the ectoderm cells are as clearly much less highly modified. When we look for a reason why the ectoderm retains to so considerable a degree its undifferentiated character, we find a sufficient one in the fact that it is largely relieved of the protective function that usually belongs to this layer in adult animals by its being itself well protected by the deep, close sub-umbrella of this medusa. And in the two facts that the buds arise in a region where the endoderm is highly modified for the digestive function, and that the ectoderm remains comparatively unmodified, appear, as I believe, an adequate reason why the ectoderm alone contributes to the formation of the buds.

Braem ('95), in puzzling over this case, suggests that the most probable explanation of the anomalous condition is to be found in the fact that the buds arise from the same layer, and not remote from the position where the sexual cells are produced. But Chun states, page 32, that "Mann trifft keine Sporen oder parthenogenetischen Eizellen an, welche durch Grösse und abweichendes Verhalten des Inhaltes sich von den übrigen Ektodermzellen abheben."

Of course, if my explanation of this case is correct, we may expect to find other instances where similar conditions and causes will have produced like results; as, for example, in the budding medusa of *Bougainvillia Niobe* recently described by Mayer ('94), which is likewise a deep-belled form, and in which the buds "spring from the gastric region manubrium." But I do not think that if in a particular instance, where the conditions are right to produce the results, they still do not appear, we should on that account be justified in concluding their inefficiency to produce them. It is highly probable that, in groups of animals which reproduce by budding, the faculty is

acquired independently by different species and at different times.

Now it is certain, both on theoretical and on observational grounds, that there would always be a tendency, and a strong one, for all the germ layers to participate in the production of the bud ; and should they be found to do so in particular cases where the conditions are such as to cause, according to my idea, one or the other of the layers to be excluded from the process, this might be due to the fact that such exclusion had not yet been fully effected because of the comparatively recent acquisition of the property of budding.

## 2. On the Question of Different Types of Budding among Tunicates.

Having now before us the facts concerning the budding in these two genera, we must compare them in order to see whether the differences must be regarded as fundamental, or whether the mode of development in the two cases is more probably a modification of a common type.

The first point to be considered is the relation of the bud to its parent. Various authors have expressed more or less positively the view that the two modes of bud origin represented by *Perophora* and *Goodsiria*, *i.e.* the stolonic and the pallial, have been derived from a common primitive type. Garstang ('95) is the most recent of these, and he has adopted this interpretation apparently with considerable confidence.

It is interesting to notice that the relation between the blastozooids of a colony in these two genera may be viewed in such a way as to give the appearance of a rather close resemblance.

If we disregard the embryozooid from which the colony has sprung in each case, and fix our attention upon the adult blastozooids only, we may reduce the entire colony to a series of individuals connected with one another by their peribranchial sacs through the stolonic septum, or cloison.

This is evident enough in *Perophora*, where it is essentially the actual state of things ; the only modification of it being

that in some instances at least, as already shown, the connec-
tion between the zooid and the stolonic septum becomes
severed.

To see how the same relations would be produced in *Good-
siria*, it is only necessary to imagine the bud to retain the
connection with its parent, and for the connecting neck, shown
almost severed in Fig. 15, Pl. XII, to become more elongated ;
in other words, to form a stolon.

Text figures 1 and 2 illustrate the scheme that is here
described, 1 representing *Goodsiria* and 2 *Perophora* ; *d* indi-
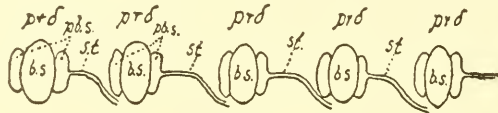


FIG. 1.
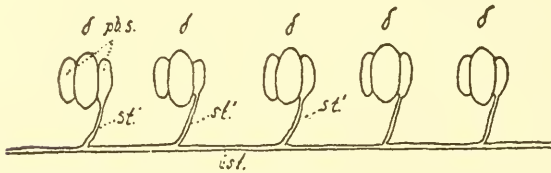


FIG. 2.

cates daughter and *p* parent ; *b.s.* branchial sac, *pb.s.* peribran-
chial sac, and *st.* stolon. The stolons in *Goodsiria* are not
connected to the daughter zooids, since we do not know
whether the connection would be to the branchial or peribran-
chial sacs.

But when we come to examine the subject more closely we
find that the likeness of the two conditions is much less strong
than it at first appears. In the first place we see that the
blastozooids of the *Goodsiria* colony would stand in the relation
to one another of mother, daughter, granddaughter, etc., while
in *Perophora* this is not so. Here the blastozooids are rather
all to be looked upon as sisters ; that is, they are all produced
by the stolon, and we have no facts to indicate that the stolon
ever arises from any zooid excepting the original embryozooid
of the colony. Another distinction that results directly from

the one just pointed out is this : in *Goodsiria* it would always be the mother ascidiozooid that would be attached to the stolonic septum by its peribranchial sac, while in *Perophora* it is the daughter zooid that is so attached.   Or, to express the same thing in another way, we do not know how any given blastozooid of *Goodsiria* would be attached to the supposed stolon from which it would arise.   We only know how it would be attached to the stolon that would arise from *it*.

In reality, then, there is a rather deep-seated difference between the processes in the two genera.   I am, however, far from affirming that the difference is fundamental.   It is not at all impossible that they may be modifications, though rather profound ones, of a common original process.   How is the question to be decided ?   The answer I wish to give with emphasis.   If it is ever to be correctly answered, it must be by considering the evidence afforded by the *blastogenesis in connection with the evidence to be derived from embryogenesis and from the comparative anatomy of the adults.*

It was my original plan to make the discussion of the question as full as the data at hand would permit ; and with this in view I have already prepared the manuscript for the anatomical comparison, and in fact have presented it in brief on another occasion.   Further reflection has, however, convinced me that it will be better not to attempt a full description until the embryological data for both genera, now almost wholly wanting, have been furnished.   We must know how the peribranchial sacs arise in the embryo in each case, and also how the first bud arises in each case, before we have a basis on which to profitably speculate.

It will be worth while to point out briefly how unsatisfactory such speculation would of necessity be in view of our imperfect knowledge of the ontogeny in these genera.

We have no observations on the origin of the peribranchial sacs in the embryo of either *Perophora* or *Goodsiria*.   Neither do we know how the first bud arises in either case.   We might infer that in these particulars *Perophora* is like *Clavelina ;* in fact, such an assumption is generally made.   But as I have shown the relation of the blastozooids to the stolon to be very

different in the two cases, it seems rather probable, at least not at all improbable, that there may be important differences between them in the two points mentioned, *viz.*, in the origin of the peribranchial sacs and the first buds.

As regards *Goodsiria* we may assume that the embryonic development is essentially like that in *Botryllus*. For my own part I fully expect that this will prove to be the case. However, we are by no means certain of it, and even if we were we should be far from clear sailing as regards the origin of the peribranchial sacs of the embryo, since Pizon insists that they arise from the endoderm of *Botryllus,* and Garstang is inclined to accept his conclusion. If the peribranchial sacs of *Perophora* arise from the ectoderm as they do in *Clavelina*, and *if* the stolonic septum of the first bud of *Perophora* arises from the pharynx as it does in *Clavelina*, and *if* the first bud of *Goodsiria* arises like the first bud of *Botryllus,* and *if* the peribranchial sacs of *Botryllus* arise from the ectoderm, then weighing all the evidence together, anatomical as well as developmental, it seems to me that the budding in the one genus is genetically independent of the budding in the other.

This view I have practically expressed before ('95a), in contending that the Compound Ascidians have had a double origin from the Simple ones.

But, as already said, I regard it as necessary that the above formidable array of ifs should be gotten out of the way before the discussion can profitably be carried farther.

And now concerning the "epicardiac tubes" that are of so much importance in connection with Tunicate budding. I have elsewhere ('95) said, regarding the origin of the pericardial vesicle in *Goodsiria*, that an epicardium is here present "as in *Botryllus*." As will be readily seen by comparing Figs. 18 and 19, Pl. XIII, with, for example, the woodcut given by Pizon ('93), page 30, of *Botryllus,* there can be no doubt that the posterior extremities of the peribranchial sacs in my figures of *Goodsiria* are the same as those marked *p.v.* by Pizon. The only difference in the two cases is in the size. The two pouches are much longer in *Botryllus* than in *Goodsiria*. Both Pizon ('93) and Garstang ('94) regard the structures in *Botryllus*

as homologous with the epicardium of *Clavelina*, *Distaplia*, *Fragroides*, and other species.

Thus the last-named author says: "In spite, therefore, of the final difference of position between the epicardial (or perivisceral) sacs of the Botryllidae and the epicardial tubes of *Distaplia* or *Clavelina*, there can be no doubt, as Pizon has maintained, that there is an exact homology between the two structures." Nevertheless, there is, in my mind, a very grave doubt that the structures in *Goodsiria*, which are certainly the same as those called epicardial sacs in *Botryllus*, have anything whatever, either morphologically or physiologically, to do with the epicardial tubes of *Clavelina*.

It is well known from the investigations of Seeliger and Van Beneden et Julin that the epicardial tubes of *Clavelina* arise from the branchial sac, and they arise in the same way in *Glossophorum* (Hjort, '95) and *Fragroides* (Maurice, '88). As Pizon and Garstang contend, the mere fact that the structures arise from the branchial sac of some species, and from the peribranchial sacs in others, would not in itself present any difficulty against regarding them as *genetically homologous* (and I take this to be the kind of homology that these authors mean, for I do not suppose they recognize any other kind). But this view would have to regard it as proven that the branchial and peribranchial sacs both arise from the endoderm in the embryozooid ; and this can certainly not be granted as our knowledge now stands.

As previously said, Pizon claims that such is their origin in the *Botryllus* embryo ; and Garstang is inclined to support the same view. Should it finally be established that this is in reality the case, then, so far as this much of the evidence is concerned, the structures may be homologous in *Botryllus* and *Clavelina*, and of course in *Goodsiria*, also, if its embryonic development here is similar to what it is held by Pizon to be for *Botryllus ;* but this is still to be shown.

But now it being conceded that, so far as concerns the evidence yet in hand relating to the origin of these structures, they may *possibly* be homologous, we must still consider what the evidence of their destiny is.

In *Clavelina*, *Distaplia*, *Glossophorum*, and *Fragroides* the epicardium gives origin either directly or through the stolonic septum to the inner vesicle, or endoderm, of the bud. In *Botryllus* and *Goodsiria* the so-called epicardial sacs have nothing whatever to do with the budding, and there is not the slightest evidence that they ever had anything to do with the process. The last part of this statement is at least true for *Goodsiria*. Here the buds arise, as reference to Figs. 9 and 10, Pl. XII, will show, far forward on the parent zooids, while the epicardial pouches are at the extreme posterior end of the animal. All they are is this: When the intestine begins to form at the postero-ventral side of the primitive inner vesicle it produces, as one might say, an obstacle in the way of the further expansion posteriorward of the inner vesicle, which, of course, is constantly growing. The notch thus produced has been already described, and is well seen in Fig. 16, *d.*, Pl. XIII.

The extensions of the vesicle on each side of this notch are the epicardial sacs of the adult animal. When the peribranchial sacs are completely formed the epicardial sacs are merely their posterior extremities.

And now a word concerning the relation of the epicardium to the heart. In attempting to show that the epicardium is the same structure in all Tunicates, and to make use of it as a basis for classifying the group, Garstang has tried to escape the difficulty of its being in some cases connected with the heart, while in other cases it is not, by supposing that such connection is secondary.

In my account of the origin of the heart in *Goodsiria* I have shown that it arises from the right epicardial pouch, and I can certainly see no reason for regarding this method of origin as secondary. In fact, it was partly this consideration that made me speak of the pouch as an epicardium in my preliminary communication.

Concerning the epicardium in *Perophora*, after describing the relation of the bud to the stolon, I have said in my preliminary paper: " It is obvious that there cannot be in *Perophora* an epicardium corresponding to the structure called by that

name in *Clavelina*, since in this species the epicardium is connected with the *branchial* sac."

Lefevre ('95) says: "No epicardium is present; in this respect *Perophora* differs strikingly from *Clavelina* and some other Ascidians." So far as the *blastozooids* are concerned these unqualified statements are, I believe, fully justified. It must, however, be remembered that we do not know how the stolon originates from the embryozooid, and until we are informed on this point I must place a certain reservation on my assertion of the entire absence of the epicardium in *Perophora*.

If we accept Garstang's view that the relation of the heart to the epicardium is secondary, then the fact that the heart arises on one side of the body, while the stolonic septum is attached to the peribranchial sac of the other side in *Perophora*, would be of little weight against supposing an epicardium to be present in this species. But I have already shown that this author's conjecture is contradicted by the evidence of *Goodsiria*, if he would still maintain that the pouches described in this species are homologous with the epicardium of *Clavelina*.

In the present state of our knowledge on this point, then, I am a long way from conceding, as Garstang thinks we must, "that these modifications of the epicardial tubes provide a sound basis for a true and genetic classification of Tunicate budding." That, *when considered in connection with various other developmental and anatomical facts*, it is of prime importance in interpreting the budding, cannot be questioned. But the attempt to make it, in itself, a basis for classifying the Compound Ascidians can hardly be more satisfactory than one-legged classifications ever are in zoölogy.

### *D.* SUMMARY OF RESULTS.

#### GOODSIRIA DURA.

1. The budding is pallial, and the buds arise far forward on the parent zooid. In no case has more than one bud been seen on the same parent.

2. No "budding zone" is recognizable in zooids on which buds are not developing.

3. The buds become wholly separated from the parent zooids at a very early stage, *i.e.* before any differentiation of organs begins; and at a considerably later time they become secondarily connected with the vessels of the test.

4. The ampullae of the testicular vessels do not produce buds.

5. The formation of the branchial and peribranchial sacs, and of the digestive tract from the primitive inner, or endodermic, vesicle of the bud does not differ in any essential particular from that of all other Compound Ascidians.

6. The common *Anlage* of the pericardium and heart is derived from the wall of the endodermic vesicle by an imperfect evagination that does not become fully separated until the ventral partitioning folds which separate the peribranchial sacs from the branchial sac have reached back to the region where the heart is forming.

7. The ganglio-hypophyseal *Anlage* arises as a gutter-shaped evagination from the dorsal side of the endodermic vesicle. As this closes off to become the hypophyseal duct, the ganglion is produced from the mass of cells that forms the last of the connection of the duct to the endodermic vesicle. The ganglion, therefore, in this species as in *Botryllus*, lies *ventral to the hypophyseal duct*.

8. The youngest sexual cells observed were found free in the body space of the buds, so that in all probability they pass from parent to bud as is the case in *Botryllus*. But in none of the material available for study were the elements present in sufficient quantity and development to make it possible to give a complete history of them. The "polycarps" appear to

be confined to a single row on each side of the endostyle, and not far from it.

<center>PEROPHORA ANNECTENS.</center>

1. The inner or endodermic vesicle of the bud is derived from the stolonic septum ; and from this are derived the branchial and peribranchial sacs, and the digestive tract in a manner in all essentials similar to that of all other Ascidian buds.

2. The peribranchial sacs of the developing blastozooid are formed in such a manner that the stolonic septum is connected to the left one of these, and not to the branchial sac, as has been hitherto supposed.

3. The pericardial *Anlage* arises almost certainly from the wall of the inner vesicle, though there remains some doubt whether or not it may be produced by an aggregation of mesenchyme cells. But if this is so it is still probable that its ultimate source is the endoderm, since the mesenchyme cells that seem to enter into it are themselves probably produced by the endoderm.

4. The ganglio-hypophyseal *Anlage* was originally produced from the endodermic vesicle as an evagination, as it is in the buds of numerous other Ascidians. This primitive method of origin is, however, found in an occasional individual only at the present time, the evagination having, in most cases, been replaced by a migration of cells.

For the most part this migration takes place at the point at which the ganglion will be ultimately situated; but it may occur at other points more or less remote from this, and the coming together of these migrating cells makes it appear as though the *Anlage* is produced from mesenchyme cells. Or, as in the case of the pericardial *Anlage*, we may consider that mesenchyme cells do participate in the formation of the *Anlage*, but that these cells are derived from the endodermic vesicle.

## In General.

1. It is now established beyond question that in some, at least (and *Goodsiria* may be taken as one of the best instances of this), of the Compound Ascidians the outer layer of the bud contributes much less to the structure of the adult blastozooids than it does to the adult embryozooids. This is most conspicuously seen in the case of the nervous system, for this is certainly produced from the outer, or ectodermic layer, of the embryo, while it is as certainly produced from the inner layer of the bud. Whether we call this inner layer endoderm or not, the fact of chief importance remains that the same layer produces most of the organs of the zooid, among which are included the digestive tract and the nerve ganglion.

2. The anomalous course of development of the bud is due to the fact that the ectoderm is at no time in the life of the bud an undifferentiated, or embryonic layer. It is from the very outset and always a fully formed organ, its function being to secrete the cellulose matrix of the test. The inner, or endodermic, vesicle of the bud is, on the other hand, in the completest sense, an undifferentiated, or embryonic layer. By this purely physiological cause the inner layer has been made to produce structures, most important of all the nervous system, which in the embryo are produced by the ectoderm.

3. Illustrations of the potency of physiological influences to profoundly change the usual course of development are found in the budding of other animals, one of the most instructive of which is the medusa *Rathkea octopunctata*, where the change has been in the opposite direction from that in the Ascidians; for here the endoderm takes no part in the formation of the bud, the whole blasto-medusa being produced from the maternal ectoderm.

4. The evidence now in hand, drawn from adult structure and from the blastogenesis strongly tends to the conclusion that the budding of *Goodsiria* and *Botryllus*, represents a type that is genetically independent of the type represented by *Perophora*. In other words, that the type of budding represented by *Goodsiria*, has originated independently of the

type represented by *Perophora*. But more knowledge of the embryology of each genus is requisite before this or any other conclusion respecting their relationships will be fully warranted.

5. In neither *Goodsiria* nor *Perophora* is there an epicardium comparable with the structure called by that name in *Clavelina* and many other Compound Ascidians.

6. The budding of *Goodsiria* greatly strengthens the conclusion justified by adult structure that the Polystyelidae and Botryllidae are very closely related. The two families will probably have to be ultimately united into one, but it is not best to do this until we know the embryology of both groups much more fully than we yet do.

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    Nov. 11, 1895.

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## DESCRIPTIVE LETTERS.

| | | | |
|---|---|---|---|
| *a.* | Anterior. | *int.* | Intestine. |
| *amp.* | Ampullae of testicular blood vessels. | *in.ves.* | Primitive inner vesicle. |
| | | *i.p.* | Internal papilla. |
| *at.sip.* | Atrial siphon. | *l.coe.* | Lacteal coecum. |
| *bd.* | Bud. | *l.d.* | Lacteal duct. |
| *bd.a.* | Bud *Anlage.* | *l.pb.s.* | Left peribranchial sac. |
| *b.c.* | Blood corpuscles. | *l.s.* | Lacteal system. |
| *br.s.* | Branchial sac. | *m.en'c.* | Male polycarp. |
| *br.sip.* | Branchial siphon. | *mes.gas.* | Gastric mesentery. |
| *br.sta.* | Branchial stigmata *Anlage.* | *mes.rec.* | Rectal mesentery. |
| *br.s.ep.* | Branchial epithelium. | *oe.* | Oesophagus. |
| *br.st'g.* | Branchial stigmata. | *ov.* | Ova. |
| *b.s.* | Body space. | *p.* | Posterior. |
| *b.v.* | Blood vessel. | *pb.s.a.* | *Anlage* of peribranchial sac. |
| *cl.* | Cloaca. | *pc.* | Pericardium. |
| *cl'n.* | Cloison, or stolonic septum. | *pc.a.* | Pericardial *Anlage.* |
| *cl.ep.* | Cloacal epithelium. | *p.f.* | Partitioning fold. |
| *d.* | Dorsal. | *p'l'c.* | Polycarp. |
| *d.f.* | Dorsal partitioning fold. | *r.c.* | Remnant of connection between bud and parent zooid. |
| *d.l.* | Dorsal lamina. | | |
| *ec.* | Ectoderm. | | |
| *ec.ves.'* | Ectodermic, or testicular blood vessels. | *rec.* | Rectum. |
| | | *r.e.o.* | Remnant of evagination. |
| *ec.ves.'* | Ectodermic vessels projecting into body space. | *r.pb.s.* | Right peribranchial sac. |
| | | *r.v.f.* | Right ventral partitioning fold. |
| *end.* | Endostyle. | | |
| *en'c.* | Endocarp. | *s.ec.* | Siphonal ectoderm. |
| *ep.pb.s.* | Epithelium of peribranchial sac. | *s.f.* | Siphonal folds. |
| | | *st.* | Stomach. |
| *f.en'c.* | Female polycarps. | *sto.* | Stolon. |
| *gl.* | Ganglion. | *t.* | Testa. |
| *gl.ev.* | Ganglionic evagination. | *t.c.* | Testa cells. |
| *ht.* | Heart. | *tr.ves.* | Transverse vessel. |
| *hy.a.* | Hypophysis *Anlage.* | *ts.* | Testis. |
| *hy.d.* | Hypophysis duct. | *v.* | Ventral. |
| *i.l.b.* 1, | | *v.f.* | Ventral partitioning fold. |
| *i.l.b.* 2, | | | |
| *i.l.b.* 3, | Internal longitudinal bars. | | |
| *i.l.b.* 4, | | | |
| *i.l.b.* 5, | | | |

## EXPLANATION OF PLATE **XII.**

### Goodsiria Dura.

*The figures were drawn with the aid of a camera lucida and a Leitz microscope,
excepting when otherwise specified.*

FIG. 1.  A colony, natural size, completely covering the surface of a piece of
seaweed.  No buds are present in this colony.  Not camera, but as faithful a
reproduction as possible.

FIG. 2.  A colony, also on a piece of seaweed.  The Ascidiozooids much less
closely crowded than in the preceding.  Buds in various stages of development
are seen, and some of the very young ones are not as near the margin of the
colony as are some of the older ones.  The ampullae of the testicular vessels are
seen, particularly near the margin of the colony.  Not camera, but as exact as
possible.   × 4.

FIG. 3.  Small fragment of a thin colony removed from its substratum and
examined as a transparent object in clove oil.   × 87.

FIG. 4.  Digestive tract with a portion of the branchial sac attached.  The
longitudinal folds of the stomach and the single spiral groove of the intestine are
seen.   × 87.

FIG. 5.  Camera sketch of a section of a colony showing sections of three
blastozooids in various stages of development.  The top of the figure corresponds
to the top or dorsal surface of the colony ; it is consequently seen that the blood
vessels of the test, *ec.ves.*, are at a deeper level than are the Ascidiozooids.
× 87.

FIG. 6.  Transverse section of an almost fully formed blastozooid, the section
being well toward the posterior end of the body.   × 120.

FIG. 7.  An optical section of two ampullae of the testicular vessels which
contain a closely packed mass, *b.c.*, of blood corpuscles.  This, together with the
thick ectodermal wall of these vesicles, give them somewhat the appearance of
young buds.  Drawn from a specimen cleared in clove oil.   × 87.

FIG. 8.  A single male "polycarp" attached to the mantle.  The short vas
deferens is here seen.   × 120.

FIG. 9.  Optical section of an ascidiozooid with a bud, *bd.*, still attached to it.
This is the zooid from which the section shown in Figs. 12 and 13 were cut.  The
direction of the section is indicated by the line *A.A.′*  The specimen was cleared
in clove oil.  Not camera, but as faithful a representation of the object as
possible.

FIG. 10.  Optical section of a zooid with a young bud, *bd.*  Cleared in clove
oil.   × 87.

FIG. 11.  Section of the earliest stage seen in the formation of a bud.  As
seen, the ectoderm is not yet modified at the point where the bud is forming, the
bud *Anlage*, *bd.a.*, being confined to the parietal wall of the peribranchial sac ;
*br.st.a.* are points where stigmata of the present zooid will form.   × 210.

Pl. XII
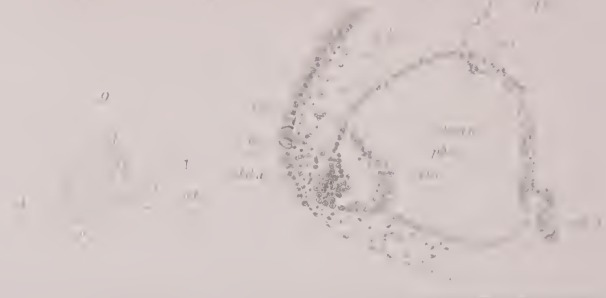
## EXPLANATION OF PLATE XIII.

FIG. 12.   Section of the budding zooid shown in Fig. 9, the section corresponding to the line *A.A.'*   × 87.

FIG. 13.   From the same series as 12, but far enough posteriorward so that the inner layer of the bud is not continuous with the maternal endoderm.   At *amp.* are seen two ampullae of the testicular vessels which are in close contact with the young bud, and the vessel, *ec.ves.*, is still more closely pressed by the bud.   × 210.

FIG. 14.   Transverse section of a bud not yet separated from its parent. × 210.

FIG. 15.   Section of a zooid with a bud, *bd.*, almost severed, there being only a remnant, *r.c.*, of the connection between bud and parent.   The bud is here entirely undifferentiated as to the organs of the future zooid.

FIG. 16.   Optical section of a young bud ; the intestine, *int.*, is established, and the folds that will ultimately separate the peribranchial sacs from the branchial sac, are barely indicated at *p.f.*   The ganglio-hypophyseal evagination, *hy.cl.*, is represented as projected on the plane of the section.   In reality it would not be seen at the focus at which the rest of the bud is shown.   The beginnings of the two so-called epicardial pouches will be observed at *X.*   Seen from the dorsal side.   × 87.

FIG. 17.   A bud somewhat more advanced in development than the preceding. The specimen was cleared in clove oil.   It is seen from the dorsal side, but is slightly rotated to the right.   The right partitioning fold, *p.f.*, is well seen.   The distribution of the blood cells in the body space is shown.   × 87.

FIG. 18.   A bud considerably more advanced than the last, the peribranchial sacs nearly complete.   Seen from the dorsal side.   Viewed as a transparent object, but not shown in optical section.   × 87.

FIG. 19.   A still older bud, the differentiation of the organs almost complete. Method of treatment and view similar to the last three.   × 87.

FIGS. 20 and 21.   Sections of a bud in which the peribranchial sacs, *br.s.a.*, are barely begun.   The sections are not quite at right angles to the antero-posterior axis of the bud, and as a consequence the sacs do not both appear on the same section.   × 210.

FIGS. 22–24 (and 25, Pl. XIV).   Four transverse sections of a bud about corresponding in its stage of development to the bud shown in Fig. 17.   Fig. 22, the most anterior of the series, passes through the position at which the branchial siphon, *br.sip.*, is soon to form.   By Fig. 23 it is seen that the folds which are to separate the branchial from the peribranchial sacs extend along the ventral as well as along the dorsal side of the primitive inner vesicle, *v.f.* and *d.f.*   In the last of the four sections, Fig. 25, no trace of the folds appears.   This section passes through the beginning of the atrial siphon, *at.sip.*   × 210.

Pl. XIII
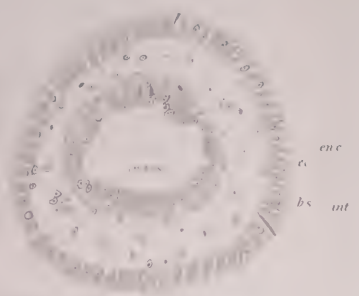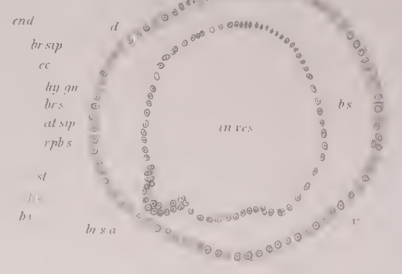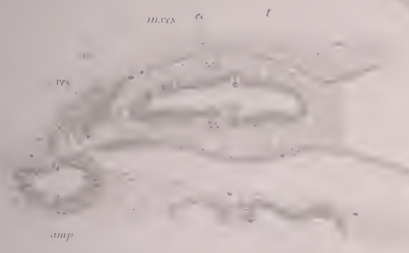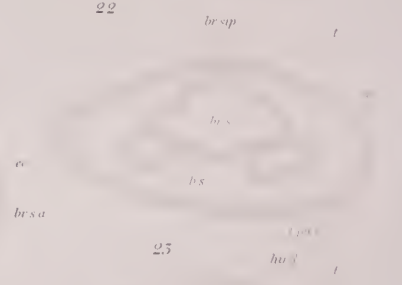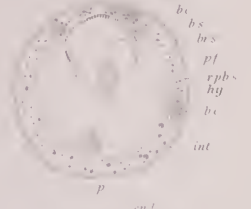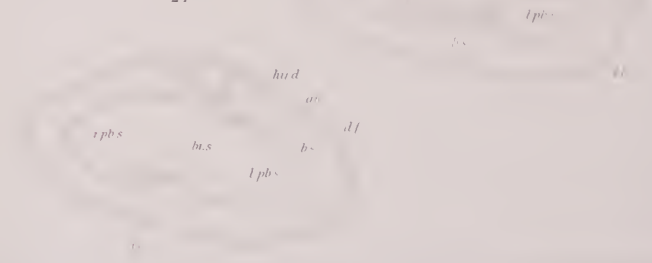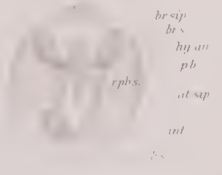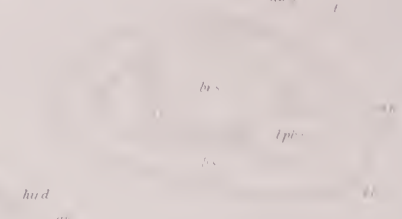
## EXPLANATION OF PLATE XIV.

Figs. 26–29. A series parallel to the last, but from a more advanced bud. The stage of development is about midway between the buds shown in Figs. 17 and 18. × 210.

Figs. 30 and 31. From the posterior end of a bud nearly as far advanced as the one shown in Fig. 19. The sections show that the branchial sac, *br.s.*, is wholly closed off from the peribranchial sacs, *r.pb.s.* and *l.pb.s.*, and Fig. 31 shows that the peribranchial sacs unite behind the branchial sac to form the wide atrium, *at.* × 87.

Fig. 32. Shows the relation of the digestive tract to the peribranchial sac before it has become pushed into the latter. × 120.

Fig. 33. Shows the digestive tract pushed fully into the peribranchial sac. × 210.

Fig. 34. Section of the digestive tract in the practically adult condition. The lacteal coecum, *l.coe.* (this lettering should point to the smaller of the two circular structures; the lithographer has moved the letters), the lacteal system, *l.s.*, and spiral fold of the intestine, pointed to by the index line, *int.*, are seen. × 210.

Fig. 35 (and 36, Pl. XV). Two sections showing stages in the development of a siphon. Fig. 35 shows that the siphon begins by an evagination of the endodermic vesicle. × 508.

Pl XIV
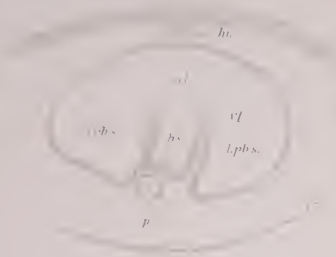
EXPLANATION OF PLATE XV.

Fig. 36.   A much more advanced stage ; shows the peculiar folding, *s.f.* and *s.f'.*, that is produced before the siphon is complete.   × 210.

Fig. 37.   Shows two of the testicular vessels, *ec.ves'.*, projecting into the body space, *b.s.*, of the developing bud.   × 210.

Fig. 38.   Shows two vessels, *ec.ves.*, opening into the body space.   × 210.

Figs. 39–42.   Relate to the origin of the pericardial vesicle.   Fig. 39 shows a transverse section passing through the posterior portion of the animal, and at *r.pb.s'.* is seen the pouch of the right peribranchial sac (the epicardial pouch) from which the pericardial vesicle is produced.   Fig. 39, × 210 ; the others, × 508.

Fig. 43.   Cross-section of a bud in about the same stage of development as the one shown in Fig. 16.   The section of the gutter-like evagination, *hy.d.*, of the ganglio-hypophyseal duct, is here very distinct.   × 210.

Figs. 44–47.   Four sections of a series passing from before backward of the ganglio-hypophyseal duct.   They are almost, but not quite, at right angles to the long axis of the duct.   Fig. 44 shows the mouth of the duct, and Fig. 47 shows that at its posterior end, or near the end, the duct still communicates with the branchial sac, *r.e.o.*

Both the ectoderm and the cells in the body space of these sections are reproduced with special care.   Figs. 44, 45, and 47, × 508 ; Fig. 46, × 720.

Fig. 50.   Cross-section of the duct and ganglion nearly fully formed.   × 508.

Fig. 51.   Cross-section of the endostyle showing a young female polycarp. × 210.

Figs. 53 and 54 (Pl. XVI).   Two early stages in the formation of male polycarps.   × 508.

Pl XV.
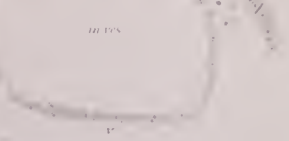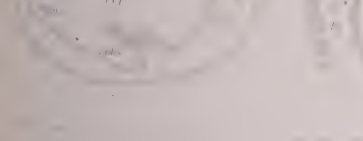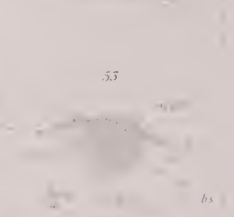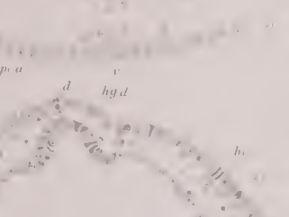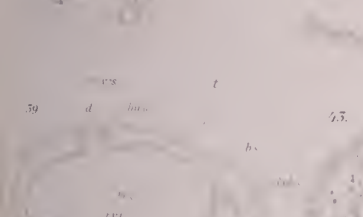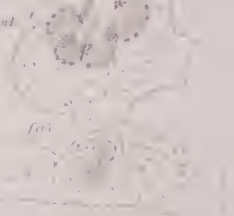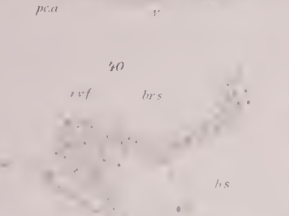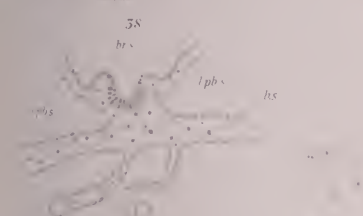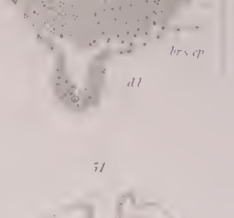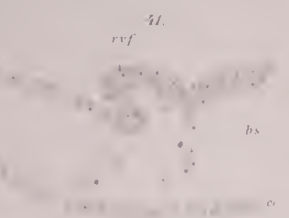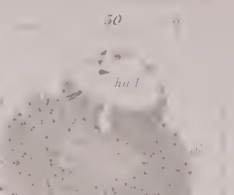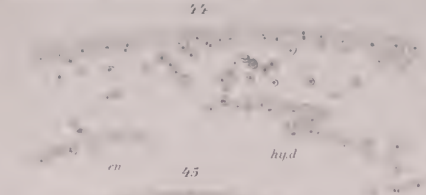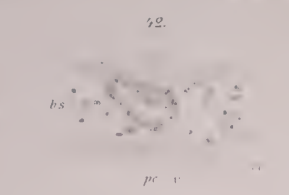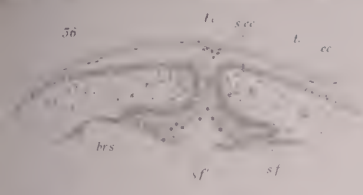
## EXPLANATION OF PLATE XVI.

FIG. 48. A longitudinal section of the ganglio-hypophyseal duct at a slightly more advanced stage than the one shown in Figs. 44–47. It will be noted that here the communication of the duct with the branchial sac, *r.e.o.*, the remnant of the evagination, is almost closed. The differentiation of the ganglion, *gl.*, from the duct and from the wall of the branchial sac is complete at its anterior end, but not at its posterior end. × 720.

FIG. 49. Cross-section of a duct and ganglion after the ganglion is fully separated, but is still very small. × 720.

FIG. 52. An ovum found alone and free in the body space. × 1200.

FIG. 55. A multinucleated mass floating free in the body space ; probably(?) an early stage in the formation of a male polycarp. × 508.

### PEROPHORA.

FIG. 56. Small portion of a colony of *P. annectens* almost entirely covering a zooid of *Clavelina*. The specimen is the fully compounded variety, and forms a thin encrusting layer on the surface of the *Clavelina*. The tips of the stolons, *sto.*, and young buds, *bd.*, are shown at the margin of the colony. Not camera. × 2.

FIGS. 57–64. From a series of transverse sections extending from before backward of *P. annectens*, illustrating the method by which the bud is connected to the stolonic partition by its left peribranchial sac. The point of connection is seen in Fig. 60. × 138.

FIG. 65. Transverse section of a *P. Listeri* bud, slightly more advanced in development than the preceding ; illustrating, likewise, the connection of the left peribranchial sac to the stolonic partition. In this section, however, the series passes from behind forward, so that the apparent reversal of right and left does not occur. × 138.

FIG. 66. Shows a case in which the zooid is fully separated from the stolon. This is the section of a complete series in which the stolon approaches most closely to the bud, but here the separation is complete. *P. annectens.* × 210.

FIGS. 67 and 68. Show the development of the pericardial vesicle. Fig. 67 shows its position with reference to the point of attachment of the inner vesicle to the stolonic septum. Both are from the same series of sections. *P. annectens.* Fig. 67, × 72 ; Fig. 68, × 330.

FIG. 69. A later stage in the formation of the pericardium, but still before its separation from the endoderm. × 720.

FIG. 70. From the same series as the last to show the relation of the forming pericardium to the point of attachment of the stolonic septum to the primitive inner vesicle. × 120.

FIG. 71. A condition of the pericardium similar to that shown in Fig. 69, but slightly more advanced in development. × 500.

Pl. XVI.
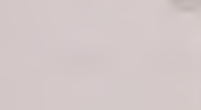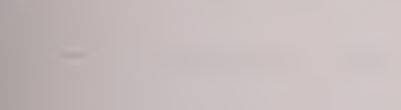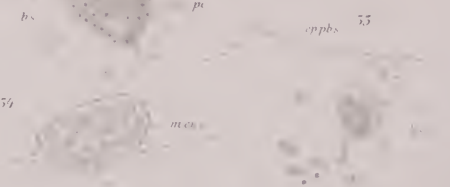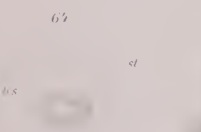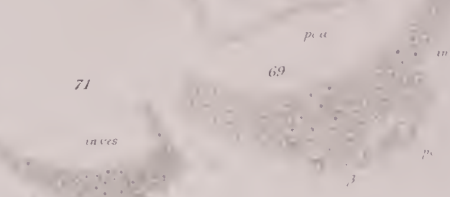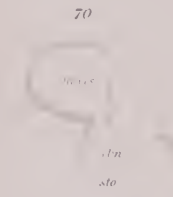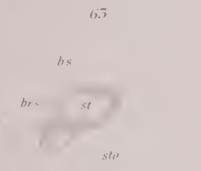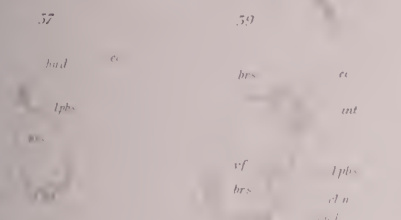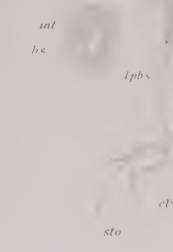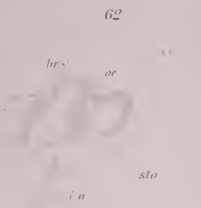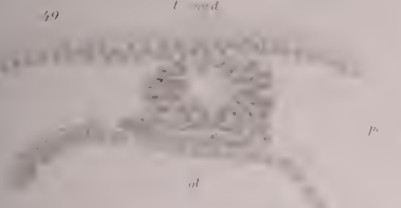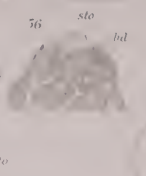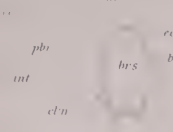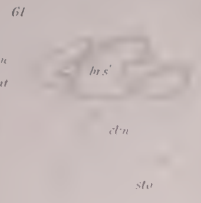
EXPLANATION OF PLATE XVII.

FIG. 72.  An early stage in the formation of the ganglio-hypophyseal *Anlage* before its separation from the endoderm.  Camera drawing as far as possible with Leitz. oc. 5, obj. 7.

FIG. 73.  Shows a stage in the formation of the ganglion somewhat more advanced than the preceding, the whole section drawn in order to show the distribution of the blood or mesenchyme cells in the body space.  × 210.

FIG. 74.  From the same series as Fig. 73, more highly magnified, showing the supposed evagination to form the ganglio-hypophyseal *Anlage*.  × 508.

FIG. 75.  Longitudinal section of the ganglion and duct, the former almost separated from the latter.  × 250.

FIG. 76.  Transverse section of the duct, with its dorsal wall considerably thickened for the formation of the ganglion.  × 508.

FIGS. 77 and 78.  Two sections in which cells appear to be breaking away from the endoderm, and passing into the body space to become mesenchyme cells. Both of these cases are far from the position where either heart or ganglion will form.  × 720.

72.

t        ec        hy.a

b.s.

in.ves

76.

gl

hy.d.

in.ves

73.

hy.a.

in.ves

pb s.a.        bs.        t

sto

cbu

77.        b.c.

b.c

74.        hy.a.

ec

in.ves        gl.ev

n.ves

75        d.sip

br.sip        gl        ec

b.s

hy.d        br.s

78.        m.ves

b.c

b.v
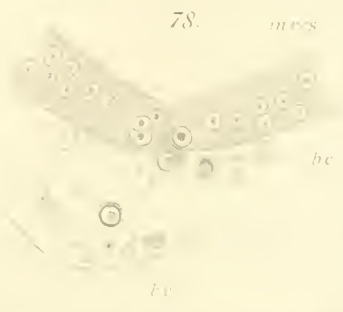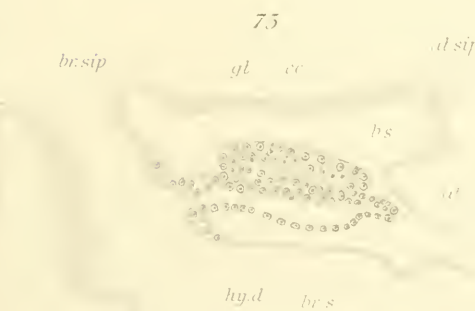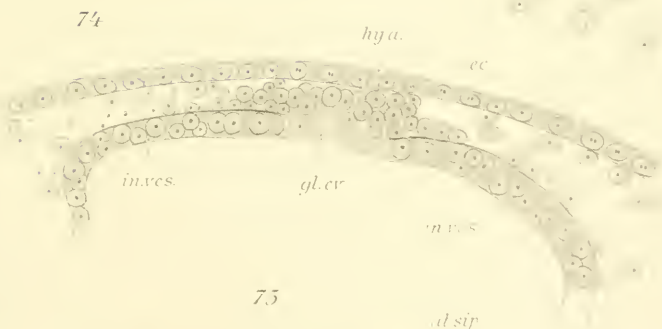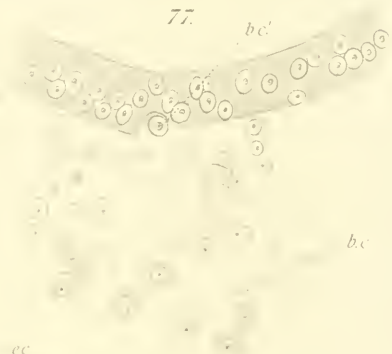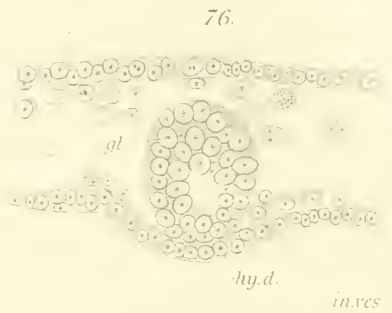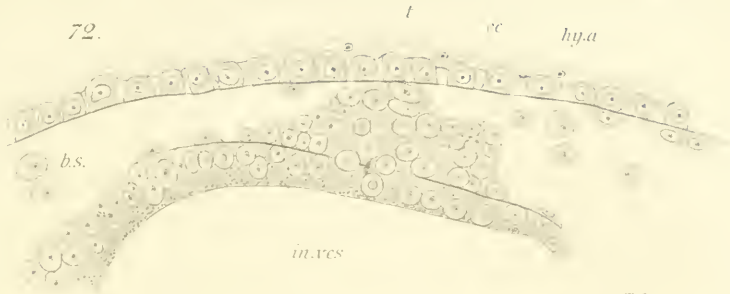
# ON THE SMALLEST PARTS OF STENTOR CAPABLE OF REGENERATION; A CONTRIBUTION ON THE LIMITS OF DIVISIBILITY OF LIVING MATTER.

FRANK R. LILLIE.

IN experiments on the power of multiple development of the ovum the result has been reached, that a portion of less volume than one-fourth that of the normal ovum does not possess the capacity of producing an *embryo* or *larva*, though it may a gastrula (see postscript); while a portion equal to one-fourth or more of the volume of the normal ovum may, under suitable conditions, produce a gastrula and finally a larva of corresponding relative bulk. This result has been attained by Loeb,[1] Wilson,[2] Driesch,[3] Morgan,[4] and Zoja.[5] Wilson found only a single larva of Amphioxus of one-fourth the normal size and that showed several defects ; and Driesch has not, so far as I am aware, mentioned any pluteus of less than one-quarter size. Morgan has recently published the results of his studies on the power of multiple development of the echinoderm ovum. In this he shows that "the volume of the smallest gastrula which can be produced from fragments of the egg falls below $\frac{1}{64}$ the volume of normal gastrulae. The volume of the fragments of the egg which produced such gastrulae, varies between $\frac{1}{40}$ and $\frac{1}{50}$ of the volume of the ovum." (Summary p. 124 *loc. cit.*) But these smallest gastrulae were unable to

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[1] Jacques Loeb. "On the Limits of Divisibility of Living Matter." In *Biol. Lectures of Marine Biol. Lab. for 1894.* Boston, Ginn & Co. Also in *Archiv für Ges. Physiologie von Pflüger.* Vol. LIX. 1894.

[2] E. B. Wilson. "Amphioxus and the Mosaic Theory of Development." JOURNAL OF MORPHOLOGY. Vol. VIII, No. 3. 1893.

[3] Driesch. "Entwicklungsmechanische Studien." III–VI. *Zeitschr. f. wiss. Zool.* Bd. LV, p. 9. 1893.

[4] "Studies of the Partial Larvae of Sphaerechinus," by T. H. Morgan, in Roux's *Archiv für Entwicklungsmechanik der Organismen.* Bd. II, H. 1. 1895.

[5] Sullo sviluppo dei blastomeri isolati dalle uova di alcune meduse (e di altri organismi) per il Dr. Raffaello Zoja; in Roux's *Archiv für Entwicklungsmechanik der Organismen.* Bd. II, H. 1. 1895.

develop further. Morgan himself says (p. 117): "The smallest pluteus which I have found measured $7 \times 8$, and the normal form in the same dish $12 \times 15$. Another larva at the beginning of the pluteus stage measured $6 \times 7$. The larvae have apparently one-eighth the volume of the normal, and correspond in size very nearly to the pluteus figured by Loeb. If, however, we compare these small larvae with the larvae derived from isolated blastomeres, the conclusion is forced upon one that these plutei have in all probability come from fragments of the egg having only about *one-half to one-fourth of the volume of the egg.*" Inasmuch as the test proposed for the limits of divisibility rests upon the capacity for complete development to an *embryo,* or *larva* properly so-called, it is only these last figures of Morgan's that demand consideration. It would seem from these that Loeb (*loc. cit.*) has made his figure, *one-eighth,* too low, not having taken in account the fact, emphasized by Morgan, that the growth of the small blastulae, gastrulae and plutei is less rapid than that of the normal.

Zoja's results on the separation of the blastomeres of the ova of certain medusae must also be considered. In the summary, p. 32, we find the following remarks: "Medusae; Die Entwicklung der getrennten Blastomeren ($\frac{1}{2}$ und $\frac{1}{4}$ Ei von Liriope mucronata, Geryonia proboscidalis, und Mitrocoma annae; $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, Ei von Clytia flavidula und Laodice cruciata) ist ganz genau in allen ihren Phasen wie diejenige des ganzen Eies." — "Es bildet sich endlich immer eine schwimmende Larva, aus zwei Gewebe bestehend, die von jener, welche aus $\frac{1}{1}$ Ei hervorgeht, nicht unterscheidbar ist, ausser in den Dimensionen."

From this it appears that a $\frac{1}{16}$ blastomere of Clytia and Laodice is able to develop into a swimming larva. But from the next statement I judge that the development cannot go farther; this is: "Bei Clytia zeigten $\frac{1}{2}$ und $\frac{1}{4}$ Ei auch die vollständig entwickelte idroide Form, und bei Liriope gab $\frac{1}{2}$ eine kleine runde Medusa, in welcher die vier primären Tentaculi normalerweise im Kreuz angeordnet waren." Thus a fourth blastomere is the smallest portion capable of complete development.

There are three possible explanations of this failure of such small parts to develop:

1. That the whole organization of the species cannot be included in so small a space. Briefly, *deficient organization.*

2. That so small a volume of matter cannot fulfill the mechanical conditions consequent on cell-division, formation of a segmentation cavity, invagination, and so forth, owing perhaps to increased surface tension (Driesch), not to mention other conceivable alterations of the extrinsic factors of development.

3. That such a small part "is not able to set free that amount of energy which would be required for its transformation into a gastrula or a pluteus." (Loeb.) [1]

The third explanation seems to me inadequate; because such masses may continue to live for a considerable period of time and display an amount of energy in *atypical* form changes and rapid ciliary movement, which would suffice to produce the phenomena of normal development, did not other factors (included in the first or second of the above alternatives) prevent. Moreover, it is well known that exceedingly minute protoplasmic bodies, very much smaller than one-eighth the echinoderm ovum, may produce a relatively enormous amount of energy: *e.g.* bacteria and spermatozoa. Finally we do not know how much of developmental energy is of intrinsic and how much of extrinsic origin. *We are limited, then, to the first two alternatives.*

Now, in the regeneration of a unicellular organism those mechanical conditions consequent on cell-division, formation of a segmentation cavity, invagination, and so forth, are not required to be fulfilled. Surface tension and other extrinsic factors of development of Metazoa have not been shown to exercise a controlling influence in the regeneration of such an organism.[2] It occured to me, therefore, that the *ciliate Infusoria*

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[1] Morgan's explanation, that the failure to develop is due to inability to produce a sufficient number of cells for the next ontagenetic stage, will come under the first or second of these alternatives, according to the general point of view.

[2] Of course it is possible for any one to maintain that extrinsic forces do control the regeneration. But the burden of proof rests upon the maker of such an assumption.

offer conditions for decision between these two alternatives. If it should be shown that a nucleated portion of the body below a certain minimal size is incapable of regeneration, the first alternative would receive support. If, on the other hand, the smallest nucleated fragments of the body are capable of regeneration with restoration of the normal form, the first hypothesis would fall, and the second tend to be established.

My material consisted of two species of Stentor, viz.: *S. polymorphus* and *S. coeruleus*. The former species occurred in immense profusion on decaying leaves of the water-lily in a small pond near Ann Arbor, the latter appeared in considerable numbers in a small aquarium which had stood in the laboratory for six or seven weeks, and contained gatherings from a swamp. This species is more favorable for experimental work than the former, because the protoplasm is transparent, enabling one to see the nucleus readily in the living animal. In *S. polymorphus* the body is rendered almost perfectly opaque by the presence of immense numbers of symbiotic unicellular Algae, the so-called zoochlorellae, which either hide the nucleus completely from view, or permit mere momentary glimpses of it. On account of the ease of procuring any desired supply of *S. polymorphus* my work was done chiefly on this form; but the results were checked on *S. coeruleus*, and were practically the same for both species.

To reach the desired result it was necessary to find or devise a method by which nucleated fragments of every possible size, beginning with a portion not much larger than a single node of the nucleus, could be produced in large numbers; for reliable quantitative results can be reached only by observation of a large number of cases of regeneration. For this purpose I tried the method of *shaking* which has yielded such admirable results with the animal ovum in the hands of Wilson, Driesch, Morgan, and others, and found it to succeed to perfection. If a number of Stentors are put in a small vial about one-third filled with water and shaken quite violently from five to twenty times (*S. coeruleus* requires to be shaken only about five times; *S. polymorphus* ten to twenty times), and then examined under a low power of the microscope, one sees that

the animals have been broken into numerous fragments of every possible size and shape. In the field of the microscope there are present at the same time naked nodes of the nucleus either single or united in groups of two or three, and parts of the body, both nucleated and unnucleated, ranging in size through every possible gradation from $25\mu$ in diameter to about $200\mu$. Most of the latter are being driven hither and thither by the action of the cilia with which they are covered; and many of them are of the most bizarre and curious shapes : T-shaped, Y-shaped, or provided with other arm-like processes, or of forms impossible to describe; but most of them are of more regular form, triangular, quadrilateral, oval, and spherical.

The moniliform character of the nucleus in these species of Stentor insures that a large proportion even of the smallest pieces receive at least some part of the nucleus. In order to satisfy myself that such is the case, I killed and stained the whole of one lot of *S. polymorphus*, which had been shaken as described, about fifteen minutes after the operation. The stained material was then mounted in balsam and measurements were made of the smallest nucleated pieces. Some of the measurements were as follows :

1. Naked nodes of nucleus, spherical or oval; $20–25\mu$.

2. A spherical piece $31\mu$ in diameter containing a single node of the nucleus. Nucleus excentric. Protoplasm a thin cortex.

3. A spherical piece $37\mu$ in diameter; contained a single node of the nucleus.

4. A spherical piece $37\mu$ in diameter; contained two nodes of the nucleus.

5. A spherical piece $40\mu$ in diameter; contained six nodes of the nucleus.

6. A spherical piece $50\mu$ in diameter; contained one node of the nucleus.

7. A spherical piece $50\mu$ in diameter; contained two nodes of the nucleus.

8. A spherical piece $50\mu$ in diameter; contained four nodes of the nucleus.

9. A spherical piece 66μ in diameter ; contained a single elongated node of the nucleus.

10. A spherical piece 69μ in diameter; contained seven nodes of the nucleus.

Other similar pieces containing one or more nodes of the nucleus were seen; some were of course not spherical, but I have given the spherical pieces as easier to compute the volume. Of larger nucleated pieces there was no lack ; they were very numerous, as one would expect. There were, of course, numerous unnucleated masses of protoplasm of various sizes, but few large pieces. In fact, the majority of the pieces below 100μ in diameter were unnucleated; but the above list shows that a good many of such small pieces contained one or more nodes of the nucleus. I did not attempt to ascertain what was the proportion of nucleated to unnucleated pieces of such small size ; but it must have been quite large, perhaps one to ten or even more.

One further remark as to my methods. When any doubt existed in my mind as to the presence of parts of the nucleus in examples noted, the specimen was killed and stained, generally in Schneider's aceto-carmine, and the actual condition of the nucleus thus determined with certainty. This of course involved the sacrifice of a great deal of material.

In consequence of the often curious and asymmetrical shapes of the pieces produced by shaking, I expected to obtain valuable results on the teratogeny of the Stentors for comparison with the results of Balbiani and Johnson, but I have been almost completely disappointed in this respect. When regeneration of a piece takes place at all, it almost always happens that a single more or less perfect animal of typical form results.

### RESULTS.

In this paper I shall speak only of results obtained on the smallest parts capable of regeneration, leaving other questions suggested by the experiments for future consideration. From numerous experiments, involving many hundreds of *S. polymorphus*, it was found that the smallest parts capable of

regeneration possess the volume of a sphere of about 80μ diameter. A lesser number of experiments on a smaller number of *S. coeruleus* yielded results almost identical. In the following list I give measurements of some of the smallest Stentors found. After measuring in a more or less expanded condition the animals were made to contract, when they assumed almost the form of a sphere; the diameter of the sphere was then measured, and this measurement was used for comparison.

1. *Stentor coeruleus.* 45½ hours after shaking. Regeneration was complete or nearly so. I could see the adoral spiral sinking into the oesophagus, the mouth, and contractile vacuole. When expanded the form was quite typical. There were two *separated nodes* of the nucleus present.

*Measurements.* None were made of the expanded animal. Diameter of contracted animal (spherical) 90μ.

2. *S. polymorphus.* 67 hours after shaking. Regeneration complete.

*Measurements.* *a.* Expanded, 257μ in length; 80μ across frontal field. *b.* Diameter of contracted animal (spherical) 80μ.

3. *S. polymorphus.* 70 hours after shaking. Regeneration complete.

*Measurements.* *a.* Expanded, 257μ in length; 84μ across frontal field. *b.* Diameter of contracted specimen (spherical) 87μ.

4. *S. polymorphus.* 96 hours after shaking. Regeneration complete. The animal was sluggish and did not expand well.

*Measurement.* Diameter of contracted specimen 75μ.[1]

I have measurements of a number of Stentors of slightly larger size than the ones given; but the smallest Stentors were very scarce. By far the greater number of nucleated parts, which possessed a spherical diameter of less than 100μ, were incapable of regeneration, or at any rate did not regenerate. However, but a single example is sufficient to show that a portion of the volume of the example in question is capable of regeneration.

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[1] My note-book expresses a little doubt about this specimen, but it was certainly under 80μ.

The volume of the smallest Stentor found was thus equal to a sphere of somewhat less than $80\mu$ in diameter. Not one of the hundreds of smaller nucleated parts regenerated, though I found one part, $71\mu$ in diameter in spherical form, which had assumed a fairly typical form of semi-contraction and possessed a single bead of the nucleus; anterior and posterior ends (or foot) were thus recognizable, but there was neither oesophagus nor adoral membranellae present. Even if we admit this as regenerated, which I do not, it does not essentially alter the final result.

My conclusion is, therefore, that nucleated parts of *Stentor polymorphus* of less volume than a sphere of $80\mu$ (approximately) in diameter are incapable of regeneration; nucleated parts of greater volume are capable under favorable conditions of complete regeneration.

The main results hitherto reached on the merotomy of the Protozoa can be summarized as follows :

1. Cytoplasm without nucleus is incapable of regeneration (Nussbaum, Gruber, Verworn, Balbiani, and others). This I can confirm. (Verworn has shown that the isolated central capsule of *Thalassicola nucleata* from which the nucleus has been removed is capable of partial regeneration, but it soon goes to pieces. Gruber has shown that if a Stentor in process of fission be transversely divided so that the posterior part receives no nucleus, this part is nevertheless able to regenerate.)

2. Nucleus without cytoplasm is incapable of regeneration. (Verworn, Balbiani.) This also I can confirm.

3. Portions of the body consisting of nucleus and cytoplasm are capable of regeneration. *To this I must add: provided that the amount of cytoplasm exceed a certain minimal volume* (which in the case of Stentor at any rate is quite considerable).

*This amounts to a demonstration of Verworn's view that regeneration in the Protozoa is due to the reciprocal interaction of nucleus and cytoplasm. Organization resides in the cytoplasm as well as in the nucleus. How otherwise are we to explain the fact that a difference in the amount of cytoplasm alone (equivalent to the difference in volume of two spheres of 80 and 70*

*micromillimeters respectively*) *determines the occurrence of regen-*
*eration ?*

As regards the bearing of the results on the limits of divisi-
bility of living matter : we are not concerned here with the
question of the ultimate constitution of protoplasm, its com-
position of any ultimate vital elements whatsoever, but merely
with the question propounded by Loeb, "What is the order of
magnitude of the smallest particle that can show all the
phenomena of life ?" In the case of the animal ovum as
already noted this is about one-fourth of its volume, if we
include development as one of the phenomena of life. Cer-
tainly development includes all the phenomena of life. In the
case of Stentor the volume is relatively considerably less as the
following calculation will show :

The volume of the smallest perfect *Stentor polymorphus,*
which I was able to produce, was equal to that of a sphere of
about 80$\mu$ diameter; the average volume of the Stentors
used in the experiments was equal to that of a sphere of
about 230$\mu$, as I determined from a series of measurements of
animals killed in a weak killing-fluid, and thus completely con-
tracted. That is, the ratio of the diameters of the smallest
and the average Stentor is about 1:3; or the ratio of volume
to volume is about 1:27. That is to say that the smallest
Stentor which can be produced is about one twenty-seventh of
the volume of the average Stentor.[1] This number is of course
a mere approximation, but it certainly will not be made any
greater by subsequent investigation, though it may be lessened
somewhat.

[8] In the case of *S. coeruleus* the figures are different: the smallest measurement
which I have of this species is 90$\mu$; the average is 280$\mu$; thus the ratio of the
smallest to the average is about 1:3 in terms of the diameter, or 1:27 in terms of
volume. I believe, however, that it would be possible to produce a smaller *S.*
*coeruleus* by working over a larger amount of material. I do not think that there
is much difference in the *absolute* size of the smallest Stentors which can be pro-
duced, whether one uses the largest or smallest normal specimens. If *e.g.* the
average size of a lot of large Stentors were 320$\mu$, the smallest specimen which
could be produced would still be 80$\mu$. The ratio of volumes would then be 1:64.
Of course this does not necessarily mean that 64 Stentors could theoretically
be produced at one time from a single one, for I doubt that the nucleus could un-
dergo that amount of division.

In any case this relation forms a striking contrast to that found in the development of the animal ovum, where a portion of less volume than one-fourth that of the ovum does not develop into an embryo (see postscript). It has been very generally found that a portion (of the two- or four-cell stage) equal to one-eighth the volume of the ovum never develops farther than the gastrula stage.

In the case of the animal ovum, again, parts slightly smaller than the minimum necessary for the complete development may undergo partial development. In Stentor we have a parallel phenomenon: parts of less than $80\mu$ spherical diameter may undergo partial regeneration, but are unable to complete it.

It seems to me that neither increased surface tension due to diminution of surface area, nor yet any other external factor, is responsible for this failure of small pieces of Stentor to regenerate. The cause lies within; and I do not believe that it is to be sought in an insufficient production of energy. For such small pieces may live for days, constantly producing and expending energy in the ordinary processes of metabolism. I am forced, therefore, to the conclusion that the organization of these parts is in some way deficient. *There is probably for each species of animals a minimal mass of definite size consisting of nucleus and cytoplasm within which the organization of the species can just find its latent expression.* This is the *minimal organization mass.*

In the case of the Protozoa the size of this minimal mass is that of the smallest part capable of complete regeneration. But I do not believe that in the Metazoa the minimal organization mass is that of the smallest part of the ovum capable of developing into a normal embryo; for undoubtedly the influence of external factors is of the greatest importance here. I would conceive then that in the Metazoa this hypothetical minimal organization mass is smaller than any part yet observed to develop into a normal embryo. Still, from my results on Stentor, I believe that it is of such a size as to be easily visible under a low power of the microscope.

POSTSCRIPT. — After sending the above article to the editor I had access to Boveri's recent paper entitled "Ueber die Befruchtungs- und Entwickelungsfähigkeit kernloser Seeigeleier und über die Möglichkeit ihrer Bastardirung," published in Bd. II, Heft 3, of Roux's *Archiv für Entwickelungsmechanik der Organismen*, Oct. 22, 1895. Boveri states that the smallest dwarf larva which he obtained came from a fragment which could not have measured more than $\frac{1}{20}$ the volume of the intact ovum "bei ungünstiger Rechnung." His conclusion is : "Das Fragment des Seeigeleies bis herab zu einer Grösse von $\frac{1}{20}$ des ursprünglichen Eivolumens besitzt die formative Wertigkeit des ganzen Eies." This is in marked contrast to the results of the other authors quoted, none of whom have found a figure less than $\frac{1}{4}$. The difference may be due in part to the fact that Boveri shook the ova before fertilization, while the other experimenters performed this or an analogous operation after fertilization ; although this does not seem very probable. If the exact proportion of the minimal organization mass to the whole ovum be a matter of any importance, very great care in the estimation of the volumes of dwarf larvae would seem to be necessary, taking into account the differences in thickness of the layers in dwarf and normal larvae, and also the relatively slow increase in volume of the former.

The figure which I have found for Stentor is but little lower than that of Boveri for the animal ovum, and this approximation suggests interesting comparisons.

# ORGANIC VARIATION AS A CRITERION OF DEVELOPMENT.

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## CONTENTS.

## INTRODUCTORY.

THE object of the present paper is, firstly, to give a tentative explanation for the origin of variation, deducible from the law of the concomitance of variation with continuing development. The attempt will then be made to show that variation is caused indirectly by change of environment, and directly by the disturbance of correlation of the organs, resulting from the change of environment. And secondly, the attempt will be made to determine whether, in a given organism, the amount (or degree) of variation, and the manner of its occurrence, can furnish us with criteria for judging its lines of development. The only postulates necessary for the treatment of the problem of variation from this point of view are : (1) the concomitance of variation with continuing development, (2) the correlation of the organs of an organism, as necessary for its existence, and (3) the influence exerted upon the organism by its environment, necessitating a degree of adaptation to its environment.

It is not my intention to review the many theories already advanced to explain the nature and origin of variation, which would be a task too extensive for the scope of my present article. There are two well-known theories, each of which has latterly been more or less modified in regard to the origin of variation : the first teaches that variation is caused more or less directly by the environment ; while, according to the second, variation is the result of an inherent tendency on the part of the organism to vary. Now, in regard to the last-named theory, it may be said with truth that, though much may be explained on the assumption of "inherent tendencies," there is no empirical proof of the existence of such tendencies ; and further, variation is not explained by the assumption of an inherent tendency to vary, until the origin and nature of the inherent tendency itself be explained. Reference may also be made to the theory of Weismann, that variation has its origin in conjugation. My own theory, as will be seen in the following pages, inclines somewhat to the doctrine of the origin of variation as caused by the influence of the environment, but is a new departure from the Lamarckian theory, inasmuch as I consider variation to be possible only under a temporary state of independence of the several organs, when their complete correlation has been disturbed by a change in the environment.

The recent admirable work of Bateson[1] has shown clearly the importance of a careful comparative study of the phenomena of variation, for the understanding of the problems of morphology. In his book he has treated principally of the phenomena of variation in their relation to certain laws of bilateral and radial symmetry ; but the problem of the origin of variation he dismisses by stating that "Inquiry into the causes of variation is as yet, in my judgment, premature" (p. 78). My reason for attempting the solution of a problem so intricate and difficult in its nature is the need of approaching the question from a new point of view ; and in this attempted solution I have endeavored to keep within the line of facts as much as possible, and to avoid making unnecessary theoretical assumptions.

[1] Wm. Bateson : Materials for the Study of Variation, treated with especial regard to Discontinuity in the Origin of Species. London, 1894.

Although the doctrine of Natural Selection will be but little
mentioned in the following pages, it is nevertheless advisable
to give a clear definition of it. Darwin states ("Origin of
Species," new American edition from sixth English edition, 1886,
p. 63) : "This preservation of favorable individual differences
and variations, and the destruction of those which are injurious,
I have called Natural Selection, or the Survival of the Fittest."
If we take this definition, and eliminate from it the sentence,
"and the destruction of those which are injurious," we have
before us perhaps a true explanation of the origin of species.
But the elimination of this passage seems necessary, since it is
a debatable question to what extent the "destruction" of the
unfavorable variations can proceed. Darwin himself discussed
the preservation and destruction of variations, and left untouched
the problem of their origin ; but in the "Origin of Species" he
makes two statements which are of interest here : "It seems
clear that organic beings must be exposed during several gen-
erations to new conditions to cause any great amount of varia-
tion ; and that, when the organization has once begun to vary,
it generally continues varying for many generations" (p. 5).
"Unintentionally he [man] exposes organic beings to new and
changing conditions of life, and variability ensues ; but similar
changes of conditions might and do occur under nature" (p. 62).
From these two quotations we may conclude that Darwin con-
sidered variation to have its origin in change of environment, —
in which view he probably followed Lamarck.

I. DEFINITION OF VARIATION ; CORRELATION OF ORGANS ;
        PROGRESSIVE AND REGRESSIVE DEVELOPMENT.

Bateson applies the following definition to variation (*l.c.*, p. 3) :
"To this phenomenon, namely, the occurrence of differences
between the structure, the instincts, or other elements which
compose the mechanism of the offspring, and those which were
proper to the parent, the name *variation* has been given." But
since cases are known in which wholly normal offspring have
descended from parents which were not normal in all respects, we
cannot consider the offspring in such cases to present variations

from the specific type, even though they may differ from their parents ; so that it is advisable to seek a more general definition. Accordingly, organic variation may be defined as growth above or below (*i.e.* beyond) a given norm ; and organic variability, the power of the individual organism to produce such variation. Thus we can only then speak of variation when at a particular point in the ontogeny the growth of a given organ in one individual is greater or less than the normal growth at that stage. It remains necessary, however, to apply distinctive definitions to the terms "normal" and "abnormal"; and, although it is not possible to give strictly distinctive definitions to such relative ideas, it is, however, generally understood that such characters are *normal* as are presented by the greater part of the individuals of a given species, and such *abnormal* as are presented by a much smaller percentage of individuals. Though no really distinctive definitions can be given, the relative meanings of normal and abnormal are sufficiently understood, which is all that our definition of variation demands. And even in cases of a more or less perfect intergradation of the degrees of development of a given organ, in a large number of individuals of a species, it seems to be always possible to show that the limits of variation of the large majority of individuals lie within a certain circumscribed compass ; so that here normal and abnormal degrees of variation may be distinguished.[1]

By the expression "Correlation of the Organs," is understood the state of mutual dependence of the organs, after their division of labor has been brought about by the process of evolution ; each has its own particular function to perform, but the fulfilment of this function is not sufficient for its existence, since rather it would be unable to perform its own function without the aid it derives from the other organs. But further,

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[1] The term *variety* is often used ambiguously, as synonymous with variation, or as equivalent to the idea subspecies (geographical race). In the strict sense, however, the term variety is applicable only to the whole individual, and not to a single organ of it, and therefore is not equivalent to variation, which is any growth above or below the normal. A variety is then, *sensu stricto*, synonymous with the term subspecies ; but in order to avoid any possible ambiguity, which has risen from the wrong use of the word variety, I shall in the following pages avoid adopting it, and shall use instead *subspecies*, or *geographical race.*

while this physio- and morphological correlation of the organs
aids each organ in the fulfilment of its proper function, it
simultaneously acts as a restraint upon the exertions of the
vital processes of the particular organ ; for the particular organ
does not functionate merely for the maintenance of its own
existence, but primarily for that of the whole organism, and
when it has fulfilled the demand of the whole, its correlation with
the other organs would cause a temporary cessation of its activity.
Thus the correlation of the organs exerts a restraint, — acts as
a regulator, upon the amount which each shall perform.    And
when the correlation is perfect, we must assume that each organ
can normally perform a certain fixed amount, and no more nor no
less.    Since the performance of a physiological function results
in morphological change, showing the direct correlation of the
function and structure of an organ, the result follows that, if
the amount of physiological action performed by an organ is
determined by the correlation of the organs, the amount of
morphological change must be determined also by the correlation
of the organs.    This fact is important for the establishment of
the deduction, which will find its treatment further on, that
variation can appear only when the complete correlation of the
organs has been disturbed.    And since the degree of perfection
of the division of labor between the several organs is propor-
tional to the amount of differentiation of the organism, it is
correct to conclude, that the completeness of the correlation of
the organs stands in a direct proportion to the degree of differ-
entiation of the organism, — the higher the organism the more
perfect is the correlation of its organs ; and *vice versa*, the
lower the organism is structurally, the less intimate is this
correlation, *i.e.* the more independent the several organs are.

    It will be well here to analyze and compare briefly the ideas,
*progressive* and *regressive development.*    Either process may
modify a given organ in regard to its structure (chemical and
morphological), its size, position, and, in meristically arranged
organs, its number.    Progressive development tends to com-
plicate or further differentiate its chemical and morphological
structure, to change its position and dimensions, and (subject
to certain limitations) to increase its number in a meristic

series ; while regressive development (degeneration, *Rück-bildung*) tends to simplify the structure, to change the position and dimensions, and (subject to certain limitations) to decrease its number in a meristic series. Since either process may produce a change of position of the organ involved, such a change furnishes no criterion whereby to judge the kind of development, until it can be determined whether the direction or amount of change of position differs according as the mode of development is progressive or regressive. And whether change of the dimensions gives us a criterion of the kind of development is also doubtful ; although, since it is the general rule that increasing complexity of structure is usually accompanied by actual increase in size, it might be concluded that an increase in size of an organ denotes frequently the action of progressive development. But since it is only a general rule, and by no means a law without exceptions, that increase in size goes hand in hand with increasing structural complexity, it would be safer to eliminate change of dimension from our criteria for distinguishing between the two modes of organic development.

We find, however, a certain criterion for estimating the kind of development, in change of structure ; for an increasing complexity of structure is distinctive of progressive development, while, on the other hand, a decreasing complexity is the sign of regressive development. Further, an increase in the number of meristically (segmentally or metamerically) arranged organs is a criterion of progressive development, as a decrease in the number of similar organs is of regressive development, — provided that, in each case, no structural changes are simultaneously taking place. This standpoint will hardly be disputed, for we consider an organism *A* to be morphologically higher than an organism *B*, when *A* possesses a larger number of organs in a given meristic series than does *B*, even though these organs are otherwise structurally equivalent in *A* and *B*.

But it is necessary to consider the case, when a progressive meristic development is acting together with a regressive structural development, or *vice versa :* as *e.g.* in the carpus of the Ichthyosauria, where the phalanges are meristically pro-

gressive, but regressive in regard to specialization, in comparison with their ancestral forms.    The question before us is, then : When in an organ there is at work a progressive structural development, simultaneous with a regressive meristic development, is the organ to be regarded on the whole as progressive or regressive ?   The answer to this is to be gained by determining whether structural complexity is of greater or of less morphological importance than is meristic change. Now the consensus of opinion among biological investigators would show that structural complexity (both chemical and histological) is of much greater morphological importance than is mere meristic development, — this assumption being, indeed, a necessary preliminary in any attempt to homologize different organisms.    For to pick out an example at random, who would venture the opinion that *Branchipus* occupies a higher morphological position than *Astacus*, simply on the ground that it possesses more numerous extremities ; and would not rather conclude that *Astacus* is the higher organism, because its extremities are structurally more differentiated ?   Therefore, if structural modification is of greater morphological importance than numerical (meristic) modification, then when progressive structural development is accompanied by regressive numerical development, the organ as a whole is to be regarded as developing progressively ; and conversely, when regressive structural development is simultaneous with progressive numerical development, the course of development of the organ is to be considered regressive.

It still remains to accentuate an apparent law, which is generally recognized, with reference to this frequent concomitance of numerical and structural development.    Apparently a progressive structural development frequently causes a regressive numerical development, as we find when, by the coalescence of previously separate meristic organs (*i.e.* through their regressive numerical development), a compound organ is produced, which is higher morphologically than was any one of the previously separate organs.    However, a numerical reduction of the units of a meristic series can proceed, and perhaps does so more usually, without

coalescence ; and such a regressive numerical development is also usually associated with a progressive structural development of those units of the meristic series which are retained. Examples of such cases may be found in abundance, and it is sufficient here to refer to the lateral line of sense-organs in the Vertebrates, where a progressive development of certain of the individual organs is concomitant with a reduction in the number of the units comprising the series. And with a view to many analogical cases, which it is unnecessary to give in detail here, since any zoölogist may recall many to mind, the law will be found to be of general application, that progressive structural development is furthered by regressive numerical development, since in this way greater centralization of the forces of growth would ensue. And although regressive structural is sometimes concomitant with regressive numerical development, as exemplified in the case of certain parapodia of sedentary Annelids, I recall no case of the concomitance of progressive structural and progressive numerical development ; but I would not imply by this that such a concomitance cannot or does not occur, but rather that such a concomitance is of so infrequent occurrence as not to render invalid the general law just mentioned. My reason for accentuating this law of the usual concomitance of progressive structural with regressive numerical development is in order (1) to characterize concisely a relation, which, although already recognized, has not yet been awarded much attention from zoölogists ; and (2) to emphasize a point which may serve as a criterion of progressive development.

By the term development is meant here any organic process of change acting in the organism ; when such a change tends to further complicate the structure, it has been termed progressive development (evolution, *Entwickelung*); when it tends to simplify the structure, regressive development (degeneration, *Rückbildung*). Speaking generally, development leads towards (1) the production of new species, or (2) towards the extinction of already present species ; obviously, development cannot be conceived as holding a species stable (*i.e.* unchanging), since development always implies an organic change.

After these preliminary explanations, we may next consider the phenomena of continuing development.

## II. Continuing Organic Development is always accompanied by Variability.

Although this postulate may seem at first sight to be a mere enunciation of a well-known biological axiom, and a *sine qua non* of the theory of development, it is nevertheless of great importance for arriving at a true conception of the nature of variation ; and although evolutionists in general will grant with Darwin that for the action of Natural Selection the occurrence of variations is necessary, yet to my knowledge no one has particularly accentuated the fact of this actual concomitance of variations with continuing development. Indeed, most biologists have accepted this fact, without a critical inquiry into its fundamental importance. In my last paper [1] I laid particular stress upon this point, by saying (p. 483) : " Now I consider this variability in the number of the eyes of the freshwater forms to be explained by the general law, that all organs (and *propter hoc* all organisms) which are undergoing progressive or regressive development tend to be variable." In the present paper I hope to substantiate the validity of this " general law " by data from another source.

### A. *Certain Criteria of Continuing Development.*

In order to prove the assumption that continuing progressive and regressive development is always accompanied by variability, it is necessary to produce a series of facts, showing that organs (or organisms) which are undoubtedly in a state of continuing development always evince variability. But, although examples of variation may be found in abundance, it is obviously difficult to prove conclusively that a given organ (or organism) is at a given time influenced by a continuing process of development. Accordingly, for each example to be cited, we

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[1] " The Derivation of the Freshwater and Land Nemerteans, and Allied Questions." Journal of Morphology, XI, 2, 1895.

must demonstrate satisfactorily that it is undergoing a process of development.

The question to be solved is then, first of all : What are our criteria of continuing development ? Progressive and regressive development having been sufficiently characterized, it remains necessary to produce criteria, whereby we can determine whether a given organ (or organism) is at a particular time dominated by a process of development, or whether the organ (or organism) is not being influenced by a particular developing agency, either progressive or regressive. We may now consider briefly three reliable criteria of continuing development, namely, (1) domestication, (2) the presence of geographical races (subspecies), and (3) migration ; no doubt other criteria may be found, but these three are sufficient for our present purposes.

(1) *Domestication* may be taken as a criterion of continuing development, since all organisms in a state of domestication are being more or less continuously selected by man, with a view to their adaptability for certain uses. The development induced by human agency is also very energetic, since man's uses for domesticated animals and plants are manifold, and since he frequently introduces changes in their environment. And further, as we know in many cases that the length of time necessary for the production of a new "breed" has been comparatively short, we must conclude that not only was the action of the development continuous, but also that it must needs have been very energetic.

(2) *The presence of geographical races* may also be considered a criterion of continuing development. A species is said to present geographical races or subspecies when in different portions of its breeding area particular forms occur, differing mainly in color and dimensions, but which are all connected together by a more or less perfect series of intergradations, and all of which may breed together fertilely. Any one at all conversant with the geographical distribution of animals or plants knows how frequently wide-ranging species are differentiated into a number of geographical races, and that the number of such races stands usually in a direct ratio to the extent, or diversification, of the

range of the species.   Now if the Darwinian doctrine of evolution
be true, all such races have descended from one ancestral form ;
but I think that we may go still further, and postulate that
wherever one geographical race grades insensibly into another,
there the agency of development must be still continuing.
For supposing *A, B, C* to be three intergrading geographical
races inhabiting contiguous areas *a, b, c.*   In area *a,* together
with the individuals of race *A,* will always occur some individuals
of races *B* and *C,* which have migrated from *b* and *c* into *a.*
Now these individuals, which have migrated from *b* and *c* into
*a,* must adapt themselves to the new environment of the area *a,*
if they would compete successfully with the individuals of race
*A ;* and thus a continuous development of a considerable num-
ber of the individuals of the species must proceed, tending to
produce favorable adaptations in the struggle for existence, —
this struggle being probably keenest where the areas *a, b,* and
*c* overlap.   It is still a bone of contention between systematic
ornithologists whether individuals of a race *B* are ever found
in the area proper to a race *A,* or *vice versa ;* but the cases
where this is so, as *e.g. Dendroica palmarum* and its variety
*hypochrysea,* are so numerous as to warrant the conclusion that,
wherever the geographical lines of demarcation between the
respective breeding areas are not strongly marked, there
must be a considerable interchange of wandering individuals.
And it seems to be always the rule, that the indefinitely broad
area of demarcation is peopled promiscuously by individuals of
the contiguous races.   Only when the lines of demarcation
are formed by high mountain ranges, deserts, or great water
expanses would there be little or no interchange of individuals ;
but when the boundaries of the areas of the several forms are
so comparatively impassable, the various forms usually do not
perfectly intergrade, and hence are to be classed rather as sep-
arate species than as races of one species.   Therefore it is cor-
rect to conclude that in a widely ranging species, split up into
a number of intergrading geographical races, a continuous
agency of development is at work, leading to the readaptation
of the migrating individuals to new environments.

Darwin ("Origin of Species") has ably argued the point

that where the adjoining areas of geographical races overlap, the struggle for existence would be keenest, so that the individuals occupying this intermediate area would in time become extinct. But he has overlooked the fact that until such extermination has been brought about, *i.e.* as long as the races continue to intergrade, the intermediate area would continue to be the vortex of development, and the sharp struggle there would itself instigate the wandering of individuals into the adjoining areas, where again they must adapt themselves to new environments. Now, when in any species the individuals occupying the areas transitional between those proper to the several races have become exterminated, the races must cease to intergrade, fewer individuals will continue to wander into other areas, and, the struggle for existence becoming less sharp, the main factor in the process of development would disappear. But, as we have shown above, when the races cease to intergrade, and become more distinctly pronounced, they can *sensu stricto* be no longer termed races, but rather distinct species. Accordingly, the presence of geographical races being correctly considered a criterion of continuing development, we should expect our data to show, as indeed they do, that, other factors being equal, species with geographical subspecies evince a greater amount of variation than do species which present no geographical races.

It is noteworthy that extensive periodical migrations act as a restraint upon the production of geographical races. And I account for this fact [1] by assuming that the migratory species, being influenced in winter by an environment to some extent different from that which it experiences in summer, must be equally adapted to both environments (*equally*, at least, if it remains under the influence of both environments for the same length of time), and hence, the winter environment exerting a restraint upon the production of adaptations suited to the summer environment alone, such a migratory species would not be capable of producing geographical races to the same

[1] In a paper which has not yet appeared, but which will be published in the *American Naturalist* for June, 1896, dealing with migration as a check upon geographical variation in birds.

degree, as would a non-migratory species with an equally extensive breeding area.

(3) *Extensive migration* may be taken as another criterion of continuing development. (By the term *extensive* migration, I mean, as will be explained later, a regular periodic migration through a considerable distance, — 30° lat. or more.) For, as was shown in the preceding paragraph, a migratory species in wandering from its summer to its winter home, or *vice versa*, is brought into contact with a different environment, necessitating a certain amount of readaptation ; therefore, there must be at work a more or less continuous process of development, leading towards readaptations. Thus, to use a well-known example, a man accustomed to spend his annual holiday abroad, on arriving at his destination experiences the lassitude preparatory to his becoming acclimated, and experiences the same physical sensations on his return. Accordingly our data should demonstrate that species which undertake periodic migrations through long distances should evince a greater amount of variation than do stationary species, other factors being equal in amount.

Other criteria of continuing development might be mentioned, but as the three already given are well founded and sufficient for the furtherance of our deductions, we shall deal but briefly with a fourth. If Wallace's theory ("Darwinism") be true, that secondary sexual characters are most accentuated in those species where the sexual impulses are strongest, so that the sexual impulse may be considered as an important if not sole agent in their production, then we might consider the presence of strongly marked secondary sexual characters as a criterion of continuing development, by regarding the sexual impulse itself as a more or less continuing impulse to development. In other words, the degree of development of secondary sexual characters would stand in direct proportion to the continuousness and energy of the sexual impulse. And since secondary sexual characters are often different in otherwise closely allied species, being as a rule the least reliable (morphologically speaking) of specific differences, they must be regarded as of comparatively recent origin in each species, and thus be considered characters prob-

ably still under the agency of a process of development. But
though this reasoning may be plausible, it is based upon Wal-
lace's assumption that the production of such characters is due
to the agency of the sexual impulse ; and rather than bind myself
to such a theory, I would leave the case still disputable, whether
or no the presence of noticeable secondary sexual characters
should be taken as a criterion of continuing development.

### B. *Data.*

It now remains to produce data in support of the thesis
that individual variation is always concomitant with continu-
ing development ; and to do this, it must be demonstrated
successively that (1) variation is always predominant in those
domestic animals which have been most carefully selected by
man ; (2) in such species as are divisible into geographical
races ; and (3) in those species which undertake extensive
periodical migrations.

Variation in domesticated animals is very marked, and espec-
ially so in those forms which have been most carefully selected
by man. It is of interest to compare the diversity of breeds
of the dog with the fewer breeds of the cat ; the former is of
greater practical use than the latter, and man has subjected it
consequently to a greater diversity of conditions of life. Whether
a greater amount of individual variation is evinced by the
domesticated animals than by their allies in a state of nature,
cannot as yet be answered with certainty, since, as Bateson
(*l.c.*) observes, the phenomena of variation in the wild forms are
not known to the same extent. Cope (" Origin of the Fittest ")
mentions the peacock and the Guinea fowl as forms which have
not been rendered variable by domestication ; but these two
may be classed as the wildest, and least carefully bred by human
selection, of any of the domesticated birds. However, without
going further into the much-discussed question of variation
under domestication, the fact is sufficient for us that animals
show considerable individual variation under domestication,
and that the amount of variation is greatest in those species
which have been most influenced by human selection ; and we

have found domestication to cause a more or less continuous development.

In order to test the correctness of the assumption that individual variation is most marked (1) in those species which possess geographical races, and (2) in those species which undertake extensive migrations, I have examined nearly all the species of North American birds with reference to individual variation in some or all of the following dimensions : culmen of the bill, wing (from carpal joint to tip of longest primary), tarsus (so-called, but really tarso-metatarsus), whole length (from tip of bill to tip of tail), and tail (from the pygostyle to tip of the longest rectrix). It was my original intention to personally undertake all the measurements, and with that object in view I commenced a series of detailed measurements upon the bird-skins in the collection of the Academy of Natural Sciences of Philadelphia. Unfortunately for me, however, this collection did not offer large enough series of individuals of all the species desired, and not having the opportunity nor time to study other large collections, — namely those at Cambridge, New York, and Washington, — I was obliged to desist from further personal examinations. In lieu, then, of such direct examination, I have taken Robert Ridgway's excellent " Manual of North American Birds, 1887 " (first ed.) as my authority for the extremes of individual variation, in regard to the dimensions specified, of the North American species of birds. And here I would express my hearty gratitude to Professor Ridgway for his liberality and generosity in allowing me to make use of his valuable data. In speaking of the measurements given in his work, Ridgway states (p. ix) : " Whenever practicable, they have been taken from large series of specimens, and the extremes given as well as the average. . . . In the case of closely allied forms, or where distinctive characters are largely a matter of dimensions or the proportionate measurements of different parts, care has been taken to measure, whenever possible, an equal number of specimens of the several forms to be compared ; and specimens in abraded or otherwise imperfect plumage, as well as young birds, have been excluded. When there is any marked sexual difference in size, the number of males and females measured

of allied forms has also been made as nearly equal as possible."
The degree of individual variation in regard to the dimensions,
according to Ridgway, is therefore based (for most of the
species) on large series of specimens of adult individuals, in
unworn plumage ; and as this distinguished ornithologist's
work is regarded as a standard by taxonomists, the accuracy of
his measurements cannot be questioned.    And as such a large
series of data is the result of years of painstaking work, I may
be pardoned for not attempting such a labor in the limited time
at my disposal.    I have taken these measurements as given by
Ridgway, with necessarily the exclusion of such extremes of
variation as were based upon a very small number of individuals,
and have computed the percentage of variation for each given
dimension, expressing the difference between the extremes of
variation as a percentage of the minor term of variation.   In this
way I have deduced the percentage of variation in the dimensions
of the larger part of the species and subspecies of North Ameri-
can birds (together with those of a considerable number of exotic
species, of casual or possible occurrence within our boundaries) ;
or, altogether, the species and subspecies of fifty-six families,
the only omissions being the following small families : *Tro-
gonidæ, Alcedinidæ, Momotidæ, Cotingidæ, Hirundinidæ, Am-
pelidæ, Laniidæ, Coerebidæ, Motacillidæ, Cinclidæ, Certhiidæ,
Sylviidæ.*    These latter families have been omitted, because
their respective scarcity of species would hardly warrant com-
parisons.   Accordingly, using the measurements given by Ridg-
way as my basis, I have computed the percentage of individual
variation for one or more of the five dimensions specified, for
the greater number (approximately 600 or more) of species
and subspecies of North American birds.   It is unnecessary to
reproduce in this paper these measurements for all the families,
which would only result in the needless occupation of too much
valuable space in this JOURNAL ; accordingly, for purposes of
comparison I will present tables of variation for those families
only, in which the diagnostic characters of most of the species
are furnished by the measurements, and for which, therefore,
the percentages of variation, based upon such necessary accurate
measurements, may be considered as accurate as possible.

Together with the degrees of variation, will be given for each species also, as briefly as possible, the range of migration and breeding area. These facts have been extracted principally from Ridgway's work (*l.c.*), and from the recent work of Witmer Stone.[1] Birds with a migration range of 30° lat. north and south, or a corresponding distance east and west across the continent, I have classed as *extensive migrants;* birds with a smaller migration range, as *migrants;* those which do not undertake regular periodic migrations, but occasionally accomplish wanderings of considerable extent, as *irregular migrants;* and finally, those which migrate not at all, or, as the meadow lark and crow, migrate through only short distances, as *residents.* Such a classification according to the range of migration is necessarily an arbitrary one ; as is shown *e.g.* by the migration of certain species from high mountain ranges to the adjoining valleys in the winter season, a case which could not be classed as an extensive migration, although each such species meets with a considerable change of environment. This classification has been used merely as a convenience for computing the relation of the amount of variation to the extent of migration of the species. In other words, the extent of migration and the breeding area have been given for each species in order to learn the laws of the amount of individual variation in its relation to the environment.[2]

[1] Witmer Stone : The Birds of Eastern Pennsylvania and New Jersey, etc. Philadelphia, 1894. I would here express my gratitude to my friend Mr. Stone for his valuable aid in helping me to determine the migration and breeding ranges of certain species; and also for the facilities offered me to study the bird collections of the Philadelphia Academy of Natural Sciences.

[2] The nomenclature adopted here for the species of birds is that employed by the American Ornithologists' Union, with the emendations contained in its supplementary lists. I was unable to consult the second edition of this work, which appeared after this paper had been sent to press.

The following abbreviations will be employed in the tables:

* = variation under 1 %.
** = variation between 1% and 1.5%.
*** = variation between 1.5% and 2%.
**** = variation of 2% or more.
R. = resident.
I.M. = irregular migrant.
M. = regular migrant.
E.M. = extensive migrant.
distr. = district.
trop. = tropical.
temp. = temperate.
G. = gulf.

c. = centre (or central).
I. = island.
int. = interior.
r. = river.
vall. = valley.
st. = state.
N.E. = New England.
A. = America.
C.A. = Central America.
Miss. = Mississippi.
B.C. = British Columbia.

n., e., s., w. = north, east, south, west (or their adjectives).

All other abbreviations for states and countries are those commonly employed in the U. S.

### TABLE I. VARIATION IN THE RALLIDÆ.

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| Rallus crepitans Gmel. | **** | *** | **** | *** | | M. Atlantic marshes n. to Long I. |
| R. c. saturatus Hensh. | *** | * | * | *** | | R. coast of La. |
| R. obsoletus Ridgw. | ** | * | * | * | | R. coast marshes from L. Cal. to Ore. |
| R. elegans (Aud.) | *** | *** | ** | ** | | M. freshwater marshes of e. U.S. |
| R. beldingi Ridgw. | ** | ** | ** | * | | R. e. coast of L. Cal. |
| R. tenuirostris (Lawr.) | **** | * | *** | | | R. c. & w. Mex. |
| R. virginianus Linn. | ** | * | * | **** | | E. M. whole N. A. n. to Hudson B. |
| Porzana carolina (Linn.) | **** | * | * | **** | | E. M. n. U. S. northward. |
| P. jamaicensis (Gmel.) | **** | **** | * | **** | | E. M. s. U. S. n. to Mass. & Ore. |
| P. noveboracensis (Gmel.) | **** | **** | * | **** | | E. M. n. U. S. to Hudson B. w. to Utah. |
| Ionornis martinica (Linn.) | * | * | ** | | | M. nearly whole trop. & warm temp. A. |
| Gallinula galeata (Licht.) | * | * | ** | ** | | M. whole trop. & temp. N. A. to B. C. |
| Fulica atra Linn. | *** | ** | * | | | M. n. Eurasia. |
| F. americana (Gmel.) | **** | * | ** | **** | | E. M. n. U. S. to Greenland & Alaska. |

## TABLE II.  VARIATION IN THE FALCONIDÆ.

| SPECIES. | | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|---|
| Elanoides forficatus (Linn.) | | ** | ** | **** | *** | | M. trop. & warm temp. N. A. |
| Ictinia plumbea (Gmel.) | | | *** | | | | R. s. Mex. to Paraguay. |
| Rostrhamus sociabilis (Vieill.) | | ** | ** | | ** | | R. Mex. to Argentine Rep. |
| Circus hudsonius (Linn.) | ♂ | | * | | * | ** | E. M. whole of N. A. |
| | ♀ | | ** | | ** | ** | |
| Accipiter velox (Wils.) | ♂ | | *** | * | *** | | E. M. whole of N. A. |
| | ♀ | | ** | ** | ** | | |
| A. cooperi (Bonap.) | ♂ | | * | ** | **** | | R. temp. N. A. & Mex. |
| | ♀ | | * | * | ** | | |
| A. atricapillus (Wils.) | ♂ | | ** | ** | | | M. n. e. N. A. n. of U. S. |
| | ♀ | | * | ** | | | |
| Parabuteo unicinctus (Temm.) | | **** | **** | **** | | | R. S. A. |
| P. u. harrisi (Aud.) | ♂ | * | ** | * | **** | | R. C. A. & s. U. S. |
| | ♀ | * | * | ** | ** | | |
| Buteo borealis (Gmel.) | ♂ | ** | ** | **** | *** | | R. e. N. A. w. to Gt. Plains. |
| | ♀ | *** | *** | * | * | | |
| B. b. harlani (Aud.) | ♂ | * | ** | **** | * | | R. G. St. & lower Miss. vall. |
| | ♀ | ** | * | **** | * | | |
| B. buteo (Linn.) | | ** | * | *** | *** | | R. n. E. Hemisphere. |
| B. abbreviatus Cab. | ♂ | **** | * | ** | * | | R. n. S. A. to s. Tex. & s. Cal. |
| | ♀ | ** | * | * | * | | |
| B. swainsoni Bonap. | ♂ | ** | ** | *** | * | | R. w. N. A. from Alaska to Argentine Rep. |
| | ♀ | *** | *** | *** | * | | |
| B. brachyurus Vieill. | ♂ | ** | ** | ** | | | R. trop. A. n. to e. Mex. |
| B. latissimus (Wils.) | ♂ | | * | **** | ** | | E. M e. N. A. to Saskatchewan. |
| | ♀ | ** | * | **** | ** | | |
| B. lineatus (Gmel.) | ♂ | **** | **** | **** | ** | | M. e. N. A. w. to Gt. Plains. |
| | ♀ | ** | * | * | *** | | |
| B. l. elegans (Cass.) | ♂ | | * | | | | R. Pacific coast of U. S. |
| | ♀ | | | * | | | |
| B. albicandatus sennetti Allen | ♂ | | *** | * | | | R. e. S. A. to s. Tex. |
| | ♀ | ** | * | ** | | | |
| Urubitinga urubitinga (Gmel.) | | | * | * | | | R. Costa Rica to Argentine Rep. |
| U. ridgwayi Gurney | | **** | * | ** | | | R. Guatemala & s. Mex. |
| U. anthracina (Licht.) | ♂ | * | ** | * | | | R. trop. A. n. to s. Ariz. |
| | ♀ | * | ** | *** | | | |
| Archibuteo lagopus (Brünn.) | ♂ | | * | | ** | | M. n. E. Hemisphere. |
| | ♀ | | ** | | * | | |
| A. ferrugineus (Licht.) | ♂ | | * | | | | M. w. U. S. n. to Saskatchewan. |
| | ♀ | | ** | | | | |
| Aquila chrysætos (Linn.) | ♂ | * | * | * | *** | | R. n. portions of N. Hemisphere. |
| Haliæetus albicilla (Linn.) | ♀ | ** | * | * | ** | | |
| | ♂ | * | ** | *** | * | | M. n. portions of E. Hemisphere. |
| H. leucocephalus (Linn.) | ♀ | ** | * | * | ** | | |
| | ♂ | **** | **** | **** | *** | | R. whole of N. A., Kamschatka. |
| | ♀ | **** | *** | ** | **** | | |

## Table II. — *Continued.*

| Species. | | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|---|
| Thalassoætus pelagicus (Pall.) | | | | | | | |
| | ♂ | | | | * | | M. Kamschatka sea-coasts. |
| | ♀ | | | | | | |
| Falco islandus Brünn. | ♂ | * | * | | | | R. circumpolar regions. |
| | ♀ | ** | * | * | * | | |
| F. rusticolus Linn. | ♂ | ** | ** | **** | * | | M. extreme n. N. A. & Eurasia. |
| | ♀ | *** | * | ** | ** | | |
| F. r. gyrfalco (Linn.) | ♂ | * | * | * | | | I. M. n. Europe & arctic A. |
| | ♀ | ** | * | *** | | | |
| F. r. obsoletus (Gmel.) | ♂ | * | * | ** | | | M. coast of Labrador. |
| | ♀ | * | ** | * | | | |
| F. mexicanus Schleg. | ♂ | * | * | *** | * | | R. w. U. S. s. to Mex. |
| | ♀ | * | * | *** | * | | |
| F. peregrinus Tunst. | ♂ | * | *** | *** | *** | | M. Europe and portions of Asia. |
| | ♀ | *** | ** | ** | ** | | |
| F. p. pealei Ridgw. | ♂ | ** | * | * | | | R. Aleutian Is. & coast of Ore. |
| | ♀ | * | * | ** | | | |
| F. deiroleucus Temm. | ♂ | | * | ** | | | R. trop. A. n. to s. Mex. |
| | ♀ | | ** | * | | | |
| F. albiguralis (Daud.) | ♂ | | **** | **** | * | | R. trop. A. n. to n. Mex. |
| | ♀ | | * | * | | | |
| F. regulus Pall. | ♂ | ** | * | * | | | R. Eurasia. |
| | ♀ | * | * | * | | | |
| F. columbarius Linn. | ♂ | * | * | * | *** | | E. M. N. A. chiefly n. of U. S. |
| | ♀ | * | * | * | ** | | |
| F. c. suckleyi Ridgw. | ♂ | * | * | ** | | | M. n. Cal. to Sitka. |
| | ♀ | * | * | * | | | |
| F. richardsonii Ridgw. | ♂ | **** | * | * | | | M. int. of N. A. from Col. northward. |
| | ♀ | * | * | * | ** | | |
| F. fusco-cœrulescens Vieill. | ♂ | ** | *** | * | | | R. trop. A. n. to s. Tex. & N. M. |
| | ♀ | ** | * | ** | * | | |
| F. sparverius Linn. | ♂ | | **** | **** | **** | | M. whole temp. N. A. |
| | ♀ | ** | *** | * | **** | | |
| F. dominicensis Gmel. | ♂ | ** | * | ** | | | R. Cuba & Hayti. |
| | ♀ | * | * | | | | |
| Polyborus tharus (Mol.) | | *** | ** | **** | | | R. S. A. |
| P. cheriway (Jacq.) | | **** | ** | *** | **** | | R. s. U. S. to Ecuador. |
| P. lutosus Ridgw. | | * | * | * | | | R. Guadelupe I. |

TABLE III.   VARIATION IN THE PICIDÆ.

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| Campephilus principalis (Linn.) | * | ** | | | * | * | R. formerly s. Atlantic & Gulf St. |
| C. p. bairdi (Cass.) | * | * | | | * | R. Cuba. |
| C. imperialis (Gould) | **** | ** | | * | *** | R. w. Mex. |
| C. guatemalensis (Hartl.) | *** | * | | * | * | R. s. Mex. to Costa Rica. |
| Dryobates villosus (Linn.) | ** | ** | | * | *** | M. e. U. S. except s. St. |
| D. v. leucomelas (Bodd.) | *** | * | | ** | * | M. n. N. A. w. to Alaska. |
| D. v. audubonii (Swains.) | ** | * | | * | * | R. s. Atlantic & Gulf St. |
| D. v. maynardi Ridgw. | **** | ** | | ** | * | R. Bahamas.      [s. to Mex. |
| D. v. harrisii (Aud.) | **** | ** | | ** | *** | M.? w. U. S. e. to Rocky Mts., |
| D. v. jardinii (Malh.) | ***** | **** | | ** | **** | R. e. Mex. s. to Veragua. |
| D. pubescens (Linn.) | **** | *** | | ** | **** | R. n. & e. N. A.   [N. M. |
| D. p. gairdnerii (Aud.) | ** | *** | | ** | *** | R. w. U. S., n. to B. C., s. to |
| D. borealis (Vieill.) | ** | ** | | ** | * | R. s. e. U. S. w. to Tex. |
| D. scalaris (Wagl.) | ** | * | | | * | R. s. e. Mex. |
| D. s. parvus (Cabot) | **** | * | | | * | R. Yucatan. |
| D. s. bairdi (Scl.) | ** | * | | | ** | R. table-lands of Mex. to U. S. |
| D. s. lucasanus (Xantus) | * | * | | * | * | R. s. L. Cal. |
| D. s. sinaloensis (Ridgw.) | ** | * | | * | * | R. w. Mex. |
| D. s. graysoni Baird | *** | * | | * | * | R. Tres Marias Is. |
| D. nuttallii (Gamb.) | * | * | | | *** | R. Cal. |
| D. arizonæ (Hargitt) | ** | * | | ** | *** | R. s. Ore. & n. w. Mex. |
| Xenopicus albolarvatus (Cass.) | | * | | * | * | R. mts. from Wash. Terr. to s. Cal.   [U. S. |
| Picoides arcticus (Swains.) | ** | * | | * | | R. n. N. A. s. to border of |
| P. americanus Brehm | ** | * | | | **** | M. n. N. A. e. of Rocky Mts. |
| P. a. alascensis (Nelson) | ** | * | | | **** | M.? Alaska to Gt. Slave Lake. |
| P. a. dorsalis Baird | **** | * | | | ** | R.? Rocky Mts. from Kodiak to N. M. |
| Sphyrapicus varius (Linn.) | * | * | | ** | ** | E. M. n. e. N. A. n. of U. S. |
| S. v. nuchalis Baird | * | * | | * | * | M.? Rocky Mts. of U. S. |
| S. ruber (Gmel.) | * | * | | * | ** | R. coast from Alaska to Cal. |
| S. thyroideus (Cass.) | **** | * | | * | * | R. w. U. S. to Rocky Mts. |
| Ceophlœus pileatus (Linn.) | **** | ** | | **** | ** | R. whole of N. A. |
| Melanerpes erythrocephalus (Linn.) | | * | | * | * | M. e. U. S. |
| M. formicivorus (Swains.) | ** | ** | | | *** | R. s. e. Mex. to Costa Rica. |
| M. f. bairdi Ridgw. | **** | ** | | ** | **** | R. Mex. & contiguous U. S. |
| M. f. angustifrons Baird | * | * | | * | * | R. s. L. Cal. |
| M. torquatus (Wils.) | | * | | * | * | R. w. U. S. e. to Rocky Mts. |
| M. elegans (Swains.) | *** | * | | | *** | R. s. & w. Mex. |
| M. carolinus (Linn.) | **** | ** | | ** | ** | M. e. U. S. w. to Rocky Mts. |
| M. rubriventris (Swains.) | | * | | | | R. Yucatan. |
| M. pygmæus Ridgw. | | * | | | | R. Cozumel I. |
| M. aurifrons (Wagl.) | *** | * | | | ** | R. n. e. Mex. & s. Tex. |
| M. uropygialis (Baird) | **** | * | | | ** | R. s. Ariz. & Cal., L. Cal., w. Mex. |
| Colaptes auratus (Linn.) | ** | **** | | * | **** | M. e. N. A. w. to Gt. Plains. |
| C. chrysocaulosus Gundl. | | * | | | ** | R. Cuba.        [Sonora. |
| C. chrysoides (Malh.) | *** | ** | | * | ** | R. s. e. Cal., L. Cal., s. Ariz., |
| C. cafer (Gmel.) | ** | ** | | * | *** | R.? w. U. S. & n. Mex. e. to Rocky Mts. |
| C. c. saturatior Ridgw. | *** | ** | | | ** | R.? coast from Cal. to Sitka. |
| C. rufipileus Ridgw. | *** | * | | | ** | R. Guadelupe I. |

### Table IV. Variation in the Tyrannidæ.

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Milvulus tyrannus (Linn.) ♂ | | *** | | **** | ** | R. Mex. to S. A. & Lesser Antilles. |
| Tyrannus tyrannus (Linn.) | | * | | ** | ** | E. M. N. A. e. of Rocky Mts. |
| T. magnirostris D'Orb. | | * | | | * | R. Cuba & Bahamas. |
| T. dominicensis (Gmel.) | * | * | | ** | *** | R. W. Indies, coast of G. of Mex. |
| T. crassirostris Swains. | * | * | | | ** | R. Mex. |
| T. melancholicus couchi (Baird) | *** | ** | | ** | *** | R. Guatemala & Mex. to s. Tex. |
| T. verticalis Say | ** | ** | | *** | * | M. w. N. A. e. to Gt. Plains. |
| T. vociferans Swains. | * | * | | * | ** | R. Guatemala, Mex., L. Cal. |
| Pitangus derbianus (Kaup) | * | * | | ** | * | R. n. S. A. to Rio Grande. |
| P. bahamensis Bryant | * | * | * | | ** | R. Bahamas. |
| Myiozetetes texensis (Giraud) | | * | | * | * | R. Colombia to n. Mex. |
| Myiodynastes luteiventris Scl. | ** | * | | * | * | R. Mex. to Panama. |
| M. audax (Gmel.) | * | * | | | * | R. Cayenne, Trinidad, Tobago. |
| M. a. nobilis (Scl.) | *** | * | | | ** | R. Costa Rica s. to Ecuador. |
| M. a. insolens Ridgw. | ** | * | | | **** | R. s. e. Mex. |
| Myiarchus mexicanus (Kaup) | **** | ** | ** | * | ** | R. Guatemala n. to e. Mex. |
| M. m. magister Ridgw. | **** | ** | * | * | ** | M. w. Mex. to s. Ariz. |
| M. crinitus (Linn.) | *** | ** | * | * | **** | M. e. U. S. to Canada. |
| M. cinerascens Lawr. | *** | ** | * | * | *** | M. w. U. S. e. to Rocky Mts. |
| M. nuttingi Ridgw. | **** | * | * | | ** | R. s. Mex. to w. Costa Rica. |
| M. brachyurus Ridgw. | * | * | * | | * | R. Nicaragua. |
| M. yucatanensis Lawr. | * | * | * | | * | R. Yucatan. |
| M. sagrae Gundl. | * | * | * | | * | R. Cuba. |
| M. lucaysiensis Bryant | * | * | * | | * | R. Bahamas. |
| M. lawrencei (Giraud) | *** | * | * | | ** | R. s. Tex. to Guatemala. |
| M. l. olivascens Ridgw. | ** | ** | * | * | * | M. w. Mex. to s. Ariz. |
| M. flammulatus Lawr. | | * | * | | * | R. s. w. Mex. |
| Sayornis phœbe (Lath.) | | * | | ** | ** | M. e. N. A. n. of Gulf St. |
| S. nigricans (Swains.) | | * | | ** | * | M. coast from Ore. to Mex. |
| S. saya (Bonap.) | | * | | * | ** | M. w. N. A. n. to Saskatchewan. |
| Contopus borealis (Swains.) | **** | *** | * | ** | **** | E.M. n. U. S. northward. |
| C. pertinax Cab. | | *** | | * | * | R. Guatemala to s. Ariz. |
| C. virens (Linn.) | **** | *** | ** | ** | *** | M. e. U. S. n. to Canada. |
| C. richardsonii (Swains.) | *** | ** | ** | * | *** | E. M. w. N. A. to int. of B. C. |
| C. brachytarsus Scl. | * | * | ** | | * | R. Yucatan & s. Mex. |
| C. bahamensis Bryant | * | * | * | | ** | R. Bahamas. |
| C. caribæus (D'Orb.) | | * | | | * | R. Cuba. |
| Empidonax albigularis (Scl.) | ** | * | * | | * | R. s. e. Mex. & Guatemala. |
| E. difficilis Baird ♂ ♀ | ** | *** | * | | ** | M. w. U. S. n. to Sitka. |
| | | ** | | | ** | |
| E. flaviventris Baird ♂ ♀ | **** | * | * | | * | E. M. n. U. S. northward. |
| | | * | | | ** | |
| E. bairdii Scl. | * | * | * | | * | R. s. & e. Mex. |
| E. salvinii (Ridgw.) | * | *** | * | | **** | R. highlands of Guatemala. |
| E. acadicus (Gmel.) ♂ ♀ | ** | ** | ** | | *** | E. M. e. U. S. |
| | | * | | | * | |
| E. pusillus (Swains.) ♂ ♀ | ** | * | ** | * | ** | E. M. w. N. A. to Sitka. |
| | ** | * | ** | * | ** | |

## Table IV. — *Continued.*

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| E. p. traillii (Aud.)   ♂ | * | *** | * | | * | E. M. n. U. S. northward. |
| ♀ | | * | | | * | |
| E. minimus Baird   ♂♀♂ | ** | ** | *** | | ** | E. M. n. U. S. northward. |
| ♀ | | * | | | * | |
| E. hammondi (Xantus)   ♂ | ** | * | ** | * | * | E. M. w. N. A. n. to L. Slave Lake. |
| ♀ | | ** | | | ** | |
| E. wrightii Baird   ♂♀♂ | ** | * | * | | * | M. w. U. S. to Rocky Mts. |
| ♀ | | * | | | * | |
| E. fulvipectus Lawr. | | * | | | * | R. s. Mex. |
| E. fulvifrons rubicundus (Cab. & Hein.) | * | * | ** | | ** | R. s. Mex. |
| E. f. pygmæus (Coues) | * | ** | *** | * | * | R. s. Ariz. to w. Mex. |
| Pyrocephalus rubineus mexicanus (Scl.) | | * | | ** | * | R. Guatemala to s. U. S. |
| Ornithion imberbe (Scl.) | * | * | * | | ** | R. C. A. to s. Tex. |
| O. i. ridgwayi Brewst. | * | ** | ** | ** | ** | R.? w. Mex. to s. Ariz. |

## Table V.   Variation in the Corvidæ.

| Species | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Pica pica (Linn.) | ** | * | ** | | **** | R. n. & c. Europe. |
| P. p. hudsonica (Sab.) | **** | *** | ** | **** | **** | R. w. N.A. from N. M. to Ariz. |
| P. nuttalli Aud. | ** | * | *** | ** | ** | R. Cal. |
| Psilorhinus morio (Wagl.) | | * | | ** | ** | R. e. Mex. |
| P. cyanogenys Gray | | ** | | ** | * | R. e. Mex. & coast of Honduras. |
| P. mexicanus Rüpp. | | ** | | * | *** | R. s. Mex. to Costa Rica. |
| Cyanocitta cristata (Linn.) | ** | ** | *** | ** | ** | R. e. N. A. n. to fur countries. |
| C. c. florincola Coues. | ** | ** | ** | *** | ** | R. Fla. |
| C. stelleri (Gmel.) | **** | ** | *** | * | *** | R. n. w. coast from Cal. to Sitka. |
| C. s. frontalis Ridgw. | **** | ** | ** | ** | ** | R. Sierra Nevada. |
| C. s. annectens (Baird) | ** | ** | * | ** | ** | R. n. Rocky Mts. |
| C. s. macrolopha (Baird) | ** | ** | * | *** | *** | R. s. Rocky Mts. to n. Mex. |
| C. s. diademata (Bonap.) | ** | * | * | | * | R. highlands of c. Mex. |
| C. s. coronata (Swains.) | ** | * | * | | *** | R. s. Mex. to Guatemala. |
| Aphelocoma floridana (Bartr.) | ** | * | *** | *** | ** | R. Fla. |
| A. woodhousei (Baird.) | ** | ** | * | ** | *** | R. middle province of U. S. s. to Mex. |
| A. insularis Hensh. | ** | * | * | * | * | R. Santa Cruz I. |
| A. californica (Vig.) | *** | ** | **** | * | ** | R. coast from s. Cal. to Ore. |
| A. c. hypoleuca (Ridgw.) | ** | * | ** | * | ** | R. s. L. Cal. |
| A. sumichrasti Ridgw. | * | * | | | * | R. s. Mex. |
| A. couchi (Baird) | * | * | | | * | R. L. Rio Grande vall. |
| A. sieberii (Wagl.) | * | ** | * | ** | * | R. s. Mex. & southward. |
| A. s. arizonæ Ridgw. | * | * | * | ** | * | R. n.w. Mex. & adjacent Ariz. & N. M. |
| Xanthoura luxuosa (Less.) | | * | | * | ** | R. e. Mex. |
| Perisoreus canadensis (Linn.) | ** | * | ** | ** | ** | R. N. E. to Minn., n. to arctic regions. |

TABLE V. — *Continued.*

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| P. c. nigricapillus Ridgw. | ** | * | * | * | ** | R. coast region of Labrador. |
| P. c. fumifrons Ridgw. | ** | ** | * | **** | *** | R. Alaska. |
| P. c. capitalis Baird | ** | * | * | *** | * | R. Rocky Mts. from Ore. into B. A. |
| P. obscurus (Ridgw.) | *** | ** | ** | *** | ** | R. n. Cal. & n. Sierra Nevada to B. C. |
| Corvus corax Linn. | ** | * | * | | * | R. Eurasia. |
| C. c. sinuatus (Wagl.) | **** | *** | *** | **** | **** | R. w. U. S. to Guatemala. |
| C. c. principalis Ridgw. | **** | * | ** | **** | ** | R. n. N. A. from Greenland to Alaska. |
| C. c. behringianus Dybowski | ** | * | * | | * | R. Commander Is. |
| C. cryptoleucus Couch. | *** | * | ** | ** | ** | R. s. w. U. S. & table-lands of Mex. |
| C. americanus Aud. | ** | ** | * | **** | *** | R. e. N. A. except arctic dists. |
| C. a. floridanus Baird | ** | * | * | | ** | R. s. Fla. |
| C. caurinus Baird | *** | * | ** | * | *** | R. Wash. Terr. to Kodiak. |
| C. ossifragus Wils. | ** | ** | *** | *** | *** | R. coast from Long I. to La. |
| C. mexicanus Gmel. | ** | * | ** | **** | ** | R. w. Mex. |
| Nucifraga columbiana (Wils.) | | ** | | * | * | R.w. N. A. from Ariz. to Alaska. |
| Cyanocephalus cyanocephalus (Wied) | | * | | *** | * | R. w. N. A. between Rocky Mts. & Sierra Nevada. |

TABLE VI. VARIATION IN THE ICTERIDÆ.

| SPECIES. | | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|---|
| Dolichonyx oryzivorus (Linn.) | ♂ | | * | | * | * | E. M. e. N. A. in U. S. w. to Gt. Plains. |
| | ♀ | | * | | * | | |
| Molothrus ater (Bodd.) | ♂ | *** | *** | ** | * | *** | M. U. S. & s. Can. |
| M. a. obscurus (Gmel.) | ♂ | ** | ** | * | * | * | R. Mex., L. Cal., contiguous U. S. |
| Callothrus æneus (Wagl.) | ♂ | * | * | * | * | * | R. Rio Grande to Panama. |
| | ♀ | | | | * | | |
| Xanthocephalus xanthocephalus (Bonap.) | ♂ | | * | | * | * | M. marshes of w. U. S. |
| | ♀ | | * | | ** | * | |
| Agelaius phœniceus (Linn.) | ♂ | **** | *** | | **** | **** | M. nearly whole temp. N. A. |
| | ♀ | ** | *** | | *** | **** | |
| A. p. sonoriensis Ridgw. | ♀ | *** | * | ** | ** | * | R. n. w. Mex., s. Cal., lower Col. val. |
| A. p. bryanti Ridgw. | ♂ | * | | * | * | * | R. Bahamas & s. Fla. |
| | ♀ | ** | * | ** | * | * | |
| A. gubernator (Wagl.) | ♂ | **** | *** | * | ** | *** | R. vall. of Ore. & Cal. into Mex. |
| | ♀ | * | * | * | ** | | |
| A. assimilis Gundl. | ♂ | * | * | * | | * | R. Cuba. |
| | ♀ | | * | | | | |

### Table VI. — *Continued.*

| Species. | | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|---|
| A. tricolor (Nutt.) | ♂ | * | * | * | * | ** | R. coast vall. from s. Cal. to w. Ore. |
| | ♀ | * | * | * | * | **** | |
| Sturnella magna (Linn.) | ♂ | **** | ** | ** | | | R. e. N. A. n. to Canada, w. to Gt. Plains. |
| | ♀ | | ** | * | * | | |
| S. m. neglecta (Aud.) | ♂ | | ** | * | * | | R. w. N. A. from Manitoba to w. Mex. |
| | ♀ | | ** | * | * | | |
| S. m. mexicana (Scl.) | ♂ | | *** | ** | ** | | R. s. Ariz. & e. Mex. to Costa Rica. |
| | ♀ | | * | | | | |
| Quiscalus quiscula (Linn.) | ♂ | * | ** | * | **** | ** | M. Atlantic slope of U. S. from N. E. southward. |
| | ♀ | | | * | | | |
| Q. q. aglæus (Baird) | ♂ | * | * | * | *** | ** | R. G. coast from Fla. to La. |
| Q. q. æneus (Ridgw.) | ♂ | ** | * | * | ** | ** | M. c. N. A. n. to N. E. |
| | ♀ | | * | | * | * | |
| Q. macrourus Swains. | ♂ | ** | * | | ** | ** | R. s. Tex. to Nicaragua. |
| | ♀ | *** | ** | | *** | *** | |
| Q. graysoni Scl. | ♂ | * | ** | | ** | **** | R. w. Mex. |
| | ♀ | * | * | | * | * | |
| Q. major (Vieill.) | ♂ | ** | * | | *** | ** | R. s. Atlantic & G. coast of U. S. |
| | ♀ | ** | * | | ** | * | |
| Q. tenuirostris Swains. | ♀ | | | | * | *** | R. c. Mex. |

### Table VII.   Variation in the Fringillidæ.

| Species. | | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|---|
| Coccothraustes vespertina (Coop.) | | * | * | | **** | *** | I. M. w. N. A. n. to B. C. |
| Pinicola enucleator (Linn.) | | *** | * | ** | * | * | I. M. n. Eurasia. |
| Pyrrhula cassini (Baird) | | | * | | | * | M.? n. Alaska & portions of Siberia. |
| Carpodacus purpureus (Gmel.) | ♂ | | * | | * | * | M. e. U. S. northward. |
| C. p. californicus (Baird) | ♂ | | * | | | * | M. coast from s. Cal. to B. C. |
| C. cassini Baird | | | * | | * | *** | M. w. U. S. from B. C. to Mex. |
| C. mexicanus (Müll.) | ♂ | | * | | | * | R. e. & s. Mex. |
| C. m. frontalis (Say) | ♂ | | *** | | | *** | M. w. U. S. from 40° lat. to Mex. |
| C. amplus Ridgw. | | ** | * | ** | * | ** | R. Guadelupe I. |
| Loxia curvirostra minor (Brehm.) | | **** | ** | *** | ** | **** | M. n. N. A. e. of Gt. Plains. |
| L. c. stricklandi (Ridgw.) | | ** | * | ** | * | * | R. s. w. U. S. & Mex. |
| L. leucoptera Gmel. | | | | | * | | M. n. U. S. northward. |
| Leucosticte griseonucha (Brandt) | | **** | *** | *** | ** | **** | M.? Aleutian, Prybilof & Commander Is. |

## Table VII. — *Continued.*

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| L. tephrocotis littoralis (Baird) | **** | ** | ** | ** | **** | M. coast mt. ranges of n. w. N. A. |
| L. atrata Ridgw. | ** | ** | * | * | ** | M. (summer range unknown). |
| L. australis (Allen) | **** | ** | ** | ** | *** | M. high mts. of Col. |
| L. arctoa (Brandt) | | | | * | | M.? n. e. Asia. |
| Acanthis hornemannii (Holb.) ♂ | *** | * | ** | | * | M. n. Greenland & e. Arctic A. |
| ♀ | * | * | * | | * | |
| A. h. exilipes (Coues) ♂ | | * | ** | | * | M. circumpolar continental regions. |
| A. linaria (Linn.) ♀ | ** | * | ** | | ** | |
| ♂ | *** | * | * | | ** | M. n. portions of N. Hemisphere. |
| A. l. holbœlli (Brehm) ♀ | **** | * | * | | * | |
| ♂ | **** | * | * | | * | M. n. coasts of Eurasia, portions of Alaska. |
| A. l. rostrata (Coues) ♀ | **** | * | * | | ** | |
| ♂ | **** | * | *** | | ** | M. s. Greenland. |
| ♀ | **** | ** | ** | | * | |
| Spinus psaltria (Say) | * | | | ** | ** | M. w. U. S. from n. Cal. to Col. |
| S. lawrencei (Cass.) | | ** | | * | ** | M. Cal. |
| S. notatus (Du Bus.) | | ** | | * | *** | R. highlands of s. Mex. & Guatemala. |
| S. atriceps (Salv.) | | | | | * | R. Guatemala. |
| S. pinus (Wils.) | | | | *** | * | E. M. n. U. S. northward. |
| Carduelis carduelis (Linn.) | **** | * | | *** | * | R. Eurasia. |
| Plectrophenax nivalis (Linn.) ♂ | * | * | | * | ** | M. circumpolar regions. |
| ♀ | * | * | | * | * | |
| P. n. townsendi Ridgw. ♂ | ** | ** | | | ** | R. Alaska, Prybilof, & Commander Is. |
| P. hyperboreus Ridgw. ♀ | * | * | | | * | |
| ♂ ♀ | * | * | | * | * | M. Hall I. |
| Calcarius lapponicus (Linn.) ♂ | | * | | ** | ** | E. M. circumpolar regions. |
| ♀ | | * | | * | | |
| C. pictus (Swains.) ♂ | | * | | * | | E. M. int. of Arctic A. |
| ♀ | | * | | * | | |
| C. ornatus (Towns.) | | * | | | | M. Gt. Plains n. to Saskatchewan. |
| Poocætes gramineus (Gmel.) | *** | *** | * | **** | ** | M. e. N. A. from Va. to Ont. |
| P. g. confinis (Baird) | **** | *** | * | ** | *** | M. w. N. A. from B. C. southward. |
| Ammodramus princeps (Mayn.) | *** | ** | ** | ** | ** | M. Sable I. |
| A. sandvichensis (Gmel.) | **** | ** | ** | * | ** | M. n. w. coast from Unalaschka south. |
| A. s. savanna (Wils.) | ** | ** | *** | ** | *** | M. n. U. S. to Labr. |
| A. s. alaudinus (Bonap.) | * | * | ** | **** | *** | E. M. w. N. A. n. to Alaska. |
| A. s. bryanti Ridgw. | * | ** | **** | | ** | R. salt marshes of San Francisco Bay. |
| A. beldingi Ridgw. | **** | ** | * | * | *** | R. salt marshes of s. Cal. & L. Cal. |

## Table VII. — *Continued.*

| Species. | | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|---|
| A. rostratus Cass. | | * | *** | *** | | *** | M. coasts of s. Cal., L. Cal., & Sonora. |
| A. r. guttatus (Lawr.) | | | | | * | | R. Cape St. Lucas. |
| A. bairdii (Aud.) | | | *** | | ** | | M. Gt. Plains from Da. to Saskatchewan. |
| A. savannarum passerinus (Wils.) | | *** | ** | * | * | ** | M. e. U. S. & s. Canada. |
| A. s. perpallidus Ridgw. | | *** | * | * | ** | ** | M. w. U. S. e. to Gt. Plains. |
| A. henslowii (Aud.) | | | * | | ** | * | M. e. U. S. to Gt. Plains, n. to Ont. |
| A. lecontei (Aud.) | | | ** | | **** | **** | E. M. Gt. Plains from Da. to Manitoba. |
| A. caudacutus (Gmel.) | ♂ | ** | * | * | | *** | M. coast from Me. to N. Ca. |
|  | ♀ | * | ** | * | | ** | |
| A. c. nelsoni Allen | ♂ | * | * | * | | ** | M. marshes of Miss. vall. |
|  | ♀ | * | * | * | | ** | |
| A. maritimus (Wils.) | | ** | * | * | ** | * | M. coast from Mass. to Tex. |
| A. nigrescens Ridgw. | | **** | * | | * | *** | R. s. e. Fla. |
| Chondestes grammacus (Say) | | | *** | | *** | *** | M. Miss. vall. n. to Mich. |
| C. g. strigatus (Swains.) | | | ** | | ** | **** | M. w. U. S. e. to Gt. Plains. |
| Zonotrichia querula (Nutt.) | | | ** | | ** | ** | M. e. Gt. Plains from Mon. to Manitoba. |
| Z. leucophrys (Forst.) | | **** | ** | * | *** | ** | E. M. high mts. of w. U. S. to Labr. |
| Z.l. intermedea Ridgw. | | ** | ** | * | *** | * | E. M. Alaska & Mackenzie r. basin. |
| Z. l. gambeli (Nutt.) | | **** | *** | **** | *** | *** | M. coast mts. of Cal. to B. C. |
| Z. coronata (Pall.) | | | ** | | ** | * | M. n. Cal. to Norton Sound. |
| Z. albicollis (Gmel.) | | | ** | | **** | * | M. n. U. S. northward. |
| Spizella monticola (Gmel.) | | | ** | | * | ** | M. Labr. & Hudson B. region. |
| S. m. ochracea (Brewst.) | | | ** | | ** | *** | E. M. Alaska. |
| S. socialis (Wils.) | | | ** | | *** | *** | M. e. N. A. to Gt. Slave Lake. |
| S. s. arizonæ Coues | | | * | | ** | ** | E. M. w. N. A. n. to 60° lat. |
| S. pusilla (Wils.) | | | ** | | *** | ** | M. e. U. S. & s. Canada, w. to Gt. Plains. |
| S. p. arenacea Chadb. | | | * | | * | ** | M. Gt. Plains from Tex. to Wyoming. |
| S. pallida (Swains.) | | | ** | | *** | ** | E. M. Gt. Plains n. to Saskatchewan. |
| S. breweri Cass. | | | * | | ** | ** | M. w. U. S. e. to Rocky Mts. |
| S. atrigularis (Cab.) | | | * | | * | * | R. Mex., L. Cal. |
| Junco aikeni (Ridgw.) | ♂ | * | *** | * | | ** | I. M. Rocky Mts. in Col. & Wy. |
|  | ♀ | * | * | ** | | * | |
| J. hyemalis (Linn.) | ♂ | ** | * | * | | * | M. Me. to Alaska. |
|  | ♀ | * | * | * | | ** | |
| J. h. carolinensis Brewst. | ♂ | * | * | * | | * | R. s. Alleghany Mts. |
|  | ♀ | * | * | | | * | |
| J. h. oregonus (Towns.) | ♂ | ** | ** | * | | *** | M. coast from Cal. to Sitka. |
|  | ♀ | * | ** | * | *** | *** | |
| J. caniceps (Woodh.) | ♂ | * | * | * | | ** | M. Rocky Mt. distr. |
|  | ♀ | * | ** | ** | | * | |
| J. cinereus (Swains.) | | * | *** | * | | ** | R. highlands of Mex. |

## TABLE VII. — *Continued.*

| SPECIES. | | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|---|
| J. c. dorsalis (Henry) | ♂ | * | ** | * | | * | R. s. Rocky Mts. |
| | ♀ | * | * | * | | * | |
| J. c. palliatus Ridgw. | ♂ | * | ** | * | | *** | R. s. Ariz. & adjacent Mex. |
| | ♀ | * | * | ** | | ** | |
| J. alticola Salv. | | * | * | * | | * | R. highlands of Mex. |
| J. annectens (Baird) | ♂ | * | * | ** | | ** | M. Ft. Bridger northward. |
| | ♀ | * | ** | ** | | *** | |
| J. insularis Ridgw. | ♂ | * | * | * | | * | R. Guadelupe I. |
| | ♀ | | * | * | | * | |
| J. bairdi Belding | | ** | * | | | * | R. mts. of s. L. Cal. |
| Amphispiza bilineata (Cass.) | | | * | | *** | * | M. s. w. U. S. & Mex. |
| A. mystacalis (Hartl.) | | | | | | * | R. s. Mex. |
| A. humeralis (Cab.) | | | * | | | ** | R. s. Mex. |
| A. belli (Cass.) | ♂ | | ** | | | ** | R. Cal. to Cape St. Lucas. |
| | ♀ | | * | | | ** | |
| A. b. nevadensis (Ridgw.) | ♂ | | * | | | *** | M. w. U. S. from Mex. to Mon. |
| | ♀ | | * | | | ** | |
| Pencæa æstivalis (Licht.) | | * | ** | * | ** | ** | R. Fla. & lower Ga. |
| P. a. bachmani (Aud.) | | *** | ** | ** | *** | * | R. s. Atlantic & G. St. |
| P. mexicana (Lawr.) | | * | ** | * | * | *** | R. Mex. |
| P. botteri Scl. | | | * | | | * | R. s. e. Mex. |
| P. cassini (Woodh.) | | * | * | * | ** | * | M. s. w. border of U. S. |
| P. ruficeps (Cass.) | | * | * | * | * | * | R. Cal. |
| P. r. boucardi (Scl.) | | *** | ** | *** | * | * | R. Mex., s. Ariz., N. M., L. Cal. |
| P. carpalis Coues | | | * | | * | * | R. s. Ariz. |
| P. notosticta Scl. & Salv. | | * | * | * | | * | R. s. Mex. |
| Melospiza fasciata (Gmel.) | | * | ** | ** | ** | *** | R. e. U. S. & Brit. Prov. n. of 40° lat. |
| M. f. montana (Hensh.) | | * | **** | * | ** | *** | R. Rocky Mts. w. to Ore. & Nev. |
| M. f. heermanni (Baird) | | *** | ** | ** | * | *** | R. int. of Cal. |
| M. f. samuelis (Baird) | | ** | *** | * | * | **** | R. coast of Cal. |
| M. f. mexicana Ridgw. | | * | ** | * | | * | R. s. Mex. |
| M. f. fallax (Baird) | | * | ** | ** | * | *** | R. Ariz. |
| M. f. guttata (Nutt.) | | * | *** | * | *** | **** | R. coast from Ore. to Vancouver. |
| M. f. rufina (Bonap.) | | * | *** | ** | *** | **** | R. coast of s. Alaska. |
| M. cinerea (Gmel.) | | ** | * | * | *** | *** | R. Aleutian Is. |
| M. georgiana (Lath.) | | | * | | ** | ** | M. e. U. S. to Labr. |
| M. lincolni (Aud.) | | | *** | | ** | **** | E. M. n. U. S. northward. |
| Passerella iliaca (Merr.) | | * | ** | * | **** | ** | E. M. G. of St. Lawrence to Labr. & Alaska. |
| P. i. unalaschcensis (Gmel.) | | *** | *** | * | * | ** | E. M. coast of Alaska. |
| P. i. negarhyncha (Baird) | | **** | ** | ** | ** | *** | R. mts. of Cal. |
| P. i. schistacea (Baird) | | **** | * | * | ** | * | M. Rocky Mts. |
| Embernagra rufivirgata Lawr. | | | * | * | ** | * | R. Rio Grande vall. southward. |
| E. r. crassirostris Baird | | | * | * | | ** | R. s. Mex. |
| E. r. verticalis Ridgw. | | ** | * | ** | | * | R. Yucatan. |
| Pipilo erythrophthalmus (Linn.) | | | ** | | *** | *** | M. s. St. to B. A. |
| P. e. alleni Coues | | | * | | *** | * | R. Fla. |
| P. maculatus Swains. | | * | *** | * | | * | R. c. Mex. to Guatemala. |

## Table VII. — *Continued.*

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| P. m. arcticus (Swains.) | ** | * | * | * | * | M. Gt. Plains to Saskatchewan. |
| P. m. megalonyx (Baird) | *** | * | * | * | ** | M. Rocky Mts. from L. Cal. to Wash. |
| P. m. oregonus (Bell) | ** | * | * | * | ** | M. coast from Cal. to B. C. |
| P. consobrinus Ridgw. | | ** | ** | *** | *** | R. Guadelupe I. |
| P. carmani Lawr. | * | * | * | | * | R. Socorro I. |
| P. macronyx Swains. | * | * | * | * | | R. vall. of Mex. |
| P. chlorosoma Baird | | | * | * | | R. s. Mex. |
| P. chlorurus (Towns.) | | ** | | ** | ** | M. Rocky Mts. n. to Ore. |
| P. rutilus Licht. | | * | | | * | R. s. Mex. |
| P. fuscus Swains. | * | *** | ** | | *** | R. Mex. |
| P. f. mesoleucus (Baird) | ** | ** | * | * | * | R. N. M. & s. Ariz. |
| P. f. albigula (Baird) | ** | * | ** | * | ** | R. L. Cal. |
| P. f. crissalis (Vig.) | ** | * | * | * | * | R. Cal. |
| P. aberti Baird | | ** | | * | * | R. N. M. & Ariz. to Col. |
| Cardinalis cardinalis (Linn.) ♂ | ** | ** | ** | | *** | R. e. U. S. n. to 40° lat. |
| C. c. superbus Ridgw. ♂ | * | * | * | * | ** | R. w. Mex. to s. Ariz. |
| C. c. igneus (Baird) ♂ | ** | * | ** | * | * | R. L. Cal. |
| C. c. coccineus Ridgw. ♂ | * | * | ** | | * | R. e. & c. Mex. |
| C. c. yucatanicus Ridgw. ♂ | * | * | * | | * | R. Yucatan. |
| C. c. saturatus Ridgw. ♂ | * | * | * | | * | R. Cozumel I. |
| C. carneus (Less.) | * | * | * | | * | R. s. w. Mex. |
| C. phœniceus Gould ♂ | | * | | | *** | R. n. coast of S. A. |
| Pyrrhuloxia sinuata Bonap. | | * | | *** | ** | R. Mex. & contiguous U. S. |
| Habia ludoviciana (Linn.) | | * | | **** | * | E. M. n. U. S. & Canada. |
| H. melanocephala (Swains.) | | * | | *** | ** | M. w. U. S. e. to Gt. Plains. |
| Guiraca cærulea (Linn.) ♂ | * | * | | | * | M. s. e. U. S. |
| G. c. eurhyncha Coues ♂ | * | * | | | * | M. w. U. S. n. to Col. & Cal. |
| G. cyanoides concreta (Du Bus) | ** | ** | | * | *** | R. C. A. to e. Mex. |
| Passerina parellina (Bonap.) | ** | * | | * | * | R. s. & e. Mex. |
| P. amœna (Say) | | * | | **** | **** | M. w. U. S. e. to Gt. Plains. |
| P. cyanea (Linn.) | | * | | **** | ** | M. e. U. S. & s. Canada to Gt. Plains. |
| P. versicolor (Bonap.) ♂ | | * | | | * | R. e. Mex. to Tex. |
| P. v. pulchra Ridgw. ♂ | | * | | | ** | R. L. Cal. & w. Mex. |
| P. ciris (Linn.) | | * | | **** | * | M. s. Atlantic & Gulf St. |
| P. leclancheri Lafr. | | ** | | ** | **** | R. s. w. Mex. |
| P. rositæ (Lawr.) | | * | | * | * | R. s. Mex. |
| Sporophila morelleti (Bonap.) | | ** | | **** | ** | R. Rio Grande to Costa Rica. |
| S. torqueola Bonap. | | * | | | * | R. w. Mex. |
| S. corvina Scl. | | * | | | ** | R. e. Mex. to Costa Rica. |
| Euetheia bicolor (Linn.) | | * | | * | * | R. Bahamas. |
| Spiza americana (Gmel.) | | *** | | *** | **** | E. M. e. U. S. to Rocky Mts. |
| Calamospiza melanocorys Stejn. | | ** | | **** | *** | M. Gt. Plains from Kan. to beyond U. S. |

## Table VIII. Variation in the Vireonidæ.

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Vireo altiloquus barbatulus (Cab.) | ** | * | | * | ** | R. Cuba, Bahamas, s. Fla. |
| V. olivaceus (Linn.) | ** | * | | *** | * | E. M. e. N. A. n. to Hudson B. w. to Rocky Mts. |
| V. flavoviridis (Cass.) | * | ** | | * | **** | R. Rio Grande to Upper Amazon. |
| V. cinereus Ridgw. | * | * | | | | R. Cozumel I. |
| V. philadelphicus (Cass.) | * | ** | | | *** | E. M. e. N. A., chiefly n. of U. S. |
| V. gilvus (Vieill.) | * | ** | * | ** | ** | E. M. e. N. A. n. to Hudson B. w. to Gt. Plains. |
| V. g. swainsoni (Baird) | * | ** | * | ** | *** | E. M. w. U. S. e. to Rocky Mts. |
| V. flavifrons Vieill. | | * | | *** | *** | E. M. e. U. S. n. of middle Sts. w. to Gt. Plains. |
| V. solitarius (Wils.) | * | * | * | **** | * | E. M. e. N. A., chiefly n. of U. S. |
| V. s. cassinii (Xantus) | ** | * | ** | ** | * | M. w. U. S., chiefly on Pacific coast. |
| V. s. alticola Brewst. | **** | * | * | | * | M. higher s. Alleghany Mts. |
| V. s. plumbeus (Coues) | *** | * | * | * | ** | M. Rocky Mt. dist. of U. S. |
| V. atricapillus Woodh. | | * | | * | ** | M. s. Gt. Plains n. to Kan. |
| V. noveboracensis (Gmel.) | * | * | * | ** | ** | E. M. e. U. S. w. to Rocky Mts. |
| V. n. maynardi Brewst. | * | * | * | | * | R. Key West. |
| V. bellii (Aud.) | * | ** | * | **** | * | M. Gt. Plains n. to Da. |
| V. b. pusillus (Coues) | *** | * | ** | * | * | R. s. Cal., L. Cal., Ariz. |
| V. ochraceus Salv. | ** | * | | | * | R. Yucatan to Guatemala. |
| V. huttoni Cass. | ** | * | * | ** | * | R. Cal. |
| V. h. stephensi Brewst. | **** | * | * | * | * | R. Mex., Tex., L. Cal., Ariz. |
| V. pallens Salv. | | * | | | * | R. w. coast of Costa Rica & Nicaragua. |
| V. vicinior Coues | | * | ** | * | * | R. s. Cal., Ariz., N. M., n. w. Mex. |
| V. gundlachi Lemb. | | * | ** | | * | R. Cuba. |
| V. hypochryseus Scl. | | * | | | * | R. s. w. Mex. |
| Hylophilus decurtatus (Bonap.) | | ** | | | * | R. e. Mex. & Guatemala. |
| H. ochraceiceps Scl. | | * | | | * | R. s. Mex. s. to Costa Rica. |

## Table IX. Variation in the Mniotiltidæ.

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Mniotilta varia (Linn.) | | ** | | **** | *** | E. M. e. N. A. from Potomac r. to Hudson Bay. |
| Protonotaria citrea (Bodd.) | | * | | ** | * | M. Gulf St. & lower Miss. vall. |
| Helinaia swainsonii Aud. | ** | *** | *** | **** | *** | M. Gulf St. & lower Miss. vall. |
| Helmitherus vermivorus (Gmel.) ♂ | * | * | | *** | *** | M. e. U. S. n. to about 40°. |
| Helminthophila celata (Say) | | * | | *** | * | E. M. n. N. A. from Rocky Mts. to Alaska. |

TABLE IX. — *Continued.*

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| H. c. lutescens Ridgw. | | * | | * | * | M. coast from s. Cal. to Kodiak. |
| H. ruficapilla (Wils.) ♂ | | * | | *** | * | E. M. e. N. A. from n. U. S. to Hudson Bay. |
| H. r. gutturalis Ridgw. ♂ | | * | | | * | M. w. U. S. from Rocky Mts. to coast. |
| H. virginæ (Baird) | | * | | * | * | M. mts. of w. U. S. |
| H. luciæ (Coop.) | | * | | *** | ** | R. Ariz., s. e. Cal., Sonora. |
| Compsothlypsis americana (Linn.) | | * | | **** | *** | E. M. e. U. S. to Canada. |
| C. nigrilora (Coues) | | ** | | ** | ** | R. lower Rio Grande vall. |
| C. insularis (Lawr.) | | * | | | * | R. Tres Marias Is. |
| C. graysoni Ridgw. | | * | | | * | R. Socorro I. |
| Dendroica tigrina (Gmel.) | | | | **** | | E. M. n. N. A. to Hudson Bay. |
| D. olivacea (Giraud) | | | | *** | | R. Tex. to Guatemala. |
| D. æstiva (Gmel.) | | ** | | *** | *** | E. M. e. & n. N. A. |
| D. petechia (Linn.) ♂ | | * | | | ** | R. W. Indies, Cozumel I. |
| D. cærulescens (Gmel.) | | * | | *** | * | E. M. e. N. A. from n. N. E. northward. |
| D. coronata (Linn.) | | * | | **** | * | M. n. U. S. northward. |
| D. audubonii (Towns.) | | ** | | *** | ** | E. M. w. N. A. n. to B. C. |
| D. maculosa (Gmel.) | | * | | ** | ** | E. M. n. N. E. to Hudson Bay. |
| D. cærulea (Wils.) | | ** | | **** | ** | E. M. Miss. vall. to Alleghanies. |
| D. pennsylvanica (Linn.) | | ** | | ** | * | E. M. e. U. S. & Canada n. of 40° lat. |
| D. castanea (Wils.) | | * | | **** | * | E. M. n. N. E. to Hudson Bay. |
| D. striata (Forst.) | | * | | *** | * | E. M. n. N. E. & Labr. to Alaska. |
| D. dominica (Linn.) | **** | ** | | * | *** | M. s. Atlantic St. |
| D. d. albilora Baird | **** | * | | **** | ** | M. Miss. vall. n. to Gt. Lakes. |
| D. blackburniæ (Gmel.) | | ** | | **** | ** | E. M. e. N. A. from n. U. S. northward. |
| D. graciæ Coues | | ** | | * | * | M. s. Ariz. & N. M., Mex. |
| D. decora (Ridgw.) | | | | ** | * | R. s. Mex. & Guatemala. |
| D. nigrescens (Towns.) | | ** | | ** | * | M. w. U. S. n. to Ore. & Col. |
| D. chrysoparia Scl. & Salv. | | | | *** | * | R. c. Tex. & highlands of Guatemala. |
| D. virens (Gmel.) | | * | | **** | * | E. M. n. e. U. S. northward. |
| D. townsendi (Nutt.) | | ** | | * | ** | E. M. w. N. A. n. to Sitka. |
| D. occidentalis (Towns.) | | ** | | ** | * | M. w. U. S. near coast. |
| D. vigorsii (Aud.) | | ** | | ** | *** | M. e. U. S. n. to Ont. |
| D. kirtlandi Baird | | ** | | ** | ** | M. |
| D. discolor (Vieill.) | | * | | *** | ** | M. e. U. S. n. to Mich. & s. N. E. |
| D. palmarum (Gmel.) | | ** | ** | **** | *** | E. M. int. of N. A., n. to Gt. Slave Lake. |
| D. p. hypochrysea Ridgw. | | ** | * | *** | ** | M. coast from Nova Scotia to Hudson Bay. |
| Seiurus aurocapillus (Linn.) | | * | | **** | **** | E. M. e. N. A. to Alaska. |
| S. noveboracensis (Gmel.) | *** | ** | ** | **** | ** | E. M. n. e. U. S. northward. |
| S. n. notabilis (Grinn.) | **** | ** | * | *** | *** | E. M. w. N. A. to Miss. r. & Alaska. |

TABLE IX. — *Continued.*

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| S. motacilla (Vieill.) | ** | * | * | ** | * | M. e. U. S. n. to Gt. Lakes. |
| Geothlypsis formosa (Wils.) | | * | ** | *** | *** | E. M. e. U. S. to s. N. E. |
| G. agilis (Wils.) | | | ** | **** | *** | E. M. Manitoba. |
| G. philadelphia (Wils.) ♂ | | ** | * | | ** | E. M. N. E. northward. |
| ♀ | | *** | | | ** | |
| G. macgillivrayi (Aud.) ♂ | | * | * | | **** | E. M. Mts. of w. N. A. n. to |
| ♀ | | * | | | * | B. C. |
| G. trichas (Linn.) ♂ | | ** | | * | | M. e. U. S. n. to Canada. |
| G. t. occidentalis Brewst. | ** | ** | ** | **** | ** | M. w. U. S. |
| G. melanops Baird | | * | * | | * | R. e. & s. Mex. |
| G. beldingi Ridgw. | * | ** | * | | ** | R. s. L. Cal. |
| G. rostrata Bryant | * | * | * | | * | R. New Providence I. |
| G. tanneri Ridgw. | * | ** | * | | ** | R. Obaco I. |
| G. coryi Ridgw. | * | * | * | | | R. Eleuthera I. |
| G. speciosa Scl. | | * | * | | * | R. s. e. Mex. |
| G. poliocephala Baird | | * | | | | R. w. Mex. |
| G. palpebralis Ridgw. | * | * | | | | R. e. Mex. & Yucatan. |
| G. caninucha Ridgw. | * | * | * | | | R. Guatemala to Costa Rica. |
| Icteria virens (Linn.) ♂ | | ** | | | *** | M. e. U. S. n. to Ont. |
| I. v. longicauda (Lawr.) ♂ | | * | | | * | M. w. U. S. |
| Sylvania mitrata (Gmel.) | | ** | | ** | * | M. e. U. S. n. to N. E. |
| S. pusilla (Wils.) ♂ | | * | | | * | E. M. n. e. U. S. northward. |
| S. p. pileolata (Pall.) ♂ | | * | | | ** | E. M. w. N. A. to Kodiak. |
| S. canadensis (Linn.) | | * | | *** | * | E. M. e. N. A. n. to L. Winnipeg. |
| Setophaga ruticilla (Linn.) | | * | | **** | * | E. M. e. N. A. |
| Cardellina rubifrons (Giraud) | | ** | | ** | ** | R. highlands of Guatemala to s. Ariz. |
| Ergaticus ruber (Swains.) | | * | | ** | * | R. highlands of e. Mex. |
| Basileuterus culcicivorus (Licht.) | | * | | ** | * | R. Veragua to e. Mex. |
| B. belli (Giraud) | | ** | | ǀ | * | R. Guatemala & e. Mex. |
| B. delatrii Bonap. | | * | | ** | *** | R. Guatemala to Panama. |
| B. rubrifrons (Swains.) | | * | | * | * | R. s. Mex. |

TABLE X.　VARIATION IN THE TROGLODYTIDÆ.

| | | | | | | |
|---|---|---|---|---|---|---|
| Oroscoptes montanus (Towns.) | * | * | * | ** | * | M. Artemisia Plains of w. U. S. |
| Mimus polyglottos (Linn.) | *** | *** | *** | **** | **** | R. 38° lat. to Mex. & Bahamas. |
| M. lawrencei Ridgw. | * | * | ** | | * | R. s. Mex. |
| M. gracilis Cab. | * | *** | ** | | *** | R. Atlantic coast from Yucatan to Honduras. |
| M. gundlachii Cab. | *** | * | ** | | * | R. Bahamas, Cuba, Jamaica. |
| Galeoscoptes carolinensis (Linn.) | **** | * | * | *** | ** | E. M. e. N. A. n. to 54° lat. |
| Mimodes graysoni (Baird) | | * | * | * | * | R. Socorro I. |
| Harporhynchus rufus (Linn.) | **** | ** | * | ** | *** | M. e. U. S. to Rocky Mts. n. to Ont. |

TABLE X. — *Continued.*

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| H. longirostris (Lafr.) | **** | ** | * | ** | ** | R. e. Mex. & s. Tex. |
| H. guttatus Ridgw. | * | * | * | | * | R. Cozumel I. |
| H. cinereus Xantus | * | * | ** | | * | R. L. Cal. |
| H. bendirei Coues | * | * | * | ** | *** | R. s. Ariz. |
| H. curvirostris (Swains.) | *** | * | * | * | * | R. Mex., s. Tex. & s. N. M. |
| H. c. palmeri Ridgw. | *** | * | ** | * | * | R. s. Ariz. |
| H. c. occidentalis Ridgw. | *** | * | | | * | R. coast of w. Mex. |
| H. redivivus (Gamb.) | **** | ** | ** | ** | *** | R. Pacific coast of Cal. & L. Cal. |
| H. lecontei (Lawr.) | **** | * | ** | * | ** | R. vall. of Colorado and Gila rivers. |
| H. crissalis (Henry) | **** | * | * | ** | ** | R. N. M., Ariz., s.Utah, s. Cal., n. L. Cal. |
| Heleodytes brunneicapillus (Lafr.) | *** | * | * | * | * | R. s. w. border of U. S. |
| H. affinis (Xantus) | ** | * | * | * | * | R. s. L. Cal. |
| Salpinctes obsoletus (Say) | **** | * | * | **** | ** | R. arid distr. of w. U. S. s. to Guatemala. |
| S. guadeloupensis Ridgw. | * | * | ** | | *** | R. Guadelupe I. |
| Catherpes mexicanus (Swains.) | **** | **** | ** | * | * | R. Mex., s. Tex. |
| C. m. conspersus Ridgw. | **** | * | ** | * | **** | R. s. w. U. S. n. to Ore. |
| Thryothorus ludovicianus (Lath.) | *** | ** | **** | ** | **** | M. n. e. Mex. & e. U. S. to 40° lat. |
| T. l. miamensis Ridgw. | ** | ** | ** | ** | *** | R. s. e. Fla. |
| T. albinucha (Cabot) | * | * | | * | * | R. Yucatan & Guatemala. |
| T. bewickii (Aud.) | ** | * | **** | ** | ** | M. e. U. S. n. to 40° lat., w. to Gt. Plains. |
| T. b. spilurus (Vig.) | *** | * | **** | ** | ** | R. coast from w. Mex. to B. C. |
| T. b. bairdi Salv. & Godm. | **** | ** | ** | *** | **** | R. table-lands of Mex. to Kan. |
| T. brevicaudus Ridgw. | * | * | * | | | R. Guadelupe I. |
| T. felix Scl. | * | ** | ** | ** | * | R. w. Mex. |
| T. lawrenci (Ridgw.) | * | * | | * | * | R. Tres Marias Is. |
| T. maculipectus Lafr. | ** | * | * | | * | R. s. Mex. |
| T. m. umbrinus Ridgw. | * | * | * | | * | R. Guatemala. |
| T. m. cannobrunneus Ridgw. | * | * | * | | * | R. Yucatan. |
| Troglodytes insularis Baird | * | * | *** | | * | R. Socorro I. |
| T. beani Ridgw. | ** | * | * | | * | R. Cozumel I. |
| T. aëdon (Vieill.) | ** | ** | *** | **** | **** | M. e. U. S. & Canada, w. to Miss. vall. |
| T. a. parkmanii (Aud.) | **** | ** | *** | **** | ** | M. w. U. S. s. to Vera Cruz. |
| T. intermedius Cab. | ** | * | ** | | ** | R. s. Mex. to Costa Rica. |
| T. brunneicollis Scl. | * | * | * | * | ** | R. s. e. Mex. |
| T. hiemalis Vieill. | *** | ** | *** | *** | **** | M. n. e. U. S. northward. |
| T. h. pacificus Baird | ** | * | * | *** | ** | M. coast from s. Cal. to Sitka. |
| T. alascensis Baird | ** | * | ** | * | *** | R. Aleutian & Prybilof Is. |
| Cistothorus stellaris (Licht.) | *** | ** | * | **** | * | M. e. U. S. w. to Gt. Plains. |
| C. polyglottus Vieill. | ** | * | * | | **** | R. e. trop. A. from Mex. to Brazil. |
| C. palustris (Wils.) | **** | *** | ** | **** | *** | M. e. U. S. & Brit. Prov. |
| C. p. paludicola Baird | ** | ** | ** | **** | ** | M. w. U. S. to Rocky Mts. |

## Table XI. Variation in the Paridæ.

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Sitta carolinensis Lath. | ** | * | * | *** | ** | R. e. U. S. & Brit. Prov. |
| S. c. aculeata (Cass.) | *** | ** | * | **** | *** | R. w. U. S. into Mex. |
| S. canadensis Linn. | | * | | *** | | M. chiefly n. of U. S. |
| S. pusilla Lath. | **** | | | ** | | R. s. Atlantic & Gulf St. |
| S. pygmæa Vig. | * | | | *** | | R. w. U. S. to Mex., e. to Rocky Mts. |
| Parus atricristatus Cass. | | ** | | | *** | R. e. Mex. to s. Tex. |
| P. a. castaneifrons Sennett | | * | | | * | R. e. Tex. |
| P. inornatus Gamb. | * | * | ** | ** | *** | R. coast from s. Cal. to Ore. |
| P. i. cinerascens Ridgw. | **** | | * | * | * | R. s. L. Cal. |
| P. i. griseus Ridgw. | **** | * | ** | * | ** | R. Rocky Mt. distr. of U. S. |
| P. wollweberi (Bonap.) | | * | | ** | ** | R. highlands of Mex. to s. Ariz. |
| P. gambeli Ridgw. | | ** | | *** | * | R. mts. of w. U. S. |
| P. meridionalis Scl. | | * | * | ** | * | R. highlands of Mex. n. to s. Ariz. |
| P. carolinensis Aud. | | * | *** | * | *** | R. e. U. S. s. of 40° lat. |
| P. atricapillus (Linn.) | | * | * | **** | * | M. e. N. A. n. of 40° lat. |
| P. a. occidentalis (Baird) | | *** | * | *** | ** | R. n. w. coast distr. of U. S. |
| P. a. septentrionalis (Harris) | | * | *** | **** | *** | R. Rocky Mt. distr. from N. M. to Alaska. |
| P. cinctus obtectus (Cab.) | | * | * | * | * | R. e. Siberia & n. Alaska. |
| P. hudsonicus Forst. | | ** | ** | *** | **** | R.? n. N. A. e. of Rocky Mts. |
| P. rufescens Towns. | | *** | *** | ** | *** | R. coast from Ore. to s. Alaska. |
| P. r. neglecta Ridgw. | | ** | *** | | ** | R. coast of Cal. |
| Psaltiparus minimus californicus Ridgw. | | ** | ** | ** | *** | R. Cal. |
| P. m. grindæ (Beld.) | | * | | | * | R. s. L. Cal. |
| P. plumbeus Baird | | * | ** | ** | * | R. Rocky Mts. from Col. to s. Ariz. |
| P. melanotis (Hartl.) | | * | * | | * | R. highlands of Mex. & Guatemala. |
| Auriparus flaviceps (Sund.) | | ** | | *** | *** | R. arid distr. of n. Mex. & contiguous U. S. |
| Chamæa fasciata Gamb. | ** | *** | * | | *** | R. coast of Cal. |
| C. f. henshawi Ridgw. | ** | ** | ** | | *** | R. int. of Cal. |

## Table XII. Variation in the Turdidæ.

| Species. | Culmen. | Wing. | Tarsus. | Whole Length. | Tail. | Migration. Breeding Area. |
|---|---|---|---|---|---|---|
| Myadestes townsendi (Aud.) | | ** | | * | ** | M. mts. of w. U. S. to B. C. |
| M. obscurus Lafr. | ** | * | * | | | R. highlands of e. Mex. & Guatemala. |
| M. o. occidentalis Stejn | * | * | * | | | R. c. & w. Mex. |
| M. o. insularis Stejn | | * | | | * | R. Tres Marias Is. |
| M. unicolor Scl. | | * | * | | * | R. highlands of s. Mex. & Guatemala. |
| Turdus mustelinus Gmel. | **** | * | * | ** | ** | M. e. U. S. to Mass. |
| T. fuscescens Steph. | *** | ** | *** | **** | **** | E. M. e. N. A. between 40° & 50° lat. |

TABLE XII. — *Continued.*

| SPECIES. | CULMEN. | WING. | TARSUS. | WHOLE LENGTH. | TAIL. | MIGRATION. BREEDING AREA. |
|---|---|---|---|---|---|---|
| T. f. salicicolus (Ridgw.) | * | ** | ** | ** | *** | M. Rocky Mts. |
| T. aliciæ Baird | **** | *** | *** | ** | *** | E. M. Labr. to Arctic coast. |
| T. a. bicknelli (Ridgw.) | * | ** | ** | *** | * | E. M. Catskill Mts. to Nova Scotia. |
| T. ustulatus (Nutt.) | **** | ** | * | ** | *** | E. M. Pacific coast n. to Sitka. |
| T. u. swainsonii (Cab.) | ** | * | ** | *** | ** | E. M. e. N. A. n. of U. S. |
| T. aonalaschkæ Gmel. · | *** | *** | * | *** | *** | M. coast from Cal. to Kodiak. |
| T. a. auduboni (Baird) | ** | *** | ** | ** | *** | M. Rocky Mts. |
| T. a. pallasii (Cab.) | **** | ** | ** | *** | **** | M. n. e. U. S. northward. |
| T. iliacus Linn. | ** | * | * | ** | *** | M. n. Eurasia. |
| Merula migratoria (Linn.) | * | ** | * | ** | * | M. e. N. A. n. to Hudson Bay & Alaska. |
| M. m. propinqua Ridgw. | ** | * | *** | ** | **** | M. w. U. S. n. to B. C., e. to Rocky Mts. |
| M. confinis (Baird) | ** | *** | * |  | * | R. s. L. Cal. |
| M. flavirostris Swains. | *** | ** | * |  | *** | R. w. & s. Mex. |
| M. graysoni Ridgw. | * | * |  |  | * | R. Tres Marias Is. |
| Hesperocichla nævia (Gmel.) |  | * |  | ** | * | M. w. N. A. n. to Behring Strait. |
| Cyanecula suecica (Linn.) | * | * | * |  | * | M. n. Eurasia. |
| C. wolfii Brehm | ** | * | ** |  | * | M. c. Europe. |
| Saxicola œnanthe (Linn.) | ** | ** | **** | *** | **** | M. n. portions of N. Hemisphere. |
| Sialia sialis (Linn.)      ♂ | * | * | * | **** | ** | M. e. U. S. w. to Rocky Mts. |
| ♀ |  | * |  |  | * |  |
| S. s. azurea (Baird)      ♂ | * | * | * |  | * | R. highlands of Mex.& s.Ariz. |
| S. s. guatemalæ Ridgw.    ♂ |  | * | * |  | * | R. highlands of Honduras & Guatemala. |
| ♀ |  | * |  |  | * |  |
| S. mexicana Swains.       ♂ | ** | ** | ** | * | *** | R. w. U. S n. to B. C., e. to Rocky Mts. |
| S. arctoa (Swains.)       ♂ |  | * |  | **** | * | M. Rocky Mts. n. to Gt. Slave Lake. |
| ♀ |  |  |  | * | * |  |

The following table (XIII) represents the percentage of species and subspecies examined, of fourteen families, evincing variation of at least 1.5% in two dimensions. Only such species and subspecies enter into the computation for which Ridgway's measurements express variation in at least three of the five dimensions ; and the percentages have been deduced separately for non-migratory, migratory, and extensively migratory species and subspecies.

Table XIII.

| Family. | Non-migratory Species. | | Migratory Species. | | Extensively Migratory Species. | |
|---|---|---|---|---|---|---|
| | Number examined. | Per cent. | Number examined. | Per cent. | Number examined. | Per cent. |
| Anatidæ | 3 | 33.3 | 31 | 35.4 | 17 | 41.1 |
| Rallidæ | 3 | 66.6 | 8 | 25 | 5 | 80 |
| Scolopacidæ | 1 | 100 | 21 | 42.8 | 22 | 50 |
| Falconidæ | 25 | 32 | 10 | 40 | 4 | 25 |
| Picidæ | 36 | 19.4 | 8 | 25 | 1 | 0 |
| Tyrannidæ | 29 | 10.3 | 13 | 23 | 8 | 25 |
| Corvidæ | 42 | 30.9 | 0 | | 0 | |
| Icteridæ | 17 | 17.6 | 5 | 40 | 1 | 0 |
| Fringillidæ | 59 | 23.7 | 64 | 26.5 | 15 | 40 |
| Vireonidæ | 9 | 0 | 5 | 0 | 7 | 14.2 |
| Mniotiltidæ | 14 | 0 | 19 | 26.3 | 24 | 33.3 |
| Troglodytidæ | 37 | 21.6 | 11 | 63.6 | 1 | 100 |
| Paridæ | 22 | 36.3 | 1 | 0 | 0 | |
| Turdidæ | 9 | 11.1 | 15 | 33.3 | 5 | 60 |

The following table (XIV) represents the percentage of species and subspecies examined, of nine families, which exhibit an amount of variation of at least 1.5% in two out of at least three dimensions examined. These percentages are computed separately: for (1) distinct species (*i.e.* without geographical races), and (2) geographical races. In this computation necessarily *Melospiza fasciata, e.g.,* is considered a race, just as is *M. f. montana.*

Table XIV.

| Family. | Distinct Species. | | Geographical Races. | |
|---|---|---|---|---|
| | Number examined. | Per cent. | Number examiued. | Per cent. |
| Anatidæ | 56 | 25 | 12 | 33.3 |
| Rallidæ | 12 | 50 | 2 | 100 |
| Falconidæ | 26 | 34.6 | 13 | 30.7 |
| Picidæ | 19 | 15.7 | 26 | 19.2 |
| Corvidæ | 17 | 23.5 | 24 | 33.3 |
| Fringillidæ | 60 | 21.6 | 74 | 32.4 |
| Troglodytidæ | 31 | 22.5 | 19 | 47.3 |
| Paridæ | 11 | 27.2 | 17 | 29.4 |
| Turdidæ | 14 | 14.2 | 16 | 43.7 |

The following four tables (XV–XVIII) give respectively the percentage of those species and subspecies examined, in each family enumerated, showing an amount of individual variation of at least 2% in the particular dimension (culmen, wing, tarsus, or whole length). Only such families are computed, for which Ridgway's data express individual variation in the particular dimension for at least ten species.

TABLE XV.

Comparative tabulation of percentage of species examined, with variation in the length of the *culmen* of at least 2%.

| FAMILY. | Number of species examined. | 0% | .5% | 1% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|
| Vireonidæ | 18 | | | | 11.1 | | | | |
| Tyrannidæ | 43 | | | | 13.9 | | | | |
| Mniotiltidæ | 14 | | | | 14.2 | | | | |
| Icteridæ | 31 | | | | 16.1 | | | | |
| Corvidæ | 35 | | | | 16.6 | | | | |
| Turdidæ | 24 | | | | 16.6 | | | | |
| Falconidæ | 64 | | | | 17.1 | | | | |
| Fringillidæ | 109 | | | | 17.4 | | | | |
| Troglodytidæ | 47 | | | | | 23.4 | | | |
| Picidæ | 42 | | | | | 28.5 | | | |
| Rallidæ | 16 | | | | | | 37.5 | | |
| Paridæ | 10 | | | | | | | 40 | |

TABLE XVI.

Comparative tabulation of percentage of species examined, with variation in the length of the *wing* of at least 2%.

| FAMILY. | Number of species examined. | 0% | .5% | 1% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|
| Vireonidæ | 26 | 0 | | | | | | | |
| Mniotiltidæ | 71 | 0 | | | | | | | |
| Paridæ | 26 | 0 | | | | | | | |
| Turdidæ | 32 | 0 | | | | | | | |
| Icteridæ | 43 | 0 | | | | | | | |
| Corvidæ | 42 | 0 | | | | | | | |
| Tyrannidæ | 63 | 0 | | | | | | | |
| Fringillidæ | 181 | | .55 | | | | | | |
| Troglodytidæ | 49 | | | 2 | | | | | |
| Picidæ | 48 | | | 4.1 | | | | | |
| Falconidæ | 89 | | | 7.8 | | | | | |
| Rallidæ | 16 | | | | 12.5 | | | | |

## Table XVII.

Comparative tabulation of percentage of species examined, with variation in the length of the *tarsus* of at least 2%.

| Family. | Number of species examined. | 0% | .5% | 1% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|
| Vireonidæ | 14 | o | | | | | | | |
| Paridæ | 20 | o | | | | | | | |
| Tyrannidæ | 34 | o | | | | | | | |
| Icteridæ | 23 | o | | | | | | | |
| Fringillidæ | 99 | | | 2 | | | | | |
| Corvidæ | 34 | | | 2.9 | | | | | |
| Turdidæ | 25 | | | 4 | | | | | |
| Mniotiltidæ | 18 | | | 5.5 | | | | | |
| Rallidæ | 16 | | | 6.2 | | | | | |
| Troglodytidæ | 46 | | | 6.5 | | | | | |
| Falconidæ | 78 | | | | 19.2 | | | | |

## Table XVIII.

Comparative tabulation of percentage of species examined, with variation in the *whole length* of at least 2%.[1]

| Family. | Number of species examined. | 0% | .5% | 1% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|---|---|
| Picidæ | 31 | | | 3.2 | | | | | |
| Tyrannidæ | 28 | | | 7.1 | | | | | |
| Icteridæ | 39 | | | | 10.2 | | | | |
| Fringillidæ | 118 | | | | 11.8 | | | | |
| Vireonidæ | 16 | | | | 12.5 | | | | |
| Falconidæ | 53 | | | | 13.2 | | | | |
| Paridæ | 22 | | | | 13.6 | | | | |
| Turdidæ | 20 | | | | 15 | | | | |
| Corvidæ | 34 | | | | 17.6 | | | | |
| Troglodytidæ | 34 | | | | | 20.5 | | | |
| Mniotiltidæ | 53 | | | | | 28.3 | | | |
| Rallidæ | 12 | | | | | | | 41.6 | |

[1] Whenever a species has been considered in the calculations of Tables XV–XVIII, for which Ridgway gives the extremes of variation in a particular dimension separately for the sexes, I have computed and added the percentage of each sex as an equivalent to that of a species. This method would hardly impede the general accuracy of the percentages.

## Table XIX.

Exhibiting variation in relation to sex, the figures in the vertical columns referring to the number of species and subspecies examined.

| FAMILY. | ♂ | | ♀ | |
|---|---|---|---|---|
| | Larger, with greatest variation. | Smaller, with greatest variation. | Larger, with greatest variation. | Smaller, with greatest variation. |
| *Culmen.* | | | | |
| Falconidæ | 0 | 10 | 13 | 0 |
| Trochilidæ | 1 | 6 | 0 | 1 |
| Icteridæ | 6 | 0 | 0 | 4 |
| Fringillidæ | 7 | 3 | 0 | 1 |
| *Wing.* | | | | |
| Falconidæ | 0 | 19 | 16 | 0 |
| Trochilidæ | 2 | 5 | 1 | 0 |
| Icteridæ | 8 | 0 | 0 | 5 |
| Fringillidæ | 13 | 0 | 1 | 9 |
| *Tarsus.* | | | | |
| Falconidæ | 2 | 18 | 8 | 1 |
| Trochilidæ | | | | |
| Icteridæ | 3 | 0 | 0 | 2 |
| Fringillidæ | 6 | 0 | 1 | 6 |
| *Whole Length.* | | | | |
| Falconidæ | 0 | 11 | 9 | 0 |
| Trochilidæ | 0 | 5 | 2 | 0 |
| Icteridæ | 5 | 0 | 0 | 8 |
| Fringillidæ | 1 | 0 | 0 | 4 |
| Totals, | 54 | 77 | 51 | 41 |
| | = 131 | | = 92 | |

## Table XX.

Differing from the preceding table in so far, that in addition to the four families therein enumerated, all species and subspecies of other families are computed, for which Ridgway has given individual extremes of measurements for each sex.

| DIMENSIONS. | ♂ | | ♀ | |
|---|---|---|---|---|
| | Larger, with greatest variation. | Smaller, with greatest variation. | Larger, with greatest variation. | Smaller, with greatest variation. |
| Culmen | 15 | 19 | 15 | 7 |
| Wing | 41 | 26 | 20 | 21 |
| Tarsus | 11 | 20 | 9 | 10 |
| Whole length | 16 | 17 | 12 | 14 |
| Totals, | 83 | 82 | 56 | 52 |
| | = 165 | | = 108 | |

### C. *Direct Inferences from the Tabulated Data.*

(*a*) It is the rule that, in genera comprising more than one species, those species which inhabit small or insular breeding areas do not evince as much individual variation in the dimensions as do species with more extensive and diversified breeding areas. This fact becomes at once apparent after a study of the Tables I–XII, when a comparison is made between species inhabiting small islands, or other restricted districts, and those which have a much wider distribution. But few exceptions are to be found to this rule.

(*b*) It is the rule that species with geographical races, when the latter differ from one another in one or more dimensions, evince a greater amount of individual variation than do species which are not divided into such races, provided that the breeding area is approximately equal in extent and diversification in both cases. Thus of the nine families tabulated in Table XIV, in all, with the single exception of the *Falconidæ*, a greater percentage of geographical races evince variation to the amount of 1.5% in two dimensions, than of species which are not split into races. If, however, the geographical races of a species differ from one another mainly in color (as *e.g.* those of *Cardinalis cardinalis*), and less or not at all in dimensions, then as a rule they do not evince a greater amount of variation in the dimensions than do species without geographical races.[1] In Table XIV, further, the percentages in favor of geographical races may still be increased, when we consider that many species which are as yet regarded as distinct, may in the future be classed by ornithologists as subspecies, — resulting in a subtraction from the left hand column of percentages, and an addition to that on the right.

(*c*) It is the rule, subject to the preceding two "laws," that migratory species evince a greater amount of individual varia-

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[1] Though I have not investigated in birds the facts of individual *color* variation, — a kind of variation of which it is obviously difficult to determine the amount, I would be inclined to conclude, *a priori*, that the races of a species differing from one another mainly in color would present a greater amount of color variation than would species which are not divisible into color races, other factors being equal in both cases.

tion than do non-migratory species ; and species which undertake extensive migrations, a greater amount than species which make migrations of less magnitude (Table XIII). This fact is conformable with our "law" *a*, since migratory species have as a rule more extensive breeding areas than have non-migratory species. Thus of the 110 species which undertake extensive migrations, entering into the computation of table XIII, 104 (94.5% inhabit breeding areas of comparatively great extent, while but 6 (5.4%) inhabit small areas.[1]

(*d*) It is the rule, that males exhibit a greater amount of individual variation in the dimensions than do females of the same species or subspecies. For of the 223 computed measurements of both sexes in Table XIX, in 131 cases (58.7%) the males show the greater amount of variation, and in 92 cases (41.2%) the females, — a difference of about 17.5% in favor of the males ; and of the 273 measurements of both sexes computed in Table XX, in 165 cases (60.4%) the males show the greater amount of variation, and in 108 cases (39.5%) the females show the greater amount, — a difference of about 20.8% to the advantage of the males.

(*e*) It is the rule that there is less variation in the length of the wing than in the length of the culmen, tarsus, or whole bird. This fact becomes at once apparent, by comparing the "curve" of variation expressed in Table XVI with the "curves" of variations of the other three dimensions (Tables XV, XVII, XVIII).

We may now consider the support given by these five "laws" to the thesis, that continuing development is always accompanied by variability.

To recapitulate briefly : it follows from the data given, that the greatest amount of individual variation occurs, as a rule, in those species occupying the most extensive breeding areas ; that of two species occupying breeding areas approximately equivalent in extent, the one divided into geographical sub-

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[1] These six species are *Ammodramus lecontei, Spizella monticola ochracea, Passerella iliaca unalaschcensis, Chen caerulescens, C. hyperborea, Turdus aliciae bicknelli ;* of these, only the first and third evince variation to the amount of 1.5% in two dimensions.

species evinces a greater amount of variation than the "stable"
species ; and that species which undertake extensive migra-
tions exhibit more individual variation, other factors being
equal, than do species which do not migrate.   Now in the first
part of this second section, we have found that the presence
of geographical races (subspecies) and migration are two
criteria of continuing development.   Therefore, the fact that
the amount of variation is greater in migratory species, and in
species which exhibit geographical races, than in non-migratory
species, or than in species which present no geographical sub-
species, is a sufficient proof for the assertion that continuing
development is always associated with variability.   In other
words, individual variation is greater in amount in those
species which we must consider under the influence of a con-
tinuing process of development than in those species which
we must consider as being influenced by no process of develop-
ment at all, or by a much less energetic development.   And as
we have found variation is as a rule more marked in migratory
than in non-migratory species, and in extensively migratory
than in less migratory species ; and further, that as a rule,
greater individual variation is present in the several races of a
species which exhibits a large number of races than in the
races of a species exhibiting a smaller number, — therefore we
must conclude that the amount of individual variation stands
in a direct ratio to the activity and energy of the operating
process of development.   In short, the logical sequence from
the facts given is plainly that the amount of individual
variation stands in a direct ratio to the degree of complex-
ity of the environmental forces which influence the organism.
A species with an extensive breeding area, or one which
meets with environmental changes in the course of its
migrations, is more variable than a species with a restricted
and little diversified breeding area, or than a non-migratory
species, which does not come into contact with new environ-
ments.

The fact that the dimensions of birds are more variable in
the males than in the females is interesting, as offering a
parallel to the case in man, where, too, the males are more

variable.[1]   Also in domesticated animals, the males are more variable (Darwin).   Why the wing should be less variable than the other dimensions, is difficult of solution on any other ground, than that the wing has but one main function, while the tarsus and the bill are put to a diversity of uses, which would result in the two latter being more variable.


### III. On the Origin of Variation.

The doctrines of Lamarck and Darwin teach that the organism is more or less adapted to its environment ; even Bateson (*l.c.*, p. 10) grants a certain amount of such adaptation, though in the main he militates against the assumption of any complete degree of adaptation.   We may then start out from the assumption, for the correctness of which there is strong evidence, that it is a biological law for the organism to be more or less completely in correlation with its environment, in order to insure its existence.   It is even permissible to go further, and assert that its chance of existence will stand in direct proportion to the degree of its adaption to the environment.   (By the term environment, which is used here in its full sense, is meant the sum total of all the external forces acting upon the organism.)   Therefore it is obvious that the chance of the organism's existence must depend upon its degree of correlation with the environment, or, in other words, with its readiness of response to the external conditions.   This law being so reasonable, and so thoroughly in accord with our modern biological ideas, it is not necessary to enter into any further discussion of its plausibility.

For the purpose of advancing further deductions as to the primal cause of variation, we may proceed from the consideration of a species which, we have reasons to conclude, is, comparatively speaking, perfectly adapted to its environment.   We

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[1] It is not impossible, that in birds, as in man, the female may be more conservative and less progressive than the male, and passing a more (physiologically) monotonous existence than the latter, is less influenced by the struggle for existence, and accordingly is less variable structurally.   This suggestion has, however, no more value than that of a mere comparison.

may take, for example, a species inhabiting a small island,
which is comparatively uniform in character throughout its
extent (in vegetation, etc.), and at all seasons of the year
(climate, etc.).    Such insular species are very numerous among
land-inhabiting animals of tropical distribution.    The environ-
ment influencing the insular species being then so uniform and
unchanging in its action upon it, the species could more easily
and quickly adapt itself to it, than if the environment were
changeable.    Taking then an insular species, which, we have
reasons to suppose, has inhabited a particular island for a com-
paratively long period, — and there are usually certain criteria
whereby we may judge whether its residence there has been of
long duration, — we must suppose that it had time to become
adapted to its environment; and if we have equally good
reasons for supposing that the character of the environment
itself has not changed, it must seem probable that its adaptation
to the environment is comparatively perfect.    Granting such a
species, accordingly, to be closely adapted to its environment,
let us consider what changes, if any, would occur in the
organism, if the environment should change.    And it may be
remarked just here, that marked changes have been recorded
by geologists and others in different districts, as is well known,
such as a surface rising or sinkage, changes in vegetation due
to a prolonged drought, the invasions of organisms strange to
the region, destruction of life caused by epidemics, etc.
And in fact, in many districts where the environment appears
to the human sense to be practically unchanging, a slow and
gradual change may nevertheless be taking place.

　　Now such an organism is adapted to its environment, at the
same time that its various organs must necessarily be cor-
related to one another.    By correlation of the organs is meant,
as was explained in the introductory part of this paper, their
mutual dependence upon, and concerted physiological action
with, each other.    Thus, in the case under question, we must
treat together the two facts : (1) the adaptation of the organism
to its environment, and (2) the physiological and morphological
correlation of its organs.    When a change of environment
occurs, *i.e.* when consequently a new and different environment

commences to influence the organism, the latter cannot at first be adapted to this new environment, but must become, so to speak, out of touch with it. This change of environment must then influence the organism, primarily, by disturbing or interrupting the correlation of its organs. For even should the change of environment interrupt directly the physiological action of only one of the organs, this particular organ would no longer be capable of acting in concerted harmony with the other organs, and thus the correlation of the whole would be disturbed. For example, if the customary food supply become exhausted, so that the organism is compelled to seek nourishment of a different kind, not only in the immediate intestinal cells must a physiological (and consequent morphological) change ensue, but also the structure and function of each organ, indirectly dependent upon the intestine's action, must become modified. And, *pari passu*, if the external forces acting upon a sense-organ assume a different direction, not only must this particular organ become physiologically modified, but indirectly also the nervous system, and all organs in functional communication with the latter; or if a certain organ become diseased, its normal action must become to some extent impeded, which would exert an influence upon the functions of the other organs. In fact, whatever organ be directly influenced by a change in the environment, a modification of the functions and structure must result in those organs which are in correlation with it.

Accordingly, after a change in the environment, a temporary disturbance of the correlation of the organs must result. When we speak in this way of an "interruption" or of a "disturbance" of the correlation, we mean that the mutual dependence of the several organs upon one another is reduced, so that they become to an inverse extent independent of one another. Now I consider that the origin of variation is to be found in this condition of temporary independence of the organs, which independence is caused by change of environment, resulting in the partial interruption of the organs' correlation. For each organ, after the disturbance of such mutual correlation, becomes more *auto-dynamic*, — more independent of the restraining influences of

the others, and consequently its own forces of growth and action may operate more freely and to greater extent than was possible in the previous condition, when it was held in check by the state of correlation with the other organs. In other words, after any change of the environment, that is, after any consequent interruption of the correlation of the organs, each organ becomes temporarily more autodynamic than it was before, and the comparatively independent action of its vital forces may result in the production of abnormalities, which are known as variations. To what extremes these variations may go in amount or extent will be considered in the next section.

The question arises at this point in the argument : why, when through the interruption of the correlation of the organs a certain degree of temporary independence of each of the latter ensues, should any of the particular organs exert this independence in the production of structural variations? Now we have seen that the chances of existence of an organism stand in a direct proportion to the degree of its adaptation to the environment ; and further, that a complete correlation of its organs is necessary before a perfect adaptation to its environment is possible, thereby its chance of existence depending upon the degree of correlation of its organs. Accordingly, when the correlation of the organs has been disturbed by a change in the environment, it has but a small chance of existence until this correlation is restored. This deduction can hardly be questioned, because it is difficult to conceive of an organism existing when the correlation of its organs is greatly impaired. It follows, therefore, that the organism must attempt, in its fight for life, to restore this correlation, which is obviously a step necessary for its becoming adapted to the new environment. Plainly, then, after the correlation of the organs has become more or less interrupted, the several organs would exert their degree of temporary independence of one another in such a manner as to restore the correlation. Bearing this point in mind, and remembering in this connection the previous assumptions of our argument, we conclude : that *organic structural variations are the morphological results of physiological exertions on the part of the organism, to restore that complete correlation*

*of its organs which had been disturbed by a change of environ-
ment.* Even in the case of a disease attacking an organism,
may we not regard any abnormal growth produced by the
afflicted organism itself, to be the structural result of vital
processes in the organism striving to restore the correlation of
its organs ? Indeed if we consider, which we must in view of
the facts at hand, that correlation of the organs is a physio-
logical necessity for the existence of an organism, then, if this
correlation be disturbed by a change of environment, we are
logically forced to conclude that the organism must make the
attempt to restore this correlation, and that structural variations
would be the result of such a physiological exertion.

A similar explanation of the origin of structural variations
can be reached also from another standpoint : when a change
of environment disturbs the correlation of the organs, the cor-
relation when restored must differ to some extent from the
previous state of correlation, since the new environment exerts
a different influence upon the functional activity of the organs.
But, as a different state of correlation cannot be conceived as
existing in the same structural unity, certain changes of struc-
ture are necessarily involved, and these we term "variations."

The statistics given in this paper on variation in birds show
that species with restricted breeding ranges and which are non-
migratory in habit are, as a rule, much less variable with regard
to dimensions — other factors being approximately equal — than
are species occupying more extensive and more diversified areas,
and which undertake periodical migrations of considerable mag-
nitude. We are justified in concluding from these facts, that
as a rule the amount of variation stands in direct ratio to the
degree of environmental diversity of the inhabited area, *i.e.* to
the amount of change of environment which influences the
organism. And, as the degree of interruption of the correlation
of the organs must be in direct proportion to the amount of
change in the environment, the greater variation in those species
of extended and diversified habitats is easily and solely explain-
able on our deduction, that the greater change in the environ-
ment should cause greater physiological independence between
the several organs ; and therefore the structural changes ensu-

ing from the physiological exertions to restore the correlation, should be greater than in species of comparatively restricted and uniform habitats which experience neither so many nor so great changes of environment.

*Recapitulation.* — Organic variation owes its origin indirectly to change of environment. For it is necessary for the existence of an organism that its organs be correlated physiologically and (consequently) morphologically, and no adaptation to its environment, a factor which is also necessary for its existence, can be brought about until the organs become correlated. Now when a change occurs in the environment, this change checks the normal action of one or more of the organs, and influences indirectly the others, so that the correlation of the organs becomes temporarily disturbed or interrupted. The degree of disturbance in the correlation of the organs, probably stands in a direct ratio to the amount of change in the environment ; and according to the statistics given above, the amount of variation certainly stands, as a rule, in direct proportion to the complexity of the environment. Naturally, when an interruption of the correlation occurs, the several organs become to such a degree independent of one another as is the extent of its disturbance : the greater the disturbance of the correlation of the organs, the more autodynamic the individual organs become. In order to adapt itself to the new environment, the organism must first restore the correlation of its organs. Now the several organs, being no longer held in strict restraint by the agency of a complete correlation, make use of their temporary degree of physiological independence in order to restore this correlation. Any structural changes resulting from the exertions of the comparatively unrestrained (independent or autodynamic) physiological forces of the organs, to restore their correlation, are organic variations.


IV. On Variation as a Criterion of Development.

In the preceding pages I have tried to analyze briefly the processes of progressive and regressive development, and from a study of the facts of variation in birds, which show that the

amount of variation stands in a direct proportion to the amount of change in the environment, to advance a theory of the origin of variation, — as due directly to the physiological exertions on the part of the organism, to restore the correlation of the organs which had been disturbed by a change of environment. This theory is sustained by the facts given here, and if it be corroborated by future studies, we may use it as a standpoint from which to review the phenomena of organic variation, as offering criteria for the study of the processes of development.

The question often recurs to the biologist engaged in comparative anatomical investigations, why in a certain species a particular organ should be structurally variable, which is eminently stable in allied species. It has, thus far, been the method of the biologist to attempt an explanation of this variability by reasons derived from his assumptions as to the phylogenetic origin of the group, and of the different forms comprising it. But may we not, conversely, acquire some understanding of the hitherto unknown, or but hypothetically conjectured, development and homologies of an organ, by starting out from the facts of the phenomena of variation themselves? This is a line of research inaugurated by Bateson, and which may in time afford important results.

First of all, it is well to recall to mind the two factors necessary for the existence of the organism: (1) its adaptation to the environment, and (2) the correlation of its organs. When the environmental forces become more complex in their action, the intimacy of the correlation of the organs being more or less in a direct proportion to the degree of complexity of these external forces, as will be shown later, — then the structural development occasioned by such a change of environment must be a progressive one, if the organism would maintain its adaptation to the environment. But if, on the other hand, the change of environment is tending toward a simplification of the previously complex action of the environmental forces, then the organism must undergo a regressive structural development in order to remain in adaptation to the environment. In other words, if the environment is becoming more complex, the structure of the organism must also become more complex in order to insure its

adaptation ; while if the environment is becoming less complex, then the structure of the organism must become less intricate. For if the environmental action become more complex while the structure of the organism remains as it was, or becomes more simple, then the latter can no longer continue to exist ; [1] or if the environment become less complex in its action while the organism's structure either remains as it was or becomes more complicated, its extinction must be brought about. These relations of changes in the environment to changes in the structure of the organism, with reference to their effect on the existence of the latter, may be graphically represented as follows :

$$
A. \text{ Increasing complexity of environment}
\begin{cases}
\begin{cases} + \text{Progress.} \\ \text{development of} \\ \text{organism} \end{cases} = \text{continued existence of organism.} \\[2em]
\begin{cases} + \text{Regress.} \\ \text{development of} \\ \text{organism} \end{cases} = \text{extinction of organism.}
\end{cases}
$$

$$
B. \text{ Decreasing complexity of environment}
\begin{cases}
\begin{cases} + \text{Progress.} \\ \text{development of} \\ \text{organism} \end{cases} = \text{extinction of organism.} \\[2em]
\begin{cases} + \text{Regress.} \\ \text{development of} \\ \text{organism} \end{cases} = \text{continued existence of organism.}
\end{cases}
$$

Accordingly, when the phenomena of variation are more clearly understood, and the direction and quantitative amount of change in the environment can be determined, then it will be possible to predict the future of a given organism.

Now variation, as I have tried to explain it, expresses a want of correlation between the several organs ; and such an interruption of the correlation can be caused only by the agency of a change of environment. Accordingly, it is permissible to state of a variable organ that it is not in complete correlation with its fellow organs ; and consequently, further, that some change is occurring, or has taken place, in the environment.

[1] A possible example of such a case is that afforded by the now extinct group of Ammonites.

Therefore, in order to explain the presence of variation in a certain species of a group not present in the same organ of closely allied species, we must compare the conditions of the environment of the one with those of the others. Thus, regarded from this standpoint alone, when variation is perceptible in organ $x$ of species $A$, but not so in organ $x$ of a closely related species $B$, we may conclude that organ $x$ of species $A$ is being influenced by some change of environment which is not affecting the corresponding organ $x$ of species $B$. May we not consider that the particular organ in $A$ is commencing to develop in a new direction, while the organ in $B$ is remaining unchanged? By this would be merely shown, however, that whenever variation is noticeable, the organ evincing it is tending towards an ultimate structural modification, due to the fact of a change of environment already taking, or having taken, place.

What light can the phenomena of variation throw upon the phylogeny of organisms? I consider that it may be possible to decide, with a certain degree of certainty, whether a given species is developing progressively or regressively at the present time, and whether in the near past it has progressed or degenerated; by basing our conclusions as to its course of development, present and past, upon the direction and degree in which the variation appears. This may seem to be a bold assumption, but if the views expressed in this paper upon the nature and origin of variation be probable, we may yet learn that the study of variation may furnish valuable criteria for estimating the facts of phylogeny. As Bateson observes (*l.c.*, p. 6), two criteria of phylogeny upon which much confidence is misplaced, namely the ontogeny and the direct study of adaptation, are by no means infallible ; so that to-day we have only the criterion of the facts of palæontology, — a criterion which Bateson fails to mention, with which we may feel ourselves secure. And this being the case, we should gladly avail ourselves of a further criterion, namely, the phenomena of variation.

In comparing the antagonistic states, progressive and regressive development (*cf.* Section I), it was found that progressive development leads towards a more complicated structural modi-

fication, and regressive development to a structural simplification of the organism. Now, as is generally conceded, species are maintained in the struggle for existence by the preservation of favorable individual variations, *i.e.* (to my mind), variations which are favorable, in as much as they tend to produce a complete correlation of the organs ; and all such structural variations are necessarily either more complex or more simple than the normal. The preservation of favorable individual variations which are more complex would result in the production of a more highly differentiated species ; and, on the other hand, the preservation of those which are less complex would result in the formation of a morphologically less differentiated species. Accordingly, we must first determine whether the variations are above or below the normal, — more complex or more simple. For if the variations are more complex, then if they should be preserved a more highly organized species would be evolved ; and if they are structurally simpler, and should be preserved, a less highly organized. Similarly, judging from the palæontological remains of a series of individuals of a now extinct species, it might be possible, after a careful investigation into the nature and amount of individual variations exhibited by them, to conclude whether a more highly or a less highly organized form, if any, had been produced. Thus we should expect, that a given species *A* occurring in the Liassic beds, presenting individual variations (osteological, *e.g.*) more complex than the normal, would be represented in the Triassic by a more highly differentiated species, if by any.

But the study of variation, thus far considered, gives us criteria for only the future development of the species, so that it remains necessary to seek criteria from the phenomena of variation for the past phylogenetic stages.

And, firstly, it is desirable to determine as far as possible what limits there are to individual variation. It seems to be well ascertained that there is a limit to such variation, but where that limit may be placed for a given organism, or what organic law fixes it, is very difficult of experimental proof. Since we find the amount of variation to be in direct proportion to the amount of change in the environment, the question is,

in other words, how great a change of environment the organism
can withstand without serious injury.    Now from a number of
carefully made experiments, as noticeably the recent observa-
tions of Davenport and Castle,[1] we find that an organism which
would be killed by a sudden change of temperature of 10° C.,
may become acclimated to that amount of increase in tempera-
ture if the change is made gradually.    This fact proves that an
organism can withstand only a certain maximum amount of
sudden change of environment, while if the change be greater
than this maximum amount, death ensues.    Still another fact is
important in this connection : lowly organized forms are, as a
rule, more *widerstandsfähig* than highly organized forms ; it
suffices, as an example, to call to mind the great changes of tem-
perature which are not injurious to certain disease germs and
swarm-spores, but which would cause the sudden death of a worm
or mammal.    From these facts we may conclude : (1) that as a
given organism can withstand only a certain maximum amount
of change in the environment, and since the amount of variation
stands in a direct ratio to the amount of change in the environ-
ment, therefore the organism can produce only a certain
maximum amount of variation ; and (2), since lowly organized
forms can withstand greater changes of environment, as a rule,
than can more highly organized forms, that the former can, as
a rule, produce a greater amount of variation than the latter
can.    And these facts coincide perfectly with the general physi-
ological law that the more differentiated the organs become
structurally, the more intimate and complex becomes their
correlation ; for more highly differentiated organisms, with a
more complex correlation of their organs, are unable to produce
variations to the same amount as can more lowly organized forms
which have a less intimate correlation of their organs.    Further,
the correlation of the organs in lowly organized forms, being
less complex, can be restored sooner after a change of environ-
ment than the correlation can be restored in more highly dif-
ferentiated forms after the same amount of change.    Thus we
find that the amount of variation depends upon the degree

[1] "On the Acclimatization of Organisms to High Temperatures," Arch. f.
Entwicklungsmech. d. Organismen, Bd. II, 1895.

of change in the environment, and upon the degree of dif-
ferentiation of the organism ; but that a certain maximum
amount of variation cannot be exceeded by the organism, and
this amount seems to differ for different organisms. Variations
must continue to be produced until the correlation of the organs
is fully restored, when the restraint exerted by this acquired cor-
relation upon the physiological processes of the several organs,
would prohibit the production of further variations. Therefore,
if the variations continue to be produced through a long period
of time, we must conclude that the correlation of the organs
has been greatly disturbed, which is equivalent to saying that
a comparatively great change has occurred in the environment.
If, however, but one considerable change has occurred in the
environment and the latter remains thereafter unchanged, then
the longer the period of time is which has elapsed since this
change, the nearer at hand will be the restoration of the cor-
relation of the organs, and consequently the less will be the
amount of variation. This will serve further to elucidate the
deduction made in a former paper[1] of mine (p. 483) : that
"the amount of variability above or below a given mean will
stand in inverse ratio to the length of time in which the devel-
opment (progressive or regressive) has acted upon the given
organ." If the change of environment be comparatively slight,
the restoration of the correlation of the organs might be fulfilled
in a single generation ; but if the change had been more marked,
this correlation might not be restored until after the lapse of
a large number of generations, during which time the produc-
tion of variations would continue, though their amount would
decrease as the time became longer.

Here, then, the phenomena of variation may furnish us with
a criterion for deducing, to some extent at least, the previous
conditions of existence, if not also the phylogenetic stages of
some organisms. For we may briefly consider, *e.g.* the fresh-
water Nemerteans, which are undoubtedly of marine origin.
In studying the comparative anatomy of this group of worms,
I was struck by the fact that while the nearest marine allies of

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[1] "The Derivation of the Freshwater and Land Nemerteans, etc.," Journal
of Morphology, XI, 2, 1895.

these freshwater species possess almost invariably four large eyes, the freshwater forms, on the contrary, have a larger number of eyes, varying from four to eight, which are also smaller than those of the marine species. How is this variability in the number of eyes of the freshwater forms to be explained? Now, variability is engendered, in our view, indirectly by change of environment; and we know that the species in question have changed their environment by migrating from bodies of salt water into freshwater rivers and lakes. (Or, in certain cases, they are inhabitants of lakes which were originally of marine character, but have become fresh.) The number of the eyes of the marine species being very stable, we conclude : (1) that the correlation of the organs in the marine forms is comparatively complete, and that, therefore, they are well adapted to their environment; and (2) that no variability being perceptible, they are neither at present giving rise to new species, nor are they themselves of recent origin. On the other hand, the eyes of the freshwater forms being very variable in number, we conclude for these : (1) that the correlation of their organs is not perfect, and hence that they are not fully adapted to their environment; (2) that this variability must have been caused by a change of environment within a comparatively recent period of time, since the variability is still continuing; and (3), that as the numerical variation of the eyes is above the normal four (the number 6–8 being in fact more frequent than 4 or 5), the species is tending to evolve a form with a greater number of eyes than its ancestors possessed (progressive numerical development in relation to the eyes).

A similar case occurs to me in regard to the individual variation in number in the rectrices (stiff tail-feathers), of certain North American species of grouse. Table XXI (placed at the end of the paper) expresses this variation for *Centrocercus urophasianus,* and for *Dendrapagus obscurus* with its two geographical races.[1] According to Mr. Clark's observations, these are the only North American species of grouse evincing such variation; the number of rectrices being stable in *Den-*

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[1] I am indebted to the kindness of my friend Mr. Hubert Lyman Clark, of Baltimore, for the communication of the facts embodied in Table XXI.

*drapagus canadensis*, *D. franklini*, and in *Bonasa*, *Lagopus*, *Tympanuchus*, and *Pediœcetes*. Now it is interesting that the subgenus *Dendrapagus* of the genus *Dendrapagus* is limited to North America, and its species are variable in regard to the number of the rectrices ; while such variation apparently does not occur in subgenus *Canace* of the same genus, which inhabits both Eurasia and North America. Of the other genera, *Bonasa* and *Lagopus* have the same geographical distribution as *Canace*, while *Tympanuchus*, *Centrocercus*, and *Pediœcetes* are limited to North America. Those individuals evincing this numerical variation of the rectrices must have been influenced by a considerable change of environment, since the variability has continued through a large number of generations. Now we can hardly suppose that this change of environment has occurred within the areas occupied by these variable species in North America, since in that case we must suppose that the other species occupying the same areas must have become similarly modified. Accordingly the species of *Dendrapagus* which presents this variation must be of comparatively recent occurrence within its present habitat ; and its variability would be caused by having changed its former habitat within a comparatively recent period, by migrating from its former area (Eurasia ?) to its present geographical position in America. On the other hand, the non-variable North American species of this genus (namely *canadensis* and *franklinii*) must be regarded either as not having experienced such a change of environment, or as having experienced it in a much remoter period, having in the meanwhile restored the correlation of their organs, *i.e.* adapted themselves to the new environment. Regarded from this standpoint, the variation in the number of the rectrices may serve to elucidate the origin and present distribution of the *Tetraonidæ*.

These considerations are offered as mere suggestions for explaining how variation can serve as a criterion of development. We have found that the data of variation offer a certain mean for determining whether the development is progressive or regressive, depending upon the fact whether the variations are above or below the normal. When the phenomena of varia-

tion are better understood, for this study is at present nearly virgin ground, we will probably be enabled to deduce from it criteria, which will offer certain and valuable aid in the study of phylogeny.    And another important line of investigation must become the study of the environment, and of the changes of the latter in their influence upon the organism.    A considerable number of experiments have recently been made in this line upon the ontogenetic stages of organisms, and a smaller number upon the adult organism ; and a careful analysis of the results of such experiments may give valuable aid in the discrimination of methods for the study of variation.

   A final word in regard to *regressive development*.    It is as yet an undecided question whether regressive development (or the action of Natural Selection during this mode of development) can result in the total disappearance of an organ, or whether a structural rudiment must remain.    According to our conclusions upon the nature of variation, the latter would be the more probable view, and for the following reasons.   For the action of a regressive development, the occurrence of variations below the normal are necessary, so that if these be preserved, a less differentiated (*i.e.* retrogressive) type of organism must be produced.   The occurrence of variations in regressive development is due, just as in progressive, to the physiological exertions of the organism to restore that correlation of the organs which had been disturbed by a change of environment.   Now if the change of environment had been a great and sudden one, and resulted in a less complex environment, the organism, in order to become adapted to the new environment, must produce structural variations which are less complex than the normal in order to bring about a less intimate and complex correlation of the organs than had previously existed.   Such variations can obviously be produced only as long as the organs continue to to be physiologically active ; and necessarily their action must cease before the organs disappear.   In other words, it is impossible for variations to lead to the total disappearance of an organ, since the very production of variations is dependent upon the physiological exertions of the organ.   Thus we must

postulate for regressive, as has been shown for progressive development, a certain maximum amount of variation, the passing of which would cause death.


TABLE XXI.   INDIVIDUAL VARIATION IN THE NUMBER OF THE RECTRICES OF CERTAIN N. A. TETRAONIDÆ.

| SPECIES. | NUMBER OF INDIVIDUALS EXAMINED. | NUMBER OF RECTRICES. |
|---|---|---|
| Centrocercus urophasianus (Bonap.) | 1 ♀ <br> 2 ♂ ♂, 3 ♀ ♀, 3 juv <br> 3 ♂ ♂, 1 ♀ | 16 <br> 18 <br> 20 |
| Dendrapagus obscurus (Say) | 1 ♂ <br> 1 ♀ <br> 6 ♂ ♂, 4 ♀ ♀, 2 (sex?) <br> 2 ♂ ♂, 1 ♀ | 16 <br> 17 <br> 18 <br> 20 |
| D. obscurus fuliginosus Ridgw. | 1 ♂ <br> 1 ♂, 1 juv. ♂ <br> 3 ♂ ♂, 1 (sex?) <br> 4 ♂ ♂, 4 ♀ ♀, 4 (sex?) | 14 <br> 16 <br> 17 <br> 18 |
| D. obscurus richardsonii (Sab.) | 1 ♀ <br> 2 ♂ ♂, 4 (sex?) <br> 1 (sex?) <br> 1 ♂ | 19 <br> 20 <br> 21 <br> 22 |


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## THE EGG OF AMIA AND ITS CLEAVAGE.

C. O. WHITMAN AND A. C. EYCLESHYMER.

### PREFACE.

THE search for the eggs of Amia, begun by Louis Agassiz, and renewed from time to time by others, was first successful May 1, 1887, when the senior author of this paper found nest and eggs in Pewaukee Lake, Wisconsin. There has been no lack of interest in the search on the part of American natural-ists, but a number of reasons conspired to delay the discovery. The nature of the breeding-grounds and the habits of Amia were little understood, and those most interested in securing the material lived at long distances from the more promising localities.

The difficulties in the way of the first discovery seem to be little appreciated by those who have recently collected these eggs and made free use of information well known to have originated with one of us, but with no acknowledgment, except to parties who were either wholly innocent of anything but bor-rowed knowledge of the subject or had acquired their first information under our guidance.

So far as we have been able to learn, no eggs of Amia had ever been collected or seen before the date above given. No one knew where to look for them, whether in deep or shallow water, under stumps and logs, or in open places, on sandy or marshy bottom. Whether they were to be found free or adhering to roots or leaves, scattered among grass and reeds or collected in nests or beds, no one could foretell. Fishermen had seen the young in swarms along the shores of many of the Wisconsin lakes, but they knew nothing of the nesting habits and could only guess at the time of spawning.

Amia is very shy and nocturnal in habit. Its nests are not designed to catch the eye. One who had never seen them might pass over dozens and fail to notice them. They are often concealed beneath supernatant grass or reeds, and appear, at first sight, like natural depressions. The eggs agree so closely in color with the ground that the inexperienced observer must get close over the nest to see them, and even then he may fail if the water is the least clouded with mud or rippled by the wind.

The male fish, which alone guards the nest, lies motionless at the approach of a boat, and often allows it to pass over him without stirring. If frightened by a jar in the boat or by the movement of the oars, he leaves the nest, but in doing so generally raises a cloud of mud, and darts off under its cover, stealthily, but with wonderful rapidity. The nest and eggs are often thus effectually concealed from view. If this behavior were something special and peculiar to the care of the nest, as one might suspect on first acquaintance, it would serve to indicate approximately its location ; but one soon learns that not every streak of mud in the wake of an Amia leads to a nest. Indeed, one may search for hours on such trails and find nothing.

Amia is fond of shallow and quiet bays, where the water first gets warm in spring, and hiding-places are easy to find among the reeds and logs. In such places they spend the day in quiet concealment. When approached in a boat pushed forward with great care to avoid noise and disturbance of the water, they may keep their places, if concealed from view, letting the boat pass by or even over them without moving. Sometimes they

steal away, under cover of plants, so quietly as to escape obser-
vation.   If suddenly surprised they dash off with such speed
that one may get no distinct image of the fish, and see only a
streak of muddy water marking its path.   A few fish escaping
in this way often leave the water of a small bay so cloudy that
the search for eggs has to be abandoned until the mud settles.

The difficulties of the search for these eggs were, then, not a
few, even in Pewaukee Lake, which is undoubtedly one of the
most favorable localities, and thus far the only one where they
have been found in abundance.   In lakes where the water is
constantly cloudy, as in the small lakes south of Chicago, the
difficulties are so great that, with all our experience in collect-
ing these eggs, we have not yet succeeded in finding a single
nest, after looking for them for two seasons.   There must be
breeding-grounds somewhere in these lakes, for Amia is abun-
dant, both young and old.

After collecting for several seasons at Pewaukee, and after
much searching in the rivers and other lakes of Wisconsin and
Illinois, we are not greatly surprised that the eggs of Amia
escaped early discovery.   But how did it happen that the dis-
covery once made was not forthwith announced, and followed
up with the usual haste to secure priority?   Although the fact
was not announced in print, no secret was made of it, and it
soon became known to many American and European natural-
ists.   As to "priority," no apology need be offered for indif-
ference.   "Priority" weighs less and less as investigation
weighs more, and, so far as the egg of Amia is concerned,
there appears to be little reason to envy either the methods or
the results of priority-seekers.

To those who have known where these much coveted eggs
could be found in abundance, and who have courteously re-
frained from helping themselves, out of respect for first claims,
a word of explanation is due for the failure to bring forth
results at an earlier date.

When Mr. Allis started his Lake Laboratory at Milwaukee,
under the direction of Mr. Whitman, it was suggested by the
latter that the eggs of Amia and Necturus would be two as
interesting subjects for embryological research as could be

found in the lakes of Wisconsin. It was decided, in case the eggs could be found, that Mr. Allis should devote himself to Amia, and Mr. Whitman to Necturus. Mr. Allis knew the lakes and the fishermen, and was thus most helpful in direct-ing to favorable localities. It was not long before the eggs of both Amia and Necturus were found. During the first season only a few Amia eggs were obtained, but the young were to be had in great numbers. It was decided, therefore, that Mr. Allis should begin his study with the development of the "lat-eral line" system, for which there was abundant material, and wait until the next season before attempting to trace the devel-opment of the egg. Mr. Allis became deeply absorbed in this study, and when the time came for collecting eggs again, the work had progressed to a point where it could not to advantage be laid aside for the tempting study of the eggs. A third sea-son came, and still there was no room for eggs, although a fairly complete series had been collected by Whitman and his assistant, Dr. Patten. At length the work on the lateral line was brought to a close; but just then Mr. Allis's health broke down, and he was advised by his physician to go to Europe for rest and for expert treatment of his eyes. From that time to the present he has found it necessary to prolong his stay, but not without maintaining a private laboratory, and, by the aid of assistants, bringing another anatomical work on Amia, of great extent and value, to completion. Beginning with the second year of his absence, Dr. Ayers had charge of the laboratory in Milwaukee, with Eycleshymer, Strong, and Nomura as assist-ants, and continued the collection of Amia eggs. A large number of elegant drawings of the egg were made by Mr. Nomura, and the various stages were systematically sectioned, and several cases of slides were prepared by Eycleshymer and Strong, ready for study the moment Mr. Allis should be able to return. The second plate of the present paper represents some of Mr. Nomura's work, directed by Dr. Ayers and Mr. Allis, and generously placed at our disposal by Mr. Allis.

The material for the study of the embryology of Amia, now in the possession of Mr. Allis, covers all stages, and the work already done upon it is considerable. In order to keep this

and related work moving, Mr. Allis kept the Lake Laboratory open for the first four years of his absence, and then closed it, with the expectation of reopening it as soon as he could resume personal direction. All the while he has been pushing forward the anatomical work on Amia (now completed and in press), and has thus prepared himself in quite an exceptional way for a thorough study of the development.

Those who know how faithfully Mr. Allis has pursued his work under unexpected difficulties, and how he has continued for years to divide his income in support of the investigations of others, and in maintenance of a national medium of publication, — those who are aware of all this may wonder that a report could get into circulation to the effect that the Amia material was being unfairly monopolized. The implication extends to all who have participated in the discovery, collection, and elaboration of this material (Whitman, Patten, Ayers, Eycleshymer, Strong, and Nomura), for all have abetted the crime, in so far as they have refrained from snatching the material themselves or assisting others in such business. The prize of priority, coupled with dishonor and theft, was not a distinction coveted at the Lake Laboratory. If to find what others have failed to find, and to devote life and fortune to its investigation, is unjust monopoly, then how might biology prosper in the exchange of footpads for monopolists?

Those who, knowing all the circumstances, have nevertheless given currency to this censure, or allowed it to pass undisputed, have something less than generous instincts to be proud of. Mr. Allis's great crime reduces itself to the misfortune of having had to submit to delay in his work, to the neglect of all tintinnabulous advertising, and to contempt for the notoriety accorded to prolific scribbling and priority hustle. To all courteous applications for Amia material Mr. Allis has freely responded to the extent that his resources and work would permit.

It remains to deal briefly with Dr. Fülleborn's mission for the collection of the eggs of Amia and Necturus. From what has already been stated, it will be clear that there were certain claims and obligations in relation to this material which were not to be entirely overlooked. Without a word of previous

notice or consultation with the parties primarily interested, Dr. Fülleborn came to us with the request for direction and aid in getting the eggs. Under the circumstances, we could only decline, explaining the reasons which stood in the way, and pointing out some of the proprieties in the case which should have received attention. It is quite possible that Dr. Fülleborn had not been previously informed of these, as was to be expected that he had been by Dr. Virchow, under whose instruction he was acting.

When, in 1893, Dr. Virchow applied to several parties for assistance in finding the eggs of Necturus and Amia, he was referred to Mr. Whitman. Learning that work on these eggs was already in progress, Dr. Virchow courteously explained that he only wished to study the "Dotterorgan," and that he would not make any use of the material which would conflict with the work on general development already begun by Mr. Whitman. With that understanding, the information desired was freely and fully given, and a pretty complete series of drawings of the egg of Necturus was shown to him.

Next came Dr. Fülleborn, under advice from Dr. Virchow, to take advantage of the information confidentially obtained by the latter, in collecting the same material for purposes well known to conflict with studies in progress here. The mystery of the "Dotterorgan" was now disclosed, and no further comments seem to be required.

## Oecological Observations.

### 1. *Previous Papers.*

There are only four works to which we can refer the reader for information on the habits, eggs, nests, and larval history of Amia. Mr. Allis [1] has given a series of drawings of the larva, to illustrate the successive changes in form and the development of the lateral-line organs. Dr. Fülleborn [2] has recently

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[1] Edward Phelps Allis, Jr.: The Anatomy and Development of the Lateral Line System in Amia Calva. *Journ. of Morph.*, II, p. 463. 1888.

[2] F. Fülleborn: Bericht über eine zur Untersuchung der Entwickelung von Amia, Lepidosteus und Necturus unternommene Reise nach Nord-America. *Sitzungsberichte der Akad. d. Wiss. zu Berlin*, XL, pp. 1057–1070. Oct. 25, 1894.

reported his experience in obtaining the eggs, together with observations on the habits, times, and places of breeding. This author describes the floating islands in Pewaukee Lake and Fowler Lake and their labyrinth of canals, and speaks as if Amia was so select in its choice of breeding-ground that its nests were to be found only in these canals. That is not the case, however, in either of the lakes just mentioned, and it is quite certain that Amia breeds in many places where there are no floating islands with canals, — for example, La Belle Lake, which Dr. Fülleborn cites as not containing a single nest.

The spawning season is said to have extended from the beginning of May to the first days of June in the summer of 1894. According to our observations, the best time for collecting generally falls between the middle of April and the end of the first week in May. We have collected eggs the last week in March and the first week in June, but these extremes mark unusually early or late seasons.

The time for hatching is estimated to vary between six and fourteen days. This is a variation wide enough to include the truth without hitting it in either direction. Surely one season's experience ought to have reduced both margins of this conjecture, and furnished an answer to the question how long a time is ordinarily required to hatch these eggs.

The nests described as "not yet filled with eggs," in which male Amiae were found, were probably nests in which the young had hatched. The observation furnishes no ground for the belief that the male alone makes the nest. The statement that the nest is always open to the sunlight does not accord with our experience, and the opinion that young and old seek deep water after the first of June is entirely erroneous. The assertion that nests containing "nur verschimmelte Eier" were still guarded by males is another error due to superficial examination. Such eggs are frequently found in nests containing newly hatched larvae, and deserted nests are occasionally occupied as convenient resting-places during the day. It does not follow because an Amia is found in a nest that it must be there for the purpose of guarding it.

The statement that the male takes its brood to the shore in order *to sun itself and them* would adorn a nursery tale better than the Proceedings of an Academy of Sciences. Dr. Fülleborn does not appear to have discovered that Amia prefers the shadows of evening and the darkness of night to the glare of the sun. Amia places its nest near the shore for warmth, not for light; it leads its young along the shore for food,* not for basking in sunlight. It is towards evening and in the early morning that the swarms of young may be seen to best advantage. As they grow older and more wary they may shift their feeding-ground, not to deep water, but to new places along the shore. Their seeming to disappear is accounted for by the fact that the individuals of a brood wander more widely apart as they grow older and require more food, and at the same time they become increasingly shy, and hide themselves beneath the banks, or whatever lies nearest, long before a boat in motion can be brought within seeing distance. The boat must come to rest and the observer must sit motionless by the half-hour if he desires to see young Amiae feeding in June. A slight jar of the water is enough to send hundreds of these young fish out of sight in a flash.

The mistake in supposing that young Amiae retreat to deep water soon after June 1 led Dr. Fülleborn quite astray on another point of considerable importance. The conclusion that the young go straight on to become more and more like the adult in both form and color is about as wide of the mark as it could be. The changes in color exhibited at different ages during the summer and autumn are as characteristic and remarkable as the changes in plumage among birds.

Dr. Fülleborn is careful to note that the young larvae with large yolk-sacs, before leaving the nest, have a distant resemblance to tadpoles, but he neglects to mention that this resemblance becomes even more striking after the yolk-sac has disappeared and after the nest has been abandoned. The larvae, shortly after leaving the nest, while moving about in a dense swarm, resemble tadpoles so closely in form, color, and

* The food of the young consists mainly of daphnia and cypris, as Dr. Ayers informs us.

motion as to be easily mistaken for them when seen at a distance of some yards, as from a boat. A stranger to the fish and its habits might wonder at the closeness of the swarm, but, if he did not stop to examine, he would take them for tadpoles.

Dr. Fülleborn was in such haste for priority that he could not stop to settle the important question as to whether the first cleavage grooves actually reach the vegetative pole of the egg. "The first grooves," he says, "appear indeed to reach the vegetative pole; but the investigation has not advanced far enough to admit of definite statements." This question might have easily been settled on the living egg, and if the material collected was so poorly preserved as not to admit of positive statements on this point, it may be more "complete" than instructive.

Only a little over four pages of this preliminary paper were devoted to Amia,— certainly enough to show the need of more careful observation.

The third paper to be mentioned in this connection is that of Bashford Dean,[3] who affirms several times that he has fully confirmed the statements of Fülleborn on the "general" and "spawning habits of Amia." Perhaps the above remarks are not superfluous after such confirmation. As Dr. Dean seems to have depended a good deal upon what "the fishermen stated," the confirmations offered are not always very confirming.

It should be said, however, that the fishermen of Pewaukee and Oconomowoc have had many opportunities for instruction since 1887, and some of them are now better informed on the habits of Amia than they were found to be at that time. Some of the statements credited to them are entirely accurate, some are crude guesses, and others pure "fish stories." The following will serve as examples :

"The fish were observed depositing their eggs as early as April 25, and before the 1st of May the spawning appeared to have been generally completed." Although no authority is quoted, this is evidently a statement based upon a fisherman's story. It so happened that we collected eggs and larvae from

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[3] Bashford Dean: The Early Development of Amia. *Quart. Journ. Micr. Sci.*, XXXVIII. February, 1896.

the same locality during the week preceding Dr. Dean's visit in the spring of 1895. On the 12th day of May, between 4.00 and 5.30 A.M., a number of broods of larvae were caught, the largest of which measured 25 mm. These must have been at least 35 days old, as a glance at our table, p. 327, will show. These facts show beyond question that eggs were laid during the first days of April.

"At the height of a ' run ' as many as a half-dozen nests, as *fishermen stated*, were found to occur within the space of a few square yards." This needs confirmation. We have never found the nests in such close proximity, — never more than four or five in a single bay, and usually rods apart. The small bays along the shore of Pewaukee Lake generally furnish ground for but one, or at most two or three nests.

"Immediately before spawning, *it is said* that the fish divide themselves into parties, each comprising a female and several males, and that they then circle about nearer and nearer the shallows." True to the extent that the female may be contended for by two or more males. They are on the "shallows" to begin with, and hence do not need to "circle about" to get nearer to them.

To say that "the fish divide themselves into parties" comports well with a fisherman's yarn, and shows how useful such yarn may be in darning up gaps in observation.

"The mode of building a nest is in some ways doubtful; *fishermen state* that the spawning party prepares it by constant circlings before the time of spawning, and this view seems entirely corroborated by a careful examination of the newly made nest; the soft weeds and rootlets appear bent and brushed aside in a way that gives it somewhat the appearance of a crudely finished bird's nest."

That the nest is built, and built with considerable care in many cases, is evident enough. "The mode of building," however, is, indeed, doubtful; no less doubtful at the end than at the beginning of the fisherman's tale.

"And it seems evident that nests are prepared sometimes well in advance of spawning, for several were noted by the writer which were occupied by the fish for a number of days

before the eggs were deposited." Dr. Dean does not give us any information as to the number or sex of the fish which he observed in these nests. From his statement, page 414, viz.: " By the time of my visit, however, the spawning season had practically ended," we are led to suggest that these nests might have contained newly hatched larvae. We have never observed either the male or female occupying a nest for a number of days, or even one day, preceding deposition.

" The mode of depositing the eggs appears to be entirely similar to that described by the present writer in the case of the gar-pike. The spawning fish leaves the nest from time to time, returning in company. The eggs and milt are emitted simultaneously. The fishes apparently rub close together, since scales are found scattered in the nest bottom, as noted by Fülleborn, and as now confirmed by the present writer." How much of this is to be understood as personal observation the author seems to avoid making clear. The words "appears" and "apparently" look more like conjecture than anything else. Leaving the nest from time to time could hardly apply to the gar-pike, which has no nest. " From time to time " suggests that oviposition occupies considerable time ; and the author conjectures that in some cases as many as twelve hours are thus consumed. In this he is probably much mistaken, as will appear later on. The occurrence of scales in the nest has an entirely different explanation from the one above suggested, as an observation made by Dr. Eycleshymer, to be related presently, will make evident.

The nest represented on page 419 is so great an improvement on nature's art that one might readily conclude that it was formed by "constant circlings." The essential thing in an Amia nest is fine rootlets or grass for holding the eggs free from mud. If the fish finds a surface adapted to its needs it may use it without making any excavation whatever. Such nests are often found along the submerged edges of the float-ing islands, or "bogs," as they are locally called. Along the shore of the lake the nests are sometimes placed beneath a log or stump, but most frequently by the side of a clump of reeds. Sometimes the fish finds rootlets only around the edges or on

one side of its nest, in which case no eggs will be found on the
bottom.    When excavations are made the depth may vary from
a few inches to a foot, depending upon how much mud and
weeds have to be removed in order to get the surface required
for the eggs.    The nest is generally from 50 to 60 cm. in
diameter, and more or less circular, varying considerably in
adaptation to the conditions of the ground.

The author's remarks on the breeding habits end with a bit
of romance funny enough to claim a place beside the story of
Dr. Estes, which will be given presently.

Dr. Dean says : " A fine nest of eggs was found to be
entirely deserted at a time when the young could not have
been older than twenty-four hours.    The closest search in and
about the nest revealed no trace of their whereabouts, although
from their larval habits it was thought that they should surely
be found attached to the neighboring weeds, or deep in the
mass of root fibres and detritus of the nest bottom.    They had
evidently left the nest in a body, and were nowhere in the
immediate neighborhood.    It was *plausibly suggested by Mr. G.
W. Kosmak, who then accompanied the writer, that they had
been taken away by the male fish, attached to him by their
sucking-discs.*    It is certain that when the male reappears it is
with a swarm of nestlings ; but they are now well grown
(⅝ to 1¼ inches)."

The earliest paper dealing with the habits of Amia to which
we have to refer is one cited by Dr. Goode from the Sports-
man's Gazetteer, pp. 324–326, 1887.[4]    It is here that the story
of Dr. Estes, above alluded to, is found.

" The best description of the habits of this fish," says Goode,
"is here quoted from the pen of Charles Hallock :

" 'They take frogs, minnows, and sometimes the spoon.
Their habitat is deep water, where they drive everything
before them.    They are very voracious and savage.    Their
teeth are so sharp and their jaws so strong that they have
been known to bite a two-pound fish clean in two the very
first snap.    They are as tenacious of life as the eel.    The

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[4] Geo. Brown Goode: Natural History of Useful Aquatic Animals, pp. 659,
660.  1884.

young, when about six inches long, make a famous bait for pickerel and pike.   To use it, run the hook into the mouth right up through the centre of the head, through the brain, cast a hundred times, catch several fish, and at the end of three to six hours he will kick like a mule.   Put one hundred in a rain-barrel and you can keep them all summer without change of water.   For the aquarium the young have no equal, and on account of the spot in the tail are quite attractive ; but nothing else but snails can live in the tank.   He will kill a lizard or any other living thing the instant it touches the water.'

"Dr. Estes says : 'I have sent these young dogfish hundreds of miles for the aquarium.   It is only necessary to keep them in water, a change scarcely being required.   The adults are the great "jumpers" of the lake.   On certain days they are to be seen in all directions jumping clean out of the water, and turning complete somersaults before again striking.   *They spawn in May and June among the grass and weeds of the sloughs*, if they can reach them in time.   As soon as the spring rise comes, usually in May and June, and connects the inland sloughs with the lake (Pepin), they run up and over into the sloughs, deposit their eggs, and remain near the beds and young just as long as they can and not be shut in by the receding water.   *The eggs hatch in eight and ten days, the parents remaining with the brood two or three weeks*, if possible, but will leave them much sooner, if necessary, to save themselves.   The young will not make any effort to escape to the lake until the next season, when, if an opening occurs, they come pouring out in countless numbers.   At this time we take them by stretching the minnow seine across the opening and raising it when full.   They are now from three to six inches long, fat and chubby.   *I come now to mention a peculiar habit of this fish, no account of which I have ever seen.   It is this: While the parent still remains with the young, if the family become suddenly alarmed, the capacious mouth of the old fish will open, and in rushes the entire host of little ones ; the ugly maw is at once closed, and off she rushes to a place of security, when again the little captives are set at liberty.*   If others are conver-

sant with the above facts I shall be very glad; if not, shall feel chagrined for not making them known long ago.' "

The author of this amusing story is evidently quite blind to the part played by his fancy. The fish has no such strange habit, and the wonder is that any sane mind could have invented such a prodigious fabrication without realizing that the whole thing was a myth. The facts from which the story was concocted are: (1) The fish sometimes opens its mouth on approach (as if threatening an attack); (2) the young suddenly disappear (in the mud and weeds of the bottom); (3) the old fish darts off and is quickly lost sight of; (4) the old and young are found a little later at a short distance from the place where they were first seen.

The young fish disappear so quickly that the observer fails to see where they go; the reappearance of the brood in company with the parent is taken as proof that he took them with him, and the open mouth as proof that they were swallowed.

## 2. *Behavior during Spawning.*

Although the early cleavage stages of Amia have been repeatedly collected, it was not until May 12, 1895, that the behavior of the fishes immediately preceding and during spawning was witnessed. The observation leaves certain points in doubt, but fills some of the gaps in the records hitherto made.

Starting at daybreak on the morning of May 12th, we soon arrived at the "bogs." After taking a number of broods of larvae, we were slowly passing through a narrow channel which in its deepest portion did not exceed 60 cm. This channel was filled with dead grass from the preceding year, while the growth of the present season was barely visible above the surface of the water. On one bank the green grass grew in profusion, on the other the dead rushes formed a thicket. While resting for a few moments a disturbance of the water not more than 3 m. distant attracted our attention; stepping to the bow of the boat we saw four large Amiae lying in a nest. Seeing us they left the nest and disappeared in the surrounding grass. Soon three of them returned, but after a few brisk movements

again retreated, this time making short circuits in the adjoining
grass and frequently thrusting their snouts above the water.
We now easily ascertained that the party consisted of two
males and one female.*   After a short interval the three again
returned, when a fierce battle for supremacy ensued between
the males.   They approached from opposite sides of the nest
and locked jaws in a most ferocious manner.   Their struggles
were so violent that a cloud of muddy water soon arose and
obscured them from view.   When the female, which during the
battle had remained concealed at the side of the nest, again came
into view, the victorious suitor rushed at her and began to bite
her sides with so much vigor that a number of scales were
detached.   The two then swam slowly about the nest, keeping
their bodies in close proximity.   At short intervals this move-
ment was interrupted by momentary periods of vigorous activ-
ity.   This lasted some five or six minutes, when they departed.
Believing this to be due to unavoidable movements of the boat,
we thought it best to retire and leave them undisturbed.

Returning after twenty minutes, and moving with greatest
care, we approached and found the male lying at full length in
the nest.   Occasionally he would move slowly around the area,
keeping up a very rapid movement of the pectoral and pelvic
fins.   We expected that our approach would frighten him ; but
not until the boat was directly over him did he show any signs
of vacating.   It was now plainly to be seen that the nest was
filled with freshly deposited eggs.   It is probable, though not
certain, that no eggs had been deposited at the time when we
first saw the fishes in the nest.   The oviposition most probably
took place after the battle of the males, and during the time
(5–10 min.) the female was in the nest with the victorious
male.   That the average period of deposition is brief can
hardly be doubted, since in most cases the eggs of a nest are
found in the same or nearly the same stage of development.
In one nest, however, we found some of the eggs in a late stage
of cleavage, while in others the embryo was already visible.

* The male is easily distinguished by a dark spot in the upper portion of the
caudal fin.  This spot is fringed by a ring of dull orange, which during the
breeding-season assumes a brilliant hue.

We inferred that two females sometimes deposit in the same nest. This view is corroborated by the fact that in this case the number of eggs was about double the average number deposited by a single fish.

The eggs were scattered over an area about 50 cm. in diameter, the greater portion being deposited upon the grass lining the sides of the nest.*

Dr. Ayers has kindly given us the following observations on the breeding habits of Amia, which supplement the foregoing statements :

" The nest of Amia is built after pairing, and during the preliminary steps to egg-laying. The nest is not a premeditated structure, but merely the result of the movements of the fish in and about the place selected for spawning during the period of sexual excitement, which ends in the emission of eggs and sperm. How long the movements continue I am unable to say positively, but from many observations I conclude that they usually last from two to three hours. The nest-forming process generally begins at early dawn, and the eggs are cast about sunrise. I have observed some cases, however, in which the eggs were deposited in the afternoon.

" Amia usually selects grassy beds on which to deposit its eggs. At the time of spawning, the dead grass of the previous season is readily swept loose by the powerful swimming movements of the excited pair, thus leaving the peaty stem and root surface for the eggs to fall upon. All loose bits of stems and leaves are swept out of the nest, and with them most of the spores of fungi and algae which were in the area.

" In May, 1892, a nest was found at 3.30 P.M. one day, in

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* In collecting the eggs we found it most convenient to clip off the blades of grass or rootlets bearing them. They can thus be easily carried alive to the laboratory, or placed at once in preserving fluids. In cutting the grass and rootlets, one may avoid the discomfort of immersing his arm by using long-handled shears. These may be improvised by making shears of two pieces of board about 1 m. long. The short ends of these are then inserted into the handles of ordinary shears and fastened by winding with wire ; the blades will thus be nearly at right angles to the wooden handles. It may not be superfluous to add that we have found it convenient to mark nests by means of stakes, to the upper ends of which were attached pieces of cloth of different colors. The value of such a guide will be apparent to those who have attempted to revisit nests.

which the pair were still engaged in spawning. Eggs were taken from the nest and at once placed under observation, and the following record made : Three sets of eggs were put in glass dishes, and Mr. Nomura, Mr. Eycleshymer, and myself observed independently the segmentation of these fresh-laid eggs. The first segmentation groove began at 5.30 P.M. The second meridional began at 6.30 in those eggs which were placed in the sun after the beginning of the first furrow, and at 7 o'clock in those eggs kept in the shade out of the sun's rays.

"The third and fourth meridional furrows began simultaneously at 7.30. The first equatorial to be seen made its appearance at 8.25, and completed the circuit at 8.40. The next set of eight peripheral meridional furrows began at 9 o'clock, and the second apparent equatorial furrow began at 9.30.

"Amiae are to be found in Oconomowoc Lake during the winter months, living in schools closely huddled together in the bottom of pockets or shallow depressions of the gravelly bed of the lake, among the water weeds. These fish may be speared from a boat, or, if the lake is frozen over, through holes in the ice. They lie so close together that occasionally two individuals are impaled on the fish spear by one throw. When thus disturbed they scatter from their resting-place, moving out a short distance, to return quickly after the first few disturbances. When alarmed by repeated thrusts of the spear, they remain away for hours at a time. Why they should be so tenacious of these resting-places will probably be explained when we know why they choose them in the first place. It is possible that the temperature of the water is higher in these places than elsewhere, owing to the entrance of spring streams into the lake bottom."

### 3. *Some Further Data.*

In some of the eggs taken from this nest, and kept under close observation, the first indication of the first cleavage appeared at the upper pole at 7.54, *i.e.*, 2 hrs. 24 min. from the supposed time of deposition. At this time the first cleavage

had already begun in many of the eggs, while in others it had not yet appeared.

At the end of 10–15 hrs., 100–150 cells have been formed in the upper hemisphere, while in the lower, 15–20 grooves are present, of which 8–12 have reached the pole.

To distinguish a stage which marks the beginning of gastrulation is somewhat difficult; if the stage shown in Cut 12 might be considered as such, the time would be 35–40 hrs. after deposition. The process covers a period of 30–40 hrs.

In 70–80 hrs. after deposition the embryo appears. On the 7th or 8th day its first movements are visible; these occur at long intervals, and last only a few moments. The periods of activity become more frequent and vigorous until the 8th or 9th day, when the envelope is ruptured and the larva escapes. The larva at this time measures 5–6 mm. Its body is pale flesh color (without pigment), while the large yolk sac is deep sepia. The larvae, as soon as they are hatched, attach themselves, by means of their adhesive discs, to the first object met, and adhere so firmly that it requires some effort to shake them loose. It is impossible at this time for the larva to move its load of food yolk. Once losing its hold, it falls heavily to the next object, where it again attaches itself.

At the time of hatching one frequently sees the male fish carefully guarding his nest, in which there apparently is not a single living egg or larva, the only evidence of their former presence being the fungus-covered débris scattered here and there. A careful search, however, among the rootlets will enable one to discover the newly hatched larvae.

During the 10th or 11th day the larva reaches a length of 6–8 mm.; pigment begins to appear in the retina and other parts of the head. The pectoral fins have shifted from the horizontal to an oblique position; the movements are less vigorous but more frequent.

In 12–15 days a length of 9–11 mm. is reached. They are now deeply pigmented with dark brown. The pectoral fins have taken a vertical position. The yolk sac has changed from the spherical to an ovoid form, with its smaller end posterior,

and is soon absorbed.    About this time the larvae are ready to
leave the nest with the male fish.    Frequently we have seen
the entire brood, generally numbering several hundred, huddled
so closely together that they formed a black mass moving along
in close company with the parent.    If disturbed, the parent
rushes off, setting the water in commotion by its movements,
and the brood scatter to conceal themselves at the bottom.    In
a short time, if one remains perfectly quiet, he may see the
larvae come forth in small groups from their hiding-places, and
perhaps the return of the parent.

The following table shows the rate of development.    The
cleavage times are based upon observations made on a single
egg, while the times recorded beyond late cleavage are based
upon data obtained by following the development of a second
egg.

| AGE IN HOURS. | LENGTH IN MM. | STAGE OF DEVELOPMENT. |
|---|---|---|
| — | — | Oviposition, beginning of |
| 2.24 | --- | First cleavage,    "    " |
| 3.28 | — | Second    "        "    " |
| 4.20 | — | Third    "        "    " |
| 5.22 | — | Fourth    "        "    " |
| 6.20 | — | Fifth    "        "    " |
| 7.25 | — | Sixth    "        "    " |
| 10. | — | Late    " |
| 15. | — | Blastula |
| 40. | — | Gastrula, early |
| 70. | — | Embryo visible |
| 160. | — | Embryo begins to move |
| 200. | 5–6 | Embryo hatches |
| 250. | 6–8 | Appearance of pigment |
| 360. | 9–10 | Yolk-sac absorbed |
| 480. | 12–14 | Pelvic fins appear |
| 720. | 17–20 | — |

## CLEAVAGE.

Our observations on the cleavage comprise : first, a study of
surface phenomena, followed continuously on the living egg ;
secondly, a comparison of corresponding stages as traced on
prepared material ; and thirdly, a study of serial sections.

In following the cleavage of the living egg we found it con-
venient to proceed as follows : The eggs still attached to blades

### Explanation of Cuts 1 to 6.*

Cut 1. — Vertical section of the mature ovarian egg, showing the calotte, the germinal vesicle, and a portion of the yolk.

Cut 2. — Vertical section of an egg at the beginning of the second cleavage. The position of the section is shown by the dotted line in Diagr. A. It shows the thickness of the calotte, the depth of the first cleavage groove (I) and the vacuolar spaces.

Cut 3. — Vertical section in the stage of the third cleavage, in the plane of the dotted line in Diagr. B. It shows the depth to which the first (I) and third (III) cleavage grooves have extended, having now cut through the calotte and become continuous with the vacuolar spaces, which have enlarged and increased in number.

Cut 4. — Vertical section of an egg in the same stage, but with the third verticals (III) arranged as in Diagr. C. The section passes near the margin of the calotte (dotted line, Diagr. C). The cleavage grooves have barely cut through the calotte, and the vacuolar spaces are less numerous than at the centre of the egg.

Cut 5. — Horizontal section of an egg in nearly the same stage. In this case one of the third set of grooves (a), instead of taking a radial direction like the others, occupies the position shown in Diagr. D. The section passes just below the calotte, and shows that the cell cut off by this groove is still in continuity with the yolk.

Cut 6. — Vertical section of an egg in the stage of the fourth cleavage, in the plane of the dotted line in Diagr. E. It will be observed that the circular groove passes somewhat obliquely, and apparently cuts off two cells at the right. An examination of another section of the same egg (Cut 7) shows, however, that centrally these cells are in direct continuity with the underlying yolk.

* Roman numerals indicate the order of the cleavage grooves.
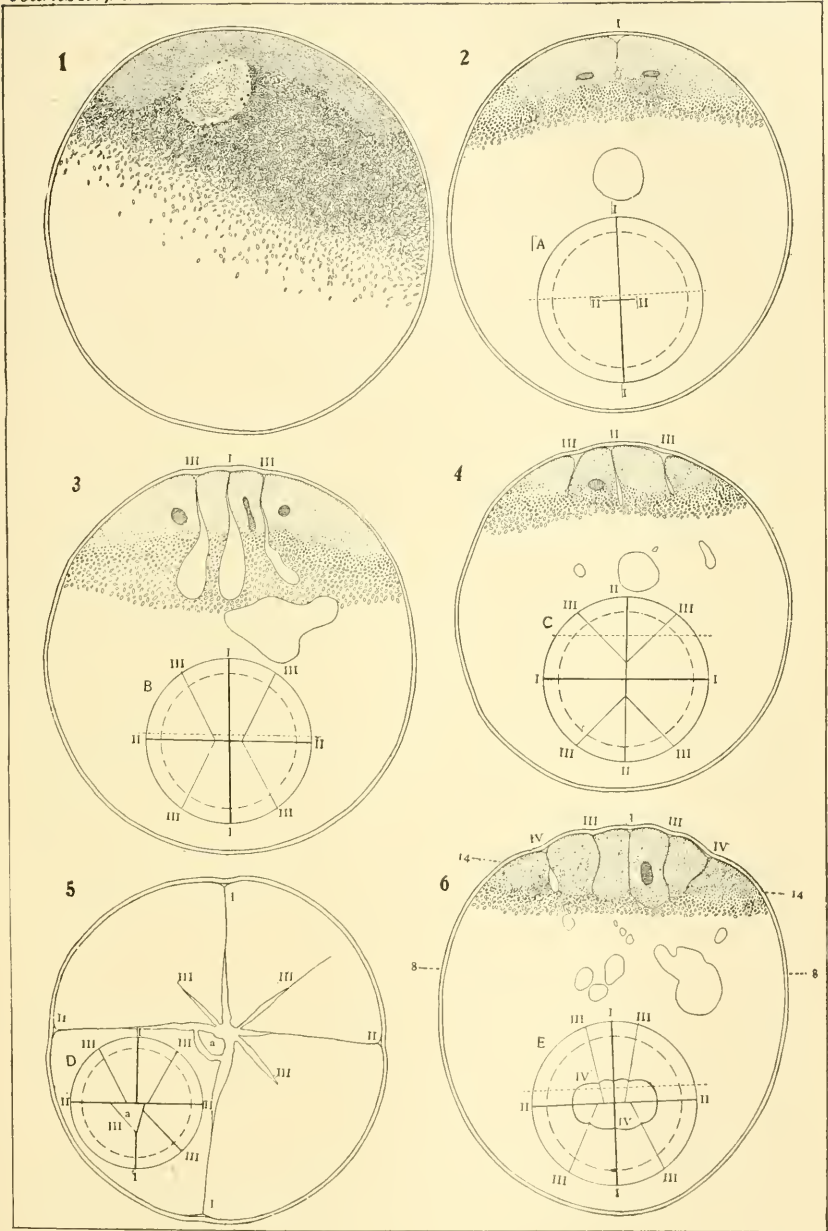
of grass or rootlets are placed in shallow watch-glasses and held in a fixed position by weighting with small pebbles. The watch-glasses are then placed upon a mirror fastened to the stage of a dissecting microscope. We could thus observe the changes occurring on opposite sides of the egg without disturbing its position.

The fixing fluids which have given the more satisfactory results are : Flemming's fluid, Perenyi's fluid, chrom-osmic, picro-acetic, and picro-sulphuric. For surface views chromosmic fixation gives most perfect pictures, the osmic blackening the cleavage grooves so that they stand out in bold relief. Another excellent method is surface staining with Delafield's haematoxylin, which may be employed with any of the above-named fixatives. For material which is to be sectioned, picro-acetic and Perenyi have given best results. Owing to the crumbling of the yolk we have been obliged to use celloidin as an imbedding mass. Serial sections by the method described by the junior author[5] have been used for the most part. Staining in section with Ehrlich's haematoxylin and Mayer's alcoholic carmine has proved most satisfactory.

The freshly deposited egg (Fig. 2) is firmly fixed to the object with which it first comes in contact by means of the threads of the villous layer, which are elongated over the lower hemisphere of the egg membrane. The egg is oval in profile view, measuring in its longest diameter, including the membrane, 2.5 to 3 mm. ; in its shortest, 2 to 2.5 mm. The upper pole of the egg, " calotte " of Fülleborn, " germ-disc " of Dean, is light yellowish brown. This calotte shades off at its margin into the dark grayish brown of the yolk. Near the centre of the calotte there is a single micropylar orifice, as shown in Figs. 21 and 22, which remains visible for some time (Figs. 12 and 16). We have frequently noticed that the margin of the calotte extends farther toward the equatorial region on one side than on the other, as shown in Fig. 2.

The calotte is already present in the full-grown ovarian egg shown in Cut 1. It is not of quite uniform thickness, but is a

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[5] A. C. Eycleshymer: Notes on Celloidin Technique. *American Naturalist,* vol. XXVI, pp. 354–358.

little thicker on one side than elsewhere. The germinal vesicle lies eccentrically beneath the deeper side, mainly in the yolk, only its upper surface projecting into the calotte. There are thus indications of a definite orientation in the egg before, as well as immediately after, fertilization.

*First Cleavage.* — In the egg shown in Fig. 3 the first cleavage appeared at 7.54 A.M. (2 hrs. 24 min. after deposition). A slight flattening of the animal pole precedes the appearance of this groove, which generally divides the calotte into nearly equal portions. The groove travels more and more slowly as it passes beyond the margin of the calotte. Its decreasing rate of progress in the egg figured is indicated by the following percentages, based upon the length of the arc traversed at successive intervals of about 1 hr. each, as compared with the entire circumference. At 8.53 (Fig. 3) the arc described equals 36% of the entire circumference ; at 9.49 (Fig. 4) 60% ; at 10.50 (Fig. 5) 77% ; at 11.55 (Fig. 6) 89% ; between 11.55 and 12.58 (Fig. 7) 100%. In most cases this cleavage is not complete until after the appearance of the fourth and sometimes even the fifth set of grooves.

The first groove is most frequently meridional, as shown in Figs. 3, 21, 22, and 23. In some cases, however, it may depart so far from a meridional that the segments formed are quite unequal (*cf.* Dean, *l.c.*, p. 425), as shown in Fig. 9. The ends of the groove may meet at the lower pole so as to form a continuous straight line, or in such a manner that an obtuse angle is formed (Figs. 14, 17). Sometimes they do not meet at all, and are only united by a portion of the second, or even third, cleavage grooves.

At the time the second groove appears the first has a depth of about one-half the thickness of the calotte, as shown in Cut 2 (made in plane of dotted line of Diagr. A). The path of this groove is definitely premarked to a point about twice this depth, terminating in an irregular vacuolar space at the level of the nuclei. The depth of the cleavage at this time strongly reminds one of the conditions seen in teleostean eggs with a perfectly defined blastodisc. The calotte certainly represents a concentration of germinal material, analogous to the blasto-

disc seen in the meroblastic egg of the teleost. One might say that the calotte represents a nascent blastodisc, — a blastodisc *in statu nascendi*, so to speak. It differs from the blastodisc, as seen in pelagic teleostean eggs, chiefly in not containing the whole, or almost the whole, of the material to be used in the formation of the embryo. The Amia egg is holoblastic, but represents an advance upon the condition seen in the egg of Acipenser, in the direction of the meroblastic egg of the typical teleost.

At this stage one or more irregular cavities are found in the upper hemisphere, between the centre of the egg and the calotte. One of these is shown in Cut 2. Just when and how these cavities arise we cannot say, but they are present in the first, as well as the later stages of cleavage. Sooner or later they become continuous with cleavage grooves, and in many cases unite in a common cavity. As these cavities appear in eggs prepared in different ways, collected in different seasons from various nests, it seems certain that they are not to be considered as artificial.

*Second Cleavage.* — The two grooves of the second cleavage usually begin at the same point of the upper pole and extend meridionally at right angles to the first groove (Figs. 4, 22, 23). Their progress is essentially the same as that of the first. In the egg shown in Fig. 4 these grooves appeared at 8.58 ; at 9.40 they had extended slightly beyond the margin of the calotte, and three to four hours later they had completely encircled the egg.

Variations in the formation of these grooves are not uncommon. The point of origin may be at a greater or less distance from the pole (Fig. 10). Instead of a right angle, acute and obtuse angles may arise, the grooves crossing in the form of an X (Fig. 11). Again, the furrows may not arise at the same point, but at points more or less widely separated, as in Fig. 12. They do not always begin at the same time ; one may precede the other by a considerable interval, reaching near the lower pole before the other has passed the equator (Fig. 14). Occasionally the two unite at the lower pole to form a continuous line running at right angles to the first ; at other times they

form acute or obtuse angles, as at the upper pole, or they may even terminate at points widely separated, as in Fig. 17. In some cases the grooves may not even reach the lower pole, but, diverging from a meridional plane, unite with the first groove at almost any point between the margin of the calotte and the lower pole.

*Third Cleavage.* — By the time the second grooves have passed just beyond the margin of the calotte the third set of grooves appear. In a majority of cases these are vertical, and occupy the positions shown in Figs. 5, 13, 15, 23, 24, and 25. They generally all depart from one or the other of the first two meridionals, thus giving rise to a distinctly bilateral appearance. In the egg followed (Fig. 5) the first of this set originated in the first meridional at 9.50. The second was formed in the adjacent quadrant, on the opposite side of the first meridional, at 9.51. The third appeared one minute later in the adjacent quadrant on the same side of the first meridional. The division of the remaining quadrant began at 9.54. The two furrows shown in Figs. 5, 6, and 7 later reached the lower pole, but the one first formed turned aside, running into the first meridional at some distance from the lower pole, while the remaining one united with the second meridional at about the level of the equator.

It often occurs that one or more of the set depart from the first meridional, while the rest depart from the second, or *vice versa* (Figs. 13 and 24). Frequently one observes the condition shown in Fig. 15, where all the grooves of this set pass in planes nearly parallel to the first or second meridional, giving rise to a bilateral form comparable with the 8-cell stage of the teleostean egg.* All these grooves may occasionally depart from a common point at the upper pole, and cut each quadrant of the calotte into two approximately equal portions, in which case the cleavage assumes a radial form such as has been described by Agassiz and Whitman [6] in the pelagic fish egg. Rarely, if ever, do these grooves divide the quadrants equally at the lower pole.

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* Dean (No. 3, p. 425) speaks of this condition as if it were the general rule.

[6] Agassiz, A., and Whitman, C. O.: On the Development of Some Pelagic Fish Eggs. *Proc. Amer. Acad. Arts and Sci.,* XX, pp. 23–75. 1884.

Cut 3 represents a vertical section of an egg at a stage just preceding the fourth cleavage. Its position is shown by the dotted line in Diagr. B. The section runs parallel with the second meridional, and cuts through two of the cells delimited by the third verticals (III). The first meridional has at this time cut entirely through the calotte and deeply into the underlying yolk. The third set of grooves have likewise extended far into the yolk. These grooves usually pass in vertical planes ; at times, however, they are more or less inclined ; we have sections in which one or more of them pass so obliquely that the cells are deeply cut, but in no case have we found any of them entirely severed from the underlying yolk. Occasionally one finds eggs in which one of these grooves (IIIa) occupies the position shown in Diagr. D, Cut 5. From surface study one might be in doubt whether this groove is vertical or horizontal. Serial sections of three such eggs show that the groove is vertical, and that the segment in each case is continuous with the underlying yolk.

In Cut 5 a horizontal section of such an egg is shown which passes just beneath the calotte. All the third cleavage grooves (III) except one (IIIa) are arranged radially, alternating with the two primary grooves. It is noteworthy that all these grooves widen into fissures which have a common vacuolar centre, giving rise to a star-shaped figure.

Cut 4 exhibits a vertical section of the same stage, but at right angles to the plane of Cut 3. The grooves on the surface of the egg are shown in Diagr. C, and the position of the section is indicated by the dotted line. The section is taken near the margin of the calotte. Sections nearer the centre of the egg show that the first and second grooves have reached a much greater depth, and have become continuous with cavities similar to those shown in Cut 3.

Dean (No. 3, p. 426) states that "the segmentation cavity takes its definite origin at this stage ; in the region of the animal pole the blastomeres are separated from the underlying yolk — the germ disc by a narrow fissure, which has been found to arise in the cleavage planes of the animal pole."

Passing over the singular construction of the second statement, which we confess to being utterly unable to comprehend, we are led to make a remark on the "segmentation cavity." It seems evident that Dr. Dean is predisposed to interpret his sections of the Amia egg in correspondence with what he finds laid down by Dr. H. V. Wilson in regard to the egg of Serranus. The discovery of a "segmentation cavity" in his Fig. 23 seems to have been thus inspired (compare this figure with Wilson's Fig. 15).

If the section shown in Dean's Fig. 23 passes in the plane indicated in his Fig. 4, the cell *a* is cut very near its central margin, and a little obliquity, either in the plane of the section or in the plane of the bounding groove, might be sufficient to account for the appearance. In other words, the section may represent only a chip from the central margin of cell *a*.

We must differ with Dr. Dean in respect to what seems to be his notion of "the segmentation cavity." The fissure, or fissures, thus designated by Dean, as a glance at his Figs. 27–30 will show, represent nothing more than ordinary intercellular spaces. Such spaces, as all the world knows, are not peculiar to any egg, and are not to be confounded with "*the* segmentation cavity." Dr. Dean says in one place (p. 424) "the segmentation cavity is practically wanting." Nevertheless he speaks later of its "definite origin," and calls special attention to it in his figures. In one case (p. 441) it is called a "flattened segmentation cavity."

Dr. Dean represents the segmentation cavity as beginning with the second cleavage but as taking "definite origin" at the time of the third cleavage. In Dean's Fig. 24, representing a section of an egg in the stage of the fourth cleavage, the "segmentation cavity" is shown as intercellular spaces, which he admits "might perhaps be regarded as artifacts." We would suggest that these spaces have a different meaning. The elongated nucleus shown in one of these cells is very clear evidence that what we have called the horizontal cleavage is in progress. At the time of the cleavage a constriction takes place at about the level of these spaces, and it is to these constrictions that they probably owe their origin. There is another

important point to be noticed in connection with this figure. The central blastomeres are represented as cut off from the yolk below. We feel confident that if the entire series be examined the cells will be found to be still continuous with the underlying yolk.

The cleavage cavity in Amia has rather an interesting mode of origin. For the first appearance of this cavity is in the form of central vacuoles, which are present as early as the first cleavage groove. The cleavage grooves, in many cases at least, expand into broad fissure-like cavities as they approach the centre of the egg (Cuts 3, 5, 15), where they become continuous with the earlier cavity or cavities. As the cleavage runs on these spaces enlarge, flow together, and thus often give rise to quite a spacious cleavage cavity, such as shown in Cuts 10, 17, 19. In some cases the cavity is so much reduced that it becomes inconspicuous.

*Fourth Cleavage.* — The fourth cleavage marks an interesting epoch, as it is the first to take a direct part in the formation of small cells at the animal pole. For the complete delimitation of the cells another cleavage is necessary, namely, a horizontal cleavage which cannot be seen from the surface and which affects only the eight cells bounded superficially by the fourth cleavage grooves, or by what we may call the circular groove. The latter usually appears midway between the pole and the margin of the calotte. It represents, strictly speaking, eight distinct grooves, one for each of the eight primary segments, but these usually run together in such a way as to form a continuous groove, encircling the pole and bounding a polar field which may have a circular form (Figs. 6, 25, 26) or a more elongated oval form (Figs. 16, 18). In one egg (Fig. 6) we have traced the order and direction of these grooves, noting also the time and place of origin as indicated in the figure.

There are many variations in this cleavage. It often happens that the eight constituent grooves run at various angles and are discontinuous, so that the polar field is bounded by a more or less irregular or zigzag line (Figs. 16, 18); sometimes one or more of these grooves take a meridional direction, as that shown in the upper part of Fig. 18.

This circular groove looks externally as if it might actually cut off polar segments, but as an examination of sections (Cuts 6, 7, 13, and 15) will show, the groove descends more or less obliquely, or centripetally, so that when completed its inner deeper boundary would be lost in confluent vacuolar spaces. As the plane of the groove probably never passes below the vacuoles, one might say that it describes an axial mass in the upper hemisphere approximating in shape an inverted truncated cone. Cut 6 is taken in the plane of the dotted line in Diagr. E. The circular groove appears to cut off two cells; this appearance is due to the section passing near the margin of the polar field. In Cut 7, which passes parallel with the second cleavage (plane of dotted line, Diagr. F.), these cells are still continuous with the yolk below. Cuts 13 and 15, taken along the dotted lines of Cut 7, further illustrate this point. It will be seen that at their bases the segments lying within the fourth cleavage grooves (IV) are likewise continuous with the yolk. Cut 8, taken at the level of the dotted line in Cut 7, shows the central grouping of the vacuolar spaces and the depth of the cleavage grooves at this time.

Dean (No. 3, p. 427, Fig. 24) figures and describes the "central blastomeres" of this stage as "separate from the underlying germ-yolk." In describing the next cleavage we shall explain how the central cells are cut off.

*Fifth Cleavage.* — The fifth cleavage comprises two distinct sets of grooves. One set appear on the surfaces of the eight primary segments in the form of meridionals. In Fig. 26 they are just beginning, and are more advanced in Fig. 27. The other set extend horizontally in the eight cells of the polar field, and of course are not visible from the surface. This cleavage thus brings about the 32-cell stage, and makes the central portion of the calotte two cells deep. The origin of yolk cells is thus not limited to the margin of the calotte, as in pelagic teleost eggs, but extends to all the cells beneath those of the polar field. These cells go on budding off cells to the germ-disc. It is rather interesting to see this horizontal cleavage appearing with the passage from the 16 to the 32-cell stage, because in the corresponding stage in the pelagic fish egg it

### EXPLANATION OF CUTS 7 TO 12.

CUT 7. — Vertical section of the same egg as that from which the section shown in Cut 6 was taken. Its position (dotted line in Diagr. F) is much nearer the centre of the egg. The section shows that the cells apparently cut off in Cut 6 are continuous with the yolk, the oblique character of the fourth (IV) cleavage grooves, and the elongated nuclei of the central cells.

CUT 8. — Horizontal section of an egg in the same stage, in the plane indicated by the line 8–8 shown in Cut 6. It shows the central grouping of the vacuoles, and the depth to which the grooves have at this time cleft the yolk.

CUT 9. — Vertical section of an egg in the stage shown in Fig. 8, Pl. XVIII. The central cells have been cut by both horizontal and vertical grooves. Nuclei are present in the yolk.

CUT 10. — Vertical section of an egg in the stage of early blastula, showing the cleavage cavity and the extent to which the yolk is now segmented.

CUT 11. — Horizontal section of an egg in the same stage, in the plane of the dotted line 11–11 in Cut 10.

CUT 12. — Vertical section of an egg in a late blastula stage. The calotte has begun to extend marginally over the yolk, and the epidermal layer is beginning to differentiate.
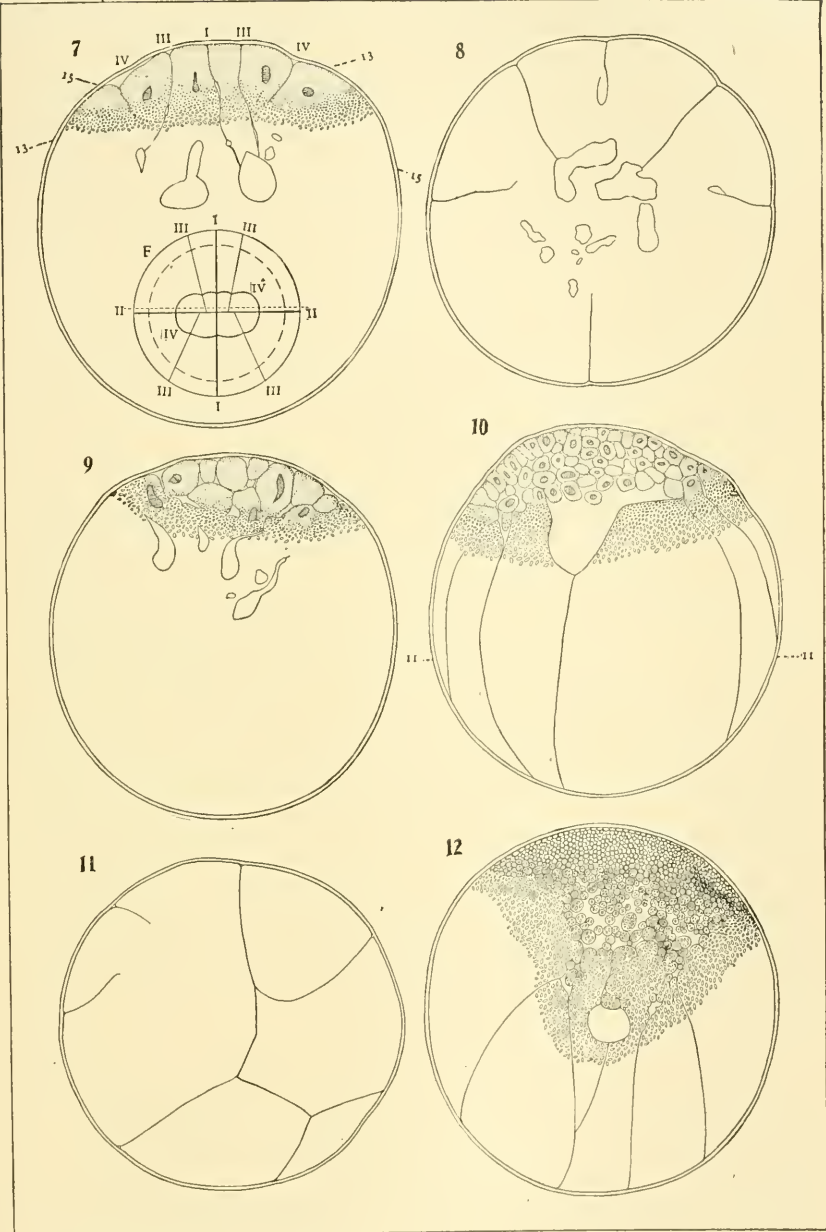
takes effect only in the four central cells of the 16-cell stage. The marginal cells are divided by verticals which correspond to what happens in the eight yolk segments of the Amia egg.

Variations in this cleavage are numerous. It often happens that one or more of the central cells are cut by grooves passing parallel with the circular. Again, some of the grooves, instead of taking a horizontal or a circular form, may pass vertically, as indicated both by surface views and the elongation of the dividing nuclei. Whether the grooves cutting the central cells shown in Fig. 7 are to be interpreted as variations, or whether they represent a part of the sixth cleavage, we are unable to say. In this egg the central grooves appeared shortly after the appearance of the grooves dividing the marginal cells, with which they soon became continuous.

Sections of this stage are shown in Cuts 9, 14, 16, and 18. In Cut 14 the plane of elongation of the nuclei in the large marginal cells confirms what has been described from surface views. In Cuts 16 and 18 the elongation of the nuclei (V) lying within the circular groove (IV) shows that the next division of these cells will take place in a horizontal plane. While this is undoubtedly the usual plane of division, there are exceptions. In each of the sections shown in Cuts 14 and 15 one cell of the eight delimited by the fourth cleavage shows its nucleus elongated in such a direction that the resulting cleavage will be circular. In other sections the elongation of the nuclei indicates that vertical cleavages may also replace the horizontal. Cut 9 represents a vertical section of an egg in the stage shown in Fig. 8. The horizontal cleavage has evidently taken place, and in addition to this another set of verticals have appeared. In this section it was impossible to trace the cleavage grooves through the yolk.

*Later Cleavage.* — The next cleavage, which might be called the sixth, is shown in Figs. 8 and 28, and consists in a general way of two sets of circular furrows, one of which appears between the first circular and the upper pole, the other between the first circular and the margin of the calotte. In addition to these, new verticals have arisen in some of the marginal segments.

Figs. 29 and 30 represent eggs of about the same stage as that shown in Fig. 28. In Fig. 29 a radial symmetry is noticeable in the arrangement of the smaller cells, while in Fig. 30 a bilateral grouping is evident. Fig. 31 represents a third egg in about the same stage, showing the position and extent of the furrows, which at this time have reached the vicinity of the lower pole. A somewhat later stage is shown in Fig. 32.

Cut 10 represents a vertical section of an egg about 10 hrs. after deposition. The calotte is now from four to six cells thick. Beneath the central portion is the segmentation cavity, which at this time has become greatly enlarged through the confluence of the vacuolar spaces. In some eggs (*e.g.*, Figs. 18, 19) at this stage the cavity is so much enlarged that it appears like that seen in Amphibian eggs. The floor of the cavity is made up of large yolk segments. A horizontal section (Cut 11) of the same stage taken in the plane of the dotted line of Cut 10 shows the number and depth of the grooves at this level ; above this level, near the equator, the grooves are more numerous.

Cut 12 represents a vertical section of an egg 35 to 40 hrs. after deposition (late blastula). The calotte, which has now begun to extend over the yolk, consists of thickly crowded spherical cells which marginally pass abruptly into the large yolk segments, while in the central portion they gradually increase in size and lie loosely scattered. The outer layer of the calotte is distinctly differentiated in that the cells are elongated and more densely granular. The entire yolk is irregularly cleft, the cells forming the lower portion are roughly polygonal and grade off into the large yolk spheres which lie at the centre.

## The Relation of the Embryo to the First Two Cleavage Planes.

The elongated form of the egg of Amia, in a closely applied envelope, prevents rotation about its minor axes. It is therefore a favorable egg for ascertaining what effects, if any, gravity

## EXPLANATION OF CUTS 13 TO 19.

CUT 13. — Oblique section of an egg in the stage of the fourth cleavage, just before the fifth cleavage. The plane of the section is represented by the dotted line 13–13 in Cut 7. The section shows on one side that the fourth cleavage has not yet cut off the central cells.

CUT 14. — Oblique section of the same stage, passing along the line 14 – 14 of Cut 6. The section shows the plane of elongation of the nuclei in the marginal segments which are soon to be divided by a set of verticals, forming a part of the fifth cleavage.

CUT 15. — Oblique section along the line 15 – 15 of Cut 7. The section shows that the circular groove (IV) becomes continuous below with the vacuolar spaces.

CUTS 16 and 18. — Vertical sections of the calotte from different eggs in the stage of fourth cleavage. The sections show the vertical elongation of the nuclei of the central cells preparatory to the horizontal cleavage which is to divide them.

CUT 17. — Oblique section of an egg in a stage a little earlier than that shown in Cut 19. The section passes in the plane indicated by the line 17 – 17 in Cut 19. The section shows an exceptionally large cleavage cavity. It also shows numerous yolk nuclei lying at the inner ends of the large yolk segments.

CUT 19. — Vertical section of a typical blastula. The cleavage cavity in this egg is also exceptionally large. Some of the large yolk segments may be seen dividing at their inner ends, the cells thus derived being continually added to the calotte.
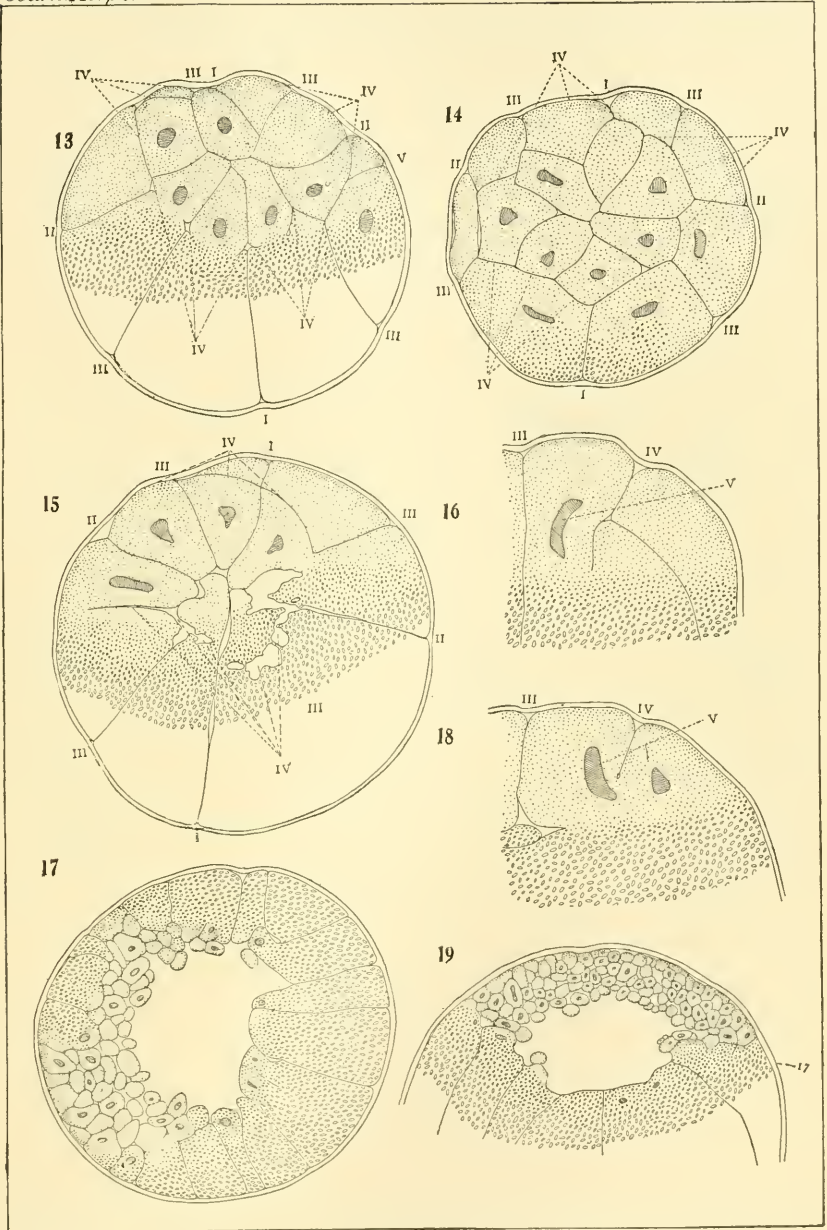
may have on the direction of cleavage, and for determining the
relation of the early cleavage planes to the median plane of

**20**
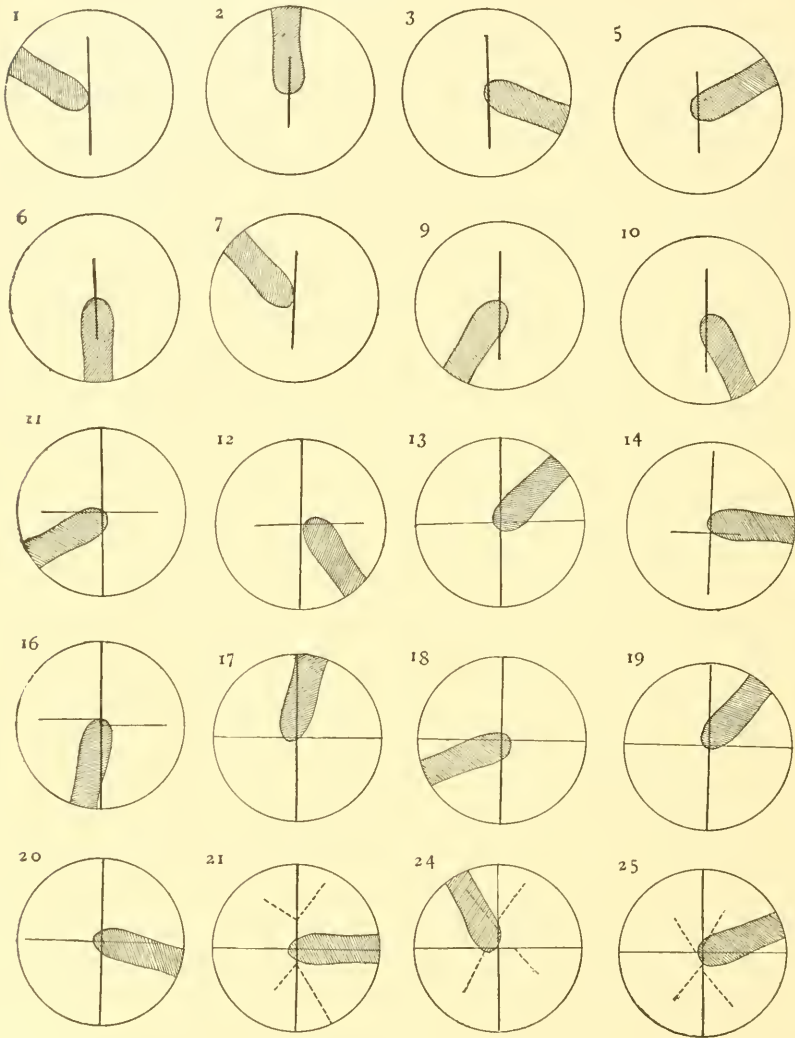


the embryo. Dean (no. 3, p. 422) found " in the early stages of
segmentation that the cleavage planes occurred in the normal
way when the position of the egg was reversed." This is true

not only for inverted eggs, but for eggs placed in any position whatsoever. It seems to follow that gravity can have no directing influence on the cleavage.

In order to ascertain whether there is any constant relation of the embryonic axis to either of the first two cleavage planes, eggs were fixed in given positions by weighting, as before mentioned, and a sketch of the early grooves was carefully made in each case. These grooves are easily identified for a long time in the lower hemisphere of the egg, even as late, in some cases, as the early stages of gastrulation. As the sketches made at successive intervals showed no movement of the egg during all this time, it seems probable that the position of the egg remained practically unchanged up to the time when the median plane of the embryo was ascertainable. In some cases accidental markings on the surface of the egg remained in a fixed position until the embryo was well defined.

Our observations were made on three sets of eggs. The results are tabulated below and illustrated by the diagrams shown in Cut 20.

*First Series.* — At 8 A.M. May 12, 1895, ten eggs in which the first grooves had just appeared were fixed in position and sketches made at successive intervals. Two of the eggs died during gastrulation, leaving eight in which the embryos were apparently normal. The results were as follows :

| Egg. | Embryo Visible. | Angle with First Groove. |
|---|---|---|
| 1 | 6 A.M. May 15 | 67° |
| 2 | 6 A.M. " | 0° |
| 3 | 5 A.M. " | 75° |
| 4 | Died during gastrulation | |
| 5 | 5 A.M. May 15 | 75° |
| 6 | 5 A.M. " | 0° |
| 7 | 5 A.M. ' " | 45° |
| 8 | Died during gastrulation | |
| 9 | 5 A.M. May 15 | 30° |
| 10 | 6 A.M. " | 25° |

*Second Series.* — At 9 A.M. on the same day, a second lot of ten eggs in which the second grooves were well under way were fixed in position. Nine of these formed normal embryos.

| Egg. | Embryo Visible. | Angle with First Groove. |
|---|---|---|
| 11 | 5.30 A.M.  May 15 | 70° |
| 12 | 5 A.M.        " | 35° |
| 13 | 5 A.M.        " | 45° |
| 14 | 5 A.M.        " | 88° |
| 15 | Died in late cleavage | |
| 16 | 5 A.M.  May 15 | 4° |
| 17 | 5 A.M.        " | 4° |
| 18 | 5 A.M.        " | 70° |
| 19 | 5 A.M.        " | 35° |
| 20 | 5 A.M.        " | 85° |

*Third Series.* — At 10.30 A.M., on the same day, a third lot of five eggs, in which the third set of grooves had begun, were fixed in position.   Only three of these formed embryos.

| Egg. | Embryo Visible. | Angle with First Groove. |
|---|---|---|
| 21 | 6 A.M. May 15 | 90° |
| 22 | Died | |
| 23 | Died | |
| 24 | 5 A.M.      " | 30° |
| 25 | 5.15 A.M. " | 70° |

The results may be summarized as follows :

1. Angles formed by median sagittal plane of embryo and first cleavage plane :

> In 10%, coincidence.
> In 10%, less than 5°.
> In 20%, greater than 5° and less than 45°.
> In 10%, 45°.
> In 35%, greater than 45° and less than 85°.
> In 10%, greater than 85° and less than 90°.
> In 5%, 90°.

2. Angles formed by the median sagittal plane of the embryo and the second cleavage plane :

> In 5%, coincidence.
> In 10%, less than 5°.
> In 35%, greater than 5° and less than 45°.
> In 10%, 45°.
> In 20%, greater than 45° and less than 85°.
> In 10%, greater than 85° and less than 90°.
> In 10%, 90°.

## Concluding Remarks.

Dr. Dean (No. 3, p. 425) states that he "*has taken especial care to verify his observations on the meroblastic character of the cleavages of Amia. During the first cleavages several hundred living eggs were examined, with a view of determining holoblastic variations. These, however, did not occur, nor were there found, even by the most favorable means of illumination, traces of what might be construed as surface furrows traversing the yolk region of the egg. In no case did a marginal cleavage pass below the rim of the germinal disc.*"

If an author can say all this of an egg that is plainly holoblastic, how can we accept his testimony against Balfour, to the effect that the egg of Lepidosteus is meroblastic? If this egg be holoblastic, its cleavage might be said to agree very closely with that of Amia. It would be of interest to know when the first horizontal cleavage occurs. Up to the 8-cell stage the cleavage grooves may have practically the same order and arrangement in Amia, Lepidosteus, Acipenser, and the teleost. In passing to the 16-cell stage we usually get four central cells, inclosed by twelve marginal ones in the teleost. This arrangement, according to Dean's figures, holds both for Lepidosteus and Acipenser. In Amia we get ordinarily the circular groove, resulting in eight small cells in the polar (central) field, surrounded by eight large cells. Thus far the three eggs agree in having no horizontal cleavage. The passage to the 32-cell stage introduces, in both Amia and the teleost, the first horizontal cleavage; but in the former we have eight cells dividing in this way, in the latter, only four. The resemblances in other respects would lead us to expect this form of cleavage at the corresponding stage in Lepidosteus and Acipenser. In that case these ganoids would agree with the teleost in having four central (polar) cells thus divided.

In the more regular forms of holoblastic cleavage, as is well known, the first horizontal, or the equatorial, as it is often called, comes in as the third cleavage. Is this cleavage, when it occurs as the third, homologous with the horizontal, which appears as the fifth in the teleost, Amia, and probably other

ganoids ?   How are we to determine homology in such a case ?
To suppose, as some authors have done, that the first horizontal
cleavage of the frog's egg is skipped in the case of the fish egg,
is nonsense.   There is no skipping in cleavage so long as the
division of the cytoplasm follows regularly the order of nuclear
division.   If nuclear division runs on unaccompanied by seg-
mentation of the egg, as in the insect egg, then we may speak
of skipping cleavage ; if cleavage does not appear until after
three or more nuclear divisions, and then begins and takes the
regular course, splitting into two, four, eight, etc., as in the
crab's egg, according to Paul Meyer, we may speak of deferred
cleavage.   In the fish egg the cleavage is neither deferred nor
skipped.   Where, then, is the homologue of the third cleavage
of the frog's egg?   If position and relation to the axes of the
future embryo are to decide homology, then there is no homo-
logue before the fifth cleavage, and no certainty of any even
then.   To assume homology between a cleavage of the 4-cell
stage and one of the 16-cell stage is to venture into a hopeless
tangle of anachronisms.   If order of succession is to be our
criterion, then first is first, second is second, third is third, and
so on, without regard to the position of the planes of cleavage
or the fate of the cells.   In either case, the attempt to homolo-
gize cleavages leads to contradictions and confusion.   Homol-
ogy that means nothing beyond the second cleavage, or that
may fail even at the first cleavage, is its own negation.   The
wide range of variation in cleavage in many eggs, its total sup-
pression in many others, and the possibility of changing its
course by artificial means, without affecting the final result, all
go to show, as it seems to us, that homologies do not depend
upon cleavage planes.   Even the first and second cleavages,
which appear, at first sight, to agree so closely in widely differ-
ent eggs, may cross the future lines of homology at various
angles.   If there is any homology in cleavages worth talking
about, it must be because they take definite and constant parts
in defining homologous organs or areas.   Something of this
sort has been claimed for the first cleavage.   On further exam-
ination the claim proves to be ill founded.   Often, as early as
the 8 and 16-cell stages, the first groove is transformed into a

zigzag line, running irregularly, now to one side, now to the other, of the median plane.

If homologies do not depend upon the form of cleavage, and may even unfold without any cleavage at all, how are we to explain the fact that in many cases (*e.g.*, annelids, molluscs) cleavage is so constant, and so early becomes coincident with lines of homology?   What determines this coincidence?   This question has been discussed by one of us elsewhere, and we need not repeat here the conclusions reached.

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## EXPLANATION OF PLATE XVIII.

All figures, excepting 8, 14, 17, and 20, drawn from living eggs. The numerals affixed to the cleavage grooves give the time in hours and minutes at which the grooves reach the points indicated by the dotted lines. The egg shown in Figs. 3–8 remained in a constant position.

Figures 9–20 are so arranged that the first cleavage groove has always the same position.

Fig. 1.  Unsegmented eggs attached to grass.  Natural size.

Fig. 2.  Profile view of the unsegmented egg, showing natural color of the egg and the villi by which it is attached.  Made by J. Nomura.  × 15.

Fig. 3.  Profile view of the egg 3 hrs. 23 min. after deposition, showing position and extent of the first vertical groove.  × 14.

Fig. 4.  Profile view of same egg at 4 hrs. 19 min.  The second verticals have extended slightly beyond the margin of the calotte.  × 14.

Fig. 5.  Profile view of the same egg at 5 hrs. 10 min., showing the position and extent of the first three sets of verticals.  × 14.

Fig. 6.  Profile view of the same egg at 6 hrs. 20 min., after the appearance of the circular groove.  × 14.

Fig. 7.  Profile view of same egg at 7 hrs. 23 min.  The fourth vertical cleavage is in progress.  × 14.

Fig. 8.  Profile view of same egg at 8 hrs. 25 min., showing additional circular grooves, one set within, the other without, the first circular groove. × 14.

Fig. 9.  Showing a case in which the calotte is unequally divided by the first groove.  × 13.

Figs. 10, 11, 12.  Views of the upper pole, showing variations occasionally observed in the position of the second verticals.  × 13.

Fig. 13.  View of the upper pole, showing variation in position of one of the third verticals.  × 14.

Fig. 14.  View of the lower pole of same egg.  × 13.

Fig. 15.  View of the upper pole, showing another variation in the position of the third verticals.  × 13.

Fig. 16.  View of the upper pole, showing a variation in the position of the grooves of the first circular cleavage.  × 13.

Fig. 17.  View of the lower pole of the same egg, showing points at which the second verticals terminate.  × 13.

Fig. 18.  View of the upper pole, showing a broken circular groove.  × 13.

Fig. 19.  View of the upper pole of an egg in a stage somewhat later than the one shown in Fig. 8.  × 13.

Fig. 20.  View of the lower pole of same egg.  × 13.

Pl XVIII

Pl XIX
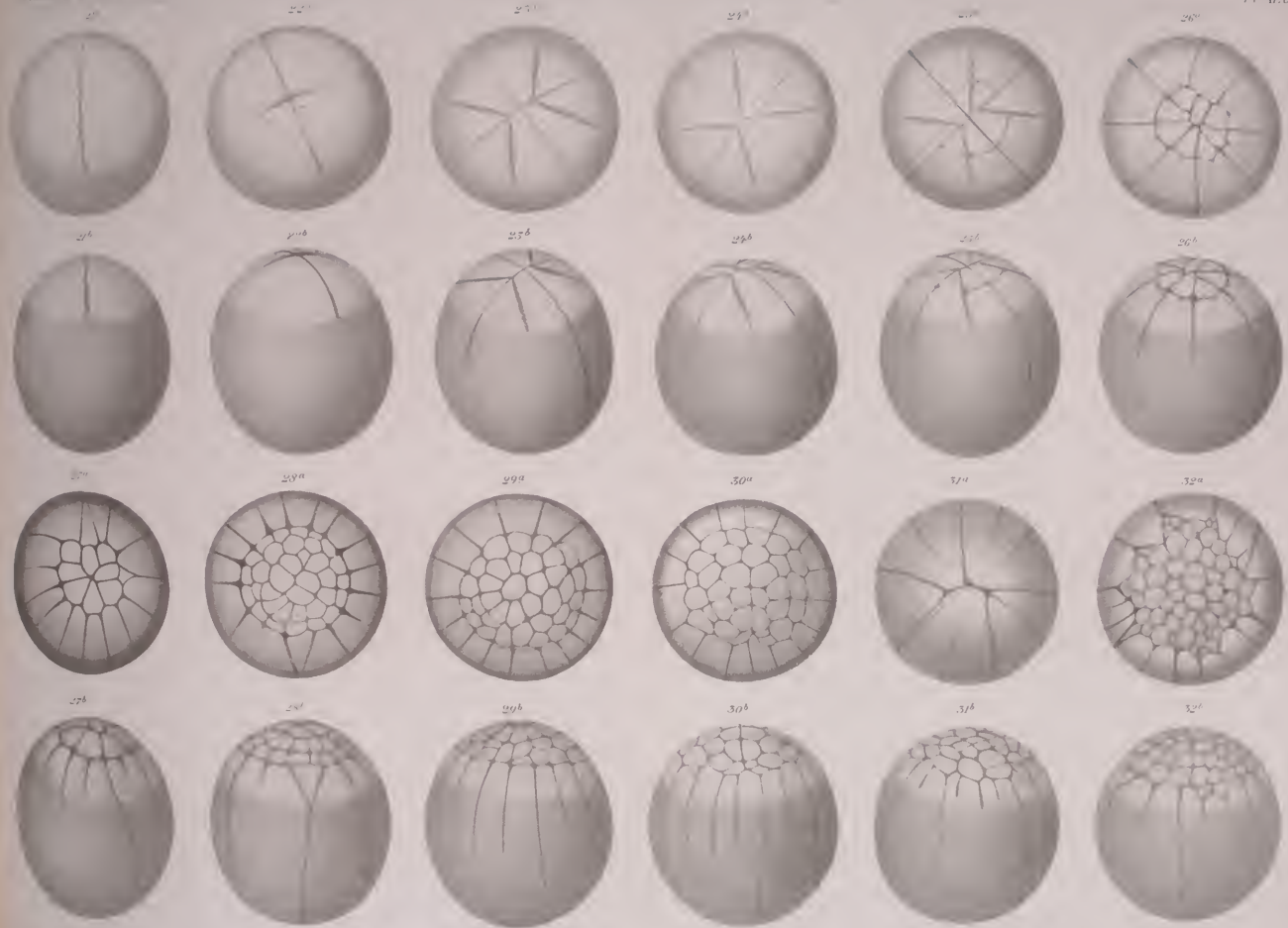
# ON THE MODES OF DEVELOPMENT OF THE MESODERM AND MESENCHYM, WITH REFERENCE TO THE SUPPOSED HOMOLOGIES OF THE BODY CAVITIES.

THOS. H. MONTGOMERY, Jr., Ph.D.

MESODERM is the term broadly applied to that embryonic cell mass situated between the primitive ecto- and entoderm. The mesoderm, *i.e.*, mesodermal cells, and not the extracellular gelatinous substance secreted in the archicoel by the ecto- and entodermic cells, is derived either from one of these two layers or from both of them. It is derived (1) from the ectoderm in the Anthozoa, Porifera ; (2) from the entoderm in Ctenophora, Polycladidea, Nemertini (in part), Nematodea, Annelida, Hirudinea (?), Sipunculacea, Chaetognatha, Balanoglossus, Echinodermata, Copepoda, Insecta (?), Mollusca, Phoronis, Ectoprocta, Brachiopoda, Entoprocta (?), Tunicata, Amphioxus ; (3) from both ecto- and entoderm: in the Nemertini (Desor's type), also perhaps in the Rotatoria and many of the Arthropoda (except Copepoda, Insecta). The origin of the mesoderm from the entoderm is thus the most usual.

The mesoderm may arise from the entoderm in three ways : (1) by multipolar delamination ; (2) by unipolar delamination ; (3) in the form of epithelial diverticula.

(1) The multipolar delamination, the so-called mesenchym production (Mesenchymbildung), consists in the separation of cells from the entodermal layer at various points on the inner surface of the latter, which become situated in the archicoel, in the gelatinous substance secreted by both ecto- and entoderm. Such mesenchym production is typical for the Nemertini, Echinodermata, and Ectoprocta.

(2) The unipolar delamination of the mesoderm from the entoderm takes place through the so-called pole-cells of the mesoderm, *i.e.*, one or two cells which divide off from the entoderm at one pole of the latter, and by the cleavages of which

the mesodermic cell layer is produced.   These pole-cells always lie in the archicoel.   Such a production of the mesoderm is typical for the Annelida and Mollusca ; further, in more or less modified form, for the Nematodea, Copepoda, and Entoprocta.

(3) Finally, the mesoderm may be produced from the entoderm, in the form of epithelial diverticula, or the so-called coelom sacks.   Such coelom sacks may be (a) evaginations of the gastrocoel, or (b) invaginations into the gastrocoel.

(a) The mesoderm is produced in the form of a coelom sack, which is an evagination of the entoderm, and the coelomic cavity of which is consequently a derivative of the gastrocoel : in the Echinodermata (the vasoperitoneal vesicle), Balanoglossus, Brachiopoda, Ascideae, and Amphioxus.

(b) The mesoderm is produced in the form of a coelom sack, which is an invagination of the entoderm, and the coelomic cavity of which is therefore derived from the archicoel, in Sagitta and Phoronis.

The three types of development of the mesoderm delineated above are not sharply distinct, since they differ from one another rather in degree than kind, and are connected by intermediate stages.   Thus we read on p. 12 of Korschelt and Heider's Lehrbuch : " So verschieden diese beiden Arten der Mesodermbildung (nämlich Urmesodermzellen, Urdarmdivertikeln) auch scheinen mögen, so lassen sie sich doch auf ein einheitliches Schema zurückführen, wenn wir annehmen, dass im ersteren Falle die Mesodermdivertikeln frühzeitig (als Urmesodermzellen) den epithelialen Verband des Entoderms verlassen, während im zweiten Falle die Mesodermzellmasse vorläufig in dem epithelialen Verbande bleibt und erst später durch die Divertikelbildung zur Lostrennung gebracht wird " (*cf.* also p. 267).   In fact, I would refer both the production of the pole-cells and of coelomic diverticula to the more primitive mode of formation of multipolar mesenchymatic migration. By the mesenchym cells remaining in contact as an epithelium, coelomic diverticula are produced, which are bipolar but multicellular in origin and character.   In the case of the two " Urmesodermzellen," I would explain, in agreement with Korschelt and Heider, the two pole-cells as two mesenchym

cells, the mesenchym tissue being here unipolar and bicellular, instead of multipolar and multicellular in origin.

Further, in meroblastic ova, from which the gastrulae are not of the invagination type, but sterroglastulae, etc. (as in the Cephalopoda, Sauropsida, Hirudinea, Turbellaria, most Arthropoda, etc.), the whole process of production is so much modified by the amount of yolk in certain of the blastomeres, that the mode of development of the mesoderm can with difficulty be referred to the three more primitive modes of development given above.

The examples given show how multifarious the development of the mesoderm is in different animal groups.

Again, there is no sharp distinction between mesenchym and mesoderm, but only a difference of degree; as is shown in a large number of the newer embryological researches.    Thus, while the term "mesenchym" is generally applied to cells of the third primitive embryonic layer which are not united together in a continuous mass, and while "mesoderm" is applied to cells of that layer when from the beginning they are in contact with one another, forming a mass of cells ("mesoderm-stripe"), we find, in the case of the production of the mesoderm through typical pole-cells, that the two cells may be or may not be in contact, but that the mesoderm cells proliferated by each construct a solid cell mass.    In fact, whether the cells of the third embryonic layer are not united ("mesenchym") or are united ("mesoderm," *sensu strictiori*) would seem to depend upon two factors : (1) the comparative size of the archicoel and (2) upon the modes of production of the third embryonic layer, which modes we have found to intergrade.    And in such groups as the Mollusca and Arthropoda, it is difficult to decide whether the third embryonic layer is to be classed as a "mesoderm" or as a "mesenchym."    Accordingly, these two terms express but extremes of a series, and morphologically cannot be sharply distinguished.

Lastly, as to the morphological value of the cavity enclosed by the mesoderm cells.    This cavity is a derivative of the archicoel in all those forms where the mesoderm is formed (1) by multipolar, mesenchymatic delamination, or (2) by pole-cells,

or (3) by invaginated diverticula of the entoderm (in Sagitta and Phoronis). It is a derivative of the gastrocoel when the coelomic sacks are evaginations of the entoderm (as in the Echinodermata, Balanoglossus, Brachiopoda, Ascideae, and Amphioxus). In the Ctenophora it is both gastrocoelic and archicoelic in origin. This cavity is called a pseudocoel when it is not lined by a continuous epithelium of cells, and a coelom when it has such a lining. The pseudocoel of a turbellarian and the coelom of an annelid are both derivatives of the archicoel; but the coelom of Amphioxus is gastrocoelic in origin. In the same animal, coelom and pseudocoel, when both cavities occur together, may communicate. From these facts we may conclude, that there is also no valid morphological distinction between a pseudocoel and a coelom, when both are derivatives of the archicoel. Whether, however, a morphological distinction can be drawn between the archicoelic coelom of an annelid and the gastrocoelic coelom of an Amphioxus, is a question which I believe has not yet been considered.

In regard to the ontogenetic derivation of the blood-vessels, or blood lacunae, their endothelia, and the free blood corpuscles, the following points are of interest. These observations are based mainly upon data given in the zoölogical text-books of Korschelt and Heider, Hatschek, and Arnold Lang.

In the Nemertini the cavities of the blood-vessels and of the rhynchocoel are archicoelic, and these are lined by endothelia, from which the floating cells are derived. And, as I have shown in a paper, "On the Connective Tissues and Body Cavities of the Nemerteans," etc., to appear in Spengel's *Zool. Jahrb.*, the gonads and their products are also archicoelic — at least in all those forms in which the gonadal sacks are then first formed, when the sexual cells become differentiated from the somatic cells.

In the Chaetopoda and the Archiannelida the cavity of the blood-vessels is archicoelic, their walls, from which the free corpuscles are derived, splanchnopleuric in origin. In the Hirudinea, to cite Korschelt and Heider (p. 222): "Von den dorsalen und ventralen Blutgefässstämmen ist angegeben worden, dass sie vom splanchnischen Blatt aus, durch Spaltung dessel-

ben, ihren Ursprung nehmen"; they stand in open communication with the pseudocoelic body sinuses, the latter having an endothelial lining in the Rhynchobdellini, but not in the Gnathobdellini. The blood corpuscles of the leeches are probably derived from the blood-vessels as well as from the so-called "parenchym" (Lankester). In the Sipunculacea, there is probably a communication between the blood-vessels and the coelom, the splanchnopleuric peritoneum of the latter being ciliated, as in the frog (Lang, p. 213).

Echinodermata: "Spaltbildungen im Mesenchym sollen die Blutgefässe der Holothurie entstehen lassen. . . . Die Blutzellen hingegen sollen sich von den Wandungen des Hydroenterocoels losgelöst und bei der Bildung jener Gefässe betheiligt haben. Diese freien Zellen, welche sich sowohl in der Leibeshöhle, wie in den Ambulacral- und Blutgefässen finden, würden also nach dieser Auffassung (Semon) nicht von dem ursprünglichen Mesenchym abstammen" (Korschelt and Heider, p. 286). In the Asteroidea: "In der zwischen Hydrocoel-, Enterocoel-, und Darmwand gelegenen Mesenchymschicht bildet sich dort ein Spalt, welcher eine Auskleidung von sehr flachen Zellen aufweist" (*ibid.*, p. 290). The blood-vessels in the Crinoidea communicate with the coelom, and arise as cavities in proliferations of the enterocoel (*ibid.*, p. 302).

In all the Arthropoda, as is well known, the blood-vessels stand in communication with the body cavities. The heart of the Crustacea is mesodermal in origin, its cavity archicoelic (Korschelt and Heider, p. 376). In Limulus (*ibid.*, p. 528), the heart develops from the mesodermal plates, and cells wander into its cavity, which had their origin in its walls. In the Scorpionidea (*ibid.*, p. 556), a segmented coelomic cavity arises in the primitive mesodermal proliferation, the walls and free corpuscles of the blood-vessels being mesodermal (is their cavity then coelomic?). As to the Araneina (*ibid.*, p. 615), it is a disputed point whether the blood corpuscles are merocytes or whether they originate from the protovertebrae; later they form a solid chord on the dorsal side of the embryo; they separate from one another again, when the heart becomes formed by a coalescence of neighboring splanchnopleuric layers; thus the

cavity of the heart is archicoelic. In Peripatus (*ibid.*, p. 711), the heart is formed by mesodermal cells, its cavity being archicoelic. The blastocoel of the Myriapoda (*ibid.*, p. 752) becomes filled with yolk, while later mesodermal cells penetrate and surround the yolk, producing a pseudocoel; these cells accomplish the formation of the blood-vessels, and perhaps also of the heart. The body cavity of the Insecta is a product of the primitive archicoel and the coelom; the heart is formed of mesodermal cells of protovertebral origin, and the blood corpuscles are also of similar derivation (*ibid.*, pp. 818, 833, 834).

In the Mollusca the archicoelic (pseudocoelic) body cavity: "stellt im Allgemeinen das Lacunen- und Sinussystem des Körpers dar, in welches sich die Arterien öffnen, und aus welchem die Venen, wo solche vorhanden sind, ihr Blut beziehen. Sie ist ohne eigene Epithelwand" (Lang, p. 792). The true coelom is much reduced, being represented only by the pericardial and gonadal cavities, and is bounded by its own epithelium. The blood-vessels (a closed system only in the Cephalopoda and certain Prosobranchs) have no endothelium (Lang, p. 780). In the Lamellibranchiata, the heart, aortae, and gill-veins are produced by the agency of mesodermal cells in the primitive body cavity; there is no communication between the blood-vessels and the pericardial cavity (Korschelt and Heider, pp. 971, 973). The heart in the Gasteropoda is derived from the pericardium, while "die Gefässe entstehen als Lückenräume im mesodermalen Zellmaterial der primären Leibeshöhle (Blastocoel), also zunächst ganz unabhängig vom Herzen" (*ibid.*, pp. 1081, 1082). In Phoronis a coelom is well developed and is bounded by an endothelium (Lang, p. 213). The blood-vessels probably arise as lacunae in the splanchnopleuric mesoderm: "während nach Cori das Gefässsystem des ausgebildeten Thieres ein vollständig geschlossenes ist, scheint in der Larve eine Communication zwischen demselben und dem Kopftheil der Leibeshöhle zu existiren. Im letzteren sollen die Blutkörperchen in Massen angehäuft ihre Entstehung nehmen" (Korschelt and Heider, p. 1184).

In the Ascideae, the early coelomic cavities give way later to a mesenchym, which fills the archicoel: "die später in die-

sem Mesenchym auftretenden Lacunen müssen ebenso wie die Blutgefässe (welche . . . einer endothelialen Wand vollkommen entbehren) als Pseudocoel betrachtet werden. . . . Indem einzelne Zellen des Mesenchyms frei werden und in das Pseudocoel gelangen, bilden sie sich zu Blutkörperchen um" (Korschelt and Heider, pp. 1289, 1290). In Salpa the heart is of mesodermal origin, and the vessels are lined with an epithelial intima : "die Blutgefässe entstehen anscheinend als Lückenräume innerhalb jenes gallertigen Bindegewebes, welches in späteren Stadien die primäre Leibeshöhle erfüllt" (*ibid.*, p. 1346).

In the Vertebrata the cavity of the blood-vessels is probably archicoelic and their walls mesenchymatic in origin; but the present views upon the derivation of the corpuscles and of the blood-vessels themselves are very conflicting.

To recapitulate in regard to the origin of the blood-vessels and blood corpuscles : the cavity of the blood-vessels (with the exception of the heart in the Gasteropoda) is apparently always archicoelic (blastocoelic), and never gastrocoelic nor coelomic. The blood corpuscles in most of the animal groups are derived from the endothelial lining of the vessels where such membrane is present, but where it is absent, from the surrounding connective tissue elements. Accordingly, since the walls of the blood-vessels may be mesenchymic (Nemertini?, Hirudinea Asteroidea, Ascideae, Vertebrata?), or may be mesodermal (Annelida, Holothuroidea, Limulus, Scorpionidea, Peripatus, Insecta, Lamellibranchiata), so the blood-vessels themselves may be either mesodermal or mesenchymic, or both (*e.g.*, Hirudinea).

The foregoing brief summary of our present knowledge on the development of the various body cavities of the Metazoa would lead to the conclusion, that particular differentiations of this cavity in one group cannot be safely homologized with similarly situated cavities in other groups. And the reason for this is not far to seek. For, in the first place, the very different modes by which the process of gastrulation takes place — a process which seems to become modified by the mechanical factors determining the previous cleavage of the egg — induces very heterogeneous formation of both archi- and gastrocoel.

Thus, though under the term "archicoel" is understood the cleavage cavity between the embryonic layers ectoderm and entoderm, it is in reality also equivalent to the space between any two neighboring blastomeres, as, *e.g.*, the cavity between two ectodermal or between two entodermal cells, or even between an ectodermal and an entodermal cell. Further, since this cleavage cavity is, in pre-blastula stages at least, in a direct communication with the outside (as is well seen in the cleavage of the Ctenophora and Tricladidea), its relation to the gastrocoel is found to be close. And in the very frequent, and perhaps most primitive, method of gastrulation, according to which the entodermal cells delaminate from the ectoderm and wander into the blastocoel, we find that the cavity enclosed by the later entoderm, and thus comparable to a gastrocoel, is, in fact, a portion of the earlier blastocoel. So, nearly all intergradations may be found between the cleavage illustrated by the triclad Turbellaria, where the blastomeres are at first isolated (with consequently a large and open cleavage cavity), and the sterroblastic cleavage exhibited, *e.g.*, by the polyclad Turbellaria or the Rotatoria, where the blastocoel is represented merely by clefts between adjacent blastomeres.

Gastrocoel and blastocoel are nothing more than spaces between or enclosed by the blastomeres, which, in early stages at least, communicate with the outside. The gastrocoel may be in certain cases a space lined by entodermal cells, divided off from the primitive blastocoel, or it may be an extraneous space bounded by such cells. In the same group of animals both modifications may occur (*e.g.*, in the Mollusca, the Crustacea, and Turbellaria).

The embryonic body cavities known as blastocoel and gastrocoel are, therefore, not morphologically distinct spaces, and only in certain cases (*e.g.*, typical invagination gastrulation) are they to be separated. For the mode of formation of both is apparently dependent upon such factors as the mechanical pressure of the yolk, etc. (other factors might well be at work, which we have failed to recognize), that is, dependent upon the process of cleavage ; and the latter process, as is well known, may present great differences in closely allied forms (as in the

Crustacea or Turbellaria), and hence no high morphological value can be attributed to it.

Obviously then the mesoderm, as well as the spaces enclosed or penetrated by mesodermal elements, cannot be granted importance in morphological classification; which deduction is in accord with a number of recent investigations, that stand in opposition to the acceptance of the germ-layer theory. For the development of the mesoderm and its cavities is, in its turn, dependent upon the cleavage and gastrulation processes. Thus a mesodermal pseudocoel as well as a coelom are usually blastocoelic; but the coelom may be also gastrocoelic in origin. Similarly there is no essential difference between formation of the mesoderm by detached and isolated cells, and by coelom sacks or epithelially united mesoderm stripes.

Accordingly, I am led to conclude that the body cavities in different animal groups cannot be homologized merely on the ground of apparent similarity of development; for the earlier development and differentiation of these cavities must be referred, directly or indirectly, to the modes of cleavage and gastrulation, and the latter, as is well known, often differ widely in closely allied forms.

The coelom of a vertebrate is frequently spoken of as being homologous with that of an annelid, since it passes through an apparently similar development. Now without stating or in any way wishing to imply that the homology of the coelom in this case is not correct, I would emphasize the point that the similarity of development is itself not an adequate reason for the homology. This standpoint should seem justifiable to any one acquainted with the facts reviewed in this paper, which tend to show how various the formation of mesoderm and its cavities are in closely related forms.

Are, then, the body cavities possible of homologization? Comparison of the modes of early development shows that the ontogeny is of little value in this connection; but it might be thought that comparative anatomy could be of avail in the search for homologies.

But, though often spoken of as such, a " body cavity " cannot be considered an organ, equivalent, *e.g.*, to a brain or a

nephridium, but rather it must be compared to a system of organs, since a number of different functions are performed by it, and since it stands in a peculiar relationship to most, if not all, of the bodily organs. The whole origin, manner of differentiation, and extent of development of the body cavity, is to a great extent dependent upon the mutual positions of the organs as well as upon their degree of correlation. Therefore, any change which causes a difference in the relative positions of the organs must also effect some degree of change in the diversification of the body cavity.

The body cavity being essentially a space or system of spaces separating and penetrating the organs of the body, a common possession of these organs, cannot be correctly homologized, for the very fact that it is not comparable to any individual organ. It would be as difficult to satisfactorily homologize these cavities in different forms as to establish homologies between states of correlation, or between mutual arrangements of parts. And in case examples are required we may consider the group of the Nemerteans, for it was a study of the body cavities in this interesting group of worms which led me to make the present examination into the question of possible homologies.

In the Nemerteans, the body cavity occurs as (1) the rhynchocoel (the large space surrounding the proboscis); (2) the cavity of the blood-vessels; (3) the perivisceral space situated between the intestine and blood-vessels on the one hand and the body muscular wall on the other. The portions of the cavity mentioned under (2) and (3) are remnants of the cleavage cavity (*i.e.*, archicoelic in origin); but whether the rhynchocoel also is archicoelic has not yet been definitely settled. Now in a previous contribution, to which I have already referred, I compared the pseudocoel in this group to the coelom of the Annelids, and for the following reasons : the Nemertean pseudocoel — that space the existence of which has, until recently, been questioned — encloses in the adult worm true mesenchym tissue consisting of multipolar cells ; it is from certain of these cells that the sexual cells are derived. Further, in all the species where the gonadal sacks are not preformed, — and this is the case in

the majority of forms, — pseudoepithelia of these primitive sexual cells arrange themselves in the form of paired and metameric sacks, which are then the gonads.   Thus the comparison holds good, that in both Annelids and Nemerteans the sexual products are derived from the lining of the perivisceral body cavity.   In the Metanemerteans the blood-vessels are completely closed, while in the lower Nemerteans they are (in the head region) in communication with the pseudocoelic spaces : in the former as in the Chaetopoda, in the latter as in the Hirudinea. Thus parts of the body cavity in the Nemerteans may be compared to a true coelom, other portions to a pseudocoel (archicoel).   The great difficulty of determining the homology of the Nemertean body cavity is simply due to the fact that it unites characteristics of a coelom and a pseudocoel — formations which, according to the mesenchym theory of Hertwig, had formerly been supposed sharply distinguishable.   So we find the difficulty is in reality owing to the fact of the impossibility of correctly homologizing such structures as body cavities ; for not only may two (supposedly) different types of cavities be present in the same species, but the two are frequently so intermingled in it as to render their recognition almost impossible in our present state of knowledge.   Thus we are not in position to state whether the Nemertean body cavity is of Turbellarian or of Annelidan character.

The general conclusion which I would maintain, then, is that body cavities in different animal groups cannot be safely homologized, either from the ontogenetic or from the comparative anatomical standpoint ; though the latter method would seem to be, in this matter at least, more reliable than the preceding. The body cavity, whether as coelom or as pseudocoel, is not comparable to any single organ or set of organs, but must be considered as a structure of approximately equal economy to all the organs.   And in its early formation and later differentiation probably most if not all of the organs take part, beginning with the blastomeres as the earliest.   Accordingly, before seeking to homologize the body cavity, the morphological value of all the organs themselves must be learned, as well as the morphological value of their mutual topography.

In my search for the homologies of the coelom, or pseudo-coel, of the Nemerteans, a search which has led me to negative results, I have been obliged to exceed the limits which I had at first intended ; but it is to be hoped that this brief critical consideration of the morphological value of body cavities in general may throw a new light on these structures.

Any one wishing to compare earlier views upon their nature may consult, outside of the text-books of Balfour, and of Korschelt and Heider, the following papers :

BALFOUR. On the Structure and Homologies of the Germinal Layers of the Embryo. *Quart. Journ. Micr. Sci.*, 20. 1880.

BÜTSCHLI. Bemerkungen zur Gastraeatheorie. *Morph. Jahrb.*, 9.

HAECKEL. Die Gastraeatheorie, die phylogenetische Classification des Thierreichs und die Homologie der Keimblätter. *Jena. Zeitsch.*, 8.

IDEM. Nachträge zur Gastraeatheorie. *Ibid.*, 11.

HATSCHEK. Studien über Entwickelung des Amphioxus. *Arb. zool. Inst. Wien*, 4. 1881.

HERTWIG, O. AND R. Die Coelomtheorie. Versuch einer Erklärung des mittleren Keimblattes. Jena, 1881.

LANKESTER. On the Primitive Cell-layers of the Embryo as the Basis of Genealogical Classification of Animals, and on the Origin of Vascular and Lymph Systems. *Ann. and Mag. Nat. Hist.*, 11. 1873.

IDEM. Notes on the Embryology and Classification of the Animal Kingdom, etc. *Quart. Journ. Micr. Sci.*, 17. 1877.

RABL. Theorie des Mesoderms. *Morph. Jahrb.*, 15. 1889.

WALDEYER. Die neueren Forschungen im Gebiet der Keimblattlehre. *Berlin. klinisch. Wochenschr.* 1885.

# SOME SPINNING ACTIVITIES OF PROTOPLASM IN STARFISH AND SEA-URCHIN EGGS.

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THE observations recorded here were begun at the Marine Biological Laboratory of Wood's Holl, in the summer of 1893. For use of an "investigator's room" and other privileges enjoyed there for the third time, I am indebted to the kindness of the Director, Dr. Whitman.

While watching the protoplasm of developing starfish and sea-urchin eggs under very high powers, certain curious filose phenomena restimulated an interest that had for years concerned itself much with the "thread-forming," "filose," or, as I prefer to call them, the *spinning* activities of the living substance, especially as found among the Protozoa, where they are of widespread occurrence outside the extremely large group of protoplasts in which they are characteristic phenomena.

The eggs in which the supposedly typical phenomena were watched were normal, so far as could be discovered by a series of comparative observations and experiments on many other specimens, some of which developed normally into quite advanced stages. Great care was used to make little or no compression on these eggs, to keep the water fresh and plentiful about them, to maintain an even temperature, and to hold them but a few moments at a time under observation.

Other cases which were carelessly dealt with, or which were immature, or over-fertilized, showed plainly an abnormal state of their substance, which was visible in spinnings of a decidedly different character from those of the normal specimens. These cases were followed closely for long periods, and normal specimens were made abnormal by heat, or pressure, or confinement, so as to learn the peculiarities of such states, and, if possible, distinguish with regard to the spinning phenomena between these and the normal condition.

The observations at Wood's Holl were made with a Beck $\frac{1}{12}$ immersion, which had been carefully chosen from a number for its flatness of field, definition, and lack of color, — with apochromatic eyepieces.

At the Zoölogical Laboratory of the Sorbonne, Roscoff, Brittany, where I was enabled by courtesy of M. Lacaze Duthiers to make a series of similar observations, the instruments used were all of Zeiss, — largest size stand, with Abbé condenser and iris diaphragm ; 4.0 mm., and 2.0 mm. immersion, lenses ; with 4–12 apochromatic oculars.

While there proved to be nothing to correct in the first observations, they were much extended and amplified by the second series whose range of magnification brought to sight a greater length of filament, also secondary spinnings, or ramifications of these, and still other primary filaments far beyond the reach of the powers before used.

The sea-urchin eggs were from two genera ; those obtained at Wood's Holl, from Arbacia ; those used at Roscoff, from the common, large, and finely colored species of Echinus found along that coast.

The Arbacia eggs were perfectly normal in their initial conditions, and in large proportion developed to a late larval stage ; they, as well as the sperm, were obtained artificially from the animals. The Echinus eggs and sperm were also obtained artificially, but, being more hardy, gave, it was thought, for that reason more reliable data than Arbacia even, although the results in the one case but confirmed those in the other.

The starfish eggs used in both series of observations were of the same genus, but of different species. In all the test cases the eggs were laid and fertilized naturally in a tank shortly after capture. Those collected at Roscoff were sometimes from two or more females and two or more males depositing at one time. The eggs were, however, protected from too great admixture with sperm by instant separation, and afterwards carefully washed and then kept in small quantities in separate, large dishes in which the water was frequently changed.

In many of these dishes it was difficult later to find any eggs which had not reached a normal, free-swimming, larval state due at about that given time. The test specimens were kept isolated of course, but compared with results given by random specimens from the larger number.

In the eggs of Arbacia, I had seen the characteristic, striated membrane produced, immediately after entrance of the sperm, by formation of innumerable, delicate, thread-like processes from a clear pellicular layer of the egg. Although the resemblance between these threads and those formed in the *tuft* of protoplasm which receives the sperm was very great, I could not convince myself that they were not perhaps of non-living matter and caused by mere exudation of a .glairy substance, which, if issuing from small, pore-like openings, could assume a thread-like shape. An appearance of partial return in single instances of the substance towards the egg, did not seem proof to the contrary, since it was thought that this would be quite possible were the substance of the nature suggested.

In the Echinus eggs, the question seemed to be solved by several cases of polyspermy, in which the egg, seen to be immature from the nuclear conditions, never formed a perfect membrane, but merely made abortive attempts to do so, the various portions of its surface having more or less success, or making, perhaps, more or less exertion, in *spinning*. Of the most typical instance of this sort, a camera lucida memorandum was made, showing the various protoplastic activities of the pellicular substance, and also the several *tufts* which mark the entrance, complete or partial, of several sperm.

The whole surface of this egg, soon after entrance of the first sperm, was covered with a rather thick and albuminous-looking material, like ectosarcal protoplasm, in which no vesicular structure of Bütschli was visible, although there was a deceptive appearance of such, caused, as it seemed, by optical sections of papillose, or short, thread-like, processes.

This clear layer seemed at first to be only partially effective in preventing the entrance of more sperm, and it responded to the attempts of these with more or less pronounced tufts, and

showed wave-like modulations of its surface outline, possibly
due to accession of more material from the egg.

About the point of entrance of the first sperm, there arose
for some distance a host of delicate threads, closely resembling,
except in their instability and their unevenness of length,
those of the normal eggs.   These threads had an optical effect
of being outspinnings of the outer clear layer of the egg, and
of the mere surface substance of this.   At other regions there
was sometimes an effect of the processes piercing this and
arising from the inner or true surface of the egg itself.   That
they did arise in some places from the outer layer was shown
by local formations of short, papillose roughenings of this,
which were seen first as mere superficial unevennesses but
which afterwards extended themselves so as to form true
threads.

That the outer layer and its products were, for the time
being at least, part of the living material of the egg, — a true,
ectosarc-like formation, — seemed proven by ramifications of the
threads in the more abnormal regions of spinning ; by unsta-
ble anastomosis with each other of such secondary, and also of
the primary, processes ; and by the general flux of their sub-
stance to and from the layer, as well as amongst each other,
in a manner most characteristic of protoplastic phenomena.
Such activities were noted in the tufts, and indeed there
were no characters optically discoverable which enabled one to
separate the two groups of phenomena in these abnormal eggs.
In normal cases the single tuft is formed from a delicate
covering of the egg, which has the same general character as
the thick, abnormal envelope, being merely more stable and
quiet.

At time of forming these areas and processes in the abnormal
type-instance the nuclear membrane had not yet been dissipated,
but lay somewhat to one side of the egg.   The nuclear area,
although perfect, showed itself to be immature by the mode of
distribution of its elements.[1]   The progress of this special egg

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[1] Certain progressive differences which mark the development or maturation of
this cell body are described in a forthcoming thesis on the living substance *per se*
in some of its more minute structural aspects.

was not followed directly, but was seen later to have had a markedly degenerating course.

Wherever such abnormal processes of membrane formation by spinning were watched, the processes differed from the normal filaments in being more irregular and unrestrained in protoplastic character ; there was less of direct thread formation, more of tufts or brush-like processes, which were unstable in progress and even returned altogether to the egg covering.

In the normal eggs the spinnings were swift, straight, smooth, direct, and continuous in their progress, neither ramifying nor returning. They seemed more homogeneous optically, and had not granules scattered along their course, as the abnormal spinnings often had for a considerable distance. Normally the threads came to be of about even length at about the same moment, while in abnormal eggs they were at a given moment unlike at almost all points of the periphery, the most perfect being formed always about the region of entrance of the first sperm.

In normal eggs, the threads, having reached a certain, but rather variable, distance from the egg, appeared to fuse at their tips and then to spread out their substance there, so as to form a ceiling film. Increase of outflowing substance soon thickened the pellicle thus made, and almost simultaneously the threads themselves proceeded to fuse along their length, either by access of material from the egg over them, their linear extension having been to a great degree stopped, or by a spreading out of their substance as they continued to be formed from the rear; or perhaps even by exudations or secretions from them.

The distance from the egg at which the threads began to fuse at their tips was variable ; when it took place at but a short distance, the film so formed was raised and extended by continued elongation of the threads, the lengthwise *filling in* beginning then or later, as the case might be.

And in this manner was a striated membrane seen to be formed about sea-urchin eggs.

*Starfish eggs.* — It has been commonly observed that, just at the region where the so-called polar globules are expelled, the

cytoplasm shows amœboid movements before, and for a short time after, these bodies are thrown off.

Under magnification of three thousand diameters, the lobe-like protrusions of the substance here were seen to be still further extended in filose processes, most like the outer portion of the *tuft* which receives the sperm, but finer and more thread-like, less protoplastically diffuse.

After the globules have been thrown off, these processes are for a short time withdrawn, while the general surface of the egg smooths itself out, restoring the general contour. For a short time only, however ; for very soon the spinnings arise again from the pellicular surface, now in the form of still more delicate threads, or rays, which extend themselves towards the egg membrane, and even at moments attach themselves to this. They also surround the polar bodies with ramifications of extreme minuteness.

At other points of the periphery similar spinnings spring up, the egg being still in the single-celled stage, and thus is inaugurated a phenomenon which from this time persists with varying freedom until shortly before the closing of the opening into the cleavage cavity, across which the polar bodies lie. Then all peripheral spinnings cease, to burst out once more for a few moments all over the pellicle when the free-swimming life of the larva is begun, after which no more filose activities were seen from the exterior of the embryo.

The threads, or rays, formed from the pellicle in the one-celled stage are of extreme delicacy in the normal egg, and in many cases are but just perceptible with the powers named, except near their point of origin in the pellicle, where they are thicker and less viscid in appearance.

They branch sometimes near their base and sometimes further out from the egg, when in their more fluid states, for their viscosity and their refractive quality vary from moment to moment. Such branches take a somewhat wandering course often, and anastomose, or interlace, with the secondary, or even at times with the primary, processes near them, so as to form unstable networks. The branches are often as thick as the threads from which they arise.

The rays do not have always a radial direction as to the egg, not even at the moment of their formation, but may make with the surface of the cell almost any angle, even so acute an one as to be actually tangential.

They are moved slowly at times, as if from a hinge, or ball-and-socket joint, at their base and point of termination in the egg pellicle.   They are seen also to bend sharply and suddenly at some point of their length, forming thus angles more or less approaching right angles.   And at all points of their length they freely interchange varying states of viscosity ; being here or there in the different filaments, or here or there at different points in the same filament, either in a state of fluid flux, or stiffly viscid to a point which may reach elastic rigidity.

The spinnings from the pellicle continue without intermission during all the internal preparations for caryokinesis, and for cell division, showing no constraint during aster formation, nor even in actual cleavage, except for a moment just before actual splitting of the surface begins, and just along the region, or coming path, of that splitting, for here they are more or less completely withdrawn.

As external cleavage begins, the rays nearest the actual splitting line show usually some agitated-looking bendings about, both from their base and along their course.   Sometimes a quivering movement runs along them, with roughenings of the ray outlines exactly as is seen often in Heliozoa.   At other times, in the earlier stages of the egg's history, such phenomena were seen from moment to moment in different rays at other regions.

The split of the first cleavage is now visible as a triangular cleft in the optical contour of the egg.   In the moment this appears, there is a visible haste in the protoplasm of the rounded, divergent sides of the two cells in process of formation, to renew the spinning activities.   The rounded optical edges may show slight amœboid modulation ; and then, rapidly starting up, a number of processes extend themselves from each opposing surface, towards the other.   This is quickly reached, for there is both haste and directness of

formation, and so the two halves become in fact re-united by
their substance before they are well separated.

Following hard upon the physical splitting of the mass apart,
similar spinnings from the newly separated surfaces spring up,
so close indeed at the heels of the cleavage, that there is often
scarcely the width of a half dozen such threads between those
most newly formed and the still fused edges of the cleft.   By
the time the first cleavage is ended, and this does not take
up many moments, the path of liquid between the sister cells
is already crossed many times by minute and most delicate ray-
like extensions, and strands, and even skeins of more tenuous
threads.

During succeeding changes of contour, when the blastomeres
round themselves up and then again flatten, and still again
become slightly concave in the centre, yet oppose a more or
less plane surface to each other, the spinnings never cease,
although the processes are in a state of general instability.

Under a lower power, even one of 1500 diameters, the filose
processes are wholly invisible, the surface of the cell presenting
an optically unbroken surface, which to still lower powers looks
smooth even.

While the cells lie opposite one another thus, there is some
ramifying and divergent spinning among the threads, and anas-
tomosis of some of the finer secondary filaments, but this is
mostly near the central, slight hollowing out of the surfaces;
those threads having their origin in the more viscous-looking,
outer portion of this plane tending rather to form bands and
strands of more refractive and direct character.

Later, when the cells begin to approach each other, the con-
necting filaments show a decided tendency to still more marked
directness, and smoothness, and viscosity of texture.   The
lateral, branching spinning becomes rarer ;  and as the cells
approach each other the band and rod-like connecting fila-
ments become shorter and gain a look of *tenseness;* and flow
between the two cells of protoplasm by way of these threads
was not noticed.

The threads shorten, and as they shorten become thicker
and more highly refractive, — that is, certain ones, for some of

the processes, notably those which spun latest, are now gener-
ally retracted.

The whole aspect of things is as if the blastomeres were
being drawn together by contraction of some of the threads.

Moderate pressure upon the cover glass, slowly increased,
tending to a mechanical forcing apart of the cells with more or
less flattening, had a most curious effect, for it seemed rather
to intensify and hasten the drawing together of the cells, and
to increase the refractiveness of the threads, intensifying, rather
than lessening, their optical characters.

The threads were not seen to be drawn out into finer and
less refractive filaments by such pressure, — as would naturally
be the case were their nature purely physical, like that of the
threads left when Bütschli forced apart masses of his viscid oil
foams, — but, as stated, if the pressure were slow and moderate,
they held and even emphasized their peculiar character.    In
finally giving way, as sometimes happened when the pressure
threatened to rupture the cell walls, they broke short off and
then rounded their ends in a viscid, mucilaginous-looking
manner before retraction, which soon took place.

Another curious fact was, that such mechanical pressure
caused the egg to withdraw many peripheral processes, but
seemed at the same time to increase, or re-stimulate, new forma-
tions from the opposing surfaces of cells.    Later, the peripheral
spinnings were renewed with even greater activity if the pressure
were taken away; or, in cases where the cells were forced apart
and the membrane remained intact, it being very plastic under
slow pressure, the peripheral spinnings were not only renewed
at points over the entire surface, but extended themselves
through unaccustomed distances until they reached the other
cells.    These results were best gotten in the 8–16 cell stage,
— which seemed peculiarly to favor them.

In the natural course of events in the typical, normal eggs,
after the blastomeres were closely apposed to, or fused with,
each other for a time, the same response to pressure was given
by the cytoplasm of the new mass.    *Pressure seemed, in short,
to increase the physical resistance of the mass to crushing stress.*
Since, if the cells so treated happened to be in that rhythmi-

cally somewhat relaxed state between cleavages, they rounded themselves up under intermittent pressure instead of flattening ; it is thought that the peripheral substance was instrumental in these changes.

After two sister cells are in close apposition to, and what may now, perhaps, be called safely, physiological union with, each other, there is offered to mechanical pressure an emphatic, and, if the pressure be slowly augmented, an increasing resistance. I found that shaking also increased the resistance to pressure. In making these pressure experiments I used a large, screw-adjustment compressorium, making marks upon the head of the screw and upon the supporting plate to estimate in a rough way the relative amounts of pressure. No doubt special devices for this purpose would bring to light an important series of differences.

After re-union of the first two blastomeres to form what may be termed *a dual mass*, matters progressed in the usual rhythmic way towards the second cleavage. During this time the spinning phenomena were persistent over the general periphery, the processes being longer and extending themselves to greater distances, which they may easily do because of increase in size of the egg membrane.

It may be difficult to convey a true idea of the extreme delicacy of these spinnings from the egg pellicle. In attempting camera drawings, I found that even a fine needle point came much in one's way in efforts to follow their outlines. There were local thickenings and differences of aspect which could not be portrayed so at all, and the chief meaning of these lay in their very evanescence. The only way seemed to be, to receive as truthful and passive a brain photograph as possible, and to translate this into terms of permanent line and shape, getting with the camera a skeleton of relative lengths and general local character of the lines of living substance.

The rays showed all the characteristic differences known in Heliozoan rays; and when one says this, much is said of many variations. They were freely produced and as freely returned to the substance of the pellicle from which they arose.

All the while, the alveolar structure, which was clearly

traceable in the protoplasm underlying the pellicle, and much finer than that found in the more central portion of the mass, remained optically undisturbed by these displacements of a peripheral substance which formed indeed the so-called pellicular film of the structures of Bütschli.

In attaining a four-celled stage, the phenomena were practically a repetition of the first set described. After fusion, the blastomeres rounded themselves up and formed a hollow mass. The central cavity of this, which was the "cleavage cavity," of course, was filled with a network of interlaced and even anastomosed filaments. And still the periphery of the now quadruple mass spun as before.

The most active portion of the common periphery seemed to be always at the rounded sides of the partial cleft which marked the junction of each two, adhering blastomeres. The most freely ramifying and anastomosing processes seemed to come always from the inner surfaces which lined the cleavage cavity, and this portion of the general substance showed greater fluidity, which extended inwards some little distance in each cell.

During the rhythmic cell-flattenings, the very active portion of the periphery just mentioned was always most extended and flattened, so that under highest powers it showed a decided prominence. This, under lower powers, might possibly be seen as a slight modulation of the rounded surface-outline.

Here the filaments attaching themselves to the opposing surface would seem to pull it somewhat out of place, and here, as in the actual cleavage, there was a tendency to formation of fusing filaments, or strands and bands.

So matters continued throughout the cleavage up to the time of closure of the opening into the cleavage cavity, over, and sometimes within, which the polar bodies lay.

At all times the cleavage cavity was crossed by a variable number of threads, connecting distant cells as well as those nearer together; each cell seeming desirous of connection, though not persistently, with all other cells. The filaments were long, more or less direct, connections which in many cases ramified during their course, sending branches to several cells, or spinning by the way a network whose filaments

interlaced with, or joined themselves to, other filaments or net-
works met with.

The greatest number of spinnings seemed to be from the cells
near the polar globules, and this region of the egg, it will be
remembered, was the first to spin as well as the most active at
all times.    In this region was shown also most tendency among
the peripheral filaments to ramification and anastomosis, espe-
cially when they crossed the path of certain spinnings from
the polar bodies, shortly to be described ; most capriciousness
in their optical qualities, and interchange of fluid and viscid
states, with greatest inclination to stiff viscidity.

At time of closure of the cleavage cavity pore, the cells
about it flatten markedly, and at the same time become irregu-
lar in optical contour ; the spinnings are then very strong and
viscid-looking, and the protruding regions are seen to be attached
by them to like regions of other cells.   There follows much the
same series of optical appearances among the filaments as when
sister cells are drawn together, and for these reasons it seemed
not unlikely that the filaments were actually instrumental in
closure of the space between the cells.

After a closure of the space is effected, the general " ecto-
derm " cells, as they multiply, become gradually flattened exte-
riorly beneath their common pellicle, so that all seeming of
intercellular clefts is obliterated.    At the same time their
inner ends protrude somewhat within the cavity, so that here
there are marked intercellular clefts.

These clefts are crossed from cell to cell by delicate, hyaline,
filose extensions of the cell pellicle; and from the ends of the
cells, which are plainly more fluid than the outer portion, are
produced other and much longer threads, which extend even
across the whole cavity, binding the most distant cells into
physiological and direct continuity.

Up to this time the external spinnings of the mass pellicle
have been progressively somewhat less profuse.   When the
blastula has a sufficient number of cells for its free-swimming
state these outer spinnings rather suddenly cease, but burst
forth again over the surface of the pellicle as hair-like pro-
cesses which quickly show irregular, contractile, or waving

motions. These become stronger, more rhythmic and organized in action, and soon form a sufficiently harmonious impulse to carry about the blastula as a free-swimming organism.

After invagination, those cells which as entoderm project, first as a plug and later as a tube, into the cleavage cavity, spun strongly and in a free protoplastic manner from their portions facing the cavity, which, like the same region of the ectoderm cells, were more fluid in appearance. These entoderm spinnings crossed the cavity to the ectoderm cells and in their course freely anastomosed in many places with the spinnings of the latter.

After the mesenchyme cells were added to the group of internal cells, these, by spinning in even more free and profusely protoplastic a manner, increased very greatly the number of intercellular connections.

It now became difficult to distinguish the various groups of spinnings from each other; in the majority of cases where anastomosis had taken place, it was impossible, because new centres of spinning were formed of accumulated substance which might have come from anywhere. The network was also most unstable from moment to moment. Yet there were many tapering processes which could be connected with their point of origin.

Larval stages were followed up to quite late periods, even to time of formation of the proctodæum; and the internal spinnings seemed at no time to pause or cease.

It should be pointed out that in the early stages of the segmenting egg the protoplasm which spins is not the least, but the most, highly organized portion of the cytoplasm. It is also the most viscous portion, as can readily be demonstrated by physical experiment.

*Polar Globules.* — Perhaps even more strange than these spinnings from the egg were similar activities shown by the cytoplasmic envelope of the chromatin granules of the polar globules.

During that short period of quiescence for the egg pellicle, which was spoken of above as following the extrusion of the

polar bodies, thread-like processes with some wave-like change of contour were noted on these little lumps, the egg being at the time under magnification of three thousand diameters.    These initial protoplastic activities were extended, and multiplied from other portions of the hyaline protoplasm enveloping the nuclear substance.

Delicate threads and ray-like processes grew out on many sides, and the shape of the whole mass suffered change from moment to moment, both from the actual displacement thus caused and from an apparent pulling here or there of the little cells by their attached filaments and strands which had formed actual connection with the egg pellicle or membrane.

It seemed to me to be from this time, *i.e.*, when the threads from the polar bodies effected reunion with the parent cell, that the egg spinnings were again renewed.    However this may be, it was certain that from this time forward, throughout the entire cleavage of the egg up to a late larval stage, the egg and these bodies were united by their individual spinnings, which formed an unstable, and at times evanescent, series of interlaced and anastomosed networks.    These more or less surrounded the polar bodies and stretched across the cleavage pore.

The processes from the polar bodies were finer in general than the average egg filament, but this was variable, and along the finest filaments would often pass little masses, relatively large in quantity, of flowing protoplasm which might collect here, or there, and form nodes as it were, and these became centres of renewed and somewhat independent spinnings.

From moment to moment, the substance composing the net-work, or its islands of protoplasm, would return wholly to the polar bodies, to be again sent out in a new direction and to assume a new form.

The globules seemed to delight, especially later on in the egg history, in forming brush-like tufts, or skeins, of finest fila-ments, which often assumed a curious, superficial resemblance to a spindle, spreading at a little distance from their point of origin and then again drawing together at their point of fusion with some ray or strand, or with the nearest egg cell.

By shortening of the filaments, the position of the globules was changed, these being drawn here or there ; and later their shape underwent strange changes which forced one to correlate them with existing thread formations. Now they would be seen as irregularly spindle-shaped, with bundles of radiating filaments arising from each end of each body ; then as spherical masses, with Heliozoan-like rays extending from all sides nearly alike ; then again as colonial groupings of separated minor masses of protoplasm connected by filaments and having the chromatin granules distributed to some extent among the larger lumps ; then again, with filaments almost entirely withdrawn, as amœboid shapes with delicate wave-like expansions of substance flowing in protean manner about the granules which were then perhaps collected together at some one point.

Delicate vacuolations of variable sizes appeared and disappeared in the main masses and in their smaller colonizing masses.

The protoplasmic granules were transported here and there along the processes, and the filaments underwent all those changes of optical quality which have been described in the egg spinnings.

The change of position and grouping of the chromatin granules were deeply interesting, for they were at times scattered, then drawn in line, then variously grouped. But I was unable to determine any coherent significance in these differences at the time, and dare not so much as guess that their arrangement was other than fortuitously and mechanically altered by flux of the surrounding substance.

The granules' presence, even singly, in any mass of the cytoplasmic spinnings certainly seemed to be correlated with more continuous, persistent, and in a way organized, displays of this sort.

From all the surrounding cells, passing filaments gave of their substance from moment to moment to the compound network which compassed the bodies about, and it was not always possible to know from what source a given portion of the network had come nor whither it was bound. It was always

possible to know some portions of the network as distinctively
the polar bodies' own.

The position of the polar bodies, with relation to their dis-
tance from the egg membrane and the egg itself, varied also
throughout the time of development up to this point.

Their substance, like that of the egg, frequently travelled
to the membrane and there adhered, spinning backwards or
anastomosing with such egg filaments as happened to be there.

At time of closure of the cleavage pore, the polar globules
were shut inside the blastula, chiefly, it seemed, by action of
the egg processes overpassing them and drawing together the
cells above them; yet possibly they assisted also by some
migratory movements of their own.

Inside the blastula, they could still be followed clearly for
some time, until at last, after being involved in the web of spin-
nings from the ectoderm cells and, in the gastrula, from the
protoplastic ends of entoderm cells, they were finally lost to
sight in the still further additions given off from the mesen-
chyme cells. After this point their fate could no longer be
followed, though it enlisted the strongest interest.

Towards the time of closure of the cleavage pore an increased
tendency to viscidity and to organized action of the spinnings
was shown by the strange geometrical positions maintained by
the lines of living substance.

In one instance where the conditions remained stable for some
moments, a camera drawing was obtained of a certain arrange-
ment which from a mechanical point of view was remarkable.
One line of very viscid-seeming protoplasm formed an arc
which would make part of a very large circle. The ends of this
terminated in the angles formed by sharp bendings of two long
rays having their origin in the two polar bodies then lying
quite apart; and their termination in two cells of the egg which
were not adjacent but separated by another cell. Thus it came
about that one saw a viscous fluid in linear extension, maintain-
ing the curve of a distinct arc, yet attached to the angles formed
by what were, mechanically, four contending directions of ten-
sion. It might have been open to one to suppose that the
angled lines were very viscid, and that the arc line was a less

viscid, hanging line of protoplasm, had not the existing condi-
tions negatived this view.    The arc line was in fact far more
dense and refractive, also thicker than the angled lines between
which it held its place ; and it did not hang.

Appearances were far more in favor of its being this line
which bent the longer lines into their angles.    Yet in such a
state of tension, how explain the curve ?    I have dwelt at
length upon this single phenomenon because it is typical of
the difficulties which everywhere beset physical and mechanical
interpretations when one brings them into contact with the facts
of the living substance.

The hyaline substance of the polar bodies frequently flowed
along the filaments of the egg, and gathering together at some
point spun characteristic brush and skein formations, or made
nodes for diverse sorts of protoplastic phenomena.

It was a noteworthy feature of the polar spinnings that they
so frequently grouped themselves in spindle-shaped bundles,
and showed a marked vesicular structure in their substance
at most times except during certain changes of viscosity.

Concentric chains and lines of vesicles gave often by their ar-
rangement the aspect of a spindle to the whole polar body, which
the outline of the mass at the same time emphasized.    In the
brush and spindle-like spinning products there was often a very
distinct chain-like arrangement of vesicles forming the threads.
These were of course the larger and more stable processes.

Description of the filose phenomena of these eggs is an
almost inexhaustible subject, and I give only the more patent
phenomena.

The activities of the polar bodies did not decrease as time
passed after their extrusion from the egg, but rather increased,
both as to amount and as to the controlled and ordered nature
of the phenomena.

In other words, instead of their losing at all their individu-
ality and becoming more like non-living and excreted substances,
they rather gained than lost in independence of action and the
vigor and vitality of their organized activities.

Whether this were due to some influence exerted upon them
by the intimate connection of the egg spinnings with them,

through which physiological relation they might possibly share in the growing individuality of the major body, it is impossible to say. I am inclined to think that although they undoubtedly show strange freedom and initiative energy, although they seem to possess all that is requisite for what would be termed from a Protozoan standpoint, a complete organism ; yet, since they are also an integral part of the egg substance and of the complex system of egg phenomena, they must share largely in the sympathetic interactions and coöperative physical and physiological phrasings of the powers of the whole material, as well as in a progressive subjugation of the whole life-machine to an ever-increasing despotism of parts.

In the Echinus and Arbacia eggs also the spinnings were seen between cells, although with far less freedom than in Asterias. The sea-urchin threads were fewer, in comparison, more direct, and less given to forming networks and side-spinnings. They arose from a more distinct, and thicker, hyaline covering of the egg, which seemed structureless under the highest powers, yet the spinnings themselves showed often a vesicular tendency and structure, albeit of the finest. Over the sea-urchin filaments granules as well as little lumps of protoplasm streamed at times. In the four-celled stage, the small cleavage cavity was crossed by a number of threads between the cells, and thereafter whenever, and for as long as, interspaces between the cells could be seen, spinnings crossed these and bound the associated cells into an intimate union.

Here, as in starfish eggs, the most active and sensitive portion in each cell was where it began to curve away from another cell.

In both starfish and Echinus eggs, after any artificial separation of the cells by such pressure as did not rupture the egg membrane, and even in some cases where this actually occurred, the spinnings were increased in number and the length of the threads was extended until connection was again established, as if indeed they sought for their lost comrades.

To the very natural question whether these filose activities of the eggs were not indeed abnormal phenomena, it is to

be answered that the same question having great weight in the observer's mind, every effort was made to learn the truth.

Thus much was made sure.   The eggs can be stimulated by additional heat, by polyspermy, by adverse states of the water such as are caused by too long confinement, by mechanical pressure, or by being fertilized when in a still immature condition, to spin far more freely.

But the protoplastic phenomena in these cases are distinctly different from those of what I was impelled to believe were normal eggs.

The character of the formations, the quantity of the egg substance, and the use made of it during the normal and abnormal states, are so different, so characteristic, that, after following a dozen or two specimens of abnormal spinnings in all grades of pathology, one feels an almost unshakable assurance in the nature of the true and normal process, and would almost be willing to diagnose the state of an egg by the very character of its spinnings in any given case.

Immature eggs being fertilized, or over-fertilized, give themselves up to uncontrolled spinning activities which in many cases end by the whole mass being drawn out of form and position and distributed through the surrounding space in a granular and unevenly vesiculate, or a partially structureless, network of substance.   The transported substance may mass itself here or there, adhering perhaps to the membrane and thence setting up from broken, or partly isolated, lumps and nodes of protoplasm new centres of activity.

Traversing relatively large spaces, often by way of invisible extensions of substance, the deported protoplasm accumulates at this or that point; and then may disappear, to come again within optical reach as a new current from nowhere flowing into or onto some filament in plain sight ; or as small lumps of substance, growing from some invisible source, suspended in an otherwise empty space, their true source being some distant filament, or the egg mass from which outflowing currents take their way, and pass at some point from one's power to trace them optically.

Mature and properly fertilized eggs when heated too much
or kept too closely confined, cease their normal, delicate spinning
to burst into profuse transpositions of their substance, which
may cause even considerable distortion of their form, or, accord-
ing to the degree of abnormality produced, may be visible only
as excess of the usual processes. If not too much affected, such
slight abnormalities may be cured and the perfect larva form as
usual.

If the adverse conditions are continued, such eggs often cease
from all further cell division and exhaust their powers in perfect
orgies of spinnings, as just described for abnormal eggs.

Protoplasm artificially pressed from normal starfish eggs in
early stages spins characteristically like abnormal unruptured
eggs and cells, not like those in normal condition. Such
activities varied also their quantity and character in correlation
with certain rhythms of difference of physical quality which
marked the living substance during development of both star-
fish and Echinus eggs. These rhythms are treated of in the
forthcoming essay mentioned above.

There was every optical evidence of actual interchange of
substance by the cells of developing starfish eggs and also
Echinus eggs by way of the filose processes; for a procession
of granules along a filament for some moments in one direc-
tion, sometimes ended by abrupt withdrawal of the filament.
Whether these granules returned later along some other fila-
ment could not of course be determined; but even so, they
would have been for a time inhabitants of the cell in which
they were left, and so also would the substance of whose pass-
ing they were clearest indications.

A most noteworthy thing was made plain by this series of
observations. The vesicular structure of Bütschli, and that
modification of it termed by him the " alveolar layer," clearly
traceable under favorable optical conditions in these eggs, exists
and remains optically undisturbed for moments at a time during
such peripheral activities as can drain the egg of considerable
amounts of its substance, yet pass undetected under the
powers which have been commonly used for observing develop-
mental phenomena.

Further, there is present and visible under these lower powers, and capable of " preservation," a perfect, pellicle-like covering of ectosarc-like substance which under quite high powers may show only such surface roughening as would pass as finely granular, or seem smooth even, and yet be extending itself all the while in hundreds of processes which are most powerful determining factors in the scheme of development.

In the case of the polar bodies as in the cell division, it becomes plain that the separation or isolation of portions of its substance by the developing egg is but an optical illusion; and that the space separation between sister cells, which has been taken to be actual and complete and unbridged by living substance, and that cavity or space between all the cells of the blastula known as the cleavage cavity ; are deceptive in the highest degree, being in fact bridged by all the cells concerned by means of extensions of their living substance.

At all times the cleavage of the mass of these eggs into portions which simulate units, is seen to be but a mask for actual continuity of the substance of the whole throughout all its subdivisions in cell form.

More than this, the cell substance is seen to have some sort of deliberateness, or purpose, in its spinning activities, for where the space between blastomeres is artificially increased far beyond the accustomed limit, it hastens to cross by unaccustomed degrees of extension, not resting till it has reached the missing masses and reëstablished union with them.

A reasonable summing up of the phenomena described, — weighted by a host of subtle, modifying and restraining evidences which in such delicate phenomena must always exist over and above the description, — would seem to be as follows.

The facts point to a physiological drawing together of the cells, rather than to any physical and chemical "cyto-tropismus":

To a physiological, rather than a physical reaction to mechanical stimulus of pressure or shaking :

To a physiological, rather than a physico-chemical, cause of the spinning activities :

To actual and physiological communion as well as physical connection between cells after and during their formation :

To some interchange of the protoplasm between cells, which may be but temporary, or may be part of the organising phenomena :

To an actual desire on the part of the cells for such physical connection and physiological communion among themselves; and a coherent attempt to regain and continue these conditions after they have been artificially destroyed :

To a continued, and to great degree independent, existence of the polar bodies after their extrusion: and to their acting as part of the coalition of cells up to a late period, yet without self-multiplication, and with marked distinctive features of their own, which are peculiarly protoplastic.

The facts assert also:

That the peripheral substance of egg and cells is freely protoplastic, despite its appearance under less magnification of being a smooth and stable pellicle :

That the ectosarcal formations of these Metazoan eggs are possessed of tactile power such as characterizes similar modifications of the substance in protoplasts :

That considerable deportation of living substance can take place from egg or cells in a manner invisible to even quite high powers (as 1500 diameters), and yet to an extent producing a measurable diminution in size of the mass from which it is carried :

That, in short, the measure of the living facts cannot be taken truly with anything less than our best optical resources, and that even these fail of perfect adequacy :

That apart from the grosser organization of the egg, which we have had spread out to some extent before us by reagents, there exists a minuter mechanism which these fail to record, or even hint to us; and that beneath the interactions of those grosser masses which seem so complete as seen under lower powers, there are supplementary and strongly causative interactions of minuter portions of the substance composing them, which are in part visible with our highest powers and which are from some points of view curiously contradictory in seeming :

That while the grosser organization acts through more definite and stable structures, the finer, and heretofore unseen,

organization acts by unstable and freely protoplastic phenomena and structures :

That while the phenomena pertaining to the organism as composed of these grosser masses and structures we call cells, are of vast importance ; the phenomena pertaining to the organism as composed of those minuter and unstable portions which go to make up the cells, and which taken together throughout the whole mass, as well as separately, go to make up the protoplasm *per se,* — *the living substance, as such ;* — are of transcending importance, since they prove to be control phenomena for the former.  In saying this I have in mind the familiar phenomena of caryokinesis, and certain unfamiliar phenomena of re-arrangements of the cytoplasmic substance which are treated of in the forthcoming paper, and which undoubtedly strengthen the expression here of these conclusions.

Here I will limit myself to saying that whatever may be the significance of the cell wall in the development of these eggs, it surely cannot be thought a separator, in either a physical or physiological sense, of the cell contents from other portions of the common mass.

If we look upon the cell wall as a part of the machinery of the embryo, the larva, the coming or immediately present organism, that is, as an organ of the mass of living substance, just as is the nuclear membrane, or any other local modification for physiological purposes, we shall probably be nearer the truth than if we keep to an earlier conception, and hold that, for the living substance, cell walls a prison make.

BALTIMORE, October, 1896.

# THE ORIGIN OF THE EGG CENTROSOMES.

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THE observations recorded in this paper were made upon the eggs of the marine annelid *Chætopterus pergamentaceus*, procured at the Marine Biological Laboratory at Woods Holl, Mass., where I was enabled to work during the past summer through the courtesy of its Director.

My best preparations were obtained by fixing the eggs with picro-acetic acid, and staining with Heidenhain's iron-alum hæmatoxylin, followed by orange G. The slides were left in the 4% iron-alum for half an hour, rinsed, and left in ½% hæmatoxylin for twelve hours. After drawing the color with iron-alum, the slides were dipped in the aqueous solution of orange G. Hermann's fluid, Flemming's fluid (weaker), and a mixture of Hermann and formalin also gave satisfactory results, though the staining was not so brilliant. Sublimate acetic usually works havoc in the region of the astrosphere. In a previous paper[1] I stated that "until the entrance of the spermatozoön, the egg remains with the first maturation spindle in the equatorial-plate stage." The earlier stages were not seen at that time.

During the past summer, however, on taking the precaution to preserve the ovaries, together with the loose eggs, immediately after dissecting them out into sea-water, I was able to obtain a complete series of stages previous to the formation of the first maturation spindle.

The cytoplasm of the smallest ovarian eggs is compact, and this gives to the eggs when stained an almost uniform dark purple color. The increase in size is due in great measure to the accumulation of yolk, the distribution of which is accompanied by noteworthy changes in the appearance of the cytoplasm. The yolk is laid down in the form of yellow-staining

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[1] Some observations on the maturation and fecundation of *Chætopterus pergamentaceus*, Cuvier. *Journ. of Morph.*, vol. X, No. 1.

granules, which are more numerous at first near the periphery of the egg. The granules are held in the meshes of a reticulum formed of beaded strands of cytoplasm, whose purple color is in striking contrast to the yellow yolk.

In most ovarian stages only a part of the cytoplasm presents the loose reticular appearance ; the rest remains as dark purple masses. Occasionally sections show but one of these masses, crescentic in outline, located near the nucleus. I presume that this is equivalent to the "paranucleus" or "yolk-nucleus" of certain authors. These masses are not homogeneous, but resolve themselves into a radially compressed cytoplasmic network, the strands of which are often clearly visible and are continuous with the more open network which contains the yolk. At first they fill the larger part of the egg outside the nucleus ; but as the egg accumulates yolk the meshes at the periphery of the masses expand into an open reticulum so as to occupy the increasing dimensions of the egg (Fig. I). Through this process of continuous fraying out, the masses become thinner and thinner and are finally completely resolved into the cytoreticulum.



Fig. I. — Section of ovary. Camera. Dissolution of the paranucleus.

The reticulum can be traced with ease to the extreme periphery of the egg, where in section one can follow a continuous beaded line entirely around the egg. The nuclear membrane is evidently a part of the same network. The general appearance of the reticulum varies with the age, the older ovarian eggs having the nodes the most pronounced. In eggs freed or about to be freed from the surface of the ovary, the nodes become less frequent and still more prominent, until at length a large portion of the reticulum is transformed into a multitude of small asters (Fig. II). Many of them, however, are by no means diminutive, particularly those in the vicinity of the

nucleus.   All gradations in size occur, but at a certain stage many of the larger ones are approximately equal both in size and distinctness.   Frequently one can count from fifteen to twenty very distinct asters in a single section.   These structures correspond closely to "secondary mechanical centers" of Reinke,[1] and I will call them the *secondary asters*.   While yet distinct from one another, they are often so near together that their rays intercross.

It is not long before two of the asters become predominant, —*primary asters*.   Their rays increase in number and length,
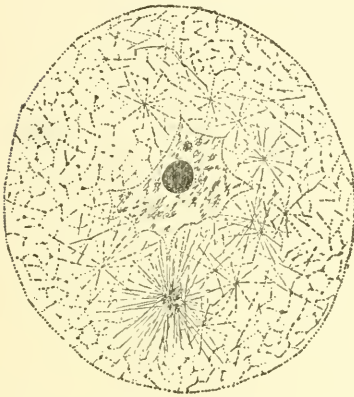


FIG. II. — Section of egg free from ovary, showing secondary asters.  Camera.
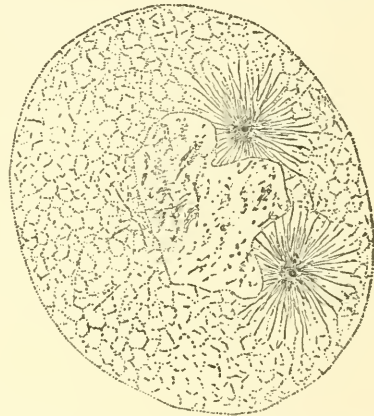
FIG. III. — Section of egg in a stage later than Fig. II.   Only the two primary centers are present.  Camera.

apparently at the cost of the secondary asters, for the latter gradually evanesce with the further development of the former, and at length the cytoplasm possesses only two well-marked centers of radiation (Fig. III).[2]   The primary asters lie near the germinal vesicle, usually about 90° apart, but their relative position is subject to considerable variation in different eggs. *They are destined to be the asters of the first maturation spindle.* Each of them has for its center a perfectly definite deeply stained centrosome (centriole), surrounded now by a lighter area from which the rays diverge.   The nuclear membrane

[1] Reinke : Zellstudien II.   *Arch. f. mikr. Anat.*, Bd. XL, p. 276.   Peritoneal cells of the larval salamander.

[2] The initial predominance and further growth of the primary asters certainly appears to be due in part to the actual coalescence of smaller asters.

invaginates in the vicinity of each aster in the manner already described by several authors.[1] It is afterwards resolved into the cytoreticulum, though the huge nucleolus and other features of the nucleus do not immediately disappear.

Definite chromosomes are seen to be attached to the achromatic fibrils of the two asters, and to take their position in the equatorial plate in the ordinary way. The centrosomes become doubled and are demonstrable in each aster as two exquisitely clear dark dots in the midst of the light yellow centrosphere. The spindle thus formed swings around to occupy a radial position at the periphery of the egg, and remains in the metaphase until the sperm enters. On the entrance of the sperm the karyokinetic activity is resumed and the maturation processes are completed.[2] Usually, before fertilization, the nucleolus of the germinal vesicle breaks up and disappears ; the last traces of it are seen in the neighborhood of the maturation spindle.

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The foregoing observations convince me that the asters and centrosomes in the *Chætopterus* ovum arise by a modification of the cytoplasmic reticulum. The phenomena of their origin and their relation to the secondary asters are similar to those described by Reinke[3] in the tissue cells of the larval salamander.

Watasé says he has " seen in the egg of *Macrobdella* a series of thirteen asters, ranging from a diminutive aster with a microsome for its center to the normal aster with a veritable centrosome." [4]

The fact that in *Chætopterus* the secondary and primary asters were formed when the eggs were transferred from the body-cavity of the worm into the sea-water, suggests a comparison of these phenomena with the production of "artificial centrospheres" in sea-urchin eggs by adding more salt.[5]

[1] Compare Wheeler: Myzostoma. *Journ. of Morph.*, vol. X, No. 1. Griffin: Thalassema. *Trans. N. Y. Acad. Sci.*, June 2, 1896.

[2] Mead : *loc. cit.*

[3] Reinke: *loc. cit.*

[4] Watasé: Origin of the Centrosome. *Biol. Lectures, Marine Biological Laboratory*, 1894, p. 285.

[5] Morgan : The Production of Artificial Centrospheres. *Archiv f. Entwickelungsmechanik*, Bd. III, Heft 3.

# DEVELOPMENT OF THE HUMAN COELOM.

FRANKLIN P. MALL.

FOUR years ago I wrote a general article on the coelom for Wood's *Handbook of Medical Science*, in which was emphasized the separation of the body cavity from the extraembryonic coelom. Since then I have had opportunity to extend my observation to the human embryo, and therefore make this communication.

Unfortunately, there are no data regarding the beginning of the coelom in the human embryo, and in all probability none will ever be found. The smallest human ovum ever seen is that described by Reichert.[1] It was obtained from a woman who had committed suicide, on account of pregnancy, forty-one days after the beginning of the last menstrual period. It was therefore presumably about thirteen days old. This ovum, which is pictured in every text-book, was 5.5 × 3.3 mm. in diameter, was surrounded by a zone of villi leaving two poles bare, and contained in its interior a mass of cells measuring 1.5 × 1.75 mm. All the space between this inner mass and the chorion is the coelom, and regarding its origin, we can no more than speculate.

During the last few years three other human ova, slightly larger than Reichert's, have been cut into sections, thus permitting a more careful study of their contents.[2] The dimensions and approximate ages of these embryos are given in the table on the following page.

It is noticeable that in the three embryos just mentioned, as well as in the remaining four of the table, the size of the whole egg does not correspond with the size of the embryo, nor with its age. I do not think that this great variation in the size of the chorionic vesicle is altogether due to the method of harden-

[1] Reichert: Abhandl. d. kgl. Akad. d. Wiss., Berlin, 1873.
[2] Von Spee: His's Archiv, 1889; Mall: Anatom. Anzeiger, 1893; Johns Hopkins Hospital Bulletin, 1893; and von Spee : His's Archiv, 1896.

TABLE OF YOUNG HUMAN OVA.

| OBSERVER. | DIAMETER OF EMBRYONIC VESICLE. | DIAMETER OF OVUM. | TIME BETWEEN FIRST LAPSED PERIOD AND ABORTION. |
|---|---|---|---|
| Mall (No. XI) . . . | 1.5 × 1    mm. | 10. × 7. mm. | 13 days |
| Reichert . . . . . | 1.75× 1.5    " | 5.5 × 3.3 " | 14    " |
| Von Spee (v. H.) . . | 1.84× 1.083 " | 6. × 4.5 " | 12* " |
| Von Spee (Gle.) . . . | 2. × 2.    " | 10. × 8.5 " | 12* " |
| Mall (No. XVI) . . . | 2.1 × 2.1    " | 18. × 8. " | 13    " |
| His (Lg.) . . . . . | 2.15× 2.    " | 15. × 12.5 " | 12    " |
| Von Spee . . . . . | 2.69× 3.    " | 15. × 14. " | 14    " |
| Janošík . . . . . . | 3. × 4.    " | 8. " | 15    " |

\* These are all of the authentic young human ova I can collect from the litera-
ture giving all of their measurements as well as the menstrual history of the mother.
In both of von Spee's cases the time between the abortion and the end of the last
period is given; in embryo v. H. the time is given as "exactly five weeks," while
in embryo Gle. "five weeks" is given. If we estimate the duration of menstruation
as five days and its frequency twenty-eight days, then the time between the first
lapsed period and the abortion is twelve days, as I have given it in the table.

ing the specimen. Just at this time the growth of the chorion
is precocious, as is also the case in the dog,[1] rabbit,[2] and
monkey.[3]

The papers by Bischoff and by Selenka are worthy of the
most careful study by every embryologist, and I take the liberty
of rearranging some of Bischoff's data on the development of
the dog. His observations are very extensive, and give us the
basis for our present ideas of the passage of the ovum into the
uterine tube after fertilization. Unfortunately, they were
made before the time of sectioning specimens, yet they are
more complete than most researches relating to this subject
published since his time.

The portion I tabulate relates to the size of the embryonic
mass or vesicle, the size of the ovum, and its approximate age.
As far as I have been able to determine, these data taken from
the dog are still the most important ones with which we can

[1] Bischoff: Entwicklungsgeschichte des Hundes Eies, Braunschweig, 1845.
[2] Bischoff: Entwicklungsgeschichte des Kaninchen Eies, Braunschweig, 1842.
[3] Selenka: Studien über Entwicklungsgeschichte des Thiere, Heft 5, Wies-
baden, 1892.

compare the human ovum. Embryologists are accustomed to state that the age of a human ovum is to be reckoned from the beginning of the first lapsed period, and I think that Bischoff's observation upon the size and growth of the dog's ovum corroborates this view. He found that the ova left the ovary during the rutting period, but the exact date could never be determined. Neither did the time of copulation determine the ovulation. As a rule, it took twenty-four hours or less after copulation for the spermatozoa to reach the ovary, and about the same time is required for the ovum to reach the beginning of the uterine tube after ovulation. So if ovulation and copulation took place at the same time, fertilization of the ovum could not take place until twenty-four hours later.

In Bischoff's tables he often rates the age of an ovum from the first or from the last copulation, or from the beginning or from the end of the rutting period. I have attempted to tabulate his specimens from all four of these dates, but in none of the attempts did the size of the ova correspond with their respective dates. Often eggs of a given date were smaller and developed to a less degree than ova presumably younger. After much difficulty I finally constructed a table in which the size of the ovum and its age correspond. A number of the ova published by Bischoff were obtained from the same animal by removing half of the uterus at one time and the remaining half the next day. In each portion a number of ova were found, and they were usually of about the same stage of development. By this method of procedure it is possible to determine very accurately the growth of the ovum from one stage to one twenty-four hours later. So, by gradually plodding through the specimens published by Bischoff, it was possible for me to correct his data completely. It is remarkable, as the table shows, how slowly the development takes place in the early stages, and about ten days are required before the ovum is one millimeter in diameter. On the fifteenth or sixteenth day the ovum is about as large as the human ovum described by Reichert (see table).

Similar results can also be obtained from the various papers published on the rabbit's embryo. Its development, however,

is considerably more rapid than the dog's, as the period of gestation is but thirty days.

Recently Selenka has given some of his results relating to the development of the monkey. The most valuable specimen relating to the early development of higher animals was unfor-

TABLE OF AGE AND SIZE OF THE DOG'S OVUM.
(COMPILED FROM BISCHOFF.)

| Age. | Diameter of Ovum. | Diameter of Embryonic Mass. | Stage. |
|---|---|---|---|
| 1 day. | .15 mm. | | 1 cell. |
| 2 days. | .14 " | | 2 cells. |
| 3 " | .14 " | | 4 " |
| 4 " | .16 " | | 12 " |
| 5 " | .16 " | | 64 " |
| 6 " | .18 " | | Mulberry. |
| 7 " | .20 " | | " |
| 8 " | .21 " | | " |
| 9 " | .28 " | | " |
| 10 " | .30 " | .07 mm. | |
| 11 " | 1. " | .16 " | |
| 12 " | 2. " | .24 " | |
| 13 " | 3. " | .43 " | |
| 14 " | 4. " | .5 " | |
| 15 " | 5. " | 1. " | |
| 16 " | 5. " | 2. " | |
| 16½ " | 6. " | 3. " | |

It has been somewhat difficult to compile this table, as Bischoff's measurements are all given in Paris lines. My measurements are taken in great part from his figures, and I think that these are very accurate.

tunately lost, but its age and dimensions are preserved for us, and are of value in the determination of the age of human ova. The ovum came from a monkey kept in confinement which was killed eight days after copulation. If we estimate one or two days required before fertilization, this ovum cannot be over seven days old. This suggests that the early stage of this variety of monkey is developed more rapidly than that of the dog.

DEVELOPMENT OF THE MONKEY. (FROM SELENKA.)

|  | DIAMETER OF OVUM. | DIAMETER OF EMBRYONIC VESICLE. |
|---|---|---|
| Semnopithecus maurus . . . . . . . . | 1.5 mm. | .3 mm.[1] |
| Semnopithecus pruinosus . . . . . . . | 6. " | .5 " |
| Cercocebus cynomolgus . . . . . . . . | 5. " | .5 " |
| Cercocebus cynomolgus . . . . . . . . | 10. " | 2.4 "[2] |

The pictures Selenka gives indicate that the development of a monkey's ovum is identical with that of the human ovum. At any rate, the few specimens Selenka publishes give results which are equal to the great number of specimens of human ova which have been published. This only indicates that many of the interesting problems relating to early human development will probably be solved by the study of the monkey's ovum. There is but little doubt now that young monkeys' ova will soon be obtained for study.

## MATERIAL EMPLOYED.

During the last few years I have obtained a number of young human embryos from physicians in different portions of the United States, and to them I am under all obligation for the present study as well as for some others which are to follow. Nearly all of the specimens which I give in a table are well preserved, and a number of them are preserved excellently. All of the specimens were stained in alum carmine, and with the exception of Nos. XVII, XLIII, and LVII were cut transversely. These three were cut in sagittal sections.

All of the specimens were hardened in alcohol, the value of which method I have repeatedly emphasized to my friends, and do continue to emphasize to those who may preserve specimens for my use in the future.[3]

[1] Not an embryonic vesicle, but only a disc.

[2] Neurenteric canal present.

[3] Embryologists usually recommend that human embryos should be hardened by placing them in dilute alcohol and then gradually increasing the strength of the alcohol. It has been my experience that by this treatment the specimen is injured by maceration due to the weak alcohol. A few years ago I emphasized the fact that the whole ovum should be placed in a large quantity of strong alcohol as soon as possible. It should be handled as little as possible before hardening it, thus preventing mechanical injury. By leaving the ovum closed the alcohol must first penetrate the chorionic and amniotic fluids before it reaches the embryo, and thus,

Nearly all of the embryos were drawn or photographed to scale and then carefully cut into sections from ten $\mu$ to fifty $\mu$ thick. I find that for purposes of reconstruction it is a mistake to cut the sections very thin. Yet in small specimens, as Nos. XI and XII, the specimens were cut thin to permit of careful

LIST OF EMBRYOS STUDIED.

| No. | LENGTH IN MILLIMETERS. | | FROM WHOM OBTAINED. |
|---|---|---|---|
| | V. B.[1] | N. B. | |
| XI | — | — | Dr. Kittredge, Nashua, N. H. |
| XII | 2.1 | — | Dr. Ellis, Elkton, Md. |
| III | 2.2 | — | Prof. His, Leipzig, Germany. |
| XIX | 5.5 | 4.5 | Dr. Williams, Baltimore, Md. |
| XVIII | 7. | 7. | Dr. Douglas, Nashville, Tenn. |
| II | 3. | 7. | Dr. C. O. Miller, Baltimore, Md. |
| IV | — | 7. | Dr. Williams, Baltimore, Md. |
| XLIII | 15. | 13. | Dr. Booker, Baltimore, Md. |
| VIII | 17. | 14. | Dr. Ritter, Brooklyn, N. Y. |
| IX | 17. | 14. | Dr. Eycleshymer, Chicago, Ill. |
| V | 18.5 | 17. | Dr. Kittredge, Nashua, N. H. |
| XLII | 18. | 15. | Dr. Wills, Los Angeles, Cal. |
| XVII | 18. | 16. | Dr. Cottrell, Louisville, Ky. |
| XXVIII | 19. | 18. | Dr. Sewall, Denver, Col. |
| VII | 19.5 | 18. | Dr. Booker, Baltimore, Md. |
| XXII | 20. | 18. | Dr. Snively, Waynesboro, Penn. |
| LVII | 23. | 20. | Dr. Howard, Cleveland, Ohio. |
| VI | 24. | — | Dr. C. O. Miller, Baltimore, Md. |
| X | 24. | 20. | Dr. W. S. Miller, Madison, Wis. |
| XLV | 28. | 19. | Dr. Douglas, Nashville, Tenn. |
| XXXIV | 80. | 60. | Dr. Ellis, Elkton, Md. |
| XLVIII | 130. | 110. | Dr. Wilson, Worcester, Mass. |

without placing the embryo first in weak alcohol, it naturally passes through the successive dilutions of alcohol before it is completely hardened.

It is very injurious to these delicate specimens to be wrapped in cotton before they are sent by mail or express. A perfect method is to place the preserved specimen in a bottle filled completely with alcohol, thus imitating the condition of a *foetus in utero*. If there is no air or cotton in the bottle containing the embryo it is almost impossible to injure the embryo by shaking it.

Since I have emphasized this method of preservation (Johns Hopkins Hospital Bulletin, 1893), I have obtained a number of specimens excellent in every respect. These specimens are not distorted, nor macerated, nor shrunken.

[1] V. B. and N. B. indicate the length of the embryo measured from the vertex to the breech and from the nape of the neck to the breech, respectively.

cytogenic studies also. In most of the specimens photographs or an additional series of sections were made of the chorion and amnion in order to study the variation of these structures.

Embryos XI,[1] XII, and II [2] were completely reconstructed in wax by the method of Born. Nos. IX, VI, and X were reconstructed in part by Born's method and finished by His's method of reconstruction. The abdominal viscera of Nos. VI, IX, X, XXXIV, XLV, and XLVIII were modeled by Born's method.

The mechanical portion of reconstruction has been simplified to a great extent by a special apparatus used in the Anatomical Laboratory,[3] which enables us to employ a modeler. The sections are projected upon a screen, to which the wax plate is attached. By working in a dark room with this apparatus it is easy to direct a modeler to draw the outlines accurately. He can then cut them out, and all that remains to be done is to pile the pieces and then blend them.

## THE COELOM IN YOUNG OVA.

All of the young human ova which have been described contain within them a cavity, lined with mesoderm ; this is the coelom, bounded by the somatopleure on the outside and by the splanchnopleure on the inside. This arrangement, as shown by a number of diagrams by recent authors, is very unlike the appearance of the blastodermic membranes of many of the lower mammals, and it is necessary therefore that we should revise our conception of the formation of the amnion in the human embryo.

The ova recently published by Graf Spee indicate that the amnion must be formed very early, and, since it is completed before the medullary grooves begin, we must admit now that it is formed much the same as it is in many rodents, *i.e.*, by apparent inversion of the membrane. When Bischoff [4] first described inversion of the membrane in guinea pigs it seemed

[1] Mall: Anatom. Anz.
[2] Mall: Journ. of Morph., vol. V.
[3] Hoen : Johns Hopkins Hospital Bulletin, 1896.
[4] Bischoff : Entwickl. d. Meerschweinschens, Giessen, 1852.

like a paradox, but, since the comparative methods of study have been introduced, inversion only means that the amnion is completed before the medullary groove begins to form. This alteration of the development of the amnion and the medullary groove makes the body of the embryo develop on a concave surface instead of on a convex one, thus apparently making the embryo inverted, as is the case in the guinea pig.

Closely associated with inversion of the blastodermic membrane is the formation of an additional layer of cells, discovered by Rauber,[1] the importance of which has been emphasized by Selenka and others. Rauber's layer is so marked in the rabbit that it was at first believed to be the true ectoderm. The fate of Rauber's layer has not been sufficiently studied to interpret it completely, and our ideas regarding it will not improbably require some revision. In many rodents Rauber's layer becomes markedly thickened on one side of the ovum, forming a support, or *Träger*, for the ovum. The relation of Rauber's layer to the *Träger* is shown beautifully by Selenka[2] on Plate XVI of his monograph.

The question which interests us here is whether the inversion of the blastodermic membrane as well as the discovery of Rauber's layer aids us in advancing a theory of the development of the germ layers of the human embryo, and thus in turn to explain the large coelom as found in all of the earliest human ova. I realize fully that any such effort will not be final, yet I believe that it will aid us to understand better the relation of the membranes as found in the human ovum.

In looking over the illustrations of the development of animals closely related to man, one is struck with the similarity of the arrangement of the membranes to those described for the human ovum by Graf Spee. One must compare only plates XXXV–XXXVIII of Selenka's[3] paper with the two plates accompanying Graf Spee's[4] to be convinced that the early development of monkeys is almost identical with that of man. Yet Selenka's researches on monkeys do not help us a great

[1] Rauber: Sitzungsber. d. Naturforcher Gesellsch., Leipzig, 1875.
[2] Selenka: Studien über Entwickl. d. Thiere, Heft 3, 1884.
[3] Selenka: Studien, *etc.*, Heft 5, Erste u. Zweite Hälfte, 1891, 1892.
[4] His's Archiv, 1889 and 1896.

deal ; they only show us that the monkey's development is like that of man. In monkeys we have also the precocious chorion and the early amnion and the large coelom between the umbilical vesicle and the chorion. The marked difference is that the amnion is attached to the chorion along its dorsal side, while in the human embryo this is only the case along the posterior end of the amnion. The attachment of the amnion along the chorion suggests that the embryonic plate separated from the exterior of the ovum along this point, as Selenka thinks he observed in a very young ovum only 1.5 mm. in diameter. Unfortunately, the most valuable specimen was injured in its preparation,[1] and Selenka did not trust himself to give any illustrations of it.

With the amnion attached at its dorsal end to the chorion, we understand why the entodermal end of the allantois must grow around an angle to reach the chorion (Selenka, Plate XXXVII, Fig. 5). Somewhat the same arrangement has been described by Graf Spee[2] in his embryo Gle., but the curve is by no means as marked, indicating that the attachment of the embryo to the chorion is along its posterior end, as shown by His[3] in his well-known diagram of the formation of the amnion.

Regarding the very early stages of monkeys and man it is better that we make comparisons with animals most nearly related to them, and now we have careful studies of the very early stages of Chiroptera at our disposal. I believe that Selenka's[4] study of the development of Pteropus edulis gives us the key for the comparison of the formation of the blastodermic membranes in mammals. Recent investigations by Duval[5] on different families of Chiroptera appear to confirm the work of Selenka on Pteropus.

In order to illustrate these points more clearly I have made diagrams of three of Selenka's figures of Pteropus. Fig. 1 is from an ovum covered completely with two layers of cells,

[1] Selenka : Studien, 1891, p. 201.
[2] Graf Spee : His's Archiv, 1896, Taf. I, Fig. 1.
[3] His: Anat. mensch. Embryonen, Theil I, p. 171.
[4] Selenka : Studien, 1892, p. 209.
[5] Duval: Jour. de l'Anatomie et de la Physiologie, 1895.

between which at one pole of the egg there is a mass of scattered cells destined to become the permanent ectoderm. The outer layer of cells has a tendency to grow into the form of villi over the embryonic disc, while on the opposite side of the
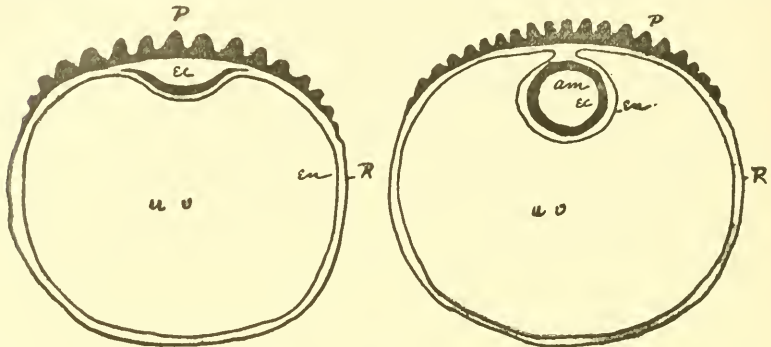


Fig. 1.

Fig. 2.
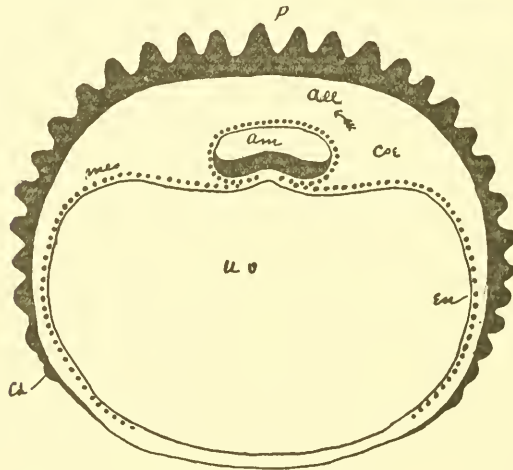
Fig. 3.

Figs. 1–3. — Diagrams of the Development of Pteropus Edulis, after Selenka. Fig. 1 is Selenka's Fig. 2, Fig. 2, Selenka's Fig. 5; Fig. 3, Selenka's Fig. 9. *R*, Rauber's layer; *P*, placenta; *ec*, ectoderm; *en*, entoderm; *ch*, chorion; *am*, amnion; *u v*, umbilical vesicle; *mes*, mesoderm; *coe*, coelom; *all*, allantois, with the arrow indicating the direction of its future development.

egg it is composed of but a single layer of cells. Since this outer layer remains well separated from the body of the embryo throughout its development, and since it holds the same position to the egg that Rauber's layer does in the rodents, I

believe it to be identical with Rauber's layer, and shall speak of it as such. According to Duval this Rauber's layer disappears over the embryonic disc in the Chiroptera much as in the development of the rabbit and the field mouse. This does not necessarily contradict Selenka's observations on Pteropus, for the house mouse begins to develop like the field mouse, but continues during the early stages in the same manner as Pteropus does.

In the next stage the ectoderm has been converted into a hollow mass of cells, Fig. 2, rather by a process of absorption than by an invagination, as I have expressed it in the diagram. The entoderm lines the whole interior of the egg, and surrounds the ectoderm of the amniotic cavity. The ectoderm of the exterior of the egg, Rauber's layer, is again thickened over the embryonic mass to form the placenta, as Selenka calls it, or the *Träger*, if we were discussing rodent embryology.

In the next stage, as expressed in Fig. 3, the mesoderm is beginning to form, and has extended completely over the amnion and partly over the umbilical vesicle. The entoderm has retracted itself and touches the ectoderm ; only the chorda dorsalis is yet to form. Between the amnion and the placenta, or the *Träger* portion of Rauber's layer, there is a marked space, and the mesoderm does not come in contact with it. The allantois grows as a bag into this space and attaches itself to the thickened part of the ectoderm, as shown by Göhre[1] in his figures. In the figure 3 accompanying Göhre's paper he shows the vesicular allantois attached to the support of the chorion (black portion of my Fig. 3) leaving on either side of the embryo a coelom. The allantois carries the mesoderm and vessels to the villi of the chorion, and these in turn are imbedded in the decidua of the uterus. In so doing the ectoderm of the chorion receives a second layer of epithelium, and I believe that this must account for the two layers of epithelium we have on the chorionic villi of the human ovum. There has been much written on the subject of the double layer of epithelial cells of the human chorion, and I think that a glance at Göhre's figures 3 and 4, on Pteropus, as well as at Selenka's figures 11

[1] Göhre: Selenka's Studien, etc., 1892, p. 218.

and 12 (Plate XXXV) and figure 6 (Plate XXXVII) on monkeys, will decide this question more definitely than all the many discussions on the human chorion put together have done.

Having now selected from Selenka diagrams and descriptions of the development of the germ layers of Pteropus, it is easier for me to give a plausible explanation of the beginning of the coelom in the human embryo. If the diagram I have given in Fig. 3 is compared with Selenka's figures 5 and 11 (Plate XXXV) and figure 5 (Plate XXXVII) of the monkey, as well as with the sections of young human ova published by Graf Spee [1] and by myself,[2] one is struck with the great similarity of the two groups of figures.

Fig. 14, given further on, is a diagrammatic outline of a longitudinal section of a young human embryo published recently by Graf Spee. It is the one marked v. H. in the table of young human ova given in the beginning of this paper. When, now, this section is compared with the transverse section of Pteropus, in Fig. 3, the only marked difference is that the umbilical vesicle in Pteropus has retracted, in order to make the arrangement of the membranes as given for the human embryo in Fig. 14.

In order to make the connection complete, I give hypothetical stages in Figs. 4, 5, and 6. Fig. 4 represents the human ovum in the two-layer stage. The outer layer, or Rauber's layer, is complete as in the rodents and in Pteropus. The inner layer, or entoderm, is also complete. Between the two is the embryonic shield, or ectoderm of the future embryo. The next figure, 5, shows the beginning of the mesoderm developing towards the tail end of the embryo, as this is the position of the primitive streak, and as the head fold of the amnion in many embryos is often only invested with ectoderm and entoderm. A stage later, Fig. 6, finds the mesoderm enveloping the umbilical vesicle completely, and also partly lining the outer layer, R, of the ovum. The cavity between the two is the coelom. At the tail end of the embryonic disc the mesoderm

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[1] Graf Spee : His's Archiv, 1889 and 1896.

[2] Mall: A Human Embryo of the Second Week, Anatom. Anz., Bd. 8, and Early Human Embryos and the Mode of their Preservation, Johns Hopkins Hospital Bulletin, 1893.

of the somatopleure and splanchnopleure are still united, and mark the place of the formation of the rudimentary allantois.

Having carried the development of the human ovum to this stage by means of hypothetical stages, based upon the development of Pteropus, I can now continue the description of the development based upon observation.

*Abnormal Ova.* — Teratologists are accustomed to view a group of abnormal states as arrested development, and in recent years a number of abnormal human ova have been studied by His,[1] by Giacomini,[2] and others. Frequently in the
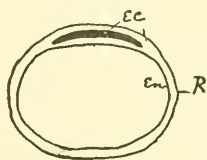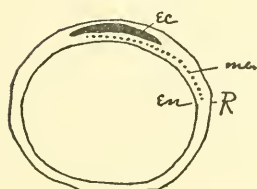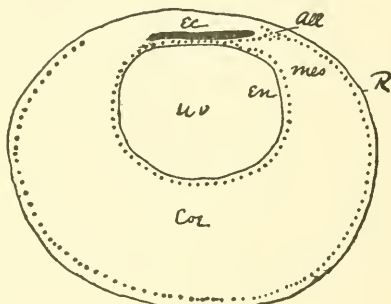


FIGS. 4–6. — Hypothetical Stages of the Early Development of the Human Ovum. *R*, Rauber's layer; *ec*, ectoderm; *en*, entoderm; *mes*, mesoderm; *u v*, umbilical vesicle; *coe*, coelom; *all*, position of allantois.

development of an ovum the embryo is destroyed completely, or, according to Giacomini, may wander out of the ovum. In these cases the ova are aborted. Frequently, however, a portion of the embryo is not developed, or it dies and the remaining portion develops for a time, and then the ovum is aborted. I have now in my collection a beautiful example of an ovum of apparently normal structure, the interior of which is lined completely with an amnion, and in place of an embryo

[1] His: Anatomie mensch. Embryonen, Heft 2, 1882, and Internationale Beiträge zur wissenschaftlichen Medecin, Bd. 1, 1891.

[2] Giacomini: Ergebnisse der Anatomie und Entwicklungsgeschichte, Bd. 4, 1895. The original papers of Giacomini are in the Archives Italiennes de Biologie, vols. XVIII–XXII.

there is only an umbilical cord.   The ovum was aborted fifty-
four days after the first lapsed period, and was 30 mm. in
diameter.   The cord was 2 mm. in diameter and 9 mm. long.
Its embryonic end seemed to be cut off abruptly, and was
covered with a small mass of round cells.   I give this example
only to show that the embryo may be entirely wanting with a
perfect cord and membranes.

A large per cent of young ova which come into the embry-
ologist's hands are abnormal.   According to Professor His's

TABLE OF ABNORMAL OVA.

| No. | Diameter of Ovum. | Diameter of Embryonic Mass. | From whom obtained. |
|---|---|---|---|
| XIII | 8 mm. | 1.4 mm. | Prof. His's No. XLIV, Leipzig. |
| XIV | 30  " | 1.5  " | Dr. Friedenwald, Baltimore, Md. |
| XX | 15  " | 2.  " | Dr. Williams, Baltimore, Md. |
| XXI | 12  " | 5.  " | Dr. Cullen, London, Canada. |
| XXXVII | 25  " | 2.  " | Dr. Gould, Philadelphia, Pa. |
| LVIII | 20  " | 6.  " | Dr. Howard, Cleveland, Ohio. |
| XXXII | 30  " | 2×9  " | Dr. Booker, Baltimore, Md. |
| XXIV | 20  " | — | Dr. Miller, Baltimore, Md. |
| XXIX | 30  " | — | Dr. Booker, Baltimore, Md. |
| LV | 30  " | — | Dr. Watson, Baltimore, Md. |

It does not necessarily follow that these embryos are all less than six weeks
old, for the menstrual history of the mother indicates that some of them must be
considerably older.   This is one source of error in obtaining the high per cent of
abnormal ova among young embryos.   The statistics will not be accurate until the
menstrual history accompanies the measurements.

experience over half of the ova less than three weeks old are
abnormal, while of those of four and five weeks one quarter are
abnormal.   In my collection there are ten abnormal ova among
twenty-six ova which are less than six weeks old.   Of these
ten specimens three contained no embryos at all, one (No.
XXXVII) contained the cord only, and six were of the
nodular form.   Of this group of six, three contained a double
vesicle with a kind of fibrous capsule, to a great extent similar
to the mesoderm of the chorion.   One of these is His's Embryo
XLIV, which is frequently described in the books as a normal

specimen, but which unfortunately is an abnormal one. My interpretation of these three specimens (Nos. XIII, XIV, and XX) is that the fibrous degeneration overtook the embryonic vesicle after it had reached the stage of Graf Spee's embryo v. H., my Fig. 14. The remaining three embryos (Nos. XXI, XXXVII, and LVIII) are of the vesicular form, and I believe them to be especially valuable for the proper interpretation of the early stages of development of the human coelom.

Nos. XXI and LVIII came to me as perfect specimens, both having been hardened unopened, the first in strong formalin and the second in strong alcohol. No. XXI was still enclosed in its decidua, and appeared to be a normal specimen until it had been cut into serial sections. The embryonic vesicle proved to be very large, and was composed throughout of two layers, an inner one giving all the appearance of the entoderm and an outer giving all the ap-



FIG. 7. — Diagram of a Pathological Ovum which represents an Early Hypothetical Stage.

pearance of the mesoderm of the umbilical vesicle of young embryos. The mesodermal layer contained within it islands of blood cells, as are also present in normal specimens. The whole vesicle was connected to the chorion with a mass of mesodermal cells somewhat as shown in the diagrammatic Fig. 7. The chorion and decidua appeared to be normal.

No. LVIII showed considerable change in the mesoderm of the vesicle and chorion, giving somewhat the appearance of fibroid degeneration rich in cells. The chorion was attached to the vesicle by a strong pedicle, as shown in Fig. 7. The vesicle itself was composed of two layers, an inner and continuous one composed of one layer of cells, and an outer and thickened layer appearing like the mesoderm of the chorion. There were no indications of blood islands. In addition to these two layers there was a third layer fairly well marked near

the pedicle and between the vesicle and the chorion. With the exception of the allantois canal, Fig. 7 is a diagram of this specimen. No. XXXVII is much like No. XXI, but it did not stain well as the specimen was a number of years old when cut.

Giacomini [1] has described a number of similar vesicles, and he expressly states that the vesicles had the structure of the



umbilical vesicle, but that there was no trace of the amnion present in any of them. A number of other vesicular forms have been described, and in general they all appear much like the two specimens I have given.

I do not think that it is rash to assert that these vesicles represent an arrested development of an earlier stage, which, due to impaired nutrition, or whatever it might have been, simply allowed the embryonic vesicle to keep on expanding. That this expansion can keep on is already shown in the sim-
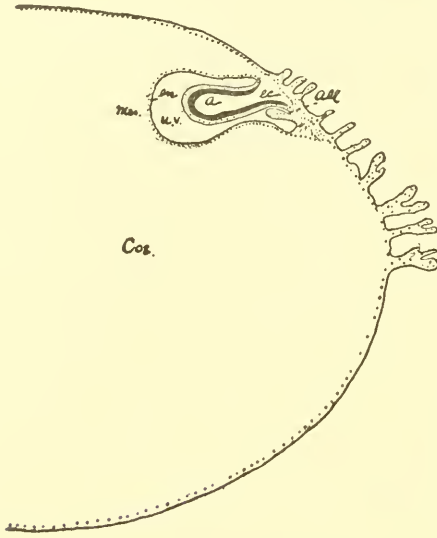
FIG. 8.— Diagrammatic Section of Half of the Human Ovum No. XI. Enlarged 10 times. The villi are drawn only on the upper side. *ec*, ectoderm; *en*, entoderm; *mes*, mesoderm; *u v*, umbilical vesicle; *coe*, coelom; *all*, allantois; *a*, amnion.

ple enlargement of the chorion after the embryo is distorted or wanting altogether. We have in these specimens a thin chorion with atrophic villi, and why can we not have an expanded and atrophic embryonic vesicle if its development is impaired? In this way I view specimen No. LVIII. It represents a much earlier stage, which has simply expanded and was ultimately aborted. In No. LVIII the embryonic vesicle must have ceased its further development a week or so before the abortion, about the time the coelom was beginning to

[1] Giacomini: Ergebnisse d. Anat. u. Entwicklungsgesch., Bd. 4, S. 636.

develop. At that time the fibrous degeneration enclosed the embryonic vesicle as well as extended around the whole chorion into all of its villi. This, then, arrested the further development of the embryo, and the embryonic vesicle simply continued to expand.

This idea is further strengthened by another ovum whose history I published on several occasions three years ago.[1] The specimen is a good one, having been preserved fairly well, and it has every indication of being normal. Since the specimen has been in my hands I have studied it over and over again, have photographed many of the sections, and have reconstructed it. At first it was very difficult for me to interpret it, but finally it appears to me that something definite can be said regarding the arrangement of the membranes and their relation to older as well as to the pathological and presumably younger specimens.

*Embryo No. XI.* —"The woman, from whom the specimen was obtained, is twenty-five years old, menstruates regularly every four weeks, the periods lasting from four to five days. She gave birth to a child Sept. 19, 1892, and had the first recurrence of menstruation December 19. The second period followed on January 25, and was very profuse; it lasted until February 1. The next period should have begun about February 22, but on account of its lapsing the patient concluded that she was pregnant, and called at my office a few days later. I did not examine her, but asked her to remain quiet and await developments, as I thought possible that she might be pregnant. On the evening of March 1 she fell and sprained herself, and during the same night had a scanty flow. The flow recurred each day, and on the 7th of March she passed the ovum. It was kept in a cool, moist cloth for twenty hours, and when it came into my hands was at once placed in a large quantity of 60% alcohol."[2]

The ovum is very large for its age, having a long diameter of 10 mm. and a short diameter of 7 mm. It is covered with villi only around its greatest circumference, having two spots with-

[1] Mall: Anatom. Anz., 1893, and Johns Hopkins Hospital Bulletin, 1893.
[2] Letter from Dr. Kittredge, April 27, 1893.

out villi, as was the case with Reichert's ovum. The villi of the chorion are from 0.5 to 0.7 mm. long and are branched.

Upon opening the chorion it was found that the germinal vesicle was situated just opposite the edge of the zone of villi. About it was much coagulated albumen, which I did not remove, and therefore could not obtain good camera drawings. The portion of the chorion to which the vesicle was attached was cut out and stained with alum cochineal and cleared in oil, but even after this treatment it was impossible to obtain any clear picture. The specimen was next imbedded in paraffin and cut into sections 10 $\mu$ thick. The series proved to be perfect. From the sections a reconstruction was made in wax, and the accompanying Fig. 8 is a sagittal section of it.

The dimensions of the different portions of the vesicle are as follows :

| | |
|---|---|
| Diameter of stem . . . . . . . . . . | 0.4 mm. |
| Length of stem . . . . . . . . . . . | 0.4 " |
| Length of vesicle . . . . . . . . . . | 1.5 " |
| Width of vesicle . . . . . . . . . . . | 1.0 " |
| Length of invagination . . . . . . . . | 0.8 " |
| Width of invagination . . . . . . . . . | 0.5 " |
| Diameter of opening of invagination . . . . . | 0.03 " |

The sections and reconstruction show that the embryonic vesicle is attached to the chorion by means of a stem (*Bauch-stiel*). The vesicle itself is composed of two layers, between which, at a distance from the stem, there are indications of blood-vessels in the middle embryonic layer. Just beside the attachment of the vesicle to its stem there is a deep, narrow invagination of all layers of the vesicle. The walls of the invagination are somewhat thicker than those of the surrounding vesicle. The accompanying figures give the arrangement of the embryonic layers in different portions of the vesicle. The invagination is in no respect artificial, as suggested by Graf Spee, as the curves are all sharp, and the layers of mesoderm and ectoderm are very definitely outlined. The ectoderm has the sharp contour of the ectoderm of other young embryos published, and gives the pictures which are familiar to all embryologists. The entoderm does not extend all around the sections as I have pictured it, but has fallen off at some points, and

this explains why the figures here given do not correspond exactly with those in previous publications. There cannot be any doubt about my interpretation of the arrangement of the embryonic layers in this specimen, nor do I think that it is abnormal. Yet this last point will be decided in the near future, I believe, and therefore it may be dropped until other young embryos are described.

Within the stem of the vesicle there is a sharply defined allantois, which communicates with the cavity of the vesicle



FIG. 9.                                    FIG. 10.

FIG. 11.                     FIG. 12.                     FIG. 13.
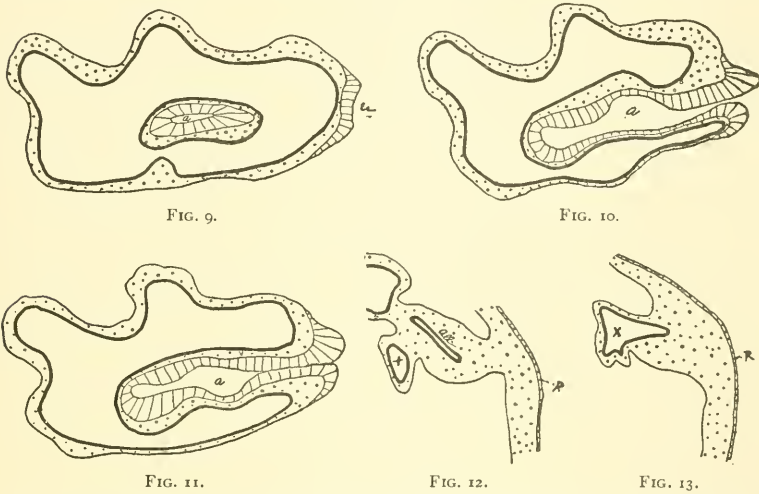
FIGS. 9–13. — Sections No. 43, 53, 68, 80, and 89 through the Embryonic Vesicle of Embryo No. XI. Enlarged 33 times. The entoderm is a heavy line, the ectoderm is striated, and the mesoderm dotted. *a*, amnion ; *X*, cavity of the umbilical vesicle extending into the stem of the vesicle ; *R*, Rauber's layer as the ectoderm of the chorion.

just below the invagination of the ectoderm. The cavity of the vesicle extends into the stem at a lower point, and it is this invagination which Graf Spee believes to be the amnion. Gladly would I agree with him were it not that there is no evidence whatever of the presence of ectoderm at this point. Throughout this invagination into the stem there are only two thin layers of cells, one of which runs over into the mesoderm of the chorion and the other into the entoderm of the vesicle. Yet in this invagination the entoderm is not detected at any point.

The ectodermic plate in the large invagination of the amnion is very broad, but not of equal thickness throughout its extent, and it ends very abruptly beyond the opening upon the surface. As the opening approaches the stem, the cells of the ectoderm are continued somewhat along its surface, as indicated by the black line in Fig. 8.

All of the space between the embryonic vesicle and the chorion is the coelom, and in this specimen it communicates with the amnion. Whether this is transient or unusual cannot, of course, be stated. Should further experience show that the amnion is closed at an earlier stage than indicated in this specimen, it would not materially affect my diagrams or observations. Graf Spee's recent observation, Fig. 14, makes him think that this is the case, but it is just as easy to interpret the formation of the amnion in Fig. 14 from that in Fig. 8 as by his theory.

The next stages in the development of the embryonic vesicle are taken from Graf Spee, and they are of importance to elucidate the changes which take place preparatory to the formation of the body cavity. In Fig. 14, which represents the younger embryo, the amnion is still surrounded completely with mesoderm, as in embryo No. XI, represented in Fig. 8. The mesoderm crosses the median line, as the sections given by Graf Spee[1] show. The dorsal side of the amnion is covered with a very thick layer of mesoderm, as the closure of the amnion in embryo No. XI would suggest.

From the stage represented in Fig. 14 it is easy to pass to the older embryo represented in Fig. 15. Now the body of the embryo is well marked, the neural folds are just beginning, and the neurenteric canal has just been formed. The chorda dorsalis is not yet separated from the entoderm, and the blood islands encircle completely the umbilical vesicle and have nearly reached the head end of the body of the embryo preparatory to the formation of the heart.

It is not very difficult to imagine the embryonic vesicle of Fig. 8 to be converted into the vesicle of Fig. 14. To be sure, the invagination in Fig. 8 seems to be much larger than neces-

[1] Von Spee: His's Archiv, 1896, Plate I, Figs. 4, 5, 9, and 10.

sary, but variations of this kind are frequently encountered in the study of embryology. In the diagrammatic outline of von Spee's embryo v. H. (Fig. 14), I have emphasized the variation of the thickness of the ectoderm lining the amnion to correspond with von Spee's Figs. 7, 8, 9 in his recent publication.
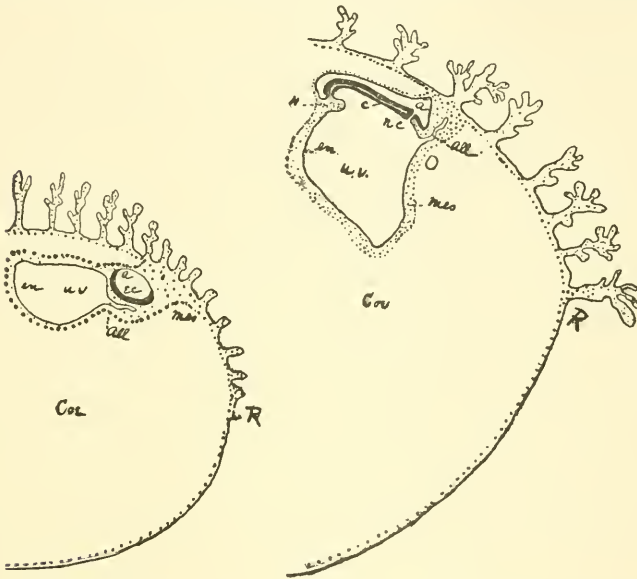


FIG. 14.                    FIG. 15.

FIGS. 14 and 15. — Longitudinal Sections of Two Young Human Ova, after Graf Spee. Enlarged 10 times. Fig. 14, Embryo v. H.; Fig. 15, Embryo Gle. Just half of the chorion is drawn, and the villi are outlined only over a portion of the ovum. *R*, Rauber's layer; *a*, amniotic cavity; *u v*, umbilical vesicle; *en*, entoderm; *mes*, mesoderm; *all*, allantois; *c*, chorda; *n c*, neurentic canal; *H*, position of heart.

His longitudinal section, from which my figure is taken, does not emphasize this point, which I consider of importance in this discussion.

In these two ova described by von Spee, the coelom is much of the same form it was in embryo No. XI, Fig. 8, and therefore needs no special comment. Yet around the head end of embryo Gle. there is a marked accumulation of mesoderm into which the heart is to grow. In the illustrations of the section of this embryo Graf Spee [1] pictures spaces in the mesoderm which he believes to be portions of the body cavity of the

[1] Graf Spee : His's Archiv, 1889.

embryo, that is, the cavity of the muscle plates, pericardial
cavity or peritoneal cavity. It is impossible to determine defi-
nitely which portion of the body cavity these spaces represent,
but I do not feel inclined to believe that what he marks peri-
cardial cavity in Fig. 23 can possibly represent it, for we are
to look for the pericardial cavity between the junction of the
pharynx and umbilical vesicle and the head end of the embryo.
This portion of the embryo is marked H in my Fig. 15, and
falls anterior to von Spee's Fig. 16. Von Spee's Fig. 16 is
the 24th section of the embryo, beginning at the head, while
his Fig. 23 is the 81st section.

The various small spaces in different portions of the meso-
derm cannot be viewed as the real origin of the body cavities
without further discussion. In the von Spee embryo v. H. there
are indications already of small spaces in the mesoderm at the
border of the ectoderm of the embryo. Similar spaces are
described by Bonnet [1] for the sheep and by Selenka [2] for the
monkey. While von Spee and Bonnet believe that these
spaces belong to the coelom, Selenka simply designates them
heart, or vascular.

The blood-vessels are intimately associated with the coelom
in their early development, and it is easy to be led into error
without an abundance of material. Drasch [3] recently has
again emphasized this relation. He has shown in the chick
that the blood islands are separated from one another by a
number of closed spaces filled only with a fluid. These spaces
soon flow together to form the large slit-like coelom of birds.
The same condition of things has been shown to be true, but
from a very different method, by Budge.[4] He injected the
blastoderm of the chick, and showed that the coelom was com-
posed of a network of spaces, which gradually flowed together
into the large coelom surrounding the embryo.

Of course in the young human embryos we have at our dis-
posal this stage of the process has long passed, but there is no
reason why a remnant of it should not exist at the point of

[1] Bonnet: His's Archiv, 1889.
[2] Selenka: Studien, etc., Taf. XXXVIII, Fig. 35.
[3] Drasch: Anatom. Anz., Bd. 9.
[4] Budge: His's Archiv, 1887.

union of the umbilical vesicle with the body. The reason I question von Spee's interpretation of these small spaces in the mesoderm in embryo Gle. is that I believe that all, or certainly nearly all, of the body cavity is formed by an incorporation of the extra-embryonic coelom within the embryo. What I have observed in human embryos as well as in the injected specimens of Budge shows that this must be true. These small spaces in the mesoderm of the body may belong to the muscle plates and the early blood-vessels, and certainly cannot play any great part in the development of the body cavity. There is no doubt whatever that the whole peritoneal cavity is simply pinched off from the coelom of the outside of the body and it is highly probable that the pericardial cavity and pleural cavities are formed in the same way. The anterior mesentery of the intestine has never existed in the human embryo, and it is therefore needless to explain its mode of disappearance.

My statements are based in great part on embryos Nos. III and XII, and since No. XII is such a perfect specimen it is well for me to describe it in greater detail. The embryo is about the same age as Kollmann's[1] embryo Bulle, which unfortunately was never fully published. No. III is an embryo given me by Professor His. This embryo had been torn from the umbilical vesicle, and was injured in different portions of the body. Yet the head end of it is fairly well preserved, and it is of value in determining the growth of the body walls covering the heart.

*Embryo 2.1 mm. long.* — The history of embryo No. XII is as follows. " The woman from whom the ovum was obtained is twenty-three years of age and has been married for three years. She is a very intelligent woman, and her statements are reliable. Her menstrual periods recur every thirty days. She had been married some time before she became pregnant, and after passing two periods aborted July 6, 1893. She was unwell the 5th of October and again on the 7th of November, this last period

[1] Kollman: His's Archiv, Supplement Bd., 1889, Plate V, Figs. 1 and 2; von Lenhossék: His's Archiv, 1891, Plate I; Kollman: His's Archiv, 1891, Plate III, Fig. 3.

lasting five days. She passed her next period and on December 18th aborted the ovum." [1]

The ovum was hardened in strong alcohol without opening it first, and when it came into my hands its dimensions were 18
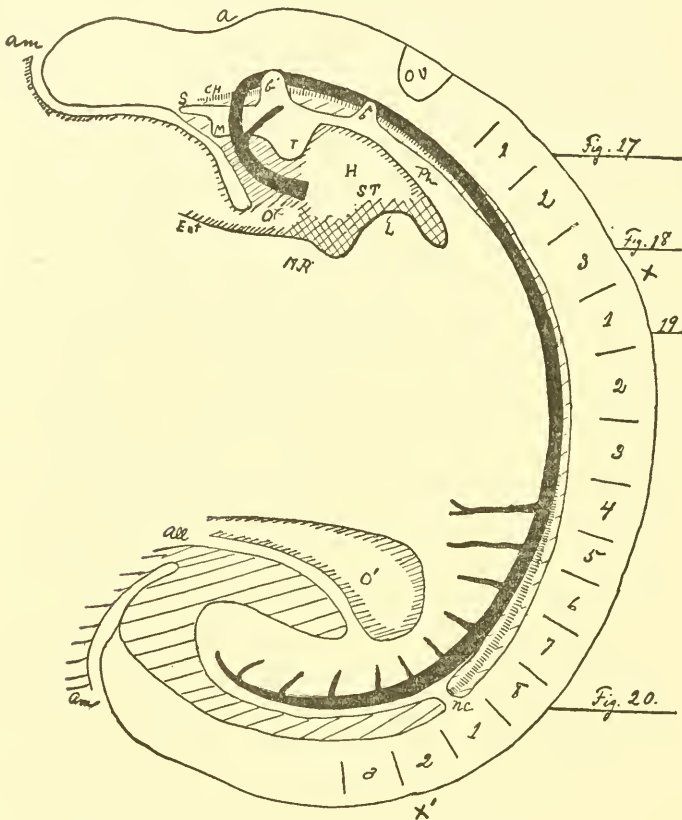


Fig. 16. — Outline Drawing of a Sagittal Section of the Model of Embryo No. XII. Enlarged 50 times. The heavy line is the aorta. The muscle plates are numbered for occipital, cervical, and dorsal regions, respectively. The mesoderm is striated. *am*, amnion; *a*, border between fore-brain and mid-brain; *x* and *x'*, extent of closure of spinal canal; *S*, Seessel's pocket; *ch*, chorda; *b'* and *b''*, first and second branchial pockets; *o v*, otic vesicle; *m*, mouth; *T*, thyroid; *H*, pericardial space; *ph*, pharynx; *ent*, entoderm; *S T*, septum transversum; *l*, liver; *n c*, neurenteric canal; *all*, allantois.

$\times$ 18 $\times$ 8 mm., that is, it was slightly flattened. It was completely covered with long villi. It was carefully opened, care having been taken not to injure the embryo in any way. The

[1] Letter from Dr. Ellis, Jan. 7, 1894.

coelom was filled with a clear fluid, and many firm shreds of a fibrine-like body which obscured the embryonic vesicle greatly. With much difficulty the embryo could be outlined, and these drawings proved to be of great service in making the reconstruction. The portion of the chorion to which the embryo was attached and the embryo were stained in carmine and imbedded in paraffin. The whole was cut into sections, at right angles to the body, 10 $\mu$ thick.

Every other section was enlarged 100 times and drawn on wax plates 2 mm. thick, and from them the model of the embryo was made. The model gives the whole central nervous system, the entoderm throughout its extent, the blood-vessels, and the muscle plates.

The shape of the neural tube is given in the diagrammatic outline. It was closed only along the middle of the body, being open in front down to the beginning of the fourth muscle plate. From the beginning of the fourth plate to the beginning of the fourteenth it was closed, and from there on again it was open. In the figure the portions between x and x′ indicate to what extent the tube is closed. In Figs. 17 and 18 the tube is nearly closed, while in Fig. 20 the tail end of the tube is just beginning to separate from the ectoderm. The cephalic end of the tube already clearly outlines the fore-brain, the mid-brain, and the hind-brain ; the constriction, Fig. 16, a, indicates the junction between the first two. On the ventral side of the fore-brain there are two marked pockets, one on either side, just behind the neuropore, which are no doubt the primary optic vesicles. It shows that in the human embryo these are fully outlined before the brain has separated itself from the ectoderm. Farther behind, very near the dorsal median line and about in the middle of the head, there is a short pocket of thickened ectoderm, the otic vesicle. Towards the hinder end of the embryo the spinal cord communicates by means of a solid band of cells with the entoderm, Fig. 20. At no point in this communication is there a canal, so it must be viewed as the last remnant of the neurenteric canal. The location is opposite the twelfth muscle plate, or in the neighborhood of what will later on be the position of the first rib. The chorda

dorsalis extends to the neurenteric canal, but not beyond it. There is no chorda in the tail end of the embryo.
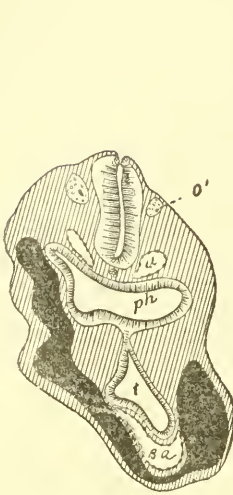


FIG. 17.
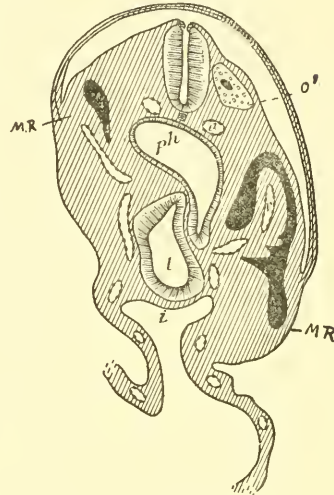
FIG. 18.
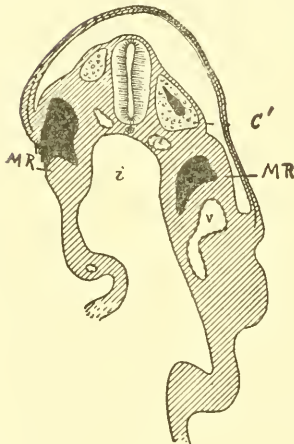
FIG. 19.
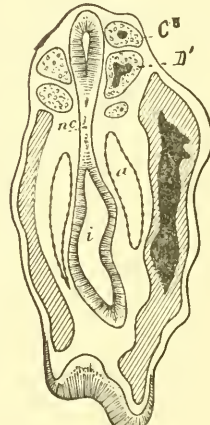
FIG. 20.

FIGS. 17–20. — Sections through Embryo No. XII, as indicated by the Lines in Fig. 16. Enlarged 50 times. The black is the coelom within the body. $O^1$ and $O^3$, first and third occipital muscle plates; $C^1$ and $C^8$, first and eighth cervical muscle plates; $D^1$, first dorsal muscle plate; $a$, aorta; $v$, omphalomesenteric vein; $t$, thyroid; $l$, liver; $ph$, pharynx; $i$, intestine; $n$ $c$, neurenteric canal; $m$ $r$, membrana reuniens.

Throughout the central nervous system, immediately about the central canal, there are many karyokinetic figures, showing

that the specimen was excellently preserved. In the greater portion of the neural tube the tissue is already marked by two zones, a central one rich in nuclei, and a peripheral containing none. This corresponds with the description already made familiar to us by His.

The general shape of the whole central nervous system is very unlike that of any other young human embryo ever published. It circumscribes the greater portion of a circle, while in the other human embryos of this size it makes more of a straight line. I think that it is probable that this specimen represents the normal, as it was not injured nor handled in any way before it was cut into sections.

The entoderm, as the figures show, is already divided into fore-gut, mid-gut, and hind-gut. The fore-gut makes the pharynx, from which there are four diverticula on the dorsal side, one on the ventral side, and two near the mouth. The four on the dorsal side mark the first two branchial pockets on either side of the embryo ; the two in front are Seessel's pocket and the entodermal portion of the mouth ; while the one on the ventral side of the pharynx is the beginning of the median portion of the thyroid gland (Fig. 17, t.).

At the junction of the pharynx with the umbilical vesicle there is a large diverticulum into the septum transversum, Fig. 18 l, the beginning of the liver.

Within the tail end of the embryo, behind the neurenteric canal, the hind-gut is enlarged considerably, and from it the entodermal canal of the allantois arises.

The whole umbilical vesicle is covered with blood-vessels which communicate with the veins and arteries of the embryo. Near the origin of the liver there are two veins which collect the blood from the umbilical vesicle and then enter the heart. These are the omphalomesenteric veins. They with a number of their branches are shown in sections in Fig. 18, v. The heart itself is broken, but there is enough of it left to show that it is bent upon itself and contains a large cavity at the point where the veins entered it. From the heart two arteries arise and pass in front of the first branchial pocket, and each follows the course as shown in black in the reconstruction.

The aortae do not unite, but each sends a number of segmental branches to the umbilical vesicle along the tail end of the embryo. These are, of course, temporary ; they may be called collectively the omphalomesenteric arteries. As the permanent omphalomesenteric artery arises more aboral than any of these, it is easy to understand that most of them must degenerate.

The sections show that there are fourteen muscle plates, all of which are hollow and do not in any way communicate with the body cavity in general. Kollman, who described an embryo of this same age, numbers them from before backward, but I think that they can be designated more definitely. Froriep[1] showed that in all amniotic vertebrates there were a number of muscle plates and dorsal ganglia formed in the occipital region, and studied their fate in the chick and in the cow's embryo. Platt[2] has also followed the order of the origin of the muscle plate in the chick, and found that the first division of the mesoderm was between the third and fourth occipital plates. The first three or four of these segments communicate in the chick, according to Dexter,[3] with the coelom, and Bonnet[4] has found also that the same is true in the sheep. Bonnet's figures (compare his Plate IV) show that a sheep's embryo of the same stage as embryo XII has muscle plates much more sharply outlined than the human. In order to locate the muscle plates more definitely I have made every effort to count the spinal ganglia in embryo XII, but with no definite result. It is impossible for me to define the spinal ganglia, as often they are represented by a few cells only, then again as a band of cells they extend over several segments. The same is true in the occipital region. Had I been able to number them definitely it would still have been impossible to number the muscle plates from them, for His[5] has shown that there is an occipital ganglion in the human embryo as well as in the lower animals.

The fact that the muscle plates reach to the otic vesicle in

[1] Froriep: His's Archiv, 1883 and 1886.
[2] Platt: Bulletin of the Museum of Comparative Zoölogy, vol. XVII.
[3] Dexter: Anatom. Anz., 1890.
[4] Bonnet: His's Archiv, 1889.
[5] His: Abhandl. d. säch. Gesellsch. d. Wiss., Bd. XXIV.

embryo XII, as well as in Kollman's embryo Bulle, indicate that the first plates must belong to the occipital region, and I have found that there are three occipital muscle plates in embryo No. II.[1] Moreover, there is every indication of a degeneration of the first two plates in XII, so on this account I am inclined to number them as they are numbered in Fig. 16. I do not think that any of them ever communicate with the pericardial cavity as Bonnet found them in the sheep. The cavities in all of the other plates are small, and they are separated by a large mass of mesoderm from the coelom. This all confirms my view.

The chorda extends from Seessel's pocket to the neurenteric canal.

There are also a few segmental ducts, some completely and some partly separated from the ectoderm, as was the case in Kollman's embryo. The ducts are small, and extend over one or two sections only, and occasionally one of them is arising at several different points between a given two segments. They are present on both sides between the first and second cervical segments, second and third segments, third and fourth segments, fourth and fifth segments, and only on the left side in the region of the fifth and sixth cervical segments.

The coelom of this embryo is especially instructive. A sagittal section of the embryo and ovum is given in Fig. 21. This embryo, when drawn connected with the ovum, is very similar to Graf Spee's embryo Gle. as shown in Fig. 15. It is very easy for us to conceive the von Spee embryo converted into this embryo, for about all the change that is necessary is that the embryo grow somewhat and bend upon itself. In so doing the attachment of the umbilical vesicle becomes smaller as the amnion encircles the body of the embryo more. The position of the neurenteric canal, the shape of the allantois, and the formation of the pericardial cavity, all show that the curving must be a normal one.

Nearly all other young embryos of this stage, or a little older, which have been published show a straighter body or even a curve in the opposite direction. I have also in my collection

[1] See also Mall: Journ. of Morph., vol. V.

two embryos of this stage, Nos. I and XV, which had been taken out of the chorion and torn from the umbilical vesicle, and both of them are straight like Kollman's embryo Bulle and His's [1] embryo L. It is difficult to conceive how my embryo XII could possibly be torn out of its membranes without straight-
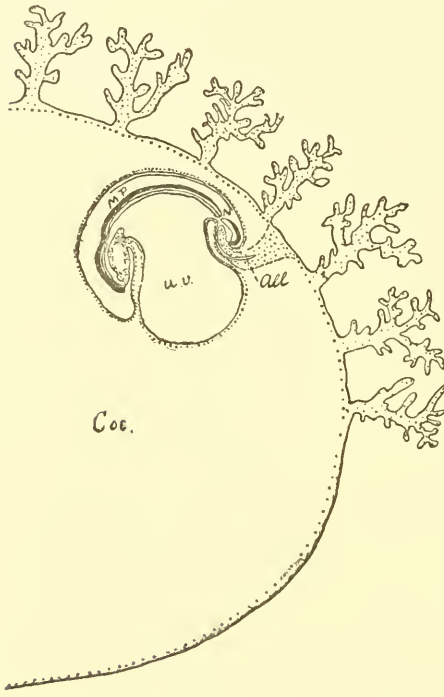


FIG. 21. — Sagittal Section of the Ovum with Embryo No. X11 Attached. Enlarged 10 times. *Coe*, coelom ; *u v*, umbilical vesicle ; *all*, allantois ; *m p*, medullary plate ; *n c*, neurenteric canal.

ening it. We need only recall our experience in hardening embryos of lower animals to be re-minded how easily a c u r v e d e m b r y o is straightened when it is handled the least bit roughly before it is hardened.

His, in his great monograph on human embryos, emphasizes a curve in the back of the embryo just the reverse of the one given in Fig. 21. I refer to embryos Sch., BB., and Lg., as well as to Minot's em-bryo 195.[2] The fact that this inverted bend in the back is not con-stant (His's Rf., for instance), and that it occurs at the time when any tension upon the umbilical ves-icle could produce it, makes me believe that it is an artifact. This view was suggested to me a number of years ago, when I was removing young dogs' embryos from the uterus, and unwit-tingly distorted a number of them in this very way before they were hardened. The middle of the back is the weakest part of the embryo's body, and the umbilical vesicle is attached to

[1] His: Anat. mensch. Embryonen, Plate VI.
[2] Minot: Human Embryology, New York, Fig. 169.

it. Under these conditions the simple weight of the vesicle is sufficient to bend the back of the embryo as pictured by His.

To return to the coelom. At the hinder end of the embryo the coelom dips into the body overlapping the hind-gut in the neighborhood of the neurenteric canal, as shown in Fig. 20. This cavity communicates with its fellow on the opposite side through an opening between the umbilical vesicle and the allantois, marked O in Fig. 16. This communication has already been described by His[1] for an embryo somewhat older. If, now, the point O in Fig. 16 is approximated towards NC, with a flexion of the embryo at the same time, this communication is easily explained. In other words, as the hind-gut is being separated from the umbilical vesicle, a groove-like portion of the coelom is also included in the body of the embryo. At the hinder portion of the embryo, on either side, the coelomic grooves extend deeper into the body of the embryo, and communicate with each other around the aboral side of the stem of the umbilical vesicle. This communication is shown well by His in Fig. I, B, Plate VI of his *Atlas,* as well as in the same figure, page 299 of Minot's *Embryology.* Excellent profile views showing this point are given in all the embryos figured on Plate IX of His's *Atlas.*

I emphasize this point in order to exclude the ventral mesentery for this portion of the embryo. The fact that this mesentery could never have existed in the human embryo is also proved by a careful examination of His's models of human embryos made by Ziegler.

As we pass towards the head in embryo XII the coelomic groove communicates freely with the extraembryonic coelom until the region of the membrana reuniens is reached. This is shown in Fig. 19, MR, with the membrana reuniens complete on one side, but not yet united on the other. The membrana reuniens extends up to the heart, and separates the pericardial cavity from the extraembryonic coelom, then crosses the ventral median line to return on the opposite side of the embryo. Throughout the extent of the membrana reuniens there is a great increase of mesodermal tissue, which encircles completely

[1] His: Anat. mensch. Embryonen, I, p. 126.

the beginning of the liver, as Fig. 18 shows. A portion of this mesodermal tissue has been described by His as the septum transversum.[1] According to His only that portion of the meso-dermal tissue is septum transversum which lies between the posterior part of the pericardial cavity (*Parietelhöhle*), the wall of the intestine, and the point where the veins enter the heart. It extends across the body, and has within it the beginning of the liver. In transverse section this region is shown in Fig. 18. Now the pericardial cavity communicates by means of a long canal on either side, with the peritoneal cavity, and the omphalomesenteric vein hangs into this, attached to a kind of mesentery, as Fig. 18 shows. Lower down, near the commu-nication (Fig. 19), there is an indication of the beginning of the umbilical vein, which unites with the omphalomesenteric vein through the membrana reuniens. The two canals which com-municate with the extraembryonic coelom are the pleural cav-ities, and the membrana reuniens aids to separate them from the peritoneal.

All of the tissues from the diaphragm to the opening of the liver duct into the duodenum arise from the septum trans-versum and the membrana reuniens ; the stomach from the fore-gut, the liver from the liver diverticulum, and the diaphragm from the septum transversum and the membrana reuniens. The Cuvierian duct must also have arisen in the membrana reuniens, in order to pass around the outside of the body cavity to reach the cardinal and jugular veins, as pictured by His[2] for the human embryo.

In the further development of the pleural and pericardial cavities the Cuvierian veins give us our best landmark, as they define the point where the pleural cavity is to be separated from the pericardial. And it really seems, as if the greater portion of the diaphragm is formed from the portion of the septum transversum on the ventral side of the vein and from the membrana reuniens, rather than from the portion immedi-ately in front of the intestine. In other words, there is a

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[1] His: Anat. mensch. Embryonen, I, p. 126.

[2] His: His's Archiv, 1881, Plate XII, Fig. 9. Also Anat. mensch. Embryonen, Plate IX, Figs. 10–12, 14.

horseshoe-shaped ridge of tissue around the neck of the embryo
to the ventral side of the pericardial and pleural cavities and
parallel to them. The median portion is composed of the
septum transversum, and each wing of the *shoe* is the mem-
brana reuniens, one on either side of the embryo. Its general
direction in this stage is parallel with the long axis of the
embryo, and within each wing there is an omphalomesenteric
vein.

*Origin of Pericardial Cavity.* — With the pericardial cavity
opening into the extraembryonic coelom on either side as a
basis, it is possible to trace back the pericardial cavity to its
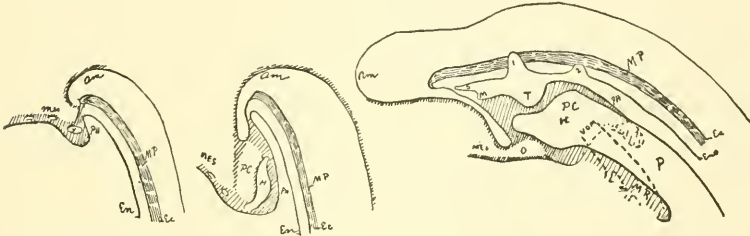


Fig. 22.    Fig. 23.    Fig. 24.

FIGS. 22-24. — Three Stages to show the Development of the Blastodermic Layers at the Head End
of the Embryo. Fig. 22, Hypothetical Stage. Fig. 23, Embryo No. III. Fig. 24, Embryo
No. XII. *V*, vein; *ph*, pharynx; *am*, amnion; *m p*, medullary plate; *p c*, pericardial
cavity; *S*, Seessel's pocket; *m*, mouth; *t*, thyroid; *1* and *2*, first and second branchial pockets;
*p*, pleural cavity; *m r*, membrana reuniens; *v o m*, omphalomesenteric vein, which is
expressed as a dotted line; *O*, communication between right and left body cavities on the
ventral side of the umbilical vesicle.

origin. Figs. 16 and 24 show that the ventral wall of the peri-
cardial cavity is composed mostly of mesoderm. This is the
portion of the membrana reuniens which is composed of meso-
derm, as the sections, Figs. 18 and 19, show. An earlier stage
is shown in the diagrammatic Fig. 23. It is taken from
embryo No. III. In this specimen, since the ectoderm of the
amnion has not reached completely around the body, as both
the sagittal and transverse sections show (Figs. 23 and 25),
it is evident that the pericardial space is first covered on
the ventral side with mesoderm and later the ectoderm is added
when the amnion begins to close over the head. In embryo
III the canals communicating between the pericardial space
and the extraembryonic coelom are not as long as in embryo

XII, and the ventral walls of the pericardial space are composed wholly of mesoderm. This indicates that the growth of this wall was first by a union of the mesoderm, which was followed by the ectoderm of the amnion to complete the body wall. The process is shown in Figs. 22–24. Fig. 22 is a hypothetical stage between Graf Spee's embryo Gle. and my embryo No. III. As the process from Graf Spee's embryo continues, the blood-vessels reach the body to form the heart, as indicated by the outlines marked v. in Fig. 22. The mesoderm of the amnion then unites with that of the umbilical vesicle, and the first pericardial space is formed. This is not wholly an imaginary stage, for it is based upon Bonnet's observations upon the sheep,[1] as well as Cadiat's upon the chick.[2] In a sagittal section of a sheep's embryo of about the same stage (Plate III, Figs. 16–20, c CB) Bonnet gives a similar fold, and after the pericardial walls are well formed he gives an illustration of a stage in which it still communicates with the extraembryonic coelom (Plate IV, Fig. 17, KC). With Graf Spee's embryo Gle. and with Bonnet's observations upon the sheep as a starting-point, it is not difficult to interpret Figs. 22–24.



FIG. 25. — Section through the Head of Embryo No. III. Enlarged 55 times. *Ph*, pharynx ; *H*, heart. The arrow in the amniotic cavity indicates the direction of the future growth of the amnion to complete the ventral body wall.

*Extension of the Amnion.* — After the stage of embryo XII is passed the amnion rapidly envelops the whole body and soon passes out over the cord. The next stage after No. XII which I have studied is No. XIX. I have very perfect photographs of this specimen, and the sections are all good, although the nervous system is macerated. The embryo has rotated in the amnion, throwing the cord to the right side with the left side towards the observer. It would have been impossible to obtain a view of the right side of the embryo without cutting the cord. The outlines of this embryo and ovum are given in

[1] Bonnet: His's Archiv, 1889.
[2] Cadiat: Jour. de l'Anat. et de la Physiol., 1883, Plate V, Figs. 1, 2.

Fig. 26. Two sections through the body are given in Figs. 27 and 28.

The amnion has become separated from the body with the exception of the part about the cord and also that along the right side of the body, over the heart. The arrow in Fig. 25 shows how the amnion on that side is extended over the ventral body wall to make the condition shown in Fig. 28. No doubt the cause of this is the rotation of the body, throwing the cord to its right side and the amnion with it. In nearly all young embryos the cord is on the right side.[1] With the exception of the four instances mentioned below, the rotation has always been so as to throw the left side of the body away from the chorion, and in all of these specimens the amnion must have swept over the body from left to right, as shown in the figures. I find a similar illustration by His in his great monograph.[2]
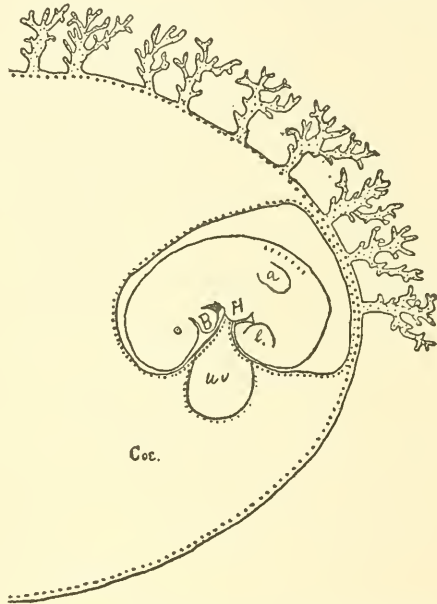


Fig. 26. — Ovum and Embryo No. XIX. Enlarged 5 times. Just half of the ovum is shown. *A*, arm ; *l*, leg ; *H*, heart ; *u v*, umbilical vesicle ; *B*, branchial arch.

*Absence of a Ventral Mesentery.* — After the septum transversum has been formed as it is in embryo XII, there is on its ventral side a pretty sharp groove, which indicates that the umbilical vesicle is being constricted at this point.

It is generally believed that the ventral mesentery of the intestine extends to the umbilicus, and that ultimately the round ligament of the liver represents its remnant after most of it has

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[1] The exceptions have been published by Waldeyer : Studien des physiol. Inst. zu Breslau, 1865 ; Janošík: Arch. f. mik. Anat., Bd. 30 ; His: Anat. mensch. Embryonen, Plate VIII, Figs. A 1–4 ; Mall : Journ. of Morph., vol. V.

[2] His: Anat. mensch. Embryonen, Plate VI, Fig. 3, No. 10.

disappeared. This theory is expressed by two diagrams in Minot's *Embryology*, page 767. As the liver begins to grow, and while the heart is being pushed down in front of it, the ventral end of the septum transversum is turned down to the umbilicus. While this is taking place the stem of the umbilical vesicle becomes relatively smaller and smaller, but there is no union between the umbilical vesicle and the septum transversum as expressed in Minot's diagram. The first stage of this
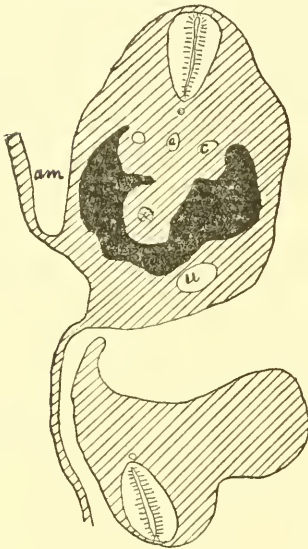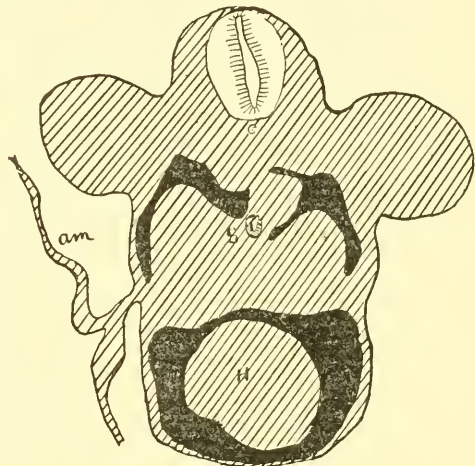


FIG. 27.                                        FIG. 28.

FIGS. 27 and 28. — Section through Embryo XIX to show the Attachment of the Amnion to the Side of the Body. Enlarged 25 times. *Am*, amnion; *S*, stomach; *H*, heart; *c*, cardinal vein; *u*, umbilical vein; *a*, aorta.

process is shown in my Fig. 24, and its successive stages are shown in His's *Atlas*, Plate IX. In all six embryos pictured on that plate the successive stages are represented, and in none of them is the umbilical vesicle attached to the septum transversum to form a ventral mesentery. From these embryos of His we can pass to embryo XIX, in which the umbilical vesicle communicates by a round canal with the intestine, and the tube is completely encircled with a space which extends to the liver, thus cutting off any possible ventral mesentery at that point. The same thing is shown, but in a later stage, in

Fig. 30, *O*, but a new process has already taken place to complicate matters.

In embryo XII there is just a beginning of an umbilical vein in the membrana reuniens. In Kollman's embryo the vein is more marked.[1] The vein extends out into the somatopleure, far away from either the intestine or the median line. This same position is again shown in His's embryos BB. and Lr on Plate IX in his *Atlas*. The left umbilical vein becomes
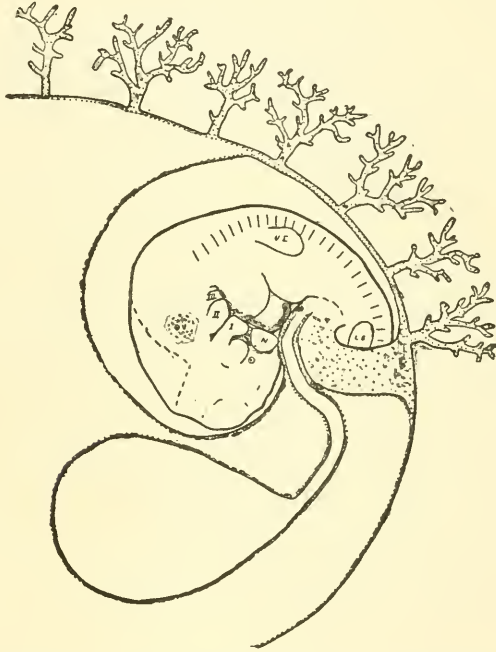


FIG. 29. — Embryo No. II Attached to the Chorion. Enlarged 5 times. Just half of the Ovum is shown. *O v*, otic vesicle; *U E*, upper extremity; *L E*, lower extremity; *N*, nose; *I, II, III*, branchial arches.

the more prominent, and as the body wall is developed more and more it moves around towards the ventral median line. This movement takes place in common with the movement of the amnion over the body from left to right, as shown in Fig. 28. In embryo No. II, however, the liver has nearly reached the umbilicus, and the vein has almost moved around to the

[1] Kollman: His's Archiv, 1891, Plate III, Figs. 2, 3, 4. V. umbil.

ventral median line, as shown both in the reconstruction and the sections (Figs. 30, 37–39). After the vein has moved around the body to its ventral surface, and after the liver moves away from the umbilicus up to the permanent diaphragm, it is easy to explain the formation of the round and broad liga-
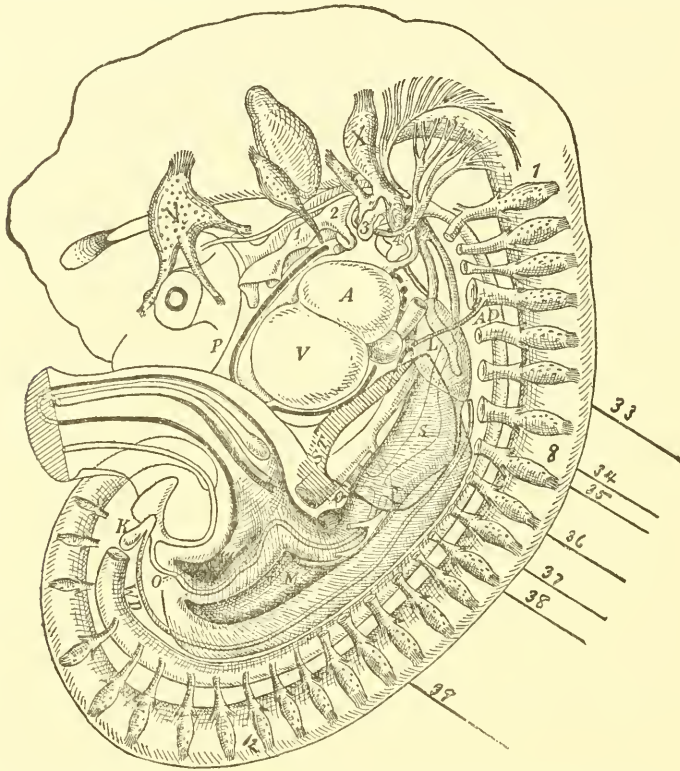


FIG. 30. — Reconstruction of Embryo No. II. Enlarged 17 times. *V* and *X*, fifth and tenth cranial nerves; *1, 2, 3,* and *4,* cast of the branchial pockets; *1* and *8*, first and eighth cervical nerves, from the fourth the phrenic arises; *12*, twelfth dorsal nerve; *A*, auricle; *V*, ventricle; *L*, lung; *S*, stomach; *P*, pancreas; *W D*, Wolffian body; *K*, kidney; *M*, mesentery; *S T*, septum transversum; *O*, openings which communicate with the peritoneal cavity of the opposite side. The black line around the heart marks the pericardial cavity.

ments of the liver as a secondary formation, but not as a remnant of a ventral mesentery. It might be called a portion of the septum transversum, as it is directly continuous with it. A ventral mesentery does exist between the abdominal walls and the liver, and only extends slightly below the liver. It is

always slightly to the left of the median line, and is in direct connection with the septum transversum (Fig. 30, *O* and *S T*).

*Coelom of Embryo No. II.* — After the body cavity is beginning to separate from the extraembryonic coelom, the next important stage is the one after the separation is complete, as from now on the adult body cavities are formed by a simple division and expansion of the cavities already within the body. This stage is represented in embryos XVIII, II, and IV. All of these embryos are nearly of the same size, the successive stages being in the order they are given. No. XVIII is somewhat distorted in the middle of the body, while No. IV is slightly macerated. No. II is a perfect specimen, and has been already described by me several years ago.[1] I shall confine my description of it to the body cavity.

The external form of the embryo within the ovum is given in Fig. 29. The position of the umbilical vesicle, as well as the extent of the amnion and the



FIG. 31. — Cast of the Body Cavity of Embryo No. II. Enlarged 22 times. *A*, position of the aorta; *V*, position of the vein; *M*, position of the mesentery; *W B*, position of Wolffian body; *P*, pericardial cavity; *L*, coelom over liver.

relation of the umbilical vesicle and amnion to the chorion, are all given. The umbilical cord is large and lies on the left side of the body, while in most embryos already published it is upon the right side. The cord is short, and midway between the embryo and its attachment to the chorion it shows a decided enlargement. The umbilical vesicle is large, measuring 5 × 7 mm., and is located between the head end of the embryo and the chorion.

The amnion has not grown very much, still leaving a great

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[1] Mall: Journ. of Morph., vol. V.

space between it and the chorion, the extraembryonic coelom (compare with Fig. 26).   Within it hangs this large umbilical vesicle, the lumen of which no longer connects with the alimentary canal.   The separation is now complete.   Around the stem of the vesicle the extraembryonic coelom communicates freely with the body cavity, as shown in Fig. 30.   This figure is from a reconstruction, and shows the general extent of the body cavity within the embryo.   It encircles the heart, and then extends to the lungs and over them and to the stomach, over the intestines, and out into the cord.   A cast of the whole cavity is also given, showing the slit on the dorsal side for the mesentery of the intestine, and the grooves on either side of this for the Wolffian bodies.   There are also grooves in the cast for the veins, and the place where the Cuvierian duct enters the heart is marked $V$.   The sagittal section of the peritoneal cavity is given in Fig. 32.   The striated line indicates where the cavity crosses the median line of the body, while the other lines outline the cavity beyond.   $Lp$. outlines the lesser peritoneal cavity.   Figs. 33–39 give the extent of the peritoneal cavity in different portions of the embryo, as indicated by the lines in Fig. 30.



FIG. 32. — Outline of Coelom in Embryo No. 11 in Sagittal Section.  The striated line indicates that the coelom crosses the median line.  *P*, pericardial space; *pl*, pleural cavity; *l p*, outline of lesser peritoneal cavity.

It is not difficult now to imagine the body cavity of embryo XII converted into the one just described.   In that embryo the heart is high in the neck on the oval and dorsal side of the septum transversum.   In this embryo it is on the ventral and oral side of the septum transversum, but still above the eighth cervical nerve.   The septum transversum has already received its nerve supply from the fourth cervical nerve, as pointed out in the early part of the century by von Bear.   This movement of the septum transversum is accompanied by a movement of all the other organs on their way into the thorax and abdomen of the future individual.   In the rotation the Cuvierian duct acts much as the fixed point about which the coelom is bent.

The figures all illustrate this beautifully. But as the heart has rolled over the liver, and the septum transversum has undergone a quarter-revolution, the Cuvierian ducts and all have moved away from the head. This is by no means the end of the excursion of the septum transversum, as its dorsal end must move down and beyond the twelfth dorsal segment (compare Fig. 30).

The pericardial cavity surrounds the whole heart, as the various figures show. The cavity is traversed only where the large veins enter, and where the aorta leaves the heart. The
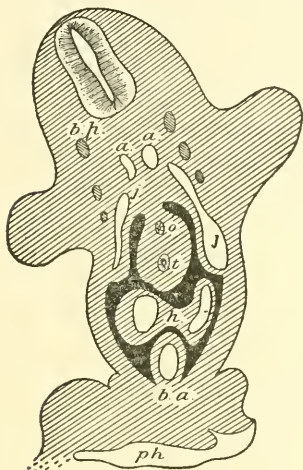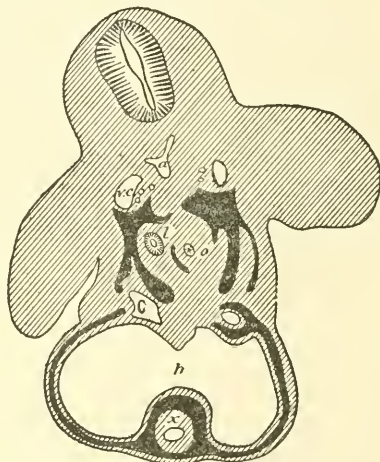


FIG. 33.    FIG. 34.

FIGS. 33 and 34. — Sections through Embryo No. II at the Points indicated in Fig. 30. Enlarged 22 times. *B p*, brachial plexus; *a*, aorta; *b a*, bulbus aortae; *ph*, pharynx; *h*, heart; *t*, trachea; *o*, oesophagus; *j*, jugular vein; *l*, lung; *v c*, cardinal vein; *C*, Cuvierian duct.

cavity completely surrounds the bulbus aortae to its origin (Figs. 32–35) in the ventricle. On the dorsal side of the heart the pericardial cavity is separated by a bridge for the transmission of the veins to the heart. Between the bulbus aortae and the entrance of the veins into the heart the pericardial cavity crosses the median line as three distinct openings, as expressed by the black areas in front of the trachea in Fig. 30. On the dorsal side of the heart on either side of the lungs the pericardial cavity communicates with the pleural cavities by means of two openings (Fig. 33), each of which is about .1 × .5 mm. in diameter. Farther on, the pleural cavities

extend as two slits which encircle the lobes of the liver and
separate them from the alimentary canal on the one hand and
from the body wall on the other (Figs. 34–37). The two
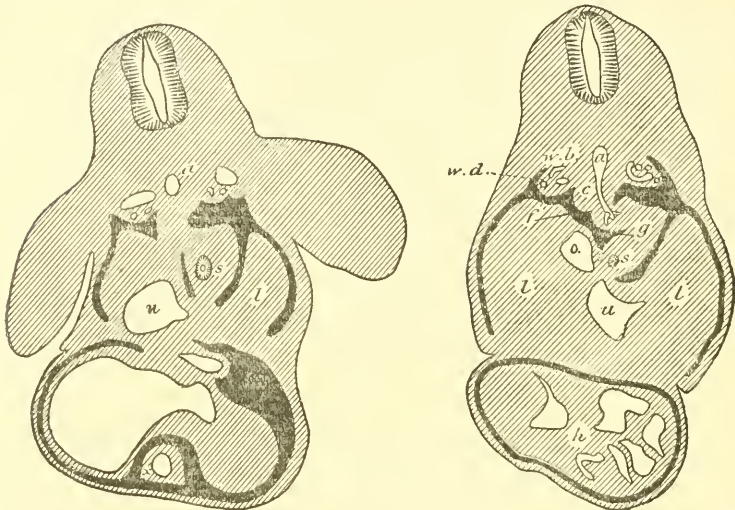


Fig. 35.   Fig. 36.

Figs. 35 and 36. — Sections through Embryo No. II. *A*, aorta ; *s*, stomach ; *l*, liver ; *u*, umbilical
vein ; *x*, bulbus aortae ; *h*, heart ; *o*, omphalomesenteric vein ; *g*, lesser peritoneal cavity ; *f*,
foramen of Winslow ; *c*, coeliac axis ; *w b*, Wolffian body ; *w d*, Wolffian duct.

pleural cavities do not communicate with each other around the
lungs, leaving for them both a dorsal and a ventral mesentery.

This appearance of the coelom about the lungs and the liver
can be explained by the lungs and liver both growing into the
two pleural cavities of embryo XII, and this has often made me
think that the membrana reuniens of embryo XII is the main
origin of what is called septum transversum in embryo II. If
this proves to be the case, then the lower end of the mem-
brana reuniens will form the ventral end of the diaphragm, and
not the reverse. A stage between embryos XII and XVIII
(Fig. 41) is required to elucidate this point.

In the neighborhood of the stomach the peritoneal cavity on
either side of it has become asymmetrical, as Fig. 36 shows.
The mesentery has become bent to the left side, leaving a
diverticulum from the right side which extends oralwards to
the tip of the lung (Figs. 34 and 35) to form the beginning of

the lesser peritoneal cavity.[1]  Further aboralwards the cavities become symmetrical again (Figs. 37, 38), and then unite along the ventral median line, as shown in Fig. 39.  The ventral mesentery shown in Fig. 38 does not extend more than a section or two beyond the liver, and is separated by a marked
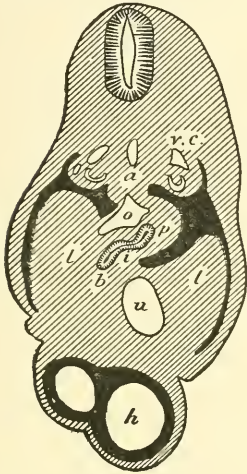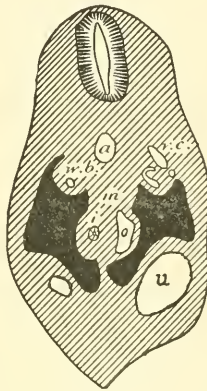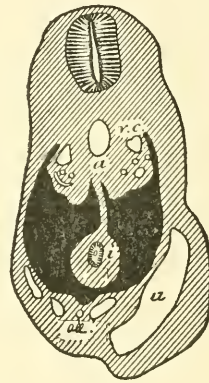


FIG. 37.　　　　　FIG. 38.　　　　　FIG. 39.

FIGS. 37-39. — Sections through Embryo No. 11.  *A*, aorta ; *v c*, cardinal vein ; *o*, omphalomesenteric vein ; *p*, pancreas ; *i*, intestine ; *b*, bile duct ; *l*, liver ; *h*, heart ; *u*, umbilical vein ; *m*, mesentery ; *w b*, Wolffian body ; *all*, allantois.

opening from the stem of the umbilical vesicle in this embryo, as is shown in Fig. 30, *O* (see also No. XII, Fig. 16, *O*).  On the aboral side of the umbilical cord the peritoneal cavities of the two sides unite in both embryos again, marked *O'* in both figures.

*Development of Body Cavity in the Chick.* — The body cavity of the chick has been carefully studied by Budge,[2] who followed its course by means of injection.  With a fine hypodermic syringe he filled the spaces forming the coelom in the order of their appearance, thus showing their extent in various embryos.  The splanchnopleure, according to Budge, may be split into two layers, one dorsal or lymphatic and the other ventral or vascular.  Drasch's[3] recent description of the early

1 Mall: Journ. of Morph., vol. V.
2 Budge: His's Archiv, 1880 and 1887.
3 Drasch: Anatom. Anz., Bd. 9.

formation of the coelom confirms this statement.   As the first blood-vessels are formed, lymph vessels appear on their dorsal side, which flow together to form a network, and accompany the primitive veins to the axial part of the germinal area.   Here the lymphatics form two spaces, one on either side of the body, which soon unite across the body on the ventral side of the heart.   In this way the primitive body cavity of birds appears at first as an H, the uprights of which are on either side of the body and the cross-piece on the oral side of the sinus venosus. In its further development the sinus venosus grows to the dorsal side of the cross-piece, thus reversing the relation of the vascular system to the coelom in this portion of the embryo.   The uprights of the H fall to the outside of the body, and are swallowed up in the formation of the amniotic folds.

According to Budge two diverticula grow from the cross-piece of the H, one on either side of the chorda, towards the tail of the embryo, to form the primitive pleuro-peritoneal cavities.   Budge's paper was published from fragmentary notes after his death, and I am sure that the above statement is not correct.   Professor His has placed at my disposal Budge's specimens, which I think show conclusively that his interpretation
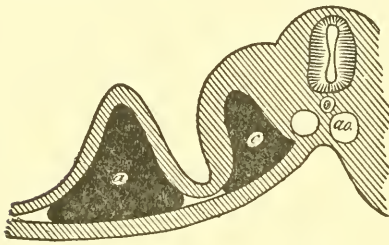


Fig. 40. — Section of a Chick to show that the Body Cavity communicates with the Extraembryonic Coelom. Although the embryo has been injected, the injection masses *a* and *c* are not continuous.

of this subject is not correct.   Most of the injections were made into the amniotic fold, which is very large in birds. Cross-sections of chicks at this stage show that the large extra-embryonic coelom communicates very freely with the body cavity, and the cross-piece also communicates freely with the cavity at the anterior end of the embryo.   This has already been described and pictured by Cadiat,[1] and recently again by Duval.[2]   Around the heart, however, the communication is freest between the extraembryonic coelom and the body cavity, and it is natural

[1] Cadiat: Jour. de l'Anat. et de la Physiol., 1883, Plate V, Figs. 1 and 2.
[2] Duval: Atlas d'Embryologie, Plate XXII, Fig. 354.

that the fluid should find its way along this channel first, and then extend into the body cavity from this point, giving pictures which in transverse section are like Fig. 40. Surface views could not decide that these cavities communicate freely; and these sections which I have studied were no doubt made after Budge had written the rough draft of his manuscript, as they are not referred to in his paper.

Although the body cavity of the bird is formed after the same manner as it is in the human embryo, there is one marked difference in the formation of the pericardial space. In the bird the mesoderm does not extend throughout the head fold of the blastoderm, leaving a portion of the ectoderm in direct contact with the entoderm. Later the mesoderm grows into this region, and at the same time the pericardial cavity extends to the outside of the body. This condition continues for a long time, allowing the pericardial cavity to communicate with the false amnion after the embryo is well formed. Duval's excellent *Atlas* shows how the pericardial cavity first communicates with the exterior of the body, and after the body walls have united the heart still lies in apposition with the liver, as there is no septum transversum. The only trace of a septum transversum that I can find in young chicks is at the point the Cuvierian ducts enter the heart. Here a bridge of tissue passes transversely to the body. The liver does not grow into it, but accompanies the single omphalomesenteric vein before it enters the heart.[1]

*Development of the Diaphragm.* — Our knowledge of the development of the diaphragm is based upon the researches of von Baer,[2] Cadiat,[3] His,[4] Uskow,[5] and Ravn.[6] Each contributed his portion : von Baer that the diaphragm is at first located high in the neck and must descend in its development, and, because of its high position at first, is innervated by a

[1] Since the above has been written this subject has been studied thoroughly by Ravn, whose paper will be found in His's Archiv, 1896.

[2] Von Baer: Entwicklungsgeschichte, 1837.

[3] Cadiat: Jour. de l'Anat. et de la Physiol., 1878.

[4] His: Anat. mensch. Embryonen, Th. I, 1880.

[5] Uskow: Arch. f. mik. Anat., 1883.

[6] Ravn: His's Archiv, 1889.

cervical nerve ; Cadiat and His recognized the mass of tissue
in the embryo which is destined to give rise to the diaphragm ;
Uskow and Ravn studied more carefully the separation of the
body cavities from one another; and the wandering of the organs
was emphasized by Uskow.

It is now no great task for me to give the development of
the diaphragm in the human embryo, for I have at my dis-
posal excellent sections, as well as definite knowledge of the
anatomy of the surrounding organs contributed by the above-
mentioned authors.

While the embryo is still straight it is very easy to locate the
various organs and their relations to one another, but through
their shifting and the flexion and extension of the embryo the
relations are constantly changing, and one must not rely too
much upon sections, or else erroneous impressions will often
be obtained. At first the heart is upon the oral and dorsal
side of the septum transversum, then on its ventral side, and
finally again on its dorsal side. At first the lungs are on the
dorsal side of the heart, then on the lateral side, and finally
also on the ventral side of it. At first the liver is on the aboral
side of the septum transversum in the head of the embryo, then
on the dorsal side of it in the cervical region of the embryo,
then as the liver is descending in its excursion it is transferred
to the ventral side of the septum and extends into the sacral
region. At first the Wolffian body extends high into the tho-
racic region of the embryo, but while it is degenerating and the
diaphragm descends, the upper part of the posterior cardinal
vein remains, while the lower part is incorporated with its vena
cava inferior, as shown by Hochstetter.[1] As the Cuvierian
ducts and cardinal vein descend into the thorax, the segmental
veins entering the cardinal veins are gradually shifted, so that
veins which originally emptied into the posterior cardinal now
empty into the anterior cardinal. While the whole process is
taking place the arteries arising from the descending aorta also
shift, as I have shown in a previous communication.[2] At that
time my collection of human embryos was very limited, and it

[1] Hochstetter: Morph. Jahr., Bd. 20, p. 563.
[2] Mall: Journ. of Morph., vol. V, p. 472.

was necessary to include some observations on lower animals to prove my point, but now I can give a complete table of human embryos in which the point of origin of the coeliac axis is recorded.

TABLE SHOWING POINT OF ORIGIN OF COELIAC AXIS.

| Embryo. | Length in Millimeters. | Origin of Coeliac Axis. |
|---|---|---|
| No. XII. | 2.1 | Opposite 4th cervical nerve.[1] |
| His's Embryo M | 2.6 | "      1st dorsal   "   [2] |
| "      "    B | 7. | "      2nd   "   "   [3] |
| No. II. | 7. | "      4th   "   " |
| His's Embryo A | 7.5 | "      6th   "   "   [4] |
| No. XLIII. | 13. | "      10th   "   " |
| No. IX. | 14. | "      11th   "   " |
| No. XXII. | 18. | "      11th   "   " |
| No. XVII. | 16. | "      12th   "   " |
| No. LVII. | 20. | Below 12th   "   " |
| Adult. | | "      12th   "   " |

[1] In the first two embryos the omphalomesenteric artery is noted, and not the coeliac axis.
[2] Compare Fig. 15, Plate VI, His's Atlas, with M4, Pl. VII.
[3] Compare Fig. 35, Plate II, His's Atlas, with Fig. 1, Plate I.
[4] Compare Figs. 79 and 86, His's Atlas, with Fig. 4, Plate I.

The table shows that the arteries arising from the ventral side of the aorta to supply the stomach and intestines are constantly shifting until their definite origin is finally reached. In these specimens the omphalomesenteric artery is shifted ahead of the coeliac axis. In embryo No. II the omphalomesenteric artery has a double origin from the aorta, which indicates that this movement may be brought about by a new anastomosis forming, which is then followed by an occlusion of the old origin. At any rate it is impossible that the whole aorta shifts with the abdominal viscera, for it is bound to the vertebrae and muscle plates through the segmental arteries.

The various sections and the reconstruction of embryo No. II show the pleural and pericardial cavities still communicating freely. The same is true in embryos XIX, XVIII, and IV.

Immediately after this stage there are no embryos in my collection, so I have no specimen in which the communications between the pleural and pericardial cavities are just closing.
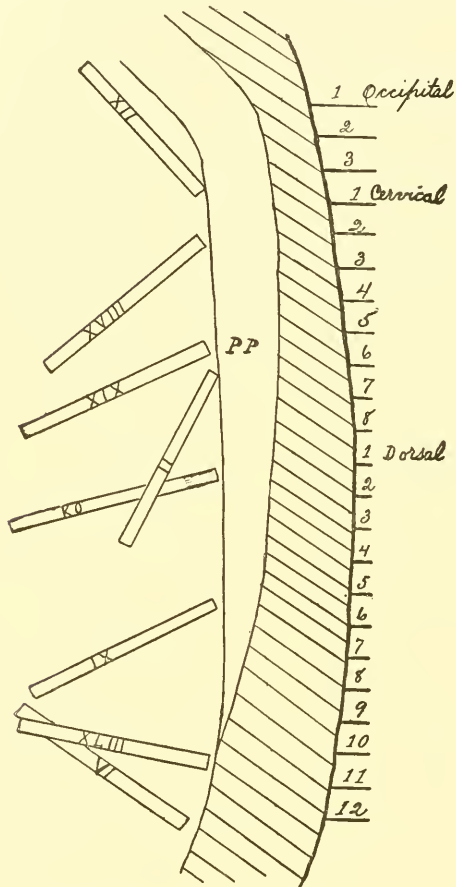


Fig. 41. — Diagram to show the Position of the Diaphragm. The numbers on the blocks indicate the embryos from which the diaphragms are taken. *KO* is His's embryo K O; *PP*, the outline of the opening between the pleural and peritoneal cavity, which is finally closed when the diaphragm reaches the tenth dorsal segment.

In embryos VIII, V, IX, and XLIII (Figs. 41–43), the pleural and pericardial cavities are separated, while the pleural and peritoneal still communicate. In the embryos with a vertex-breech measurement exceeding 17 mm. the pleural and peritoneal have been separated completely.

The separation of the pleural from the pericardial cavity is dependent upon the complete development of the diaphragm. At first the septum transversum and the membrana reuniens are
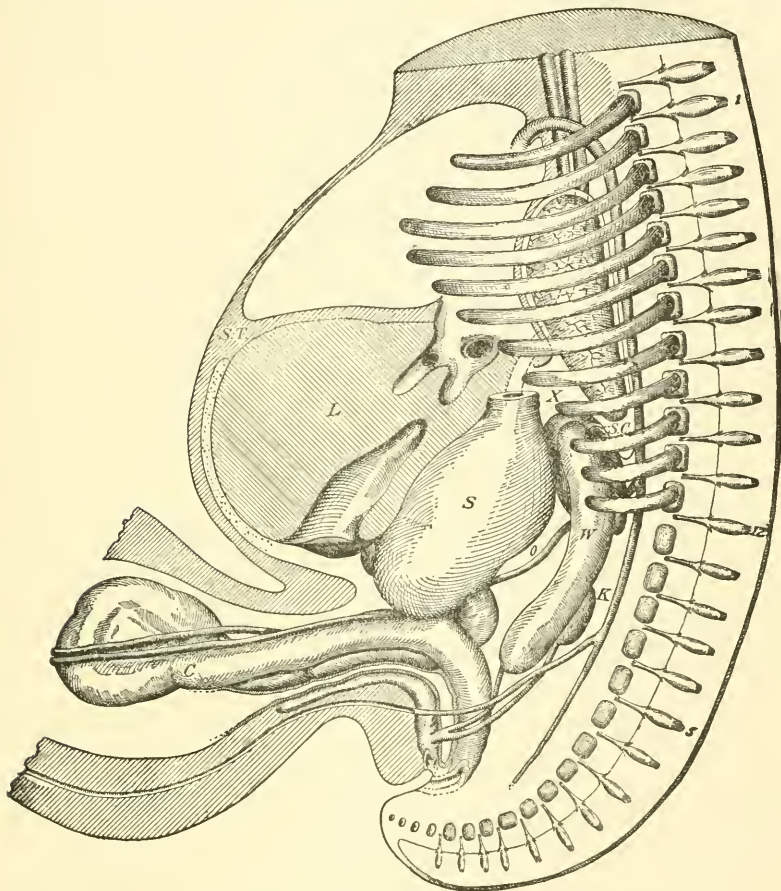


FIG. 42. — Reconstruction of Embryo No. IX. Enlarged 17 times. *S T*, septum transversum; *L*, liver; *S*, stomach; *C*, caecum; *W*, Wolffian body; *K*, kidney; *1–12*, dorsal ganglia; *o*, omphalomesenteric artery. The ventral mesentery of the liver is dotted, as it is only a thin membrane; *S C*, suprarenal capsule; *X*, point of communication between pleural and peritoneal cavities.

on the ventral side of the pleural cavity, and both are still located within the head. As the septum transversum descends into the body it is next located on the dorsal side of the heart. In other words, the dorsal end of the septum transversum has not moved as rapidly as the ventral end, and thus the whole

mass of tissue has turned a quarter revolution.  This is accompanied by the extreme flexion of the head, as represented in embryo No. II.  At this time the septum transversum has descended to the lower part of the cervical region.  Now the septum begins to turn in the other direction again, for with the
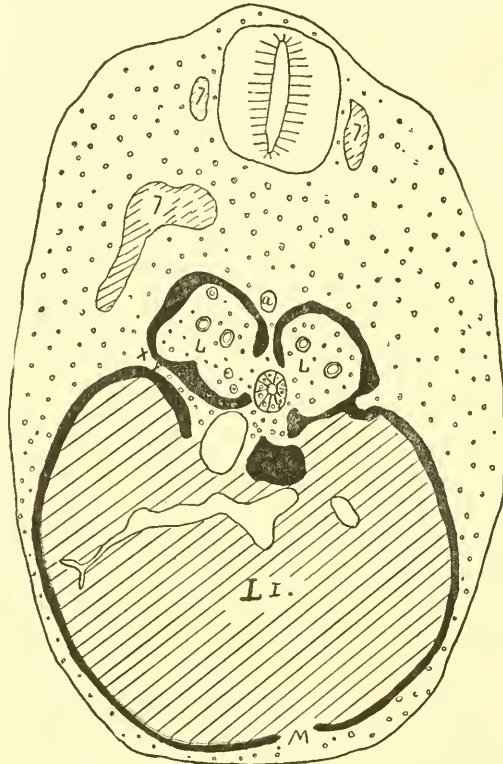


Fig. 43. — Section through the Point of Communication between the Pleural and Peritoneal Cavities in Embryo No. IX.  Enlarged 15 times.  *7*, seventh rib; *L*, lung; *Li*, liver; *M*, ventral mesentery of liver; *a*, aorta.  The diaphragm is complete on one side, *X*, while it is incomplete on the other.

development of the neck the ventral end of the septum becomes the fixed point and the dorsal end moves more rapidly.  The successive stages in the movement of the septum are best shown in the diagrammatic Fig. 41.

Fig. 42 shows the septum transversum on the ventral side of the stomach and the pleural cavity communicating with the peritoneal at the point X.  The Wolffian body and the supra-

renal capsule, which is very large, have receded markedly, and the pleural cavity already forms a pocket on the dorsal side of them.   A sagittal section through this region, somewhat
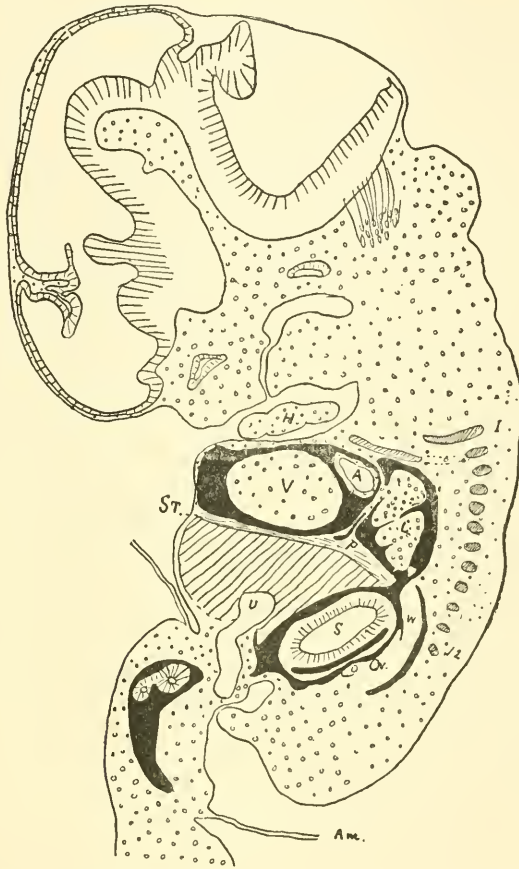


FIG. 44. — Section through Embryo No. XLIII.   Enlarged 8 times.  *H*, hand ;  *A*, auricle ;  *V*, ventricle ;  *L*,  lung ;  *S  T*, septum transversum ;  *P*, phrenic nerve ;  *U*, umbilical vein ;  *S*, stomach ;  *W*, Wolffian body ;  *Ov*, ovary ;  *A m*, amnion ;  *1-12*, ribs.

distant from the median line, is given in Fig. 44.   A transverse section of the embryo pictured in Fig. 42 is given in Fig. 43. This section is just at the point above the opening, and shows the communication between its pleural and peritoneal cavities closed on one side, but open on the other.   There is a ridge on the side of the cavity which projects between the lung and

the liver and continues down to the suprarenal capsule. This ridge has been well described by Ravn,[1] who gives an excellent illustration of the opening with the ridge encircling it.
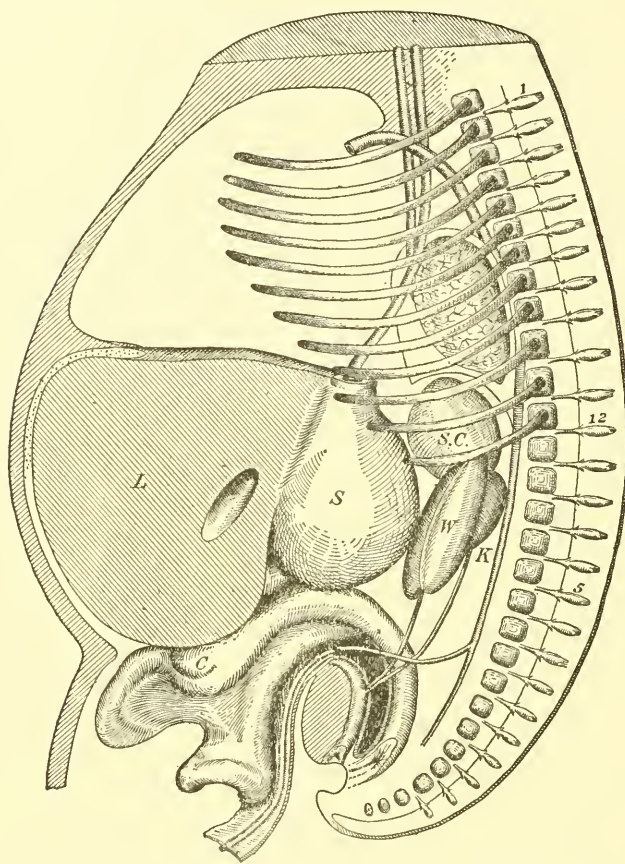


FIG. 45. — Reconstruction of Embryo No. X. Enlarged 8 times. *1–12*, dorsal ganglia; *S C*, suprarenal capsule; *W*, Wolffian body; *K*, kidney; *L*, liver; *S*, stomach; *C*, caecum. The dotted area on the ventral side of the liver indicates the extent of the ventral mesentery of the liver.

In all the embryos in which the pleural and peritoneal cavities still communicate, the vena cava does not yet communicate with the posterior cardinal vein.

Fig. 45 is from an embryo slightly larger than the one from which Fig. 42 was taken. The pleuro-peritoneal communica-

[1] Ravn: His's Archiv, 1889, Plate X, Fig. 16.

tion has just closed by the walls of the ridge having grown together; the extent and shape of the pleural cavity is much as it is in Fig. 42. The Wolffian body is smaller, and the kidney and suprarenal capsule have come together.

The story, then, is brief: as the diaphragm descends, its dorsal end is in apposition with the suprarenal capsule, and finally, when the capsule approaches the twelfth rib, a ridge of tissue which also includes the capsule unites with a ridge from the septum transversum, and the opening is closed. These two ridges, however, are portions of one and the same ridge, as they form a circle and in section appear as two ridges. The circle is closed much after the fashion of tying up a bag.

All of the abdominal organs, with the exception of the kidney, descend; and the descent is not completed until the pelvis is formed to admit some of them. In the stages pictured nearly all the small intestine lies in the umbilical cord, as is the case in many mammalian embryos. In embryo X (Fig. 45) a large portion of the liver also projects into the cord. I have also observed a hernia of the liver in another embryo somewhat larger. I do not consider the form of embryo X altogether normal, but this was not noticed until the reconstruction was complete.

Closely associated with the closing of the pleuro-peritoneal opening is the development of the coeliac ganglion. In these young embryos it is extremely large, and can be outlined already, while the septum transversum is still high in the thorax. As the septum descends, the various communicating branches of the nerves are caught up with the coeliac ganglion and dragged along. This accounts for the high origin of the splanchnic nerve.

Fig. 46 (embryo VI) shows that all the tissues are becoming more definitely outlined, and the whole structure is firmer than in embryo X. The organs of the abdomen are more firmly clustered together, and the intestine has become more convoluted. The lung is much larger, and the pleural cavity extends to the ventral wall of the embryo, obscuring wholly the outline of the heart. In general it confirms everything given in Fig. 45.

Minot[1] has stated that the pleural cavities are to be considered a portion of the septum transversum, because they lie on the dorsal side of it. From what has already been said above it will be seen that I consider the septum transversum the
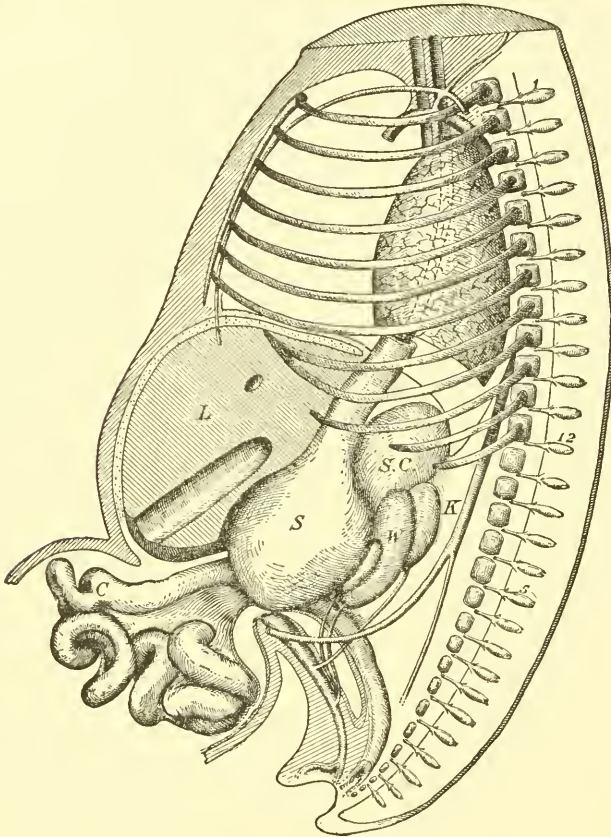


Fig. 46. — Reconstruction of Embryo No. VI. Enlarged 8 times. *1–12*, dorsal ganglia ; *S C*, suprarenal capsule ; *K*, kidney ; *W*, Wolffian body ; *S*, stomach ; *C*, caecum ; *L*, liver. The dotted area on the ventral side of the liver indicates the extent of the ventral mesentery.

mass of tissue between the pericardial cavity, the pleural cavities, and the opening between the two sides of the peritoneal cavity immediately below the liver, marked *O* in Figs. 16 and 30. This tissue includes the membrana reuniens, which is really the wings of the septum transversum as described by His. In my

[1] Minot : Human Embryology, p. 483.

account I have employed the term membrana reuniens where-ever possible to avoid confusion, and have usually employed the terms septum and primitive diaphragm as synonyms.

There are developed within the region of the septum trans-versum the whole liver, including its ventral mesentery, the lesser peritoneal cavity, the stomach, and the suprarenal capsule. This same region which I have marked out by these three boundaries as the septum transversum is still sharply defined in the adult. The point *O* in Figs. 16 and 30 is still as defin-ably marked as ever by the round ligament, foramen of Wins-low, and the duct passing from the liver to the duodenum. The round ligament is developed by the umbilical vein shift-ing around the side of the abdominal walls into the ventral mesentery of the liver, and then when the liver is retracted from the umbilical cord, the vein and mesentery remain as the round and broad ligaments respectively.

*Lesser Peritoneal Cavity.* — I have already discussed the lesser peritoneal cavity in a separate paper,[1] and find that I can confirm all that I have stated at that time. I can only add that the portion of it extending up under the lung degenerates, while the omental sac is growing rapidly. I have also found that it is extremely easy for the omentum to find its way over the large intestine. At the time this takes place the large in-testine is in the median line, while the stomach and the omen-tum are on the left side of the body. After the intestine is retracted from the cord the caecum falls over to the right side of the body, while the descending colon is shifted to the left side, and the omentum then comes to lie on the ventral side of the transverse colon.

*Expansion of the Body Cavity and Obliteration of the Extra-embryonic Coelom.* — After the pleural and pericardial cavities are separated from each other it is very easy to follow their further development. In embryo II, Fig. 47, the heart is still upright, and a transverse section of it is also transverse to the lung. The pleural cavity lies wholly on the dorsal side of the pericar-dial, Fig. 32. In the next stage, as the lungs descend more and more, the heart is tilted over so that its base is towards the

[1] Mall: Journ. of Morph., vol. V.

lung and its apex away from it, as in embryo IX, shown in Figs.
42 and 48.    The pericardial space has now become separated
completely from the pleural, although both have grown at
about the same pace.    From now on the pleural space grows
more rapidly than the pericardial, as shown in Fig. 49.    I have
a number of embryos which represent intermediate stages
between embryos IX and XXII, and all of them confirm the
idea that the pleural space develops first and then is followed
by a growth of the lung.    Fig. 50, which is a section of
embryo No. XLV, shows a marked increase in the size of the
lung, but the heart and pericardial space are of about the same



FIG. 47.                    FIG. 48.                        FIG. 49.
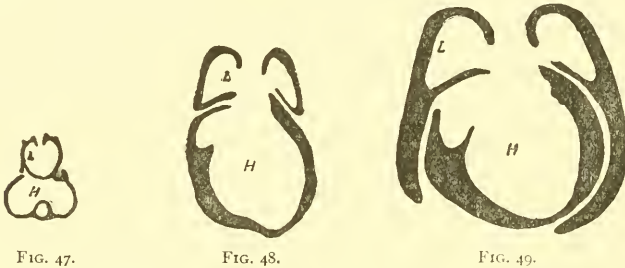
FIG. 47–49. — Outlines of the Pleural and Pericardial Cavities to show their Relative Position and
       Size.   Enlarged 7 times.   Fig. 47, Embryo No. II ; Fig. 48, Embryo No. IX ; Fig. 49,
       Embryo No. XXII.   *H*, position of heart ; *L*, position of lung.

size as in embryo XXII.    A much later stage is shown in
Fig. 51.    The scale of enlargement is only half that of Fig. 50,
and when this is considered it is again seen that the heart has
not grown very much but the lung has developed enormously.

    It is therefore seen that at first the pericardial cavity is on
the oral side of the pleural, then on the ventral side, and is
finally enclosed by the pleural cavity growing over it.

    The growth of the pleural cavity over the pericardial accounts
for the location of the phrenic nerve in the adult.    In Fig. 47
the nerve passes to the septum transversum from the lateral
body wall and it is gradually separated from it by the descent
of the septum and by the growth of the pleural cavity between
the nerve and the body wall, thus locating the nerve in a mem-
brane, as Figs. 48 and 49 will readily explain.

    The expansion of the peritoneal cavity is by no means as
simple.    In it there are many bands and mesenteries, as

well as a marked shifting of the organs.  With the descent of the testis a portion of it is cut off to form the tunica vaginalis.

In embryo II the peritoneal cavity is extremely simple, as the figures show, — a simple cavity on each side, communicating the one with the other by means of two openings, one above and one below the omphalomesenteric duct.  Later, as the diaphragm descends more and more, the liver rotates, and its lobes soon fill the peritoneal cavity, while the intestine develops out into the cord.  The Wolffian body, sexual glands, and suprarenal capsule fill the dorsal side of the cavity and the rudimentary pelvis.  The whole development of the intestine takes place within the cord, and finally it is drawn into the embryos when it is about 30 mm. in length.  By what process this takes place I am unable to determine, but it must take place very rapidly, for I have never seen a human embryo in which it is only partly retracted. In the pig's embryo, however, I have found the stages in which the intestine is in process of retraction.



Fig. 50. — Outline of Pleural and Pericardial Cavities in Embryo No. XLV. Enlarged 7 times.



Fig. 51. — Outline of Pleural and Pericardial Cavities in Embryo No. XXXIV.  Enlarged 3½ times.  *H*, position of heart; *L*, position of lung.

The liver now fills nearly the whole cavity, and extends down to the pelvis, and in embryo XXII projects over the ovary and is in contact with the rectum.  As the intestines are retracted from the cord the liver is relatively higher and higher, for the expansion of the abdominal walls is now greater below the umbilical cord than before, giving more space in this region for the intestine which displaces the liver.  In embryos XXXIV and XLVIII the intestines have been studied, and it was found that they were still located in the ventral portion of the peritoneal cavity, as there is no pelvic cavity large enough

to hold any portion of them. This question will be discussed
in the next paper.

The extraembryonic coelom has only a short existence, as it
is already completely obliterated in embryo No. XXII. This
embryo came to me in an unopened ovum, and on this account



FIG. 52. — Embryo No. XXII within the Ovum. Enlarged 3 diameters. The villi of chorion are
outlined on one side of the ovum only. The umbilical vesicle, *u v*, has become shifted
around to the dorsal and right side of the embryo. The outline is made from a photograph,
and is correct in detail with the exception of the attachment of the chord to the chorion. This
in reality attaches itself to the chorion immediately to the right of the embryo.

is extremely valuable for this purpose. This embryo is about
six weeks old, so, reasoning from it, the union of the amnion
and chorion takes place earlier than is generally believed. In
embryo No. XLIII, which is about five weeks old, the amnion
has expanded over the whole embryo and has nearly reached
the chorion. The earlier stages are given in the sagittal sec-
tions. They show that the amnion is nearest the chorion at
the caudal end of the embryo in the earliest stages, and soon

the two unite at this point.   As the embryo grows, the union
of amnion and chorion extends.   At the end of the fourth
week the extraembryonic coelom is still very large ; at the end
of the fifth week the space between the embryo and chorion is
divided equally between the amnion cavity and the coelom ;
at the end of six weeks the extraembryonic coelom has
disappeared.

BALTIMORE, May 5, 1896.

# A CONTRIBUTION TO THE STUDY OF VARIATION.

### (SKELETAL VARIATIONS OF NECTURUS MACULATUS *RAF.*)

#### HERMON C. BUMPUS.

*" Both heredity and variation are in urgent need of causal explanation."*— Roux, Wheeler.

In this communication, based upon the comparative examination of skeletons of Necturus, an effort is made to answer the following questions:

I. What is the per cent of homœotic variation [1] in the attachment of the pelvic arch to the axial skeleton ; is there true meristic variation, and is homœotic associated with meristic variation ?

II. Is there a ratio between the absolute length of the animal and the number of vertebræ ?

III. Why does the variation tend towards forward rather than backward homœosis ?

IV. Can an explanation be given for the frequent occurrence of oblique or unsymmetrical sacra ?

V. Is the position of the pelvic arch dependent upon the ordinal position of some one segment (sacrum) of the vertebral column, or is its position determined by the location of some topographical point ?

VI. Are there variations in the position of the pectoral arch, and are these correlated with variations in the pelvic arch ?

VII. Are there other skeletal variations associated with pelvic variation ?

[1] Bateson ('94) states that " Homœotic variation in the spinal column consists in the assumption by one or more vertebræ of a structure which in the type is proper to vertebræ in a different ordinal position in the series. Examples of this are seen in the . . . occurrence of a vertebra, normally lumbar, in the likeness of a sacral vertebra, having its transverse process modified to support the pelvic girdle. . . . In using the expression, Homœosis, . . . we may speak of the variation as occurring from before backwards or from behind forwards, according as the segment to whose form an approach is made stands in the normal series behind or in front of the segment whose variation is being considered. The formation of a cervical rib on the VII vertebra is thus a backward Homœosis for the VII vertebra thus makes an approach to the characters of the VIII," etc.

VIII. Are variations more frequent in males than in females?

IX. Are there anatomical grounds for the theory of vertebral intercalation?

## METHODS.

Until within a few months the examination of a large series of skeletons involved the expenditure of considerable time for their proper preparation, the destruction of valuable anatomical material, the occasional loss of certain cartilages, and the too frequent misplacement of disjointed parts. The truly wonderful discovery of Röntgen, however, has placed in the hands of the anatomist an economical means for the most critical examination of the bones, *in situ*, without the use of macerating fluids or the scalpel, and a means readily applicable, without injury to the most valuable museum specimen or even to the living animal.

The one hundred alcoholic specimens on the comparative examination of which this paper is based, belong to the museums of Brown University and the Boston Society of Natural History. Each animal, properly numbered, was bound in a proper position to a thin piece of board, and the board with the specimen attached was then placed upon an envelope of black paper containing a common photographic plate. When large plates were used as many as ten animals were exposed at the same time.

Immediately over the table on which lay the photographic plate, and approximately 500 mm. distant, was suspended a Crooke's tube of the Elihu Thomson pattern, rendered fluorescent by means of a Tesla coil. The exposure was invariably five minutes. When the tube became dim it was warmed with a Bunsen flame until the return of the brighter illumination. The plates were developed with the ordinary "pyro" developer.

The negatives, taken so as to show both the dorsal and lateral views of the vertebral column, were so clear that the ultimate joints of the tail could be readily counted. The amphicœlous structure of the vertebræ and the minute pores of the bones were often exhibited with remarkable detail. A

few specimens showed the opaque fish-hook, and thus betrayed the mode of their capture.

## Section I.

(1) What is the per cent of homœotic variation in the attachment of the pelvic arch to the axial skeleton?

This question was considered by G. H. Parker ('96), who based his conclusions on an examination of twenty-seven specimens, cleaned by a preparator, in none of which could the total number of vertebræ be determined. Of these specimens two were found with oblique or unsymmetrical sacra. In nineteen cases the sacrum was developed from the XIX vertebra and in six from the XX vertebra. Arranged in the form of a per cent table, Parker's results were as follows:

The pelvis is attached to the XIX vertebra in 70% of 27 specimens.
" " " " " " XX " " 22% " " "
" " " " obliquely " 7% " " "
" " " " abnormally " 29% " " "

An examination of a larger number of specimens shows that the number of variable individuals may be considerably increased. On Plate C the pelvic appendages are represented by short lines crossing the vertical ordinates on the abscissas of the XVIII, XIX, and XX vertebra.

The pelvis is attached to the XIX vertebra in 64% of 100 specimens.
" " " " " " XX " " 28% " " "
" " " " obliquely " 8% " " "
" " " " abnormally " 36% " " "

The pelvis, then, is attached abnormally in 36% (28 + 8) of the 100 specimens. This variation should, in the light of the 127 specimens thus far tabulated by Parker and myself, be corrected by uniting the two sets of figures. This union gives the remarkable variation formulated in the following table, *viz.*, an average of 35%, and offers an example of excessive variation in animals not domestic.

The pelvis is attached to the XIX vertebra in 65% of 127 specimens.
" " " " " " XX " " 27% " " "
" " " " obliquely " 8% " " "
" " " " abnormally " 35% " " "

(2) Is there true meristic variation?

Bateson ('94, p. 102) states that "numerical change may be brought about in the series of vertebræ by two different processes : first, by variation in the total number of segments comprising the whole column, in which case the variation is truly meristic ; and, secondly, by variation in the number or ordinal position of the vertebræ comprised in one or more regions of the column, not necessarily involving change in the total number of segments forming the whole series, and in this case the variation is homœotic." He further states that though the latter form of variation may be associated with the former it is rarely possible in any particular case " to distinguish clearly whether such a change has occurred or not," because the terminal joints of the caudal vertebræ cannot be readily enumerated.

The application of the Röntgen rays often so clearly defines the centers of ossification of even the terminal caudal vertebræ that I have attempted to plot the several series on Plate C, though in a few cases, due to the imperfection of the specimens, tails in process of regeneration being quite frequent among the larger individuals, the number of the last few vertebræ have not been determined with certainty. Such specimens, five in number, are designated with ? over the *estimated* terminal joint.

An examination of the plotted curve of the vertebral columns on Plate C will reveal an extremely irregular line passing from Specimen 1, with 45 vertebræ, to 2 with 44, 3 with 47, etc. (Plate B, Specs. 1 and 2). There is, then, considerable meristic variation, and the second of the questions considered in this section is answered in the affirmative. Since it is possible to enumerate the caudal vertebræ, we are in a position to answer the third question, *viz.:*

(3) Is homœotic associated with meristic variation?

On Plate C the dotted ordinates indicate the specimens which are homœotic, and, taking the specimens in blocks of twenty, let us see if the homœotic specimens of each block have a greater variation in the number of vertebræ than do the normal specimens.

In the first block of twenty specimens the average number of vertebræ for each animal is 45.7. The sum of the departures from this mean in sixteen normal specimens is 22.4, giving in this block an average departure from the mean, for each specimen having the pelvis on the XIX vertebra, of 1.4. The four homœotic specimens, however, show an average departure of 1.6, *i.e.*, the amplitude of variation among the homœotic specimens is greater. The second, third, and fourth groups of twenty also reveal greater amplitude of variation on the part of homœotic individuals, though the last group is exceptional, the homœotic specimens showing less variation than the normal. The tabulated results are as follows:

The mean amplitude of meristic variation of the normal specimens

| | | | | |
|---|---|---|---|---|
| In the first 20 | is 1.4 | and of the homœotic specimens is | | 1.60. |
| In the second 20 " | .94 | " " " " | " " | 1.97. |
| In the third 20 " | 1.35 | " " " " | " " | 1.50. |
| In the fourth 20 " | 1.52 | " " " " | " " | 1.81. |
| In the fifth 20 " | 1.66 | " " " " | " " | 1.19. |
| In 100 " | 1.37 | " " all the " | " " | 1.61. |

Though in this table there is considerable irregularity in the ratios, it seems to the writer that the final figures, 1.37 and 1.61, are sufficient to warrant the conclusion that specimens having abnormally placed sacra (homœotic) do present a considerably increased meristic variation, and aside from the fact that there are twice as many homœotic specimens on the right as on the left of Plate C.

Is the converse true? Do specimens presenting extremes in meristic vertebral variation tend towards homœosis?

An examination of the facts in the case brings out most striking results. There are only two specimens whose vertebræ are reduced to the abnormal number of forty-three, and both of these are homœotic. Of the nine examples bearing forty-four vertebræ, only 33% are homœotic. Of the twenty-one specimens bearing forty-five vertebræ, only 19% are homœotic. As the vertebræ now leave the normal and tend towards the other extreme, homœosis becomes more frequent, as will be observed by reference to the table.

Of  2 specimens with 43 vertebræ, 100% are homœotic.
"   9    "    "  44   "    33% "    "
"  21    "    "  45   "    19% "    "
"  18    "    "  46   "    29% "    "
"  14    "    "  47   "    36% "    "
"  16    "    "  48   "    44% "    "
"  11    "    "  49   "    45% "    "
"   5    "    "  50   "    60% "    "
"   4    "    "  51   "    50% "    "

A profile curve, or "curve of frequency" (Galton, '89), illustrating the relative distribution of the several columns according to their vertebral enumeration, is drawn in dots at the right of Plate C, the length of the ordinates being determined by the number of the specimens having respectively 43, 44, 45, 46, 47, 48, 49, 50, and 51 vertebræ. If now the respective per cents of homœotic individuals in each series be represented by a red curve on the same ordinates, we will observe that as the first curve ascends to its culminating point the second curve descends, the ordinate 45 bearing the highest point of the first curve and the lowest point of the second. To the right of this ordinate the curves converge in approximately the same way as they primarily diverged.[1]

The conclusion that specimens presenting extremes of meristic vertebral variation tend towards homœosis is irresistible, and the third question of the first section is answered in the affirmative.

## Section II.

Is there a ratio between the absolute length of the animal and the number of the vertebræ?

As before mentioned, the animals are arranged on Plate C, the smaller toward the left and the larger toward the right. The curve of relative lengths, drawn in red, passes from the specimen No. 1 at the left, with a length of only 193 mm.,

[1] The small dotted curve in red is drawn on the following scale. On abscissa 43 a dot is placed ten squares above the line which forms the right margin, and these ten squares are taken as representing the 100% of homœotic individuals having 43 vertebræ. The curve then follows the respective altitudes of 33%, 19%, 29%, 36%, 44%, 45%, 60%, and 50% on the abscissas of 44, 45, 46, 47, 48, 49, 50, and 51. I regret that the print does not make the course of this curve perfectly clear.

toward the larger specimens at the right, and of course with a constantly increasing altitude.[1]    It will now be profitable for us to draw upon Plate C a line which shall represent the mean of the very irregular curve of length in terms of vertebræ.  If the specimens having a larger number of vertebræ are irregularly distributed, this line will lie horizontally, but if numerical vertebral increase tends to occur in larger animals, the line will tend to rise from left to right.   Suppose now we take the means used in Section I for the several blocks of twenty specimens, and, using them for our ordinates, we plot the curve of mean numerical vertebral variation.   We will find it to lie between the abscissas of 45 and 48 (see dotted line on Plate C). The curve shows a *regular* rise from left to right, following the flowing curve of absolute lengths in millimeters, there being an increase of about three vertebræ in the entire series.   The question at the beginning of this section is answered, then, in the affirmative.

But the simple addition of three vertebræ, each less than one millimeter in length, at the caudal end of the animal, cannot account for the increase of over one hundred millimeters in the total length of the larger specimens.   The increase in length is not then to be explained by the addition of new segments behind, after the method of growth of developing arthropods and worms, but is interstitial, the individual vertebræ increasing in length with age ; though of course it does not follow that length is an absolute criterion of age.   Under varying conditions growth may be more or may be less rapid.

Whether the slightly increased number of vertebræ is due to multiplication of vertebræ after sexual maturity, or is an index of unusual embryonic vitality, or is predetermined in the egg, I will not now attempt to answer, though the application of the X-rays to the developing young will definitely settle the first of these, *viz.*, the question of vertebral multiplication after sexual maturity, and the frequent occurrence of smaller and average specimens possessed of nearly the maximum number of vertebræ might indicate early extraordinary vitality, predetermination, or both.

[1] The red figures on the right of the plate indicate millimeters.

It will be observed that the rise in the curve of relative lengths is quite abrupt until the specimens are 252 mm. long, when the ascent is gradual until the specimens are 330 mm. long, from which point to the longest specimen in the series the ascent is again rapid. The curve then shows that the larger number of specimens are between 252 and 323 mm. in length. If the lengths are examined in profile, as shown by the red curve on the right of Plate C, it will be observed that the culminating point of constancy is around that specimen which has a length of 260 mm.

As the sum total of opportunities for death are directly dependent upon the length of existence, it is not surprising that the upward trend of the curve is considerably more abrupt than the descent of senescence. I am inclined to look upon the absence of specimens measuring between 245 and 255 mm., causing the depression in the ascending curve, as accidental, though it is barely possible that the depression signifies some change in the rate of growth, consequent upon the approach of sexual maturity (Minot, '91); or it may be dependent upon the season of capture. I am also at a loss to explain the depression in the curve of descent in the neighborhood of specimens having a length of 280 mm.

Before leaving this section it should be noted that the proportion of homœotic specimens is not the same among the shorter as among the longer specimens. Of the first fifty specimens, but ten are homœotic, while of the second fifty twenty-five present cases of forward homœosis.

It has already been shown that the mere addition of a few terminal caudal vertebræ cannot alone account for the increased size of homœotic specimens. The fact, then, appears, if our specimens are not exceptional, that this homœotic departure is a character of increasing frequency among such animals as have met with success in the struggle for existence. If this is the case, the present species of Necturus may be considered as undergoing a process of "mutation," since the causes of transformation are acting with considerable "uniformity upon large numbers of individuals" and "in a definite manner" (Scott, '94).

## Section III.

Why does the variation tend towards forward rather than backward homœosis?

Bateson says "the attempt to apply to numerical variations the conception of variation as an oscillation about *one* mean is not easy, difficulty arising especially in regard to the choice of a unit for the estimation of divergence; . . . to judge from the scanty indications available, it seems that in cases of numerical change, variations to numbers greater than the normal number and to numbers less than it, are not generally of equal frequency. Probably no one would expect that they should be so " ('94, p. 571).

It has already been noted that in 28% of the one hundred salamanders examined, the sacral ribs are borne on the XX vertebra, while in not a single specimen are they symmetrically borne on the XVIII. Most certainly numerical variations towards a greater number, than the normal, of pre-sacral vertebræ are more frequent than variations towards a smaller number.

The positions of the oblique or unsymmetrical sacra also indicate pre-sacral increase. In only one case among the eight examples is the territory of the XVIII vertebra invaded (Plate A, Spec. 62), and this invasion marks the only encroachment upon the domain of this vertebra in a total of 127 specimens examined by Parker and myself,—an interesting fact when it is considered that the XX vertebra, no further removed *morphologically*, presents a total of 35 invasions.

Of course the easiest way of disposing of this question is to look upon the variation as atavistic. The "ancestral type" was possessed of a larger number of pre-sacral vertebræ, and the "mud-puppy" of to-day, by its anatomical variations, kindly indicates, in approximately 35% of its representatives, the character of its quasi-progenitor.

But if 35% of the present individuals tend towards pre-sacral multiplication, *i.e.*, tend to assume ancestral characters, we must not deny to these potentially progenitorial individuals the same tendency to vary that is possessed by their young, *i.e.*, 35% of the thirty-five should have an *added* increase of the pre-sacral

region, and thus about twelve specimens should have sacral
ribs on the XXI or XXII vertebra. Such a disposition of the
sacral ribs does not occur, though its absence may be attributed
to a presumed tendency of the parent form towards "fixity"
or "specific stability."

Rejecting the theory of atavistic variation, I think the for-
ward homœosis of the sacral vertebra may be explained on
other grounds.

In the first place, the origins of the processes that bear the
ribs, as will be noted by reference to Pl. A, Spec. 87, arise not
from the middle, but from the posterior end of each vertebra,
making it a much shorter distance from the transverse process
of the XIX to the territory of the XX, than from the trans-
verse process of the XIX to the territory of the XVIII trunk
metamere. It will thus seem more probable that variations
occurring in the course of ontogenetic development will fall on
the side of nearer proximity ; and the Anlage of the limb, com-
pounded though it may be by contributions from several trunk
segments, will depart less from its normal position when it
finally falls within the territory of the XX than when it falls
within the territory of the XVIII vertebral segment. This
explanation rests upon the assumption that the territory of
the XX metamere once invaded, the secondary processes of
growth within that segment will arrange for the accommoda-
tion of the sacral rib at its normal place, even though its
Anlage was first located in the anterior portion of the segment.[1]

A third and possibly valid explanation is offered by anatomy
alone. The XVIII, XIX, and XX segments are not, in the
adult at least, of equal length. The Anlage of the limb would
be obliged to vary considerably more, in a linear direction, in
order to influence the XVIII than it would to influence the
XX vertebral segment.

Though these two possible explanations of the more fre-
quent occurrence of forward homœosis are here given, they
are both dependent upon an assumed variation in the position
of the Anlage of the appendage with respect to some one

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[1] The occurrence in certain fishes of pelvic fins, which lie in front of the pecto-
ral fins, is in this connection of especial interest.

"normal" vertebral segment, *viz.*, the XIX. We assume, moreover, that when the relative position of the appendage is once determined in the embryo of Necturus it is determined for life ; since there is no evidence to show that there is a "migration" of the parts of the pelvic region after the first rudiments are laid down. Rosenberg ('**76**), however, claims a true pelvic migration for Homo, involving an emancipation of sacral elements for the production of coccygeal vertebræ, and Credner ('**86**) claims a distal shifting of the pelvic arch in the fossil amphibian Branchiosaurus. There are grounds, moreover, as will be shown in Section V, for the belief that the very process of local differentiation of the embryonic cells, for the final production of the appendage peripheral to the vertebral axis, occurs in a position in no respect dependent upon the position of any one vertebral segment, but dependent rather upon the general proportions of the embryo as a whole. The determination of the loci of the successive vertebræ and their early differentiation exerts no determining influence on the position of the appendages. But the Anlage of the appendage once determined, influence from it will direct the growth of proper sacral elements in the nearest vertebral segment, be it the XVIII, XIX, or XX.

It should furthermore be noted that when the Anlage of the appendage does not fall within the territory of the normal metamere (XIX), its vertebra, which has presumably been producing sacral ribs in two-thirds of the specimens since some remote geological period, does not show the slightest sign of sacral differentiation, but is exactly like the other neighboring trunk vertebræ.

## Section IV.

Can an explanation be given for the frequent occurrence of oblique or unsymmetrical sacra ?

In eight per cent of the specimens examined the sacrum is not a single vertebra, but is composed of two halves, each belonging to different metameres (Plate A, Spec. 62). The legs, moreover, in these specimens, leave the body at points not directly opposite.

Those who attempt an explanation of this phenomenon on the principle of intercalation, excalation, or of pelvic migration meet with most provoking difficulties. Why should only one lateral half of a vertebra be intercalated, giving rise to a body segment which bears a single rib on one side and two ribs on the other? Or why should the process of the formation of sacral ribs involve portions of two vertebræ rather than one, and thus produce an asymmetrical sacrum?

The difficulty is partly obviated if we admit that the differentiation of the sacral vertebra is the result of centripetal influence exerted by the growing Anlage of the appendage. If the first rudiments of the appendages are not laid down exactly opposite each other, and there are many reasons, as, for example, the curvature of the embryo, the pressure of neighboring eggs, etc., why their primitive positions might vary, an unsymmetrical or oblique sacrum would result.

It is a remarkable fact that of the eight unsymmetrical specimens, all but one, No. 62, have the left half of the sacrum in advance of the right, *i.e.*, the axis of the sacrum is sinistrodextral.

I have been quite unable to find any valid explanation for this peculiar uniformity, unless it is based upon the curvature of the embryo. Dr. Whitman has informed me that the young of Necturus, as it lies upon the surface of the egg, is curved, but I have not seen the embryos or larvæ, and I do not know whether the curve is lateral or dorsal-ventral, or even if it is fairly constant in its trend. An attempt to correlate the position of the sacral axis with the slightly asymmetrical position occupied by the paired viscera has proved futile.

## Section V.

Is the position of the pelvic arch dependent upon the ordinal position of some one segment (sacrum) of the vertebral column, or is its position determined by, and is the sacrum the resultant of, the location of some topographical point?

I do not know that an attempt has ever been made to compare the distribution of the vertebræ and the location of the

appendages with the general dimensions of the vertebrate body, though the radiographs of Necturus, procured by the X-rays, make the necessary process of measurement extremely simple.

For a standard of measurement I have considered the distance from the first to the XXX vertebra, no matter what the actual length of the animal may be, to be represented by 100 ; the XXX vertebra being selected because the terminal portions of the animals are liable to injury. I have taken the intervertebral space between the XIX and XX as the locus of a variable, and I find by making accurate measurements on the negatives that the position of this intervertebral space varies in different animals in a very appreciable way. In certain animals the trunk, the part lying anteriorly to the selected intervertebral space, is relatively longer, while in others the caudal portion is relatively longer. The distance from the XX to the XXX vertebra may in some specimens (Nos. 4, 67, 95) be only 29% of the entire measured length, while in others it may be as great as 35% (Nos. 58, 61, 74).

On Plate C these relative measurements of the several specimens are given, each ordinate representing the length from the first to the XXX vertebra, and the upper of the two black lines that cross the plate represents the fluctuating intervertebral septum between the XIX and XX vertebræ. In by far the larger number of cases the caudal region is one-third, 33%, of the length. Specimen No. 1 has a trunk slightly longer and a caudal region correspondingly shorter than the average. The trunk of No. 2 is shorter and the tail longer. No. 3 is like No. 2. No. 4 has an uncommonly long trunk and short tail, etc.

If this curve is followed across the plate, it will be noted that the first nineteen vertebræ may suffer regional expansion and contraction, with correlative contraction and expansion of the caudal region, and to such an extent as to give an amplitude of variation to the dividing line between the XIX and XX vertebræ, amounting to $\frac{6}{100}$ (35% minus 29%) of the average distance between the I and XXX vertebræ. The amplitude of variation in the column itself, absolutely independent of the limbs, is sufficient, then, to include within

itself $\frac{6}{100}$ or $\frac{1}{17}$ of the measured distance. But the verte-
bræ in the region of the selected intervertebral spaces are, in
length, considerably less than $\frac{1}{17}$ the measured distance;
so that the oscillations of the dividing line between the XIX
and XX vertebræ would be such as to carry it back and forth
over a distance greater than one vertebra anteriorly and one
posteriorly.

If we should string a series of thirty short segments of rub-
ber tubing upon a cord, and then, after placing a card between
the XIX and XX, subject the entire series to slight pressure,
we would have what might roughly correspond to the column
under consideration. The card, representing the selected in-
tervertebral space, can be forced forward or backward over a
certain amplitude, according to the compressibility and elas-
ticity of the rubber rings. Suppose, while the card is at rest, we
hold two fingers of the hand in such a position as to represent
the rudiments of the appendages, lying on either side of the
nineteenth ring. Now when the card is forced anteriorly the
fingers lie no longer opposite the XIX, but opposite the XX
vertebra. A forward homœosis has taken place illustrative
of what happens in a large per cent of the specimens of
Necturus.

But do our data warrant the assumption that with the com-
pression of the anterior vertebræ there is a concomitant varia-
tion in the position of the hind limbs? When specimens tend
toward elongation of the caudal region, do the legs appear on
the XX, and when the reverse obtains do they tend to arise
from the XIX vertebra?

A further examination of the plate will answer in a most
conclusive way.

Specimens No. 58, 61, and 74 have the greatest elongation
of the posterior vertebræ, and in every one of these specimens
the sacral ribs are borne by the XX vertebra.

In other words, in all the specimens presenting extreme
compression of the anterior vertebræ there is concomitant
variation in the position of the hind limb.

Arranged on the abscissa of 34% (Plate C) are thirty-two
specimens, and, if our theory is to be supported, a less number

of specimens should present homœotic variation than was the case with the three just mentioned, belonging to the abscissa of 35%. A reference to the plate will show that a less number, only fifteen specimens (46%), have homœotic sacra.

A still smaller number of homœotic specimens should be found on the abscissa of 35%, and, in fact, of the forty-four specimens arranged on this line but thirteen present cases of forward homœosis, 29%.

An even greater diminution in the number of homœotic specimens should be found on the abscissa of 32%, where we count fourteen specimens, only two of which, barely 14%, are homœotic.

On the abscissa of 31% among four examples there is only one that is homœotic, and among three examples on abscissa of 30% there is also only a single homœotic individual.

There are thirty-five specimens below the mean abscissa of 33% and twenty-one specimens above. The former exhibit eighteen cases of forward homœosis, *i.e.*, 51% are homœotic ; the latter exhibit only four, *i.e.*, 19%.

This effort to prove that the sacral ribs are developed as the result of centripetal influence bears directly upon the proposition of Bateson " that individuality should not be attributed to members of a series which has normally a definite number of such members," for it is shown that the normal XIX vertebra owes its specialized form to the molding influence of surrounding parts, and not to some inherited directive influence upon one particular vertebra. It shows that a " redistribution of differentiation " may and frequently does take place.

The appearance of the appendage at a definite topographical point, without respect to the location of certain segments of the neighboring axial area, is in harmony with the view of Professor Whitman ('93) that the real unity, both in development and in adult stages, is the organism as a whole, " that the organism dominates cell formation."

The view here advanced also receives direct support from the facts of embryology, as shown by the following extract from Weidersheim's " Grundriss " ('93): " Mit anderen Worten — und ganz dieselben Gesichtspunkte ergeben sich auch für

Reptilien, Vögel und Säuger, und sie gelten auch ebenso gut bei allen Vertebraten für die hintere Extremität — es handelt sich um ein festes, die ganze Vertebraten-Reihe beherrschendes Gesetz, dass der Anstoss zur Entwicklung des Gliedmassen-skeletes stets von der Peripherie ausgeht, und dass sich die centralen (Gürtel-) Theile erst secundär unter dem formativen Einfluss der freien Gliedmasse entwickeln."

One of course feels some regret that there is not a suffi-cient number of examples of backward homœosis for the basing of a conclusion in regard to the association of backward homœ-osis with trunk elongation. The only specimen which shows this unusual homœotic tendency is No. 62, and on a reference to Plate C it will be seen that its XIX intervertebral septum is located on the mean abscissa of 33%.

## Section VI.

Are variations in the relative position of the pectoral arch associated with variations in the relative position of the pelvic arch?

The pectoral arch, located near the anterior end of the body and closely associated with the head and visceral skeleton, would be expected *a priori* to show a lesser amplitude of varia-tion in respect to the segments of the vertebral axis than is shown by the pelvic arch. In front of it are only three verte-bræ, and the limits of vertebral elasticity, if I may use the term, are correspondingly restricted.

On the other hand, the shoulder girdle is not articulated to any one vertebra, and those intimate relations between bones which we have been taught are so potent in determining their position and character, do not here exist, and were it not for the tensile influence of the myotomes the enclosed vertebræ might slide back and forth through the arch *ad libitum.*

Since the girdle thus lies imbedded only in muscle tissue, its normal position in relation to the embraced vertebræ is rather difficult to determine from specimens which have been killed without special care. My observations, however, con-vince me that the halves of the arch do not always occupy the same position with regard to the vertebral segments.

The small ossified scapulæ are often very clearly shown on both the lateral and dorsal radiographs, and it is not a difficult matter to get an approximate idea of their relation to the neighboring vertebræ. They ordinarily lie opposite the middle of the fourth vertebra, though they may lie opposite the intervertebral space which separates the third vertebra from the fourth, and less frequently opposite the space which separates the fourth from the fifth. They are represented on Plate C by the small cross-lines occurring between abscissas three and four. Let us examine the extreme cases, and see if there is a correlation between pelvic and pectoral variations.

Specimens 12, 28, 30, 31, 35, 38, 39, 43, 52, 73*, 79*, 92, 94* all show the scapulæ to lie opposite or near the third intervertebral space, while specimens 23, 24*, 37*, 53*, 58*, 68*, 71, 77, 78*, 83*, 89*, 90, 95*, 98 show a tendency towards the approach of the fourth intervertebral space.

Now of the first series of thirteen specimens there are only three cases (indicated by an *) of forward homœosis of the sacrum. In the second series, however, of fourteen specimens, there are nine cases of forward homœosis.

When one stops to consider that the total number of homœotic specimens is approximately only one-half the number of normal specimens, and that the chances of scapular variation are hence twice as liable to gather around normal individuals, the figures in the previous paragraph have an increased significance.

We can conclude that variations in the relative position of the pectoral arch *are* associated with variations in the relative position of the pelvic arch, and that both variations are causally connected with some common factor. I believe that factor to be the variation of the relative length of different vertebral regions, as considered in the fifth section.

## Section VII.

Are there other skeletal variations associated with pelvic variation ?

A critical examination of the skeletons of Necturus, made for the purpose of detecting a possible tendency on the part of

homœotic individuals towards variations in other directions, leads to the conclusion that homœosis is only an index of general instability on the part of the skeleton as a whole.

(1) This principle was illustrated in Section VI, where it was shown that variations in the position of the sacral vertebra are accompanied by variations, in a definite direction, in the pectoral region.

(2) The principle was also illustrated in Section I, when it was shown that homœotic specimens tend towards numerical increase in the number of vertebral segments. — Before passing on to other possible illustrations, let us examine this present phenomenon more in detail.

According to Bateson's law, forward homœosis, involving one vertebra, should yield a column of *one* more than the normal number of vertebræ. But if we examine the curve of lengths in terms of vertebræ, on Plate C (the lower of the curves drawn in black), we shall find that whereas the normal specimens average only 45 vertebræ, the homœotic average not *one* more, 46, but *two* and *three* more, 47 and 48, and that the amplitude of variation, moreover, is much greater among the latter than among the normal specimens. It is clear, then, that there is an added increase in the variation of the vertebræ among homœotic specimens.

(3) A third illustration of the principle of general variability was given in Section V, where it was shown that abnormalities in the relative lengths of the anterior and posterior portions of the body tend to gather about homœotic individuals.

(4) Dr. Parker ('96) has made use of the first hæmal arch in his comparisons of the vertebral columns of Necturus, showing that its position is subject to considerable variation. Let us see if homœotic specimens present greater or less variation in the position of this arch than do the normal examples.

Of sixty-three normal specimens giving satisfactory radiographs of the first hæmal arch,

10 examples (16%) have the arch attached to the XXII vertebra.
51    "    (81%)  "    "    "    "    " " XXIII   "
2    "    (3%)  "    "    "    "    " " XXIV   "

and of thirty-five homœotic specimens,

1 example  (3%) has  the arch attached to the XXII vertebra.
31 examples (88%) have "    "      "     " " XXIII
 3    "      (9%)  "   "   "     "      " " XXIV

Our figures show that in both normal and homœotic speci-
mens the point of constancy is the XXIII vertebra, and,
moreover, that the homœotic specimens are slightly less liable
to vary than the normal, the former presenting 88% of cases of
stability against 81% presented by the normal specimens.

It would seem that the production of the first hæmal arch is
then a function of the XXIII vertebra, a function of the axial
portion of the embryo, and that the development of the hæmal
arch proceeds entirely independent of the position of the
neighboring appendages, for in 81% of the normal specimens
there are three intervening vertebræ between the sacrum and
the first hæmal arch, while in nearly all of the homœotic speci-
mens there are but two.

Of course if we assume that the normal position of the
first hæmal arch is on the fourth vertebra behind the sacrum,
we can then show that the homœotic specimens present a
greater range of variation than normal individuals. But this
assumption would be unjustified.

(5) Specimens may frequently be found whose caudal verte-
bræ are provided with abnormal processes. These abnormalities
may appear as bi-lobed or double neural plates, or double neural
spines. Similar malformations may affect the hæmal arch
(Plate B, Spec. 79).

If our belief in the general variability of the skeleton is to be
justified, these minor variations should be of more frequent occur-
rence among the homœotic than among the normal specimens.

Among thirty-six homœotic specimens there are twenty
which present vertebral variations in the caudal region, while
of sixty-four normal specimens there are only twenty-six that
show the same local variability.

It should not be forgotten that imperfect processes of regen-
eration may be responsible for the large number of caudal
malformations, though this does not explain why homœotic
individuals should be more frequently captured during the
regenerative process.

I wish that alcoholic " mud-puppies " presented some striking coloration, like the spots of Diemyctylus or Amblystoma, so that one might detect any tendency towards color variation in specimens presenting skeletal instability ; few animals, how-ever, are more alike externally than preserved specimens of Necturus.

## Section VIII.

The question may arise: Are variations in the position of the pelvis correlated with sex, or, if not directly dependent upon the sex, does one sex show a greater tendency towards skeletal variation than the other ?

It is frequently the case when a large number of individuals of a single species are collected that the males predominate, but in the present collection, as will be seen by counting the indices of sex at the top of Plate C, there are thirty-seven males and sixty-three females. There should be, then, if varia-tion is equally distributed among males and females, nearly twice as many abnormally placed pelves among the females as among the males. In fact, however, of the thirty-six homœotic specimens, only nine occur among the males, while twenty-seven, three times as many, occur among the females.

It is, I think, quite generally believed that males are much more subject to variation than are females. Montgomery ('96) in his recent paper on " Organic Variation " states that " the dimensions of birds are more variable in the males than in the females," and that " it is not impossible that in birds, as in man, the female may be more conservative and less progressive than the male." This of course recalls Brooks ('83) and Geddes and Thomson ('90), though we should not forget that males are fre-quently larger among birds and mammals, and thus offer an increased amplitude of variation, and that males are, moreover, frequently provided with many and variable secondary sexual characters whose development is directly dependent upon the activity of the proper sexual glands, and are hence subject to considerable variation.

Among Amphibia, however, it has been many times shown that the male may assume certain of the duties which are

generally considered to be attributes of the female, and it is barely possible that this affectation by the male is only an expression in habit of a general condition of physical conservatism.

## Section IX.

Are there anatomical grounds for the theory of vertebral intercalation ?

If we accept the theory that there is a fixed intimate interrelation between the appendage, girdle, and some one supporting vertebra ; that all are parts of one developing area, parts bound together by some intangible law of correlation, the occurrence of the XX vertebra as the support of the pelvic appendages can be explained only by the theory of vertebral intercalation of Albrecht and Baur.

If I understand the theory correctly, it is the function of one particular vertebra — or where more than one vertebra enters into the formation of the sacrum, of certain particular vertebræ — to give attachment to the pelvic arch, and no matter what secondary alterations may occur before or behind, this vertebra or these vertebræ will tenaciously hold to their prescribed and inherited function.

After examining the descriptions of several examples of intercalation given by Baur, in an earlier number of this journal ('**91**), I fail to see that they necessarily support his theory.

In the first place, Albrecht and Fürbringer are acknowledged to differ in their interpretation of the Belgian python, and the mention of vertebral and costal asymmetry in Pelamis and Cimoliasaurus does not render the intercalation theory more probable.

The case of the gavial, described by Baur and considered by Parker to show "very conclusively that, in place of one vertebra, two or parts of two may arise," is equally inconclusive. Baur writes : " It is well known that the typical number of the pre-sacral vertebræ in the living Crocodilia is twenty-four; there are two sacrals; the first caudal is peculiar by being convex. In a specimen of *Gavialis gangeticus* I found twenty-five presacral vertebræ. As in all living crocodiles, the first caudal

vertebra is bi-convex; but in this case it is the twenty-eighth, in the other the twenty-seventh. Is it not evident, therefore, that at some place between the occipital condyle and the first caudal a new vertebra has been inserted? By careful comparison I find that this new vertebra has been intercalated between the ninth and tenth."

Dr. Baur does not tell us how he was able to pronounce this an intercalated segment, leaving it to be presumed, however, that a new vertebra possesses certain diagnostic features. Moreover, if perfectly formed sacral ribs may appear through discontinuous variation on the XX vertebra of the "mud-puppy," I see no reason why the new first caudal of the abnormal gavial should not produce a bi-convex rather than a procœlous centrum.

If we could only have the relative trunk and tail measurements of these specimens with abnormal vertebræ, and could compare them with the normal, we could soon tell whether a strange vertebra had been wedged into the regular series, making the trunk abnormally long, or whether the arrest in the development of a trunk vertebra had not resulted in the abortion of certain anterior vertebræ, drawing into the sacral region certain primarily caudal elements.

The Helodermas mentioned by Baur, on page 335, would form excellent material for such a comparative study, since the four specimens exhibit four variations in the numerical position of the first caudal vertebra. But in the absence of a large number of Helodermas, let us examine again our Necturus material and see if we cannot find vertebræ that might be looked upon as intercalated.

Specimen No. 46 shows a slight reduction in the lengths of the fifth and sixth vertebræ, and specimen No. 61 shows a similar reduction in the twelfth and thirteenth. In both cases, the posterior of the affected vertebræ is slightly smaller than the anterior.

If during the process of embryonic growth there was a disturbance of the regular formation of the body segments, we can easily see that this disturbance might have resulted in the interruption of the growth of certain pre-sacral vertebræ. The

general proportions of the respective vertebral regions would thus be altered, and a tendency would result for the XIX segment to be laid down in a relatively more anterior position. The sacrum, in other words, would tend towards forward homœosis. The two specimens just mentioned should, then, present forward homœosis, and both do.

On Baur's ground, also, both specimens should show forward homœosis, but on his ground that portion of the body lying in front of the normal XIX segment should show, in addition, an increase in absolute length proportional to the numerical increase of the vertebræ. In the specimens at hand such a proportional increase in the length of the trunk region does not take place. Specimen No. 46, though possessed of an additional pre-sacral vertebra, has its sacrum, so far as the general proportions of the body are concerned, in the normal position, *i.e.*, on abscissa 33%, as will be observed by reference to Plate C ; while No. 61, far from having a longer pre-sacral region, has this region remarkably short ; indeed, in the one hundred specimens of which proportional measurements have been taken, only two show an equal amount of pre-sacral compression.

Again, according to the theory of intercalation, if the first hæmal arch occurs normally on the XXIII vertebra, four joints behind the sacrum, on the occasion of the introduction of an additional pre-sacral vertebra, the pelvis should be simply forced backward with all its chattels. This, however, does not occur, — the hæmal arch does not change its position from the XXIII vertebra.

On the theory of regional compression and expansion, which we have advanced, the hæmal arch, a function of the axial rather than of the appendicular areas, should tend in forwardly homœotic specimens to approach the pelvic arch, *i.e.*, in homœotic specimens the number of vertebræ intervening between the sacrum and the first hæmal arch should tend towards reduction. This is what actually occurs. (See Section VII, p. 473.)

The assumption that the embryonic pelvic girdle travels backward and forward over a fixed vertebral column has been

questioned both by Bateson and Parker, though believed by Rosenberg and Credner. With Parker one can agree that the " sacral region has the power of developing sacral ribs at several points on both right and left sides." But Parker gives us no reason for the abnormal position often taken by certain sacral ribs, and does not explain why a particular vertebra (or vertebræ) produces sacral ribs, and why others, potentially able to produce sacral ribs, produce ribs of the ordinary sort. If several vertebræ are endowed with this power, why do we never find nicely formed sacral ribs passing out into the surrounding tissue as if searching for some ilia with which they might articulate, and why are the ilia not occasionally attached to ribs of the ordinary triangular type?

The sacrum, pelvis, and appendages are not intimately associated parts of the body that represent one complete whole, and the definitive location of the sacrum is probably due to centripetal influence derived from the budding appendage. Intercalation in the sense of the introduction of new segments does not take place, and what have been given as examples of intercalation are probably only imperfectly formed body segments.

Brown University, Providence, R. I.
        Nov. 3, 1896.

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## EXPLANATION OF PLATE A.

The figures are printed *directly* from the original radiographs, and, so far as size and general structure are concerned, are faithful reproductions; but the exquisite details and sharp lines of the originals are unfortunately obscured.

SPECIMEN 62 is peculiar in that the pelvis is oblique. The figure also shows the location of the scapulæ. The animal was placed in a supine position upon the photographic plate.

SPECIMEN 62*, the same animal seen from the side.

SPECIMEN 87. This figure shows a symmetrical pelvis, and indicates the position of the scapulæ.

*cimen No. 62.*



*cimen No. 62.*



*imen No. 87.*

Specimen No. 62.

Specimen No. 62'

Specimen No. 87

EXPLANATION OF PLATE B.

SPECIMENS 1 and 2, the smallest in the series, gave, on the original photographic plate, the total number of vertebræ with remarkable clearness. They have greatly suffered in the process of reproduction.

SPECIMEN 79 is especially interesting in that certain of the caudal vertebræ are provided with double neural and hæmal spines.

Specimen No. 1.

Specimen No. 2.

Specimen No. 79.

Specimen No. 1.

Specimen No. 2.

Specimen No. 79.

## EXPLANATION OF PLATE C.

The vertical lines, ordinates, indicate the several specimens from 1 to 100. Entire lines represent normal, broken lines represent homœotic specimens. The numbers at the left, in ascending order from 1 to 55, indicate the number of vertebræ and the ratio of the length from the XXX to the XX, and XIX to the I vertebra.

The irregular line, crossing the plate transversely from 45 on the left, represents the length of the animals in terms of vertebræ, and the more regular dotted line follows its mean course.

The irregular line extending across the upper part of the plate represents the relative variation in the ratio of tail-length to body-length.

The red curve, running obliquely across the plate, indicates the variation in absolute length, and is represented in profile on the right. The figures in red indicate millimeters ; thus, specimens 71 and 72 are 308 mm. in length.

*Pl. C.*

*Pl. C.*

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## THE CRANIAL MUSCLES AND CRANIAL AND FIRST SPINAL NERVES IN AMIA CALVA.

### EDWARD PHELPS ALLIS.

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### CONTENTS.

#### IV. Muscles Innervated by the Postvagal or Occipital Nerves, and the Nervi Occipitales.

#### INTRODUCTION.

Much the larger part of the purely investigative work on which the present memoir is based was done in 1886, in connection with a work on which I was at that time particularly engaged: "The Anatomy and Development of the Lateral Line System in Amia Calva." It was done simply to better understand the serial sections on which I was working, and the notes and sketches once made were put away as of no special value to any one but myself, it being assumed that the several memoirs already published on the cranial nerves and cranial muscles of Amia (van Wijhe, Sagemehl, McMurrich, Wright) were so full and complete that any further publication on the subject would be not only unnecessary but superfluous.

In 1889, after the publication of my earlier memoir, the notes and sketches put away in 1886 were taken up for more careful examination as a study preliminary to an investigation of the central origin of the peripheral nerves. A little study and consideration convinced me that such frequent reference would have to be made to my own work, rather than to that of others, that it would be much better for my purpose, even at the risk of considerable repetition, to make and publish a brief but full account of my results before beginning the research I especially

contemplated. I therefore began at once the preparation of the final dissections needed for illustration in such a publication. The work was hardly begun when I was obliged to discontinue it and all other similar work for several years. Mr. Jujiro Nomura, who was attached to my laboratory as artist, and who was simply to have made the drawings from my dissections, then undertook to make the dissections also, under my direction. On this work he has been engaged continuously since that time, accompanying me from place to place, where considerations of health have obliged me to go. The length of time he has been engaged on the work only proves but too well that it has been no simple matter, even where the number and position of the nerves and muscles were definitely known, to find them, trace them to their origins, and lay them bare *in situ*, sufficiently clean and clear for a drawing to be made of them.

As the work has progressed it has repeatedly been found necessary to study details, and to include in the investigation whole subjects, not contemplated in the beginning. The work has, accordingly, gradually lost the preliminary character it was intended to give it, and has become a somewhat extensive memoir. It nevertheless, in its general conception and arrangement, still retains many of the characteristics of the preliminary work originally contemplated, for it remains, as it was intended to be, an accumulation of facts and references grouped so as to be conveniently used as a basis for further work.

The drawings used in illustration are all by Mr. Nomura. They all represent actual dissections, made mostly by Mr. Nomura, but continually controlled by me, not only on the dissection itself, but also by comparison with the results obtained in my own earlier work both on the adult and on serial sections of larvae. Nothing is shown in the adult that was not controlled in larvae, and everything found in larvae has been sought for until found, or accounted for, in the adult.

Most of the drawings represent conditions found in a single fish, but certain of them are combinations of dissections made on two different heads; such combinations being made only when the results so given could not possibly be misleading. Whenever there has been the slightest doubt as to any arrange-

ment of the parts concerned, another complete dissection has been made and the drawings changed to agree with it, or discarded and made over again. No pains have been spared to have them correct. Nevertheless I do not doubt that omissions, if not errors, will be found in some of them, especially in those first made.

The numerous serial sections used in the work were prepared for me at my laboratory, the Lake Laboratory, Milwaukee, at first by Dr. W. Patten, under the direction of Dr. C. O. Whitman, and afterwards by O. S. Strong and A. C. Eycleshymer, under the direction of Dr. Howard Ayers. Several of the dissections used for control, especially those of the occipital region, have been made by Dr. Julius Dewitz. Dr. Dewitz also made the final dissections used for Figs. 35 and 58.

In the nomenclature adopted I have followed as closely as possible van Wijhe, Sagemehl, and Vetter. New names have been used only when absolutely necessary for descriptive purposes, for I fully agree with Sagemehl (No. 104, p. 180, note 1) that radical changes will have to be made, even in names already in general use, when the development and anatomy of the vertebrate head is better known.

In the literature referred to I have had to limit myself to such memoirs and publications as I could readily procure, circumstances obliging me to live where access to libraries, even to my own, was impossible. Certain of the references are made from notes made years ago, others are to abstracts only of the works concerned. All references to Scomber and Gadus, and all those to Perca and Esox, where special reference to Vetter has not been made, refer to work now being done, in my laboratory here, by Dr. Dewitz and Mr. Samuel Mathers.

PALAIS CARNOLÈS, MENTON.

## I.   THE ORBIT, THE MUSCLES OF THE EYE, AND THE
## ASSOCIATED NERVES AND ARTERIES.

### 1.  Eye-Muscle Canal.

Amia has, as already described by Sagemehl (No. 104, p. 214), a well-developed "Augenmuskelkanal" (*emc*, Fig. 11, Pl. XXI), bounded in front and behind by transverse ridges of the cartilaginous base of the skull.   The posterior ridge lies near the hind edge of the petrosals, and bears on its summit the transverse processes of those bones.   The anterior ridge is a specially developed structure (*w*, Figs. 9 and 11, Pl. XXI), called by Sagemehl a transverse " Wulst," and by Shufeldt (No. 115) a transverse " bar " of cartilage.   This bar is sharply marked off from the rest of the basis cranii by the Augenmuskelkanal behind, and in front by a narrow but comparatively deep groove (*ct*) which extends transversely from orbit to orbit. At each end of the bar there is usually, but not always, a small ossification, the basisphenoid of Bridge (*B,S*, Figs. 13, 14, and 15, Pl. XXII), which in one large specimen, contrary to Sagemehl's statement (No. 104, p. 215), extended in part through the cartilage of the base of the cranium, so as to be seen on its under surface.   In the same specimen the basisphenoid on the left side of the head extended forward across the transverse interorbital groove, bridging it or being perforated by it.   In all the specimens examined the two ossifications were of unequal size, and they never touched each other at any point, a median region of cartilage always separating them.

At about the middle of the median edge of each basisphenoid there was in all the specimens examined a deep indentation, forming, with the adjoining cartilage, a canal (*icc*, Figs. 9, 10, 14, and 15, Pls. XXI and XXII) through which, and not through the hypophysial fenestra as stated by Wright (No. 133, p. 495), the internal carotid artery on each side entered the cranial cavity.   From this canal, near its ventral opening, one, or sometimes two, small canals run forward and laterally through the bone into the tranverse interorbital groove, or into the extreme hind end of the orbit near it.   From the free lateral edge of the bone the superior, inferior, and internal recti

muscles arise, and along its postero-lateral edge, which is slightly grooved, the external rectus lies in its passage from the orbit to its origin, close to the middle line of the head near the hind end of the eye-muscle canal. In none of the specimens examined did the muscle arise, as stated by Sagemehl, immediately behind the transverse " Wulst " or bar. It arises from the cartilaginous floor of the eye-muscle canal, or in part also from the median rib or ridge of the parasphenoid, which fills the median longitudinal fissure in that floor. This fissure (*hfn*, Fig. 10, Pl. XXI) is the hypophysial fenestra of Sagemehl and Wright. The process of the parasphenoid that fills it, although differing somewhat in position, may be the homologue of the one which in Cyprinoids is said by Sagemehl to replace the basisphenoid of other teleosts (No. 107, p. 575).

In larvae of Amia, even in those 50 mm. in length, the internal carotid canal is represented by a foramen only which perforates the basal plate of the future orbital opening of the eye-muscle canal. The internal carotid artery, having issued from this foramen, runs upward and forward along the lateral surface of the base of the transverse cartilaginous bar of the chondrocranium, traversing an open space between the basal plate of the skull and the overhanging, upper, lateral edge of the bar. The anterior corner of this overhanging edge projects upward and forward, and the artery, having reached the anterior face of the bar, below and median to its projecting dorso-anterior corner, turns outward and upward along the dorsal surface of that corner, not having again entered the cartilage at any place. While traversing the open space along the lateral surface of the bar, its sends or receives a communicating branch from the efferent pseudobranchial artery, the branch giving rise, undoubtedly, to the small branch canal found in the basisphenoid of the adult; though in the adult the communicating artery itself was not traced. The three recti muscles which, in the adult, arise from the lateral surface of the basisphenoid, arise, in larvae, from the under surface of the dorsal edge of the transverse bar. The basisphenoid of Amia is therefore probably, in large part, a membrane bone developed in connection with the insertions of certain of the recti musles.

The roof of the eye-muscle canal in the adult is formed, as described by Sagemehl, by the horizontal processes of the petrosals, and by a tough, glistening membrane which extends forward from the front edges of those processes to the transverse bar of cartilage. The membrane, however, in all the specimens I examined, formed a much less important part of the roof of the canal than in the specimens figured and described by Sagemehl. The processes of the petrosals, the united front edges of which have a concave outline, cover nearly, if not quite, one half of the canal, and the space between their front edges and the transverse cartilaginous bar is largely occupied by an important sack-like depression or pit (*ph*, Fig. 26, Pl. XXV), described by Sagemehl as a slight depression only. In this pit are lodged the hypophysis cerebri and saccus vasculosus. The rounded front end of the hypophysis projects forward slightly beyond the anterior edge of the pit, and almost touches the transverse "Wulst," while the saccus vasculosus projects backward beyond the hind edge of the opening of the pit and lies, in large part, under the projecting, overhanging processes of the petrosals in a backward, pocket-like extension of the pit.

The tough membrane that forms part of the roof of the eye-muscle canal extends upward, on each side, and forms the median wall of what Sagemehl has called the upper, lateral chamber of the canal. In front of the canal the membrane extends forward across and beyond the transverse bar of cartilage, closely attached to its upper surface. Here, on each side, it extends upward from the lateral edge of the basisphenoid and the cartilage of the basis cranii in front of it, to the lower edge of a thin, projecting plate or fin of the alisphenoid, and to the lower and posterior edge of the orbitosphenoid in front of and continuous with that fin, thus filling the optic fenestra. Immediately in front of the transverse bar the membrane covers and takes part in the formation of a transverse pad of tough, dense tissue which extends from orbit to orbit (Fig. 26, Pl. XXV), covering and filling the interorbital groove. The top of the pad projects backward so as to slightly overhang, and the hind edge thus formed has a concave outline. Under this overhanging, curved hind edge, on each side, at the

lateral end of the pad, the optic nerve, which runs outward, forward, and slightly downward, along the upper, shelving surface of the cartilaginous bar, pierces the lining membrane of the optic fenestra and enters the orbit.

The hind edge of the optic fenestra (*ofn*, Figs. 9 and 11, Pl. XXI) is formed by the pedicle of the alisphenoid, the bone being always, in the specimens I have examined, cut out behind by the large trigeminal foramen, so as to leave a pedicle, and not simply perforated by it, as described by Sagemehl. The pedicle thus formed rests externally upon a process of the lateral wing of the parasphenoid, and internally upon a cartilaginous base which forms part of the side wall of the eye-muscle canal. Between this cartilaginous base and the pedicle externally, and the lining membrane of the eye-muscle canal and the thin, projecting plate or fin of the alisphenoid internally, there is formed a tall and relatively narrow opening by which the eye-muscle canal communicates with the orbit. Along the bottom of this opening, which may be called the orbital opening of the canal, the rectus externus enters the main, lower portion of the eye-muscle canal, and in its upper part the trochlearis, oculomotorius, and ophthalmicus profundus issue from the upper, lateral chamber of that canal and enter the orbit.

The upper, lateral portion or chamber of the eye-muscle canal (*emc'*) lies in a recess in the side wall of the cranium, the recess extending from the pedicle of the alisphenoid back to that process of the petrosal that forms the front wall of the utricular fossa. This chamber has in addition to the tall, narrow opening just described, which is common to it and the main eye-muscle canal, five openings leading to the outer surface of the cranium, namely : the large facial foramen (*ffr*) through the petrosal ; the small foramen for the external carotid (*ecfr*) immediately below and in front of that foramen and through the same bone ; the otic canal (*ofc*) leading to the top of the skull through the cartilage in front of and above the petrosal ; the large trigeminal foramen (*tfr*) through the edge of the alisphenoid, immediately behind and lateral to the pedicle of the bone ; and the smaller foramen for the ophthalmic nerves (*opfr*) through the alisphenoid immediately above and

in front of the trigeminal foramen and separated from that foramen by only a thin layer of bone.

Immediately below the upper, lateral portion of the eye-muscle canal there is, in the side wall of the main canal, an oblong opening, the palatine foramen (*pfr*), through which the palatine branch of the facialis leaves the cranium. This foramen is closed externally by the base of the lateral wing of the parasphenoid, and leads into a canal between that bone and the base of the cranium. This latter canal, which may be called the palatine canal (*pc*, Fig. 17, Pl. XXII), begins behind the palatine foramen, at an opening found in the angle formed by the hind edge of the wing of the parasphenoid and the lateral edge of the body of that bone, the opening being almost but not entirely enclosed in bone (*icfr*, Fig. 17, Pl. XXII). Beginning at this point the canal runs at first medianward and forward to the hind edge of the palatine foramen, then almost directly forward across that foramen and onward toward the anterior end of the head, lying in part between the cartilaginous base of the skull and the parasphenoid and vomer, and in part in those two bones. Immediately in front of the anterior edge of the palatine foramen a branch canal is sent forward and outward to the orbit, the opening of the canal lying in the angle between the anterior edge of the wing of the parasphenoid and the lateral edge of the body of the bone in front of the wing. Immediately in front of this canal a second branch canal is given off, the internal carotid canal, which pierces the basis cranii and enters the cranial cavity along the median edge of the basisphenoid as already described.

Through the posterior opening of the palatine canal the internal carotid artery and the pharyngeal branch of the glossopharyngeus enter the canal and join the palatinus facialis (No. 133, p. 494). The opening may therefore be called the internal carotid foramen, although it is only indirectly the point of entrance of that artery to the cranium. Through the first branch canal the posterior branch of the palatinus facialis enters the orbit. The external opening of this canal may therefore be called the foramen of that nerve (*ppffr*, Fig. 17, Pl. XXII). Anterior to this point and anterior to the ventral opening of

the internal carotid canal, the palatine canal lodges the anterior branch of the palatinus facialis, the pharyngeal branch of the glossopharyngeus and a small branch only of the internal carotid.

## 2. Carotid Arteries, Hypophysis Cerebri, and Saccus Vasculosus.

The carotid arteries in the young of Amia have been described by Wright (No. 133, p. 494). With the results obtained by him, my work both on the young and on the adult is mainly, but not entirely, in accord.

The efferent artery of the first branchial arch (ea. I, Fig. 28, Pl. XXV, and Figs. 61–63, Pls. XXXVI and XXXVII) as it bends inward and backward to join the dorsal aorta, gives off two vessels, the common carotid artery (*cc*) directed forward, and the hyo-opercularis (*hop*) directed forward and outward. From near the root of this last artery a small branch is sent backward and upward, the remaining and larger portion of the artery continuing forward and outward until it reaches the facial foramen, which it crosses under the issuing truncus hyoideo-mandibularis facialis. It then turns upward, outward, and backward above the nerve, and running backward above the adductor hyomandibularis, separates into two main parts, the hyoid and opercular arteries. The former joins and accompanies the truncus hyoideo-mandibularis facialis and its branches ; the latter, destined to supply the inner surface of the operculum, separates into two parts, one of which runs backward above, and the other backward and downward below, the opercular process of the hyomandibular. As the main hyo-opercularis artery passes forward across the facial foramen, under the facialis, it sends a branch upward in front of that nerve, through the foramen into the upper, lateral chamber of the eye-muscle canal. Branches are there given to tissues in the canal, and the artery then, running upward and forward lateral to the trigemino-facial ganglion, issues through the trigeminal foramen with the ramus buccalis facialis and truncus maxillaris trigemini, which nerves it accompanies in their further outward course. No branches could be definitely traced into the cranial cavity proper or the auditory labyrinth, as described by Wright (No. 133, p. 495).

The common carotid artery, soon after its origin from the first aortic arch, and just before reaching the lateral wing of the parasphenoid, separates into two parts, the internal and external carotids.

The internal carotid (*ic*) enters the palatine canal between the parasphenoid and the basis cranii, and after crossing the palatine foramen, turns upward into the internal carotid canal, and enters the cranial cavity under, or median to and in front of, the optic nerve of its own side. As it leaves the palatine canal a small branch is sent forward in that canal, and as it traverses the internal carotid canal another small branch is sent outward and forward in the small branch canal to join the efferent pseudobranchial artery, as already described on page 493.

Having entered the cranial cavity, the internal carotid artery, in the adult, turns backward and then outward and forward under the optic nerve. At this point it separates into three parts, an optic, an anterior or olfactory, and a posterior portion. The optic portion runs outward with the optic nerve, enclosed in the membranous sheath enclosing the nerve, and supplies, according to Wright, the ventral half of the choroid gland. The olfactory portion runs directly backward to about the level of the hypophysis, where it separates into two parts, both of which turn inward and then forward above the optic nerve and supply the anterior parts of the brain and brain cavity, one of the branches running forward under the olfactory nerve toward the nasal sack. The third, or posterior, portion of the artery runs at first directly backward, median to the olfactory portion. Reaching the level of the auditory labyrinth, it gives off a large branch, and, turning inward behind the lobus inferioris, joins its fellow of the opposite side, thus forming a circulus cephalicus, from the median point of which a branch is sent forward and another backward under the brain.

In embryos the optic chiasma lies in front of the internal carotid canal ; and the internal carotid artery, after issuing from its canal, runs upward and forward along the lining membrane of the optic fenestra, behind the optic nerve. In this part of its course it becomes so flat and thin that I could not definitely

determine from my sections whether it lay along the inner or the outer surface of the membrane. When it reaches a point slightly above and behind the optic nerve, it either perforates the membrane from its inner to its outer surface, or sends an important branch inward through the membrane, and then turns sharply outward and slightly downward above and behind the optic nerve, and, lying close to and behind the nerve, along its open edge, and at no place inside the nerve, as Goronowitsch (No. 50, p. 443) seems to have found in the adult, enters the eyeball with it.

The external carotid (*ec*, Fig. 28, Pl. XXV) enters the upper, lateral chamber of the eye-muscle canal through the small external carotid foramen which lies in the petrosal immediately below and in front of the large facial foramen. In the eye-muscle canal it runs upward and forward under or in front of the trigemino-facial ganglion, and near the front end of that ganglion separates into two parts. One of these parts, the ophthalmic portion of the artery (*ecop*), issues from the canal and enters the orbit through the ophthalmic foramen with the ophthalmic branches of the facialis and trigeminus; the other or mandibular portion (*ecm*) issues through the trigeminal foramen with the truncus trigeminus, which it accompanies. Immediately outside the cranium the mandibular portion gives off the afferent pseudobranchial artery (*aps*), which runs backward to the uppper surface of the pseudobranch. From the lower surface of the pseudobranch the efferent pseudobranchial artery (*eps*, Figs. 22–28, Pls. XXIV and XXV) arises and runs forward under the external carotid, or under the mandibular branch of that artery. Immediately in front of the lateral wing of the parasphenoid it crosses above the ramus palatinus posterior facialis, as that nerve issues from the palatine canal by its foramen, and, turning inward and forward, passes through a small foramen (*epsfr*, Figs. 9–15, Pls. XXI and XXII) in the turned-up lateral edge of the thin layer of cartilage that forms the bottom of the passage leading from the eye-muscle canal to the orbit, and enters that passage opposite the basisphenoid. Here, in larvae and probably also in the adult, a communicating branch is sent to the internal carotid, the branch corresponding to and

representing the anastomosis of the two arteries described by Wright in Lepidosteus (No. 133, p. 484). Continuing forward the efferent pseudobranchial artery crosses under the rectus externus, turns upward behind and above the rectus inferior but under the nervus oculomotorius, and reaches the orbit. It then turns outward and forward, internal to and then above the inferior branch of the oculomotorius, and lying immediately behind the optic nerve, enters the eyeball with that nerve, behind and a little above it, through the large opening in the cartilaginous sclerotic (*op*, Fig. 24, Pl. XXIV). It is the arteria ophthalmica of Sagemehl (No. 104, p. 203), and gives rise, according to Wright (No. 133, p. 495), to the dorsal part of the choroid gland. During its course through the orbit it is enclosed in a tough fibrous envelope, which, in the large fish used for illustration, had become, either naturally or through the action of reagents, semi-cartilaginous in character and appearance, and formed a tough, elastic, tube-like structure extending from the cranium to the eyeball. Sagemehl found no trace of a choroid gland in Amia (No. 106, p. 116).

Two other vessels (*ov* and *ov'*, Figs. 22–27, Pls. XXIV and XXV) extend from the cranium at the hind end of the orbit to the eyeball, and, in the one large fish, they were, like the arteria ophthalmica, semi-cartilaginous in appearance. They are both of them veins, though they have, even in larvae in sections, much the appearance of arteries. The smaller of the two, *ov'*, issues through a perforation in the sclerotic near its outer edge, between the insertions of the rectus superior and the rectus externus, and runs downward, backward, and inward between those two muscles to join a large vein, the orbital sinus of Parker in Mustelus, which lies along the side of the skull immediately below and internal to the ophthalmic branch of the trigeminus. From this orbital vein, distal to the point where it is joined by the vein *ov'*, a branch, the anterior cerebral vein of Parker in Mustelus (No. 83, p. 712), was traced in embryos through the cartilage of the side of the skull into the brain cavity, where it was distributed to the fore-brain and the olfactory nerve. In the adult this branch was not traced; the foramen by which it leaves the skull was, however, always found, lying some-

times through the cartilage immediately in front of the alisphe-
noid, and sometimes through the anterior edge of that bone
(*acvfr*, Figs. 9–11, Pl. XXI).   In embryos the vein *ov'* could be
traced inside the sclerotic outward and forward toward the optic
lens, accompanied by the ciliares longi.   After it has joined the
orbital vein, the latter enters the upper, lateral chamber of the
eye-muscle canal through the large orbital opening of that canal,
lying external to the profundus ganglion and dorsal to the ciliares
longi.

The second and larger of the two optic veins, *ov*, issued, in
the large specimen used for illustration, by two main openings
and other smaller ones through a porous portion of the sclerotic
lying immediately below and lateral to the optic perforation.   It
arises from the choroid gland and other structures in the interior
of the eye, one branch being traced through the pigment layer
of the retina to its inner surface.   Having issued from the eye-
ball it runs inward and backward immediately below the opticus,
and enters the orbital opening of the eye-muscle canal, passing
above and in front of the inferior branch of the oculomotorius,
between the rectus superior and the rectus inferior, and above
the efferent pseudobranchial artery.   Its further course was not
traced in the adult.   In 40 mm. and 50 mm. specimens it sepa-
rates near the origins of the internal and inferior recti into two
parts, one of which continues backward below the rectus exter-
nus while the other turns upward in front of that muscle and
joins or is joined by the orbital vein.   The united veins then
run backward, at first below the trigemino-facial ganglion, then
internal to it, and finally above it, the larger portion of the vein
passing through the ganglion.   It then issues through the facial
foramen and joins the jugular vein.   The other portion or branch
of the vein *ov*, the portion continuing backward below the rectus
externus, reaches the external surface of the lining membrane
of the eye-muscle canal and continues inward and backward
along that membrane to the hind end of the hypophysis, where
it turns inward behind that organ, between it and the saccus
vasculosus, and joins the corresponding vein of the opposite side
of the head.   That part of the vein that lies in the orbit is
unquestionably the structure considered by Sagemehl (No. 104,

p. 203) as the homologue of the eyestalk in selachians, and that part of it that lies under the base of the brain, behind the hypophysis, is the "hintere Quer-Anastomose" of Gaupp in Anguis and Lacerta (No. 41, p. 571). The transverse anastomosis in Anguis and Lacerta lies above the hind end of the hypophysis, between it and the base of the brain, but as the hypophysis in Amphibia grows backward from its point of attachment to the gut (No. 71, Figs. 6 and 7), and in Ganoids forward (No. 69, Figs. 13–16), this difference of position of the vein is probably apparent rather than real. In larvae of Acipenser a vessel that has, in the median vertical plane of the head, exactly the position of the vein *vo* in larvae of Amia is considered by von Kupffer (No. 69, p. 11) as the artery of the mandibular arch. It may be such in Amia also, the choroid gland representing the degenerated respiratory organ of that arch, as has been suggested by Dohrn, I believe, though I do not find the reference. The artery disappears in older larvae of Acipenser (No. 69, p. 25).

The base of the brain in Amia, above and in front of the transverse anastomosing vein *vo*, is, in 50 mm. specimens, flat and thin, with a large, median, much-plaited fold projecting into the cavity of the infundibulum. At the middle of its length the lateral edges of the fold almost touch, leaving a slit-like opening into its relatively large interior, which is filled in sections with what is apparently glandular tissue and a dense mass of blood corpuscles. Immediately below and in front of the fold, and hence immediately under and in contact with the base of the brain, is the hypophysis, a flattened oval mass of tissue similar to and continuous with that that fills the fold. It is as wide as the underlying hypophysial fenestra, is everywhere separated from the mouth cavity by the parasphenoid, and is everywhere limited and defined by membrane excepting toward the brain and where it comes in contact with the vein *vo*, at its posterior end and along the posterior portion of its lateral edge. At these places the tissue of the organ is apparently directly continuous with masses of blood corpuscles in the vein. The saccus vasculosus begins behind the vein, and in its posterior portion ramifications of the central cavity give to it a glandular appearance.

In 12 mm. and 14 mm. specimens the median fold in the base of the infundibulum is not yet formed, but there is already a slight thickening of the tissue, indicating its position. The hypophysis has much the appearance that it has at 50 mm., but there is less evidence of glandular formation. The vein *vo* lies entirely behind the organ, instead of behind it and along the posterior portion of its lateral edge, as at 50 mm., and is, as at that age, directly continuous with it. The base of the brain, above the vein, is formed of a single layer of cells, as is also the saccus vasculosus, which is, as in Acipenser (No. 69), a simple sack-like prolongation arising from the lower posterior limit of the base of the infundibulum and running directly backward, its hind edge being wedged under the front end of the medulla. It is narrow in front, widens considerably as it extends backward, and contains a large central cavity, but gives at this age no indication whatever of a glandular structure or of association with such a structure. Immediately dorsal to it there is another posterior prolongation of the infundibular cavity which turns upward in front of the front end of the medulla and ends there blindly. It is the saccus dorsalis infundibuli of von Kupffer in Acipenser (No. 69, p. 19), and from it arise laterally the cavities of the lobi inferiores, as in Acipenser.

In the adult (Fig. 64, Pl. XXXVIII) there is a true lobus infundibulum, not shown by Goronowitsch (No. 50) in his figures, and there is also a large and long saccus vasculosus (*sv*) not shown by him, the part called by him the saccus being the lobus infundibulum or part of that structure. The lobus infundibulum lies between and below the lobi inferiores (*li*), the hypoaria of Gage (No. 40, p. 281), and its posterior portion is trilobate in outline. The median of the three lobes contains the posterior prolongation of the infundibular cavity, described above, and the two lateral lobes contain each a slight lateral prolongation of that cavity.

In the base of the median lobus there is a median slit-like opening which leads directly into the anterior end of the saccus vasculosus, which is here formed of a single layer of cells lined externally by a large venous sinus of a semi-circular or horse-shoe shape. At the hind end of the slit

the lips of the opening project downward as two columns, which
then turn backward and, as large nerve cords or strands, extend
backward along the upper part of the saccus, lying between the
upper ends of the venous sinus, and forming the upper wall of
the central cavity of the structure. Diverticula of this central
cavity are found along nearly its entire length, extending out-
ward into the venous sinus. They are short in the anterior
portion of the organ, but long and much convoluted in the
posterior portion, where the organ becomes a large glandular
structure. Into this glandular formation nervous strands or
fibres extend from the upper, nervous wall of the structure, and
finally, toward the hind end of the organ, the strands and cen-
tral cavity become entirely lost in the general tissue. No
slightest indication of a canal or canals extending into the
mouth cavity was found, and the organ seems to be devoted
solely to a glandular secretion destined to supply the central
cavity of the brain, as Waldschmidt states to be the case in
Polypterus (No. 126, p. 318). That part of the hypophysis
shown by him in sections seems, however, to be the saccus of
Amia, his "feinere Gefüge" indicated by the letter *d* being
in part, at least, the nervous portion of the organ. The nerve
cells shown by Bickford in Calamoichthys (No. 11) seem to
correspond in position to the blood corpuscles of the venous
sinus in Amia. In Amia no nerve cells associated with the
organ could be identified.

The hypophysis, in the adult (*hy*, Fig. 64, Pl. XXXVIII), is
somewhat heart-shaped, viewed from below, the point of the
heart directed forward. On the sides it extends upward along
the sides of the anterior end of the lobus infundibulum, this
part of the lobus containing the cephalic projection of the cen-
tral cavity of the brain described by Gage. In the floor of
this cephalic projection is the median fold found in the base
of the infundibular cavity in larvae. Canals from the hypophy-
sis open into the infundibular cavity and into the hypophysis
nerve strands and fibres are sent from the brain, the nervous
supply being large and important, as is also that of the glandu-
lar part of the saccus.

The vein *vo* of larvae is found in the adult (*vo*, Fig. 64, Pl.

XXXVIII), crossing under the front end of the hypophysis immediately internal to the rectus externus and immediately behind the transverse " Wulst " of cartilage that marks the front end of the eye-muscle canal. From the vein a median branch is sent backward and upward into the hypophysis. Whether this branch communicates directly with the venous sinus along the base and sides of the saccus vasculosus, or passes through the hypophysis to supply that organ, could not be definitely decided. Probably the former. The hypophysis and saccus are thus in all probability both glands secreting, from venous blood, a fluid destined to fill and supply the central cavities of the brain.

In small larvae the hypophysial fenestra is covered externally by the parasphenoid as in the adult, and the hypophysis lies directly upon that bone. The saccus vasculosus lies directly upon the inner surface of the base of the skull, and the transverse ridge on that base, which, in the adult, forms the posterior limit of the eye-muscle canal, does not exist. The processes of the petrosals that form the roof of the canal have also not yet been formed, and the hind end of the saccus vasculosus is separated from the base of the brain by membrane only. The eye-muscle canal of the adult, therefore, does not exist in embryos or young larvae. It is, however, represented by the space in which the vein *vo* traverses the median line of the skull, and by the space along the sides of the hypophysis and saccus in which lie, on each side, the vein *vo* and the rectus externus.

In larvae 50 mm. in length the saccus begins to present a glandular appearance and the processes of the petrosals, represented by cartilages, are well developed ; the hind end of the saccus being separated from the brain by their united edges. The space enclosed by these processes, the future eye-muscle canal, is much larger than the saccus, and the space around that organ is filled with loose tissue such as that described by Waldschmidt in Polypterus and shown by Bickford in Calamoichthys. No trace or indication whatever of a transverse lymph sinus in this space could be found. The eye-muscle canal in all probability does not, therefore, as Sagemehl was led to conclude, owe its origin to such a sinus. It is a space

formed around the saccus vasculosus into which the rectus externus on each side has crept, following the vein *vo* or the nervus abducens, and the orbital opening of the canal is the fused foramen of those two structures, or, as in Amia, of those two foramen and the foramen of the trochlearis, oculomotorius, and profundus as well. That a muscle should enter the canal of its nerve certainly seems more natural than that it should enter one serving simply for the passage of a lymph canal. In Mustelus, where there is no eye-muscle canal, the rectus externus arises (No. 123, p. 81) close to the opening of the canal for the abducens. In larvae of Axolotl (No. 117, p. 16) the side wall of the skull is pierced, behind the optic foramen, by a larger opening, which is in part filled with muscle "Anlagen." Through this opening pass also a blood-vessel and a bundle of nerve fibres.

Although no trace of a transverse lymph sinus was found in the eye-muscle canal, such a sinus was always found, in larvae, extending from orbit to orbit between the internal carotid canals and the optic chiasma, and connecting the periorbital lymph spaces. This sinus is represented in the adult by the interorbital groove already described on page 406, but whether there is a transverse lymph passage at this point in the adult or not I did not determine, as my attention was not called to the point in the earlier stages of this work, and lately I have had no material suitable for its investigation. The groove is, however, certainly the canalis transversus of selachians, and Gegenbaur's description of the canal in Galeus and Squatina (No. 44, p. 76) could almost be taken for that in Amia, the "Sattellehne" in the former being considered as the posterior edge of the transverse Wulst or bar in the latter. The carotid canal or canals, however, lie in selachians in front of instead of behind the canalis transversus. The presence of this latter canal, together with an eye-muscle canal in Amia, shows definitely that the latter is not derived from the former as Gegenbaur (No. 44, p. 78) and Sagemehl (No. 104, p. 217) were led to suggest.

### 3. Nervus Opticus.

The optic nerves (*o*, Figs. 22–27, Pls. XXIV and XXV) in the adult have a well-developed chiasma and a long tractus or pedicle, on each side of the head, connecting the chiasma, along the side of the brain, with the lower anterior end of the optic lobe.

Whether the chiasma is entirely free from the base of the brain or not was not investigated. Each nerve has, as Goronowitsch states (No. 50, p. 443), the appearance of a much-plaited plate, folded upon itself so as to form a cylindrical or oval, nearly closed gutter. The open line between the free edges of the plate lies along the lateral and posterior edge or surface of the nerve, and, proximally, the free edges of the plate open and embrace the tractus. The fibres contained in the upper of these free edges, in part at least, run upward along the anterior edge of the pedicle and do not enter the chiasma or cross to the opposite side of the brain. The same is possibly true of other fibres of the nerve. The fibres along the median and anterior edge of the nerve run directly into the corresponding ones of the nerve of the opposite side.

The nerves in the adult run outward and forward into the extreme hind end of the orbit, with the recti muscles. In young specimens they run outward, nearly at right angles to the axis of the body, a little in front of the place of origin of the superior, inferior, and internal recti, and not far from the middle of the orbit, as in adult selachians. They lie immediately behind the tough, dense pad of tissue already described as extending transversely from orbit to orbit. The position of this pad, and its appearance in sagittal sections of the young, correspond closely to that of the mass of fibres, *a.c.*, described and figured by Balfour and Parker, in Lepidosteus (No. 6, p. 378, and Fig. 45). These fibres in Lepidosteus are considered by them to be nervous, and to be probably equivalent in part to the anterior commissure of the brain.

The brain in the young of Amia entirely fills the cranial cavity; in the adult it occupies a portion only of the middle and hind part of that cavity, the remaining and larger portion

of the cavity being filled by fatty tissue (No. 105).   This marked
change in the relative size of the cranial cavity and brain takes
place mostly in post-larval stages, the unequal development of
skull and brain being most marked anteriorly.   By it the floor
of the anterior part of the skull, and its perforations, are carried
forward relatively to the brain, and the optic nerves at their
origin are, on the contrary, pulled relatively backward ; so that,
while in the young they issue in front of the canalis transver-
sus and internal carotid canals, they lie in the adult either
directly above the internal openings of the carotid canals or
even behind them.   The olfactory branches of the carotids
must, therefore, first run backward under the optic nerves and
then forward above them to reach their destination.


### 4. Nervus and Lobus Olfactorius.

The lobus olfactorius, Goronowitsch, or bulbus olfactorius,
Sagemehl, is in the adult Amia a well-rounded mass (*lol,* Figs.
25 and 64, Pls. XXV and XXXVIII), lying close to the middle
line of the head, on either side, immediately above the trans-
verse bar of cartilage marking the front end of the eye-muscle
canal, immediately in front of the optic chiasma, and imme-
diately behind the pad of tissue behind and under which the
nervus opticus enters the orbit.   Its diameter is about one and
one half times that of the nervus olfactorius, and it contains, as
Goronowitsch has stated, a small lateral extension or diverticu-
lum of the ventricle of the fore-brain.   On its upper surface,
in well-preserved specimens, there are three slight furrows, one
running medianward and backward from the lateral surface of
the lobus, near its anterior end, one laterally and backward
from the median surface of the lobus, and the third directly
backward along the median surface, beginning at the point
where the second furrow begins, near the root of the nervus.
These three furrows thus correspond fairly well with the
three furrows shown by Burckhardt on the so-called tuber-
culum olfactorium of Protopterus (No. 16, Figs. 3 and 4).
The first two enclose a slightly marked anterior region of
the lobus.

The lobus lies partly in front of, and partly under, the median two thirds of the corresponding hemisphere of the fore-brain, the upper and lateral surfaces of which, in their posterior portion, show well-marked convolutions, much stronger than Gage's figures and reference to them (No. 40, p. 296) would seem to indicate.   Ventrally the lobus extends further backward than on its dorsal surface, and there is no line of demarcation between it and the base of the fore-brain beyond it, as Gage has already stated (No. 40, p. 297).   On the sides and dorsal surface, on the contrary, it is sharply limited and defined by a deep fissure or furrow, which, on the median side of the lobus, lies just in front of the lamina terminalis, and separates that structure, externally, from the lobus.   The fissure, therefore, at this point is the fissura prima.   In the remaining portion of its course it seems to be the sulcus olfactorius of Burckhardt in Protopterus, but it is evident to the most superficial observation that the fissure and sulcus of Amia have not the position that they have in Protopterus, and that the lobus of Amia contains elements not found in the tuberculum of Protopterus.   Lee's conclusion (No. 72, p. 5) that the latter structure is in reality a lobus, strictly comparable to the lobus of other animals, may therefore be questioned.   It seems more probable that it is a bulbus partly separated from the fore-brain.

In embryos of Amia the sulcus olfactorius extends across the ventral surface of the brain, and a continuous groove is thus formed, running backward and outward across the dorsal surface and backward and downward across the lateral surface of the brain, then across its ventral surface, and then forward and upward along its median surface between the lobus and the lamina terminalis.

The olfactory diverticulum is, in 12 mm. and 14 mm. specimens, short and broad, extends outward and forward from the median or median and upper portion of the basal ganglion of Goronowitsch, and has at its anterior and lower portion a pointed prolongation, apparently the true recessus olfactorius, extending forward into the lobus.   In its hinder portion, exactly where Studnička (No. 122) describes and figures the cornu posterior of the ventriculus lateralis in Petromyzon, it has a sharply

marked angle, or even a slight pointed recessus, directed out-
ward or outward and backward. The whole diverticulum thus
seems to correspond to the ventriculus lateralis of Studnička
in Petromyzon, with the posterior horn rudimentary, instead of
wanting, as he states it to be in ganoids and teleosts. The
lobus in Amia thus seems to correspond internally, as well as
externally, to the lobus and something more of Petromyzon,
Dipnoi, and Amphibia, — to the prosencephalon, probably, or to
part of it, as Gage has suggested (No. 40, p. 297). The origin
of olfactory nerve fibres at the hind end of the prosencephalon
in reptiles (Edinger, quoted by Gage, No. 40, p. 262) would
seem to support this supposition.

Between the olfactory lobes, and extending downward and
backward to the commissure *cm* of Gage (No. 40, p. 282), that
is, for the full length of the lamina terminalis, there is in the
bottom of the ventricle of the fore-brain a long and narrow
median prolongation, directed forward and downward. At the
upper, anterior end of this median diverticulum there is a slight
recessus directed forward. In the lamina terminalis at this
point there is no process properly so called, the recessus being
apparently formed, as sagittal sections show, by a thinning of
the substance of the lamina. The lamina here is slightly con-
vex externally, but less so than in its ventral portion. In trans-
verse sections the anterior end of the recessus is often cut so
as to show a slit-like lumen, surrounded or nearly surrounded
by an egg-shaped layer of cells, the small end of the egg directed
upward and wedged in between the lobi olfactorii. The process,
if it can be called such, is the lobus olfactorius impar, and its
cavity the recessus olfactorius impar of von Kupffer (No. 69),
or, as it has been later named by Burckhardt (No. 17), the reces-
sus interolfactorius or recessus neuropricus.

The nervus olfactorius (*ol*, Figs. 21, 26, and 64, Pls. XXIV,
XXV, and XXXVIII) arises from the front end of the lobus,
and, in the adult, is as long as, or even one half again as long
as, the brain. It runs directly forward, for fully two thirds of
its course in the cranial cavity. It then enters the olfactory
canal (*olc*) and, turning slightly outward, issues by the olfactory
foramen (*olfr*, Figs. 8 and 9, Pl. XXI) on the floor of the olfac-

tory pit (*olp*) beneath the nasal sack.   Sagemehl says of it that
it consists of from seven to ten loosely connected bundles of
fibres, between which there is to all appearance no interchange
of fibres.   I find it to consist of three principal bundles, two
large ones, one on each side of the nerve, united dorsally for
about one half their length, and a much smaller ventro-median
one (Fig. 64, Pl. XXXVIII), lying in the open triangular space
between the ventral ends of the other two.   Between the two
lateral bundles there is dorsally, for one half their length,
frequent interchange of fibres, and the two bundles break up
distally into several bundles.   Between the smaller ventro-
median bundle and the lateral ones there is also an inter-
change of fibres, but it is much less important than that
between the larger bundles.   This median ventral bundle, in
whole or in part, seems to be the "hitherto undescribed cranial
nerve" of Pinkus in Protopterus (No. 88).

In the adult the ventro-median bundle of the olfactorius is
distributed, so far as macroscopic observation can show, to the
nasal epithelium at the extreme anterior end of the nose.   In
embryos such is also its apparent distribution, though I have
never been able in my preparations to trace it definitely to that
tissue.   During the larger part of its course, in 30 mm. to
50 mm. specimens, it is easily distinguished from the lateral
bundles by the presence of the large round cells which Pinkus
describes in Protopterus.   Near the anterior end of the ol-
factorius, however, these cells disappear, and the fibres into
which the bundle breaks up cannot be distinguished from the
other terminal branches of the main nerve.   All the fibres
of the bundle apparently enter the nasal tissue, and neither
in embryos nor in the adult do I find such a mass of cells
at the end of the nose as Pinkus describes in Protopterus.
They may possibly represent in Protopterus a rudimentary
condition of the intermaxillary gland described by Gage in
Diemyctylus.

Between the ventro-median bundle of the olfactorius and the
lateral bundles there is, in large larvae as in the adult, frequent
interchange of fibres.   Toward the root of the nerve in such
larvae the large round cells that characterize the bundle dis-

appear, as they do toward its anterior end, and the fibres of the
bundle enter, in large part, with the fibres of the lateral bundles
into the anterior end of the lobus.    A very small bundle, how-
ever, continues backward outside the brain membranes to the
hind end of the lobus.    There in all my series of sections but
one it was lost.    In that one series what seemed to be the nerve
entered the base of the brain lateral to the anterior end of the
recessus interolfactorius, that is, in Amia, in front of the lamina
terminalis, or sulcus olfactorius, and hence into the lobus olfac-
torius and not into the fore-brain proper.    This root of the
olfactorius, or nerve if it be a separate nerve, is found in the
adult in the same position as in the young, but in none of my
specimens could I trace it into the brain or find where or how
it ended proximally.    It becomes delicate and disappears in the
membranes of the brain, broken doubtless in preparation.    Dis-
tally it could be separated from the ventro-median bundle of the
olfactorius further forward than was possible in sections of lar-
vae, but it finally ran into that bundle, as it did in the young,
and could not be separated from it.

In 12 mm. larvae the large cells found in the ventro-median
bundle of older larvae are collected into a compact mass lying
on the ventral and median surface of the olfactorius at about
the middle of its length.    The mass has a well-defined limiting
membrane, and forms a knob-like structure on the outer surface
of the olfactorius, the nerve and mass much resembling in
arrangement and general appearance the nervus oculomotorius
and associated ciliary ganglion.    The cells of the mass are
exactly similar in appearance to those of the ciliary ganglion,
and it may therefore be, as that ganglion is said to be in whole
or in part, a sympathetic ganglion, possibly the sphenopalatinum
of higher vertebrates, though I found no connection whatever
between it and the nervus trigeminus.    The main olfactorius,
at this age, is often distinctly double at its base, that is, it arises
by two roots, one of which, in sections, is lateral and dorsal and
the other median and ventral.    With the median of the two
roots the nerve or bundle "n" of Pinkus is associated.    In
the adult the separation of the two roots is not distinct.    In
sagittal sections of the adult brain a triangular space is, how-

ever, found near the median ventral surface of the olfactorius indicating a partial separation into two bundles.

Wiedersheim (No. 128, p. 256) in a figure of the brain of Rana esculenta shows two roots of the olfactorius, but gives no special description of them in the text. Lee (No. 72) describes and figures a similar arrangement in Spelerpes fuscus, and states, on the authority of Miklucho Maclay and von Rohon, that the olfactorius is frequently found double in selachians. Whether the two roots so described are the two main portions of the root in Amia, or one of them the small root "n" and the other the main double root, I am unable to judge.

No ganglion cells other than those described in the ventro-median bundle were found in any part of the olfactorius in any of the specimens examined. Whether these cells in the adult and the ganglionic mass in young larvae are the olfactory ganglion of Beard (No. 9, pp. 878 and 884) or not I am unable to state, not having seen his complete and later work. I am also unable to judge whether they are, or are not, a remnant of the ganglionic mass from which the nervus olfactorius is said by Holm (No. 58) and Chiarugi (No. 18) to develop, my investigations being confined to embryos already too advanced for the purpose.

The olfactory canal in Amia (*olc*) is considered by Sagemehl (No. 104, p. 217), it seems to me erroneously, as simply an anterior extension of the cranial cavity. It is separated from the anterior end of the orbit by a thin wall of cartilage through which, at about the middle of the canal, from its side and bottom, there is a large opening (*onfn*, Fig. 25, Pl. XXV) leading into the extreme front end of the upper part of the orbit. This opening in Amia seems to have entirely escaped Sagemehl's notice, although in Lepidosteus, in the Characinidae, and in teleosts in general it and its relation to the nervus olfactorius are described at considerable length by him (No. 104, p. 219, and No. 106, pp. 68 and 72). In Catostomus he did not find it, and he says of it that it has no physiological importance whatever (No. 107, p. 566). It lies in Amia internal to and under the antorbital process, directly above the obliquus superior near its origin, and through it a large venous vessel passes from the olfactory pit

into the orbit. The opening, however, is much larger than is
necessary simply for the passage of the vessel, and it therefore
forms a true fenestration of the side wall of the orbit, compara-
ble in every respect to the larger and more important one
described by Sagemehl in other fishes. Sagemehl gives no
name to the opening, referring to it in the text as the fenestra-
tion ff, letters which do not appear in the figures. I have called
it the orbito-nasal opening or fenestra. That part of the olfac-
tory canal that lies in front of it is formed by the fusion of
two canals, the olfactory canal proper and another canal, quite
probably the homologue of the orbito-nasal canal of selachians
(No. 44, p. 73). That canal in selachians is traversed by what
from Gegenbaur's description is apparently, and in Callorhyncus
(No. 116, Fig. 1, and No. 59, p. 265) and Mustelus (No. 123,
Fig. 1), judging from the figures alone, by what is certainly the
ramus ophthalmicus profundus; but neither Gegenbaur, Stan-
nius, Hubrecht, nor Tiesing mentions or shows the passage of a
vein with the nerve. In Torpedo and Raja, Tiesing (No. 123,
Figs. 2 and 3) shows the profundus passing with the super-
ficialis through what Gegenbaur calls in the former the pre-
orbital incisur and in the latter the preorbital canal. What
passes through the orbito-nasal canal, the orbital opening of
which is shown by Tiesing in Torpedo but not in Raja, is not
indicated. In Amia there is no ophthalmicus profundus, but
in Polypterus that nerve is found and it passes (No. 93, p. 394)
from the orbit to the cartilaginous nasal capsule through a
" canal in the ectethmoid bone, which it occupies together with
the ophthalmicus superficialis, the obliquus superior muscle and
a considerable vein." That there must be some canal or
channel in elasmobranchs for the passage of this vein so large
and important in Amia, and that it is probably the orbito-nasal
canal, seems sufficiently evident to warrant the use I have made
of the name.

The development of the olfactory nerves and their canals in
Amia is as follows. In specimens less than 10 mm. in length
the preorbital processes have not yet been fully formed, and the
orbit on each side is directly continuous with the olfactory pit ;
the two regions being separated by an elevation, the beginning

of the future process, through which there is a large open channel which, owing to the shape of the base of the brain and basis cranii, runs decidedly upward as well as forward, almost at right angles to the olfactory nerve. Following this stage the outer edge of the open channel near its posterior end grows upward, then inward, and then inward and downward toward the inner edge of the channel with which, when the larvae is about 10 mm. in length, it has coalesced, forming a narrow arched bridge over the posterior end of the channel. Under the bridge thus formed from its inner wall, the former inner edge of the open channel, the oblique muscles have their origin, and through the large opening or canal, formed by the bridge, a large venous vessel passes from the nasal to the orbital regions of the skull. Across the anterior, open end of the canal the olfactorius runs, nearly at right angles to it. The canal and the open channel in front of it therefore represent the extreme, upper, anterior end of the orbit, the orbito-nasal fenestra, and that part of the olfactory canal that in the adult lies in front of that fenestra. In later stages the preorbital process thickens, grows upward along the side of the brain and then backward, forming the beginning of the roof of the orbit. This is the condition in larvae 12 mm. and 14 mm. in length. The lobus olfactorius at these stages lies practically in front of the preorbital process, for the transverse vertical sections that, in 12 mm. specimens, pass through the process, pass also through or behind the recessus interolfactorius. The side wall of the skull in front of the process has at these ages not yet been formed, but, immediately in front of the lobi, a vertical transverse ridge, or a median pier, marks the anterior end of the cranial cavity. The olfactory nerve, on each side, arises from the lower, outer, anterior surface of the lobus, and runs downward, outward, and slightly forward along the shelving lateral edge of the basis cranii.

In larvae 20 mm. in length the preorbital process on each side has grown forward, enclosing the orbito-nasal canal, and that canal now opens on the shelving olfactory region of the basis cranii under or immediately in front of the anterior end of the lobus olfactorius. Where it opens in front of the lobus a true olfactory foramen is formed or indicated, lying between the

opening of the orbito-nasal canal and the anterior end of the lobus. The orbito-nasal canal in such specimens, at its anterior end, turns upward almost at right angles to the olfactory nerve, and immediately under and lateral to that nerve. In still later stages the olfactory nerve is enclosed above and in front of the opening of the orbito-nasal canal, and the anterior part of the olfactory canal of the adult is formed. The lobus olfactorius during the same period recedes, with the brain, from the anterior end of the cranial cavity, and the long intracranial portion of the nervus olfactorius is formed. There is never the slightest indication of a tractus olfactorius.

The development of the olfactory parts in Amia thus agrees exactly with that in the trout, as described by Sagemehl (No. 106, p. 76), and in the adult Amia, as well as in embryos, its nervus olfactorius lies in a certain limited part of its course exposed in the orbit. Amia, therefore, in this respect belongs definitely to Sagemehl's third or teleostean type, and it and all ganoids and teleosts are in all probability derived directly from his cyclostome type and not from the selachian type, as he concludes (No. 104, p. 219). In those teleosts that have a tractus olfactorius I agree with Lee that they owe it, as do the elasmobranchs, to special mechanical conditions that have tended to hold the anterior end of the lobus in its embryonic position near the nasal membrane. The separate development of the orbito-nasal and olfactory canals, instead of their fusion as in Amia, would seem to be either among those conditions or a secondary arrangement resulting from them.

### 5. Muscles of the Eye, Nervus Oculomotorius, Nervus Trochlearis, and Nervus Abducens.

The obliqui muscles (*os* and *oi*, Figs. 21–27, Pls. XXIV and XXV) arise, in the adult, close together from the side wall of the orbit at its extreme front end immediately below the orbito-nasal fenestra. A slight depression, occupying the space between that fenestra and the floor of the orbit, marks the place of origin, and is unquestionably the small beginning of the anterior eye-muscle canal of certain teleosts (No. 107, pp. 563 and 566). In

larvae from 12 mm. in length upward the muscles have, as already described, a similar origin, arising immediately below, or in the younger larvae immediately posterior to, the fenestra.

The rectus externus (*re*) arises, as already stated, near the hind end of the eye-muscle canal. The three other recti muscles (*rs, rit,* and *rif*) arise from the lateral face of the basisphenoid, and hence at the external opening of, or partly within, the orbital opening of the eye-muscle canal. In larvae the internus has the most anterior origin, the inferior next, and the superior the most posterior. The rectus inferior is said by Sagemehl (No. 106, p. 87) to arise in the Characinidae in the anterior portion of the eye-muscle canal.

The obliquus superior is innervated by the nervus trochlearis (*tr*), which pierces the lining membrane of the upper, lateral chamber of the eye-muscle canal, and enters that chamber in front of and slightly above the main trigeminal root and the root of the ophthalmicus profundus (Fig. 26, Pl. XXV). It lies, while in the chamber, internal to the ophthalmic branches of the facialis and trigeminus, and leaves it through the upper end of the large orbital opening of the canal. It then runs forward along the inner wall of the orbit, dorsal to all the recti muscles, and separating into two nearly equal parts, each of which again separates into two parts, it enters the obliquus superior on its upper surface, near the middle of its length. In its course through the orbit it lies below and internal to the ophthalmic branches of the facialis and trigeminus, above and internal to the profundus ganglion, and above the oculomotorius. No branches of it except those entering the muscle were found, nor were there any communicating or connecting branches to or from any of the other nerves or ganglia. The same is true of the nerve in the smallest larvae examined ; the nerve, however, often passes in sections so close to the portio ophthalmici profundi that it is impossible to say that there is there no interchange of fibres. No ganglion or ganglion cells were found at any point in the nerve, either in the adult or in larvae.

The rectus externus is innervated by the nervus abducens (*ab*), which runs forward and then forward and outward in the

cranial cavity, a comparatively long distance from its point of origin on the under surface of the brain, to the front edge of the horizontal process of the petrosal. There, immediately behind and internal to the place of exit of the main facial nerve, it pierces the membranous roof of the eye-muscle canal, and lying in a semi-circular foramen (*abfr*, Fig. 11, Pl. XXI), at the angle formed by the horizontal process of the petrosal and the process of that bone that forms the front wall of the utricular fossa, enters the eye-muscle canal. Turning downward it separates into two main parts which, separating again into two or more parts, enter the externus while that muscle is still inside the canal.

The rectus externus is sometimes double through part of its length, one part lying, relatively to the eye, directly external to the other.

Schneider (No. 112, p. 10), after describing the origin and intracranial course of the abducens in Acipenser, says that that nerve in all ganoids enters the orbit, as it does in Acipenser, at the front under corner of the Gasserian ganglion, and that, in its further course, it lies in all, Amia included, under the ophthalmicus trigemini, but over all the other branches that arise from the trigeminal ganglion and have a forward course. Just what is meant by "over all the other branches" is not entirely evident from this statement taken alone, but in his Fig. 5*a* he shows the arrangement of all the nerves concerned in Lepidosteus. In that figure the abducens, after leaving the inner, anterior surface of the Gasserian ganglion, crosses, from within outward, under the r. ophthalmicus superficialis trigemini and over, that is across the upper surface of, the truncus maxillaris trigemini and r. buccalis facialis. It cannot, therefore, be doubted that this is the further course ascribed to the abducens in all ganoids. This, it will be seen, is markedly at variance with what is found in embryos and in the adult of Amia, and I can in no way account either for his observation or his statement. As I differ as markedly and unaccountably from him in certain other of his statements, I have not been able to accept any of his results without much reservation.

The rectus superior, rectus inferior, and rectus internus are all innervated by the nervus oculomotorius (*ocm*). This nerve pierces the lining membrane of the cranial cavity opposite and above the optic chiasma, and enters the orbit through the upper end of the large orbital opening of the eye-muscle canal. Lying dorsal to all the recti muscles at their origin, it separates into a superior and an inferior portion. The superior portion (*ocms*), dividing dichotomously, supplies the rectus superior alone, one branch going to the upper and the other to the under surface of the muscle, each branch dividing dichotomously before reaching the muscle. The inferior, or main portion (*ocmi*) of the nerve runs forward and downward between the rectus superior and the rectus externus, and behind or external to the rectus inferior. It then turns forward below the rectus inferior and rectus internus, and sends two branches, arising close together, if not from a common root, one to the upper and the other to the under surface of the former muscle, and two branches, arising some distance apart, each of them double, to the latter. The nerve then separates into two parts, which again divide dichotomously and enter and end in the obliquus inferior, entering the muscle on its under and upper surfaces.

The inferior and internal recti were, in the specimen used for illustration, connected about midway of their length by two or three muscle strands, which arose distally in each muscle — that is, toward their insertions — and formed a festoon connecting them. In another specimen the two muscles arose as a single muscle, which soon separated into two parts.

**6. Review and Comparison of the Muscles and Nerves of the Eye.**

In Amia the obliquus superior is innervated by the trochlearis, the rectus externus by the abducens, the rectus superior by the superior branch of the oculomotorius, and the rectus inferior, rectus internus, and obliquus inferior, in the order named, by the inferior branch of the oculomotorius. The inferior branch of the oculomotorius runs forward and downward behind and below the rectus superior, above and in front

of the rectus externus, behind and below the rectus inferior, and below the rectus internus.

In all other ganoids, both chondrostean and holostean, the same arrangement is found ; at least I so interpret the descriptions given by van Wijhe (No. 129) of the muscles and nerves in Acipenser sturio, Polypterus bichir, and Lepidosteus osseus, and these three fishes may be assumed to represent all ganoids. Schneider says (No. 112, p. 12) that in Acipenser, Scaphirhynchus, and Amia the inferior branch of the oculomotorius, after passing between the rectus superior and rectus externus, continues forward in the "Kegel" formed by the recti muscles. This would seem to mean that the oculomotorius runs forward above the rectus inferior and rectus internus, but in his Fig. 1, showing the arrangement in Acipenser, the nerve passes below the rectus inferior, as it does in van Wijhe's Fig. 3 of the same fish. In Amia my work leaves no doubt as to the arrangement ; the nerve runs below the two muscles.

The same arrangement of the nerves and muscles of the eye is probably found in all teleosts. The obliquus superior and rectus externus are certainly innervated, respectively, by the trochlearis and abducens, as in ganoids, and the remaining muscles by the oculomotorius, but that this nerve has, in all teleosts, the same distribution and the same relation to the muscles, as in ganoids, cannot be definitely determined from the literature. Most references simply say that the four muscles are innervated in the usual or well-known way. In Amiurus, where, according to Wright (No. 132, p. 365), the inferior branch of the oculomotorius runs *over* the inferior and internal recti, it was found in its usual position *under* those muscles in the one specimen that I examined.

In the Chondropterygii, or Elasmobranchii, a somewhat different arrangement is found. In these fishes the superior portion of the oculomotorius supplies the rectus internus as well as the rectus superior, the inferior portion of the nerve supplying only the rectus inferior and the obliquus inferior. In Holocephala the rectus internus arises near the front end of the orbit, at some distance from the other recti muscles, and the nerve supplying it runs forward above the opticus

(No. 113, Fig. 12, and No. 116, Fig. 1). In Plagiostomata the internus arises with the other recti at the hind end of the orbit, but both muscle and nerve lie above the opticus, while in ganoids and teleosts they both lie below it. In the young of Scyllium the rectus internus is innervated by an anterior branch of the superior division of the oculomotorius (No. 77, p. 88). In the adult Scyllium this anterior branch may become somewhat widely separated from the branch to the superior rectus, two superior branches thus arising from the main nerve, — the proximal one of the two going to the rectus internus (No. 113, Fig. 10). In Mustelus laevis the oculomotorius, after sending branches to the internal and superior recti,
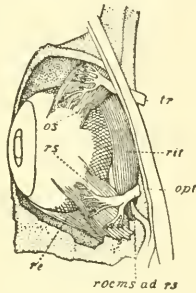


CUT 1. — Top view of left eyeball of Galeus canis. *opt*, ophthalmicus superficialis trigemini ; *os*, obliquus superior ; *re*, rectus externus ; *rit*, rectus internus ; *rocms ad rs*, branch of superior division of oculomotorius to rectus superior ; *rs*, rectus superior ; *tr*, trochlearis.

perforates, according to Schwalbe (No. 113, p. 185), the rectus internus near its upper posterior edge, while according to Tiesing (No. 123, p. 79) it perforates the rectus superior. It then runs downward behind the rectus inferior, and forward below that muscle, and ends in the obliquus inferior. In Galeus canis I find a similar arrangement (Cut 1). In Scyllium catulus (No. 113, p. 187) the inferior division of the oculomotorius penetrates the rectus superior near its hind edge, and in Raja batis (No. 113, p. 188, and No. 123, p. 80) it runs down behind that muscle, in both cases turning forward below the rectus inferior. In Chimaera, judging from Schwalbe's figure alone (No. 113, Fig. 12), it runs down in front of the rectus superior, while in Callorhynchus (No. 116, Fig. 1) it apparently lies behind that muscle.

These different relations of the oculomotorius to the internal and superior recti, in elasmobranchs, are due to and are caused by the gradual shifting from before backward of the origins of all the recti muscles, and also of the place of exit of the oculo-motorius from the cranium. As a result of this shifting the internal and superior recti, at their origins, either traverse, or are traversed by, the issuing nerve. The nerve does not traverse the inferior rectus also, by which it may, as in Galeus canis (Cut 2), be pulled backward and much diverted from its course, simply because, crossing the muscle as it does midway in its length, such a process is, or at least would seem to be, impossible without destroying or disturbing functionally the muscle.
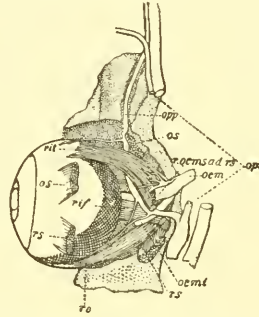


Cut 2. — Same as Cut 1 with rectus superior and obliquus superior muscles removed.  *ocm*, oculo-motorius; *ocmi*, inferior branch of oculomotorius; *opp*, ophthalmicus profundus trigemini; *rif*, rectus inferior.

In Petromyzon (No. 37, pp. 38, 58, and 70) the trochlearis innervates the superior oblique, which arises from the mem-branous hind wall of the orbit, and the abducens innervates the inferior as well as the external rectus. The trochlearis, as it issues from the cranium, lies dorsal to the abducens and apparently dorsal also to the main trunk of the trigeminus, for Fürbringer, speaking of the roots of the three nerves and their relations to each other as they issue from the cranium, says of the trochlearis, that it is "der am oberflächlichsten gelegene." If such be its position it must, as it issues from the cranium, run backward and outward above and across the other two nerves. It must therefore lie dorsal to the r. ophthalmicus trigemini, a position confirmed by Ahlborn (No. 1, p. 21). This ophthalmic nerve in Petromyzon does not, therefore, probably

contain any fibres that are homologous with those that form
the r. ophthalmicus superficialis trigemini of Amia, teleosts,
and selachians, for in all these latter fishes the trochlearis lies
under the nerve.    It is more probably the homologue of the r.
ophthalmicus profundus of other fishes, that nerve, when found,
lying always under the trochlearis, as will be shown later.

The abducens in Petromyzon arises (Ahlborn) close in front
of the ventral, motor root of the trigeminus, not ventrally near
the median line as in other fishes, runs upward inside the cranial
wall, and issues (Fürbringer) dorsal to — but, as shown in his
Fig. 19, behind — the trigeminus.    It must, therefore, acquire
this position by crossing first the ventral and then the lateral
or posterior surface of the trigeminus while the two nerves are
still inside the cranium.    That it runs upward internal to, that
is anterior to, the trigeminus, and then crosses the upper sur-
face of that nerve from before backward, seems wholly improb-
able.    The descriptions, however, are not concise.    It lies below
the trochlearis, sends a short branch to the rectus externus, and
then continues downward and outward behind that muscle to
the rectus inferior, in which it ends.    The rectus superior is
innervated by a posterior branch of the oculomotorius, sent out-
ward, according to Fürbringer, below the ramus ophthalmicus,
a wholly unaccountable position if the ramus ophthalmicus be
the ophthalmicus profundus of other fishes, as its relation to the
nervus trochlearis seems to indicate.    The rest of the oculomo-
torius, the inferior division of the nerve, runs forward under
the opticus and there separates into several branches, all of
which enter the obliquus inferior, that muscle being called by
Fürbringer the obliquus anterior.    The branches all enter the
muscle on its outer surface, according to Fürbringer's text, but
on its upper or inner surface, as his Fig. 19, giving the arrange-
ment in Petromyzon marinus, plainly shows.    Of the several
branches that enter the obliquus, two pass through it to supply
the rectus internus, which lies immediately below the obliquus,
and arises not far from it near the front end of the orbit.

In Dipnoi the muscles of the eye are small and the obliqui
are said to be wanting in certain species.    In Protopterus
annectens, where all six muscles are found (No. 88, p. 281),

they are innervated as in elasmobranchs, that is, the rectus
superior and rectus internus both by the dorsal or superior
branch of the oculomotorius.

In Amphibia the muscles of the eye are innervated, accord-
ing to Schwalbe, in Urodela, as they are in elasmobranchs, and
in Anura, as they are in ganoids and teleosts (No. 113, pp. 196,
197, and 200). In Salamandra the trochlearis and the branch
of the oculomotorius to the rectus superior are often more or
less completely fused with the r. nasalis trigemini, and by
Fischer (quoted by Schwalbe) they are described as branches
of that nerve. The branch of the oculomotorius to the rectus
internus is always independent, and lies above the r. nasalis,
according to Schwalbe. According to von Plessen and Rabino-
vicz (No. 92) it would seem to lie in Salamandra maculata, below
that nerve. By these authors both oblique muscles in Sala-
mandra are said to be innervated by branches of the ramus
nasalis, and they failed entirely to find a nervus trochlearis. In
Amblystoma the oculomotorius and all its branches are shown
by Herrick (No. 55) external to, and hence above, the nervus
opticus and ramus ophthalmicus trigemini, in a side view of
the head, but below both these nerves in a top view. In one
of the figures the terminal branch of the nerve innervates the
rectus inferior and obliquus inferior, while in the other the
obliquus is innervated by what seems to be the second branch
of the nerve, this branch innervating also the rectus internus.
From the descriptions of the nerves it cannot be told which
of the figures is correct, or if either of them is. Schwalbe's
descriptions seem, therefore, to be the most reliable and must
be relied upon for the whole class.

In Apoda an arrangement similar to that in Urodela is indi-
cated in Fig. 72, Tab. XIX of the Sarasins, thus confirming
what the Sarasins conclude from other reasons ; that is, that
Apoda is a subdivision of Salamandrina (No. 108, p. 29).

In both Urodela and Anura a small muscle is found, the
retractor bulbi, not found in fishes. Schwalbe says that this
muscle in Salamandra is innervated by a branch of the inferior
division of the oculomotorius, given off before the branch to
the rectus inferior (No. 113, p. 198). In the sheep, calf, and

dog, where the muscle is also found, he finds the lower portion of it innervated by a similar branch given off close to the branch to the rectus inferior (No. 113, pp. 218 and 220). This innervation of the retractor bulbi does not agree with that given by others. Wiedersheim says (No. 128, p. 339) that it is innervated by the abducens. Huxley says (No. 60, pp. 67 and 74) that in all vertebrates " the muscles of the nictitating membrane, and the retractor bulbi, or musculus choanoides, when such muscles exist," are innervated by the abducens, and that the musculus choanoides when it exists " lies within the four recti, and is attached to the circumference of the posterior moiety of the ball of the eye." Mivart also (No. 81), in the cat, gives the innervation of the choanoid muscle by the abducens, and Hoffmann (No. 56, p. 209) says that in Lacerta the rectus externus and retractor bulbi develop from the same somite. Schwalbe's statement may, therefore, be considered as due to some error in observation. It, however, finds some support by what Herrick finds in Amblystoma, for after stating (No. 55, p. 200) that the abducens separates peripherally into two branches, one of which goes to the retractor bulbi, he adds, " Although the two branches of the sixth nerve are indistinguishably blended when they leave the Gasserian ganglion, yet it would seem that the branch destined for the m. retractor bulbi ought not to be assigned to this nerve, but to the trigeminus." Herrick further states that, in Salamandra maculata the retractor is innervated by the oculomotorius, the statement being probably made on the authority of von Plessen and Rabinovicz (No. 92, p. 7).

In reptiles, birds, and higher vertebrates the six muscles of the eyeball are, according to Schwalbe, innervated as in Anura, for in speaking of the latter he says: "Das Innervationsschema der Augenmuskeln der Anuren weicht in nichts von dem bekannten der höheren Wirbelthiere ab." In these higher forms there may be a musculus choanoides, as already stated, and there is usually a levator palpebrae superioris, innervated, according to Schwalbe, when it exists, by a branch of the superior division of the oculomotorius.

It is thus evident that the muscles of the eye in vertebrates, although performing similar functions, are not homologous

structures. It is also evident that this want of homology is found entirely in those muscles that are innervated by the oculomotorius and abducens, that is, in those muscles that are said to arise in all the Gnathostomata from van Wijhe's first and third somites. As the several muscles that operate the eyeball have been developed from the muscle masses in these two somites, different arrangements have arisen, which, if it be assumed that reversions have not taken place, must indicate totally distinct and different lines of descent. The several arrangements characteristic of these lines may have arisen independently and simultaneously from the undifferentiated condition, or some one or two primary arrangements may have first so arisen, and from them, by later development, the existing arrangements. This latter method of development seeming the more probable, I have attempted to construct a prototype from which existing arrangements might have been derived.

The superior rectus in ganoids, teleosts, and Anura has unquestionably been developed from a muscle mass which has, in Elasmobranchii, Dipnoi, and Urodela, given origin to two muscles, the rectus superior and rectus internus. As the rectus internus in Holocephala arises near the front end of the orbit, at some distance from the place of origin of the rectus superior, it may be safely assumed that it represents a condition more primitive than that found in the Plagiostomata, where the two muscles arise together at the hind end of the orbit; for a muscle once having arisen with the other recti at the hind end of the orbit, would not, in all probability, have changed that place of origin for the apparently less advantageous one near the front end of it. It may, therefore, be assumed that the rectus superior of ganoids was formed by the fusion of the two muscles of elasmobranchs, rather than that it, by splitting, gave origin to the latter. The elasmobranch type would, therefore, be more primitive than the ganoid type, and, as a consequence, the internal and inferior recti of the latter would be formed by the splitting of the inferior rectus of the former, instead of by their fusion giving origin to it. We therefore conclude that in the prototype of all vertebrates, the Cyclostomata excepted, the superior branch of the oculomotorius inner-

vated two muscles, a rectus superior and a rectus internus, and that the inferior branch of the nerve also innervated two muscles, a rectus inferior and an obliquus inferior.

Turning now to the Cyclostomata we find that in Petromyzon the rectus internus arises near the front end of the orbit, and is innervated by branches of the oculomotorius which first pass through the obliquus inferior near the middle of its length. This manner of innervation renders it highly probable that the muscle could not have been derived from, or have given origin to, any of the recti muscles known in other fishes, for even if the muscle should acquire an origin with the other recti, the inferior branch of the oculomotorius would still lie wholly above it, a condition not known, so far as I can find, in any other animal. The position and innervation of the muscle both indicate its derivation, in Petromyzon, from the outer, under surface of a muscle or muscle mass giving origin to it and to the obliquus inferior. The embryonic condition of such a muscle mass is described by von Kupffer (No. 70) in Ammocoetes. It is said by him to be the lateral and lower portion of the subcerebral part of the trabecular arch, that arch being the first, or most anterior, of the visceral arches. This same muscle, or muscle mass, must, in the prototype of the Gnathostomata, have given origin to the obliquus inferior and rectus inferior, the two muscles innervated by the inferior branch of the oculomotorius. The outer muscle of the two that so develop in the Gnathostomata must, however, have become the obliquus, instead of the inner one, as in Petromyzon ; and the nerve innervating the two muscles, in the Gnathostomata, must have run downward along the posterior edge of the mass, instead of through it, as in Petromyzon. The inner muscle of the two, the inferior rectus, as it separated from its companion and acquired an origin with the other recti or the hind end of the orbit, would then have pushed the inferior branch of the oculomotorius before it, keeping it always posterior to and below it, as it is always found.

That the inferior rectus, in the Gnathostomata and higher vertebrates, arises as above supposed, is strongly indicated by the early development of the muscles of the eye in elasmo-

branchs and in Apoda, as given by van Wijhe, Platt, and the
Sarasins. In Scyllium and Pristiurus, van Wijhe concludes
(No. 130, p. 14) that it arises mainly from the dorsal wall of the
first head cavity, but, in part at least, from an anterior process
of the ventral wall of that cavity, — the obliquus inferior arising
from a second posterior process of this same wall, and the rec-
tus superior and the rectus internus entirely from its dorsal
wall. In Acanthias vulgaris (No. 90, pp. 83, 86, and 103) Platt
says that the inferior oblique arises from the dorsal wall of a
posterior process or constriction of the premandibular or first
head cavity, the inferior rectus from the posterior portion of
the dorsal wall of the remaining portion of the cavity, and the
superior and internal recti from its anterior portion. In her
figures the rectus inferior seems to lie immediately above the
obliquus inferior, and the inferior branch of the oculomotorius
runs outward and downward behind it. In larvae of Ichthyo-
phis the rectus inferior overlaps internally the obliquus inferior
(No. 108, Fig. 72). Whether the head cavities here referred to
belong to the somites or to the visceral arches is unimportant
for my purpose, provided the muscles of the eye in the Cyclo-
stomata and Gnathostomata are of homologous origin, which
no one would, I think, venture to question.

We thus see that, in so far as the muscles innervated by the
inferior branch of the oculomotorius are concerned, the Cyclos-
tomata and Gnathostomata represent two totally distinct and
different lines, and that they must both have descended from a
prototype in which there was but a single muscle, or muscle
mass, innervated by the nerve in question. Assuming a single
muscle to have been first formed it was probably an obliquus,
that muscle being still, in the adult Petromyzon, the largest of
all the muscles of the eye. We accordingly arrive at a proto-
type for all fishes, in which the oculomotorius would innervate
but three muscles, the superior and internal recti by a supe-
rior branch running forward above the optic nerve, and the
inferior oblique by an inferior branch running downward in
front of the rectus superior, and then forward below the opti-
cus. This would leave the inferior and external recti to be
innervated by the abducens, as they are in Petromyzon, and

the superior oblique by the trochlearis, as it is in all fishes. This last muscle is developed from van Wijhe's second myotome, and accordingly lies, primarily, posterior to all the other muscles. Its position, therefore, in Petromyzon, where it arises from the membranous hind wall of the orbit and is inserted on the under surface of the bulbus, probably represents a primitive condition. The position toward the front end of the orbit, found in all other fishes, must then have been acquired, as Fürbringer suggests, by the gradual shifting of the origin of the muscle along the upper edge of the orbit. Nothing in the arrangement of the nerves of the orbit would have interfered with this change of position *if* the r. ophthalmicus in Petromyzon be the r. ophthalmicus profundus of other fishes, nor would the muscle, during the process, have been ever functionally at a disadvantage. What is apparently an intermediate stage in this shifting process is shown in Callorhynchus, where the muscle still arises from the upper edge of the orbit dorsal to the ophthalmicus superficialis, — the trochlearis, which innervates the muscle, lying, as it should, below that nerve. Other intermediate stages are probably presented by the Monotremata, for Göppert (No. 46) finds, in Echidna, fibres of the obliquus superior arising from the frontal near the Trochlea, that is, from about the place where the whole muscle arises in Callorhynchus ; and in both Ornithorhynchus and Echidna he finds the muscle arising less deep in the orbit than in other mammals.

In Fig. 12, Pl. XXII, the arrangements of the muscles and nerves of the eyeball found in the several orders of the Ichthyopsida, and assumed to be found in the several prototypes of those orders, is represented diagrammatically. From the prototype of all vertebrates there are two lines of descent, the impulse leading to each being the splitting of the large inferior oblique. In one line, the outer of the two muscles thus formed becomes a rectus internus, and the earlier muscle of that name, innervated by the superior branch of the oculomotorius, disappears, or fuses with the rectus superior, giving rise to the arrangement found in Petromyzon. In the other line, the inner of the two muscles formed becomes a rectus

inferior, and the earlier inferior rectus, innervated by the abducens, either fuses with the rectus externus, thus giving rise to a holocephaloid arrangement of muscles and nerves, or, if Huxley's statement as to the innervation of that muscle be accepted, persists as the retractor bulbi, thus giving rise to the arrangement found in Urodela. Assuming this last arrangement to be the one first formed, there would arise what may be called a proto-urodelian type from which there are three lines of descent. One of these lines leads to the Holo-cephala and Plagiostomata, in both of which the retractor bulbi disappears, and in the latter of which the rectus internus acquires an origin with the other recti at the hind end of the orbit. A second line leads to the Ganoidei and Teleostei, where the retractor bulbi likewise disappears, probably by fusion with the rectus externus, as the frequently double muscle and double nerve in Amia indicate, and where a still further change is brought about by the formation of a new internus by the splitting of the rectus inferior. As this new muscle develops, the old internus disappears ; thus leaving but one muscle innervated by the superior branch of the oculomoto-rius. A third line leads to Amphibia and the higher verte-brates, the Dipnoi either arising from this line or from the line leading to elasmobranchs. The amphibian part of this third line separates into two parts, one leading directly to Urodela with but little change, and the other to Anura, where a new internus is formed, as in Ganoidei and Teleostei, the old inter-nus disappearing as in those fishes. In the higher vertebrates the arrangement is as in Anura, but there is often a levator palpebrae superioris, innervated, when found, by a terminal branch of the superior branch of the oculomotorius. This muscle thus seems to be developed from the disappearing rectus internus of the prototype, and the line leading to the higher vertebrates must accordingly lie between those leading to the two divisions of Amphibia, just as the line leading to Amphibia lies between those leading to the two great divisions of Pisces.

In Ichthyophis a retractor tentaculi has been formed from one of the muscles of the eye (No. 108, p. 200), probably from

the rectus internus, and not from the retractor bulbi, as the Sarasins suggest.    Ichthyophis, therefore, presents a condition intermediate between Urodela and Anura, and may represent the beginning of the line leading from Amphibia to Sauropsida. Burckhardt (No. 16) also places Ichthyophis intermediate between Anura and Urodela, but derives it from Protopterus, the Sauropsida being derived from Ceratodus and representing a totally different line.    In a later work I believe he has stated that it represents, in the arrangement of the different parts of the brain, a type leading directly to Sauropsida and the higher vertebrates.    I do not, however, find the reference.

In the higher vertebrates the retractor bulbi may persist or it may disappear, probably by fusion with the rectus externus, as indicated by the occasional double muscle in man, and also by its innervation in man, the abducens entering the orbit between the two heads of the muscle (No. 100, vol. II, pp. 290 and 291).

In this ancestral tree of the muscles and nerves of the eyeball the several lines of descent are sharply and positively defined, but they are based on most insufficient and perhaps inaccurate data.    The important assertion that reversions have not occurred must also be granted.    If that be allowed, the other assumptions, or rather deductions, are not important or improbable, and the schema becomes an ancestral tree of vertebrates.    In this tree it will be noticed that the lines leading to the higher types of each class resemble each other in that the superior branch of the oculomotorius in such lines innervates but one of the muscles of the eye ; that in the lines leading to the lower types it always innervates two ; and that the line leading from one class to the next higher arises between the lines leading to the two types of the class ; that is, from the so-called proto-urodele type upward there has been in the development of the muscles of the eyeball but one impulse, if it may be so called, leading to the formation of the arrangements found in higher types, and not repeated ones.

The forms indicated in the schema as intermediate ones are the Holocephala, Polypterus, Ichthyophis, and the Monotremata, and the grouping of the several orders is exactly the same

as that given by Hasse (No. 52, p. 84, and No. 53, p. 296), based on the development and structure of the vertebral column, and by Maurer (No. 78, p. 476), based on the development of the muscle cells and muscle fibres. It differs from that recently given by Dollo (No. 23), in that he derives the Dipnoi and Amphibia directly from the "Ganoïdes crossoptèrygiens," instead of from some form intermediate between the teleostomes and elasmobranchs.

### 7. Ramus Ophthalmicus Profundus Trigemini.

The root of the ophthalmicus profundus in the adult Amia arises from the median or anterior side of the root of the trigeminus, at a considerable distance from the base of that root, but still inside the cranial cavity (*rp*, Figs. 26 and 27, Pl. XXV, and Figs. 38 and 39, Pl. XXX). It pierces the lining membrane of the lateral chamber of the eye-muscle canal, and enters that chamber in front of the root of the trigeminus, but behind the trochlearis and oculomotorius. At the front end of the chamber, or in the passage leading from it into the orbit, it enters the profundus ganglion (*gp*) which lies near the upper end of the passage, internal to the pedicle of the alisphenoid, and external to the thin projecting plate of that bone to which the lining membrane of the eye-muscle canal and of the optic foramen is attached. The ganglion, in the adult, is somewhat arrowhead in shape, with the point of the arrow directed backward where the radix profundi joins it. It lies immediately below the trochlearis and above the oculomotorius, but neither in the adult nor in larvae could any connection with either of those nerves be found. In larvae the ganglion lies so close to the oculomotorius that it is difficult to determine whether there is or is not any connection between them. None could be established in any of my sections.

From the upper, anterior corner of the ganglion the portio ophthalmici profundi of van Wijhe (*popp*), which may be single or double, arises ; from the lower corner the radix longa (*rl*) ; between these two, from the front edge of the ganglion, close to the base of the portio ophthalmici profundi two ciliary

nerves, the rami ciliares longi (*cl*) ; and between them and the radix longa a nerve which was not satisfactorily traced in any of the dissections made. When this last nerve was found, it always accompanied the ciliary nerves as they ran forward and outward between the external and superior recti. Beyond that point it was always lost, appearing sometimes to fuse with the ciliary nerves, and at others to disappear in the general tissues.

The portio ophthalmici profundi runs upward and forward above all the muscles of the eye, and joins the ophthalmic branch of the trigeminus while that nerve still lies under the overhanging cartilaginous roof of the orbit. The two nerves then fuse, the fusion being so complete that the two could not be separated in dissection.

The two rami ciliares longi run outward, upward, and forward above the rectus externus, and below the rectus superior, and then join and become attached to the vein *ov'*, which runs from the orbital sinus to the outer edge of the eyeball. They lie along the upper, posterior side of the vein, and pierce the cartilaginous capsule of the eye internal to and below the point where the vein leaves it ; that is, near the insertion of the rectus superior, between it and the rectus externus. In larvae the nerves could be traced outward, along the inner surface of the sclerotic, towards the lens and cornea.

The radix longa runs downward and forward toward the inferior branch of the oculomotorius, which it accompanies, closely applied to it, in its course between the superior and external recti. Immediately beyond those muscles it enters a small ganglionic swelling, the ciliary ganglion (*gc*), which lies directly in its course and, like it, is closely applied to the oculomotorius. Between the radix longa and the oculomotorius no connecting or communicating fibres were found ; between the ciliary ganglion and the nerve such fibres, representing the radix brevis, were always found. From the ganglion, and as a direct continuation of the radix longa, a large nerve, the ciliaris brevis (*cb*), runs outward and forward along the outer surface of the envelope enclosing the efferent pseudobranchial artery, and enters the capsule of the eye with that artery through the opening for the optic nerve. No sym-

pathetic fibres could be traced to the ganglion, nor could I find any indication of a branch running forward along the oculomotorius to and beyond the inferior oblique muscle, such as Ewart describes in sharks and rays (No. 30).

In larvae, the branch of the profundus ganglion found in the adult between the ciliares longi and the radix longa was not found, a third portio ophthalmici profundi connecting the profundus ganglion with the ophthalmicus superficialis trigemini was, however, often found; always much shorter than the other portions, and always running directly outward from the ganglion to the nerve, instead of outward and forward. This third branch was never found in the adult, owing doubtless to the fact that the dissection at this point is exceedingly difficult. A tough membranous tissue surrounds all the parts, and six dissections, at least, of this particular region had to be made before one sufficiently complete for illustration had been obtained.

The ciliary ganglion in larvae always appears in transverse sections as a well-rounded mass attached to the upper, outer surface of the inferior branch of the oculomotorius, relatively much larger than in the adult, and enclosed in a thick fibrous-like envelope. It contains small cells, similar in appearance to those found in the ganglion on the under surface of the olfactorius, and not at all like those found in the profundus and the other cerebral ganglia. No ganglion cells were found in any part of the oculomotorius itself, and the ganglion cells described in that nerve in the adult of other forms, and considered by different investigators as the ganglion or ganglions of the oculomotorius, are in all probability simply parts of the ciliary ganglion of Amia scattered along the ciliary nerves or roots, as Ewart finds them in sharks and rays (No. 27), instead of being collected into a distinct and definite mass, as in Amia. I do not mean by this to express any opinion as to the origin of the cells, for the disposition of the parts in the youngest stages of Amia that I have examined might be taken to indicate an origin from or in connection with the profundus ganglion alone, or from that ganglion and the oculomotorius combined.

### 8. Review and Comparison.

In Laemargus (No. 26) the profundus arises by a root which is fairly separate and distinct from the root of the trigeminus, but connected with it by several small communicating branches. In Raja (No. 26) the roots of the two nerves are more intimately connected. In both Laemargus and Raja, and in Torpedo also (No. 28), the ganglion of the profundus is apparently wholly separate from and unconnected with the trigeminus and its ganglion, for Ewart does not mention any communicating branches in any of those fishes. He, however, says, in another publication (No. 27), that in skates the profundus ganglion is frequently connected by a communicating branch with the Gasserian ganglion. In sharks other than Laemargus, Ewart, in his preliminary communication, does not state definitely whether there is or is not a separate profundus ganglion; in his complete work, in a diagram indicating the distribution of the cranial nerves in selachians in general (No. 29, Fig. 2) he, however, shows an entirely separate ganglion and root. Beard says (No. 8, p. 570) that in sharks the ganglion (mesocephalic) and its root are found distinct in early stages of development, but in later ones both become fused with the ganglion and root of the trigeminus.

Ewart, in his preliminary publication, describes but one nerve arising from the profundus ganglion in Laemargus. It is the r. ophthalmicus profundus. From it a small branch passes outward above the rectus superior, in the place, therefore, where the portio ophthalmici profundi is found in Amia, and then, from the main nerve, two or three ciliary nerves, presumably the ciliares longi and ciliares brevi combined, arise and, running forward between the rectus superior and rectus externus, enter the eyeball. No ciliary ganglion is described, but delicate communicating fibres are said to pass from the inferior branch of the oculomotorius to the ciliary nerves. The main nerve is then said to bend inward between the obliquus superior and the rectus internus, and end in the cutaneous and subcutaneous tissues in the snout. In its forward course small branches are sent outward, in front of the eyeball, and others to the skin

and tissues of the snout.    In his later publication (No. 29, p. 75), which seems to give the distribution of the nerves in sharks in general rather than in Laemargus in particular, Ewart describes a ciliary ganglion lying at the junction of the profundus and oculomotorius fibres.    From this ganglion the ciliares brevi arise, the ciliares longi arising from the main r. ophthalmicus profundus, as in Laemargus in particular.    The profundus is here said to pass under the rectus internus instead of over it, between it and the obliquus superior, as in the preliminary publication.    This earlier statement is, therefore, probably an error.    It apparently never fuses with the r. ophthalmicus superficialis trigemini, and Ewart says it has a distribution similar to that in Raja and in Amia.    No reference is given, in this particular instance, indicating his authority for the statement regarding Amia.    It is, however, probably based on my earlier work (No. 3), as definite reference is made to that work in describing the nerve in Raja (No. 32, p. 90).    The nerve referred to in Amia is, therefore, probably the portio ophthalmici profundi, and not the ramus ophthalmicus profundus.

In Scyllium, Schwalbe (No. 113) says that the profundus perforates the rectus superior near its hind edge and then runs forward under the rectus internus and obliquus superior, passing, however, in its course through the sclerotic, a fact confirmed by Marshall and Spencer (No. 77) in the embryo.    In Hexanchus it apparently has a similar course (Gegenbaur). In Galeus (see Cut 1) it lies above the rectus superior, running downward in front of that muscle, through the rectus internus, near its hind edge, and then forward below the rectus internus and obliquus superior.    In Mustellus, Schwalbe gives it a similar course, but Tiesing (No. 123) says that it issues below the rectus superior and lies below that muscle, below the internus, and below the obliquus superior.    Whatever its relations to the muscle of the eye, it always lies behind, or external to, and then below, the superior branch of the oculomotorius, and above the inferior branch of that nerve; that is, it crosses the upper surface of the oculomotorius distal to its superior branch, and then runs forward always under the trochlearis.

In embryos of Acanthias vulgaris, Mitrophanon (No. 80, pp. 178 and 179) describes both a ramus ophthalmicus superficialis trigemini and a ramus ophthalmicus profundus trigemini.  The former arises from the anterior root of the trigeminal Anlage, in connection with the Gasserian ganglion.  It enters distally into connection with the nervus trochlearis.  The profundus arises from an ophthalmic prolongation of the posterior root of the trigeminal Anlage, sends a delicate branch, the portio ophthalmici profundi, to the epidermis, and then enters the ciliary, or mesocephalic, ganglion.  From this latter ganglion a ganglionic mass separates the ganglion of the oculomotorius, and from this ganglion the primitive nervus oculomotorius is developed.  The ciliary ganglion, here described, is certainly the profundus ganglion of Amia, as the name mesocephalic indicates, but its origin from the posterior root of the trigeminal Anlage seems to differ from the origin of the ganglion in Amia, as will be shown later.  The ganglion of the oculomotorius seems, with equal certainty, to be the ciliary ganglion of Amia. The presence of both a superficialis and a profundus trigemini, and of a portio profundi also, is of interest.  The relations of the three nerves to the muscles of the eyeball and to the oculomotorius are not given.  The nervus trochlearis is, however, said to pass *above* the ramus ophthalmicus superficialis facialis, which is exceptional.

In Raja, taken by Ewart as a type for skates in general, the ciliary nerves arise in part directly from the profundus ganglion and in part from the ciliary ganglion (No. 26, p. 535 and No. 27, p. 289).  The ophthalmicus profundus in the same fish lies, according to Schwalbe, below the usual two recti muscles and the obliquus superior, and always below the trochlearis and below the superior branch of the oculomotorius.  Tiesing gives it a similar course in the Batoidei examined by him, adding to what Schwalbe has stated, the fact that it lies dorsal to the eyestalk.   No ciliary nerves arise, according to him, either from or in connection with it ; they arise from the oculomotorius.

In Callorhynchus the ciliary ganglion and ciliaris brevis are not given by Stannius ; the ciliaris longus is said by him to arise from the ophthalmicus profundus, as in sharks.   In

Chimaera, Schwalbe does not give either the ciliaris longus or brevis, but he describes a ciliary ganglion on the inferior branch of the oculomotorius. In both Chimaera and Callorhynchus the r. ophthalmicus profundus crosses the orbit below the superior and internal recti and below the superior oblique, and it may even lie below the inferior oblique, if Stannius' figure is correct. It always lies, as it does in the Plagiostomata, below the trochlearis and below the superior branch of the oculomotorius, and Schwalbe even shows it in his Fig. 12 as lying below the inferior branch of that nerve, unquestionably an error.

In elasmobranchs the ophthalmicus profundus, its ganglion, and its ciliary branches are thus seen to present several somewhat different arrangements. The differences in these arrangements are, however, only apparent; and they are all easily explained by the more or less complete fusion of the profundus ganglion and its root with the ganglion and root of the trigeminus, by the adhesion, or more or less complete fusion, of the ciliares longi with the ophthalmicus profundus, or even with the ophthalmicus superficialis trigemini at its base, and by the more or less complete fusion of the ciliary ganglion with the inferior branch of the oculomotorius.

In most teleosts (No. 116, p. 39) a ciliary ganglion with two distinct roots, a radix longa and a radix brevis, is found, and from the ganglion the ciliaris brevis, single or double, arises. The ciliaris longus, as in elasmobranchs, varies in its origin. It may arise from the profundus ganglion, as in Trigla; from the so-called r. ciliaris, a nerve which arises from the Gasserian ganglion close to the r. ophthalmicus superficialis trigemini; or from the latter nerve itself, as in Salmo.

In no teleost, with the single recorded exception, so far as I can find, of Trigla (No. 116, p. 25), is there a separate profundus ganglion and root. Both ganglion and root are apparently always completely fused with the ganglion and root of the trigeminus. In Trigla the profundus root arises from the trigeminal root, as it does in Amia, and its ganglion is separate and distinct from the trigeminal ganglion, also as in Amia. From the ganglion arise a ramus ciliaris longus and a radix longa. No other

branches arising from it are given, and there is accordingly no ramus ophthalmicus profundus in Trigla. That nerve, as a separate nerve, is also not described, so far as I can find, in any teleostean forms excepting Trichomycterus and Clarias, in which fishes it is described by Pollard (No. 97, pp. 392 and 398). As Pollard describes no ramus ophthalmicus superficialis trigemini in any siluroid, it is exceedingly probable that the nerve described by him as a profundus is in reality the superficialis, its position in Trichomycterus being in that case most unusual. Pollard's nomenclature or observation, in this instance as in some others, seems to vary somewhat from my own, and to lead him to somewhat different conclusions. In all siluroids, excepting the two above named, he says that "the ophthalmicus profundus may only be represented by some fibres running along with the ophthalmicus superficialis of the Facial" (No. 97, p. 398). By most other writers it is assumed that the ramus ophthalmicus profundus trigemini has fused completely with the superficialis trigemini, which, so far as the course and position of the nerve are concerned, would amount to the same thing as the statement made by Pollard. Regarding such a fusion in individual cases and in general, several authors have expressed some doubt. To me it seems impossible. How a nerve that lies below the trochlearis and below the superior branch of the oculomotorius could fuse with a nerve lying above both those nerves without inclosing the two nerves in the single nerve so formed, it is difficult to imagine. It seems much more probable that the ophthalmicus profundus of elasmobranchs is entirely wanting in teleosts, and that the profundus elements in the latter are represented in the former by some branch or branches of the nervus profundus, its ganglion or its root, which lie above the two nerves concerned. Such a branch is found in Laemargus in the small branch which runs upward and forward above the rectus superior (No. 26, p. 527). In teleosts and ganoids this small branch must undergo a special development as the rest of the nerve disappears, becoming the portio ophthalmici profundi, which may be found as a partly separate nerve, as in Amia, or as fibres completely fused with the ophthalmicus superficialis, as

in teleosts. A confirmation of this supposition is found in the arrangements presented by ganoids other than Amia.

In Polypterus, van Wijhe (No. 129, p. 260) describes two commissural branches connecting the profundus ganglion (called by him the ciliary) with the ophthalmicus superficialis, and one connecting it with the oculomotorius. He also describes a large nerve, the r. ophthalmicus profundus, which, arising from the profundus ganglion, runs forward under the rectus superior and obliquus superior, and joins the ophthalmicus superficialis at the front end of the orbit. Pollard (No. 93, p. 394) practically confirms van Wijhe's observations, for he describes, (1) two commissural branches to the ophthalmicus superficialis, considered by him as a branch of the facialis; (2) the main nerve, which has the general course given by van Wijhe, but passes through the obliquus superior; and (3) a branch which joins the "motor nerves," these latter nerves being presumably the oculomotorius, though he does not so state. The main nerve in Polypterus thus corresponds exactly in position with the nerve of the same name in elasmobranchs, and with the small nerve which, arising from the profundus ganglion in Amia, runs forward under the superior rectus and is lost in the general tissues. The commissural branches to the ophthalmicus superficialis correspond with the portiones ophthalmici profundi in Amia, and the commissure to the oculomotorius to the radix longa.

In Lepidosteus there is a profundus ganglion and a portio ophthalmici profundi (No. 112 *x* and *b*, Fig. 5*a*) as in Amia, but in Lepidosteus the portio profundi and its ganglion lie, most unaccountably, if Schneider has made no error, immediately below and not above the main trunk of the oculomotorius (No. 112, p. 18). There is in Lepidosteus no r. ophthalmicus profundus, unless that nerve is represented in one of the three nerves called by Schneider "ciliary," which have become fused with, and have an apparent origin from, the superior and inferior branches of the oculomotorius.

In Spatularia, according to van Wijhe (No. 129, p. 249), the ramus profundus "issues under the origin of the rectus superior," and then, running forward above the other recti muscles, separates into two parts, one of which joins the

ophthalmicus superficialis, and the other enters a canal in the front wall of the orbit median to the origin of the obliquus superior.   The relations of these two nerves to the other nerves of the orbit is not given by van Wijhe, and no ophthalmic branch of the facialis is described.   Collinge also (No. 19, p. 516) gives no ophthalmic branch of the facialis, but, as he describes three dendritic systems along the supraorbital canal, and gives no branch of the facialis that could in any probability innervate the sense organs that must be associated with them, it seems probable that the nerve called by him and van Wijhe the r. ophthalmicus superficialis trigemini is in part the ophthalmicus facialis.   The origin of the r. oticus from the superficialis, as given by Collinge, sufficiently proves that the latter nerve contains elements destined to supply the sense organs of the lateral canals, and those elements must belong to the facialis. The sensory canal system of Spatularia does not, therefore, in its innervation present such exceptional interest as Collinge has ascribed to it.

In Acipenser, van Wijhe figures and describes a ramus profundus lying dorsal to the trochlearis, and dorsal to all the muscles of the eye.   In a later footnote (No. 129, p. 230) he says that the nerve so described is probably the ramus superficialis trigemini, and as nothing more is said of the profundus, the existence or non-existence of that nerve is left somewhat in doubt.   Branches are sent from the nerve so described to the glands of the "Augenmuskelschlauche," and the nerve does not join his ramus ophthalmicus superficialis, which is doubtless the ophthalmicus facialis.   Goronowitsch, who seems to have published without having seen the later footnote of van Wijhe, rather confirms than otherwise the non-existence of a true profundus.   According to him, the trigeminus 1 arises by two roots which never entirely fuse (No. 50, pp. 478 and 481). From these two roots three nerves arise, the rami maxillares inferior and superior and the ramus ophthalmicus profundus. This last nerve runs forward dorsal to the trochlearis and above the muscles of the eye, as described by van Wijhe. Schneider states that the ciliary nerve arises from the oculomotorius, a nerve which never has, according to van Wijhe

(No. 129, p. 229), any connection whatever with any other
nerve.

It is evident from the above references that the work on
ganoids needs some revision. It is also evident that Polypterus,
and probably Spatularia also, represent, in the arrangement of
the ophthalmic nerves, a condition intermediate between that
found in elasmobranchs and that in Amia and the teleosts. In
selachians there is a relatively small ramus superficialis trige-
mini, or no such nerve at all, as in Torpedo (No. 22, p. 62); a
large ramus profundus ; and in Acanthias embryos and skates
a portio profundi, represented in the former by a separate
nerve, and in the latter by communicating branches from the
profundus to the Gasserian ganglion. In Amia there is a large
ramus superficialis, a remnant only of a ramus profundus, but
a portio profundi well developed. In teleosts the remnant
even of a ramus profundus has disappeared, and the portio
profundi is completely fused with the ophthalmicus superficialis.
The superficialis and profundus seem to vary in relative impor-
tance directly as the number of terminal buds found on the
top of the snout and head. In bony ganoids and teleosts
those buds are there found in great quantity (No. 79, p. 69),
while in elasmobranchs they are much less abundant. In
Amia they are innervated by the superficialis trigemini (No. 3,
p. 513) and in elasmobranchs they must be innervated in the
same manner, for Ewart says that the profundus takes no part
whatever in their innervation. The profundus in elasmobranchs
is distributed, in part, to the tubes extending from the ampullae
to the skin (No. 26, p. 527). The disappearance of these organs
in Amia and the teleosts may account for the great reduction
of the profundus in these fishes, while the retention of a closely
related organ, the nerve sack, in cartilaginous ganoids (No. 79,
p. 38) may account for the possible retention of the nerve by
them.

In Protopterus (No. 88, p. 296) there is a large ophthalmicus
profundus and no ophthalmicus superficialis trigemini. From
the profundus three important branches are sent upward and
forward to the top of the head and snout, where they become
associated with the ophthalmicus facialis. They, therefore,

undoubtedly represent the portio ophthalmici profundi of Amia, or that nerve and the superficialis trigemini also. Under these branches, but, so far as can be judged from Pinkus' Fig. 1, above the remaining main portion of the nerve, the nervus oculomotorius and nervus abducens run, from within outward, to the muscles of the eye. In the text Pinkus says that the oculomotorius leaves the profundus on its ventro-lateral side. The position of the main profundus relative to the superior and inferior branches of the oculomotorius is, therefore, left somewhat in doubt, as is also its relation to the abducens.

In Petromyzon the ramus ophthalmicus trigemini lies under the nervus trochlearis, but, if Fürbringer's figure is correct, over the superior branch of the oculomotorius. There is no other ophthalmic nerve, and that the terminal buds in Petromyzon are homologous with those in Amia seems from Merkel's description an open question. The " Nervenhügel " that he describes in Petromyzon seems to resemble the " Nervensäcke " rather than the " Nervenhügel " of ganoids, and the nerve in that case in Petromyzon would probably be the profundus, as its position below the trochlearis indicates, rather than the superficialis, which, in all other fishes, lies always above that nerve.

In Salamandra and Rana (Schwalbe) the ramus nasalis trigemini lies under the trochlearis, under the rectus superior, and under the nerve supplying that muscle. In Salamandra it lies also under the branch to the rectus internus. It, therefore, corresponds exactly in position to the ophthalmicus profundus of selachians, as Schwalbe has himself pointed out (No. 113, p. 200). In both Salamandra and Rana a ciliary ganglion is described by Schwalbe as a ganglion of the oculomotorius, connected in Rana with the ramus nasalis by a very delicate radix longa, and probably so connected in Salamandra also, although Schwalbe did not find the nerve. In Salamandra, and also in Menobranchus and Siredon, ciliary branches arising from the ramus nasalis are described in addition to those arising from the ciliary ganglion. In Rana and Salamandra rami frontales are described, arising from the base of the ramus nasalis and considered by Schwalbe as the equivalents of the

ophthalmicus superficialis trigemini in fishes. This latter nerve as a separate and distinct nerve is not described, and in Amphibia there are no terminal buds on the top of the head (No. 79, p. 76). Strong (No. 121, p. 108), in larvae of Rana, finds the ramus ophthalmicus trigemini arising from a partially separate anterior, ventral and median portion of the Gasserian ganglion. The nerve runs forward between the two divisions of the oculomotorius, and gives off its first branches after passing that nerve. It lies apparently below the abducens, but the description is not precise (No. 121, p. 134). Strong does not describe a ciliary ganglion, a profundus ganglion, or a radix longa. He, however, says that sympathetic fibres are connected with certain of the trigeminal branches (No. 121, p. 119).

In the adults of Amphibia and higher vertebrates a profundus ganglion is not described, so far as I can find. It is, however, described in certain embryos. In late embryonic stages of Lacerta, Hoffmann (No. 56, p. 205) describes it as a more or less separate and distinct ganglion, called by him the ganglion ophthalmicum. From it the ciliary ganglion is developed. From the profundus ganglion proper, after this differentiation, there arise a ramus frontalis and a ramus nasociliaris, and from the latter a ramus ciliaris connecting the nerve, and hence its ganglion, with the ciliary ganglion. The ramus nasociliaris is thus the ophthalmicus profundus of Ichthyopsida ; the ramus ciliaris is the radix longa, and the ramus frontalis is the portio ophthalmici profundi of ganoids and teleosts, or branch 1 of Ewart in Laemargus. The ciliary ganglion is connected with the inferior branch of the oculomotorius by a short radix brevis. As in Amphibia, so in Lacerta, there is no ramus ophthalmicus superficialis trigemini, for from the posterior of the two trigeminal ganglia described by Hoffmann, that is, from the entire ganglion of the trigeminal outgrowth minus the profundus ganglion, there arise only the so-called second and third branches of the trigeminus, that is the superior and inferior maxillary nerves.

In embryos of Torpidonotus natrix the oculomotorius and trochlearis are shown by Grosser and Brezina (No. 51) in one

reconstruction, Fig. 8, median to, that is ventral to, the ramus nasociliaris trigemini and its ramus frontalis. In another reconstruction of an older embryo, Fig. 9, they are shown lateral to, that is dorsal to, the same nerves. One or the other must be wrong.

In the guinea pig the ophthalmic nerve arises, according to Chiarugi (No. 18, p. 507), from a distant ophthalmic ganglion which soon fuses with the ganglion of the fifth nerve. This ganglion is unquestionably the profundus ganglion of Amia, and the ophthalmic nerve, therefore, the ophthalmicus profundus.

In a five-months human embryo Ewart (No. 27, p. 290) finds vestiges of a profundus ganglion, lying under cover of the inner portion of the Gasserian ganglion. After describing it he states his conviction that "the ophthalmicus profundus of the elasmo-branch is represented in man by the so-called nasal branch of the ophthalmic division of the fifth nerve."

The arrangement of the ophthalmic nerves and their associated ganglia in reptiles and the higher vertebrates seems, therefore, to be exactly the same as in Amphibia, if a certain allowance be made for the indefiniteness or insufficiency of the observations and descriptions. In these higher forms, and in certain fishes as well, sympathetic branches are traced to the ciliary ganglion, and several investigators state that the ganglion is in all probability a sympathetic, and not a spinal, or cerebro-spinal, ganglion. Retzius (No. 103) states positively that it is such in mammals, and as it is known as the ganglion ophthalmicum in man, that name should certainly not be given, as proposed by Hoffmann, to the ganglion of the profundus where that ganglion is found as a separate ganglion, as in fishes and in Lacerta.

In the schema (Fig. 12, Pl. XXII) showing diagrammatically the relations of the oculomotorius to the muscles of the eye, I have also endeavored to show the relations, as above described, of the ophthalmicus profundus and ophthalmicus superficialis trigemini to those muscles and to the oculomotorius. From it it will be seen that in the disposition of these nerves, as well as in the manner of innervation of the muscles of the eye, the arrangement found in Amphibia and higher vertebrates

is naturally derived from some such arrangement as that shown
in the hypothetical proto-urodele type which lies intermediate
to and below the two great divisions of Pisces.

## II. MUSCLES INNERVATED BY THE TRIGEMINUS AND FACIALIS, AND THE NERVI TRIGEMINUS, FACIALIS, ACUSTICUS, AND LINEAE LATERALIS VAGI.

The muscles included in this group are in part innervated by
the trigeminus alone, in part by the facialis alone, and in part
by nerves formed by the fusion of branches of both those
nerves. Whether, in the latter case, the muscles concerned
are innervated by the facial elements, or by the trigeminal, I am
unable to determine; the muscles of the group are, therefore,
placed for convenience in three separate sub-groups.

### 1. Sub-group 1. Muscles innervated by the Trigeminus alone.

#### a. *Adductor Mandibulae.*

The adductor mandibulae (Figs. 29–42, Pls. XXVI–XXX)
is a large and complex muscle having an extended surface of
origin and several different insertions. It has three main divi-
sions: a superficial one $A_2$, an inner or deeper one $A_3$, and a
mandibular one $A\omega$.

The superficial portion $A_2$ is much the largest of the three.
It lies immediately underneath the postorbital bones, and is
exposed when those bones and the thick tough dermis extend-
ing from their hind margins to the outer edge of the preoper-
culum are removed. Its outermost fibres present a fan-like
appearance, radiating approximately from the coronoid process
of the mandible and spreading out through somewhat more than
a right angle. The lower, posterior fibres run, from their origin,
forward and slightly upward, and the upper, anterior ones for-
ward and downward, the most anterior ones turning inward at
the front edge of the muscle and running downward and slightly
backward along its inner surface.

The muscle has an extended surface of origin. Its super-
ficial fibres arise, above, from the postorbital process and

from the under surface and outer edge of the squamosal ; behind and below they arise from the entire outer, anterior face of the preoperculum, excepting only its outermost edge which gives attachment to the dermis covering the side of the head. A few fibres often arise from the under surface of the upper postorbital bone along its upper edge. Its deeper fibres arise from the outer surfaces of the hyomandibular, quadrate, and symplectic, the surface of origin lying immediately in front of the preoperculum and extending somewhat under the lower end of that bone and forward onto the hind edge of the mandible. This inner, deeper portion of $A_2$ is intimately connected, particularly below, with the inner, deeper portion, $A_3$, of the adductor. A part of $A_2$, often found as a somewhat separate bundle, arises from a slightly raised portion of the outer surface of the hyomandibular immediately behind the middle portion of that bone. This surface is well defined above by the lower, nearly horizontal, border of the dilatator operculi ; in front by the line of attachment of the strong metapterygoid membrane, which extends across the open space between the hyomandibular and the median process of the metapterygoid and covers the insertion of the posterior portion of the levator arcus palatini ; behind by the front edge of the preoperculum ; and below by the external opening of the facial canal through the hyomandibular, and by the slight groove extending from this canal downward and backward to the point where the hyoid branch of the facial nerve passes under the preoperculum. It is below and in front of this groove that the superficial muscle $A_2$ is, at its origin, so intimately connected with $A_3$.

Although continuous and single at its origin, the superficial muscle, $A_2$, always shows, toward and at its insertion, indications of a separation into three portions, a lower or posterior one, $A_2'$, a middle one, $A_2''$, and an upper or anterior one, $A_2'''$. The middle portion, $A_2''$, sometimes shows a further but much less marked separation into two parts, as shown in Fig. 40. Between the three main portions, at their insertion, the surfaces of separation are clean and distinct, the actual separation of the muscle into three parts is, however, wholly artificial. The surfaces of separation lie in the direction of two planes intersecting at

the top of the coronoid process of the mandible, the planes
extending obliquely into the muscle from its outer surface, but
not passing entirely through it excepting at or near its inser-
tion.   The separation, therefore, on the external surface of the
muscle extends much nearer its origin than in its deeper por-
tions.   The plane between the upper and middle portions of
the muscle extends downward, backward, and inward; that
between the middle and lower portion almost directly down-
ward and inward; so that the line separating these two parts
on the outer surface of the muscle runs almost directly back-
ward from the tip of the coronoid process.   The lower or pos-
terior division of the muscle overlaps the middle one and so lies
partly superficial to it, and the middle portion in the same way,
but to a much greater extent, overlaps and lies superficial to the
upper or anterior one.   The separation between the lower and
middle divisions is much more complete than that between the
middle and upper ones.

The outer and upper fibres of the lower portion, $A_2'$, have
their insertion on the outer surface of the supra-angular, near
its hind edge, and on that hind edge from the tip of the coro-
noid process to the lower end of the bone.   At the top of the
coronoid process they extend almost onto a strong tendon or
ligament (No. 76, p. 126), which has its origin there and is
inserted on the inner surface of the maxilla at about its ante-
rior third.   The connective tissue sheath covering this portion
of the muscle at its insertion is continuous with that covering
the ligament, so that, before it is removed, the ligament has
strongly the appearance of being the tendon of the muscle.
The remaining deeper and lower fibres of $A_2'$ separate from the
outer ones and are inserted on the inner surfaces of the artic-
ular, the supra-angular, and the coronary cartilage.   This por-
tion of the muscle is continuous at its lower, ventral margin, and
toward its origin, with the lower fibres of $A_3$, the two muscles
sometimes enclosing between them, intimately connected with
them, a special bundle of fibres (Fig. 37, Pl. XXIX), which arise,
partly tendinous, from among their fibres, and, joining the man-
dibular portion of the adductor, $A\omega$, at its hind edge, are in-
serted continuous with that muscle on the inner surface of the

articular in its lower, anterior part. This is the only connection $A_2'$ has with $A\omega$, for although its deeper portion, where it passes to its insertion on the inner surface of the coronary cartilage, lies closely upon the outer surface of $A\omega$, there is no interchange of fibres between the two muscles, and the r. maxillaris inferior trigemini lies between them, as shown in Fig. 31, where $A_2'$ has been entirely removed.

$A_2''$, the middle portion of the muscle, is larger than either of the other divisions. Some of its outer, upper fibres are often inserted on the inner surface of the dentary, at the coronoid process and in front of it. The remaining fibres pass in large part directly into the ramus of the mandible, and there form the outer portion of the outer division, $A\omega'$, of the mandibular muscle. A part of them, however, including the deepest and lowermost fibres, are inserted along the lower, ventral edge, and on the outer surface, of a broad tendon or fascia, $A_2 \, A\omega'$, which is formed on the inner surface of $A_2$, where it contracts to pass into the mandible (Figs. 41 and 42, Pl. XXX).

$A_2'''$, the upper, anterior division of $A_2$, consists mainly of those fibres of the adductor that arise from the postorbital process, the surface of origin on this process being the deeper, lower, portion of the cap-like piece that forms the upper, outer angle of the ossification (No. 3, p. 480). It also contains those fibres of $A_2$ that arise from the under surface of the squamosal, immediately behind this cap-like piece, and some or all of those that arise from the under surface of the upper postorbital. The muscle is inserted entirely along the upper, dorsal edge, or on the outer surface of the upper half of the tendon $A_2 \, A\omega'$. Although continuous at its origin with the upper, outer fibres of $A_2''$, and at its insertion on $A_2 \, A\omega'$ with the deeper fibres of that muscle, it is, nevertheless, wholly distinct and separate anteriorly where there is no interchange of fibres whatever, the fibres of $A_2'''$ going entirely to the tendon $A_2 \, A\omega'$, and those of $A_2''$ entirely to the inner surface of the dentary.

In larvae of from 20 mm. to 50 mm. in length, $A_2'''$ arises entirely from the cartilage of the postorbital process, the surface of origin lying below the postfrontal bone, in front of the plane of the dorsal opening of the spiracular canal, and imme-

diately dorsal to the origin of the levator arcus palatini, the surface of origin of which muscle extends a little in front of it. The muscle lies on the inner surface of $A_2$, does not come to the level of the outer surface in any part, is nearly vertical in position, and the fibres of $A_2''$ run downward and forward across it at a considerable angle. Its fibres, which are all inserted along the upper, anterior edge of the tendon, or fascia, on the inner surface of $A_2$, are long in front and short behind where they gradually vanish, the fascia here extending upward to the origin of the muscle. At this point $A_2''$ begins, arising as a thin sheet, partly tendinous in front and wholly so behind, where it lies superficial to the dilatator operculi. This fascia, rather than muscle, is attached in front to the upper, outer edge of the cartilaginous rib that forms the lateral boundary of the anterior diverticulum of the temporal groove, which rib is, in the adult, inclosed in a V-shaped process on the under surface and outer edge of the squamosal (No. 104, p. 188). As this V-shaped process has not, at this age, begun to form anteriorly, the origin of the fascia in that part is entirely from the cartilage. Posteriorly, where the process is represented by a single bony fin or plate along the outer edge of the squamosal, the line of origin of the fascia extends onto that fin, that is, onto the lateral edge of the squamosal. In the adult this fascia has become muscular, and the surface of origin of the muscle is a narrow line extending along the outer edge, and along the under surface of the squamosal, back as far as the upper end of the preoperculum. This uppermost portion of the muscle is in the adult thin and partly tendinous. It lies immediately superficial to the levator arcus palatini and dilatator operculi, and passes almost abruptly, at the lower edge of the dilatator, into the thick fleshy lower portion of the muscle.

The inner, deeper division of the adductor, $A_3$, lies immediately underneath $A_2$. Although a relatively small muscle, it has a large surface of origin, arising from the hyomandibular below the external opening of the facial canal, from the outer surface of the quadrate down to the articular swelling at its distal end, from the outer surface of that part of the metapterygoid that lies behind the front edge of its median, dorsal pro-

cess, from the entire outer surface of that process, and also by
a few fibres from the outer surface of the metapterygoid mem-
brane, some of the outermost of these last fibres overlapping the
membrane and arising from the outer surface of the hyoman-
dibular beyond it.   $A_3$ is continuous below, or ventrally, with
$A_2'$, as already described.   Its fibres converge strongly and are
all inserted on a broad tendon or fascia, $A_3\ A\omega''$, formed where
the muscle passes into the ramus of the mandible.

The mandibular portion of the adductor, $A\omega$, is that part of
the muscle that lies inside the hollow of the ramus of the man-
dible.   It can be separated into two parts, $A\omega'$ and $A\omega''$, which
are distinct and separate in origin and insertion.   There is,
however, no smooth and even surface of separation between
these two parts, as there is between $A_2$ and $A_3$.   On the con-
trary, the surfaces are rough and uneven, muscle fibres, or
bundles of one division, projecting into and interlocking with
those of the other, so that they must be carefully pulled apart
to effect a separation of the muscles.   There is, however, no
interchange of fibres between the two divisions, each being
confined strictly to its own region of origin and insertion.

The larger, outer portion, $A\omega'$, has its origin from the broad
tendon $A_2\ A\omega'$.   Its fibres arise from the outer surface of the
tendon, near the hind end of the mandible, and pass directly,
with the outer fibres of $A_2''$, to their common insertion on the
entire inner surface of the dentary and the anterior part of the
angular.   The arrangement is such as would arise if a single
broad muscle, arising at or near the preoperculum and having
its insertion inside the ramus of the mandible, thus represent-
ing $A\omega'$ and the greater part of $A_2$, should become contracted,
and in part tendinous, near the middle of its length.

The inner, deeper portion, $A\omega''$, of the mandibular muscle is
much smaller than the outer one.   It arises entirely from the
tendon or fascia formed on the outer surface of $A_3$, and is inserted,
as a flat, thin muscle, along the upper surface of the posterior half
of the longitudinal cartilaginous rib which, as described by van
Wijhe (No. 129, p. 281), projects horizontally from the lower
edge of Meckel's cartilage into the hollow of the ramus of the
mandible.   Considered together with $A_3$ it forms in outline, on

a smaller scale, a fair copy or reduplication of the outer muscles $A\omega'$ and $A_2$ taken together. The tendon $A_3\,A\omega''$ has its front edge posterior to that of $A_2\,A\omega'$ and, although lying closely against the inner surface of that tendon, is wholly separate fror and disconnected with it, except at one point near the hind edge of the splenial. There the front edges of the two tendons are connected by a short tendinous band, which extends between the points where they are joined, $A_2\,A\omega'$ by the tendon of the first division of the levator maxillae superioris, and $A_3\,A\omega''$ by the tendons of the second and third divisions of that muscle. The tendon $A_3\,A\omega''$, where it passes across the hind edge of the splenial, is strongly attached to a tough band of dermis formed along that edge, so that this becomes in a measure a point of attachment for the two divisions of the adductor $A_2$ and $A_3$, and also for the first three divisions of the levator maxillae superioris.

### b. *Levator Maxillae Superioris.*

In addition to the adductor mandibulae, properly so called and above described, there are four muscles intimately associated with it, and in teleosts and selachians considered, in part, as parts of it. They have been called by McMurrich, in Amia (No. 76, p. 122), the second, third, fourth, and fifth divisions of the levator arcus palatini. They are, however, so evidently derived from the levator maxillae superioris of selachians, and from a muscle that Vetter (No. 124) has called $Add\beta$, a part of the adductor mandibulae, in Acanthias and Scymnus, that it has seemed best to reject the names used by McMurrich, and to call the muscles the first, second, third, and fourth divisions of the levator maxillae superioris. It seems best, also, to change slightly the order of numbering used by McMurrich, and to consider his third division of the levator arcus palatini as the first one of the levator maxillae superioris ; his second division as the second ; his fourth division as the third ; and his fifth division as the fourth.

The fourth division of the muscle ($Lms^4$, Figs. 21–42) is short, stout, and conical in shape, with the base of the cone below at its insertion on the upper surface of the palatine bone near its outer, hinder edge. At its apex or origin the muscle was,

in most of the specimens examined, double, arising by one head from the under surface of the posterior extremity of the antorbital bone, and by the other from the anterior and under face of the prefrontal process this process being pyramidal in shape, as described by Sagemehl (No. 104). Where the muscle was single in its origin it arose entirely from the antorbital, and in specimens of about 20 mm. in length it had apparently this origin only. Where it has a partial insertion on the antorbital, it must, in contracting, depress that bone and so diminish the size of the nasal cavity, thus having some functional connection with the nose.

Immediately posterior to the insertion of this muscle a strong ligament arises from the outer edge of the ectopterygoid. It runs backward closely attached to the inner surface of the loose dermal fold that extends from the edge of the palatine arch to the maxilla, and which may be called the supramaxillary fold (No. 3, p. 489, and Fig. 20), and disappears on the under surface of the dermis behind the superficial fold or crease that marks the hind edge of the mandible, its fibres running downward and backward somewhat parallel to that crease. Another ligament arises from the under surface of the anterior of the two infraorbital bones, and running backward and downward closely attached, as the preceding one is, to the dermis between the maxilla and the palatine arch, disappears on the under surface of the dermis behind the hind margin of the mandible and behind the first ligament.

The third division of the muscle, *Lms*[3], arises in full-grown fishes immediately behind the antorbital process from the upper surface of the palatine bone near its hind edge. In nearly all the fishes examined some of the lateral and upper fibres of the muscle continued forward above and across the ligament that binds the palatine arch to the prefrontal ossification, and had their origin on the posterior face of that bone. The muscle is a long one, of flat oval section. It runs downward, outward, and backward along the upper surface of the palatine arch until it reaches the angle or bend in the upper surface of the metapterygoid, where it turns downward and contracts abruptly into a long, slender tendon. This tendon continues downward

and backward, in the direction of the muscle, to the anterior
edge of the large tendon $A_3 A\omega''$, which it joins near the hind
edge of the splenial.    Here it joins and usually fuses com-
pletely with the tendon of the second division of the muscle,
$Lms^2$.    The united tendons, as a broad flat band, continue
backward and then downward in a curved line along the outer
surface of $A_3 A\omega''$, closely attached to that tendon, but in no
way fused with it.    They have their own particular insertion
on a small ossification which lies immediately behind the hori-
zontal projecting rib of Meckel's cartilage, and has been called
by Bridge ossicle $c$ (No. 15, p. 618).    This is the only insertion
of the muscle evident in larvae.    In the adult, however, what
seemed to be a second tendon of the muscle was always found.
It lay on the inner surface of $A_3 A\omega''$, as shown in the
figures (Figs. 41 and 42, Pl. XXX), and had its insertion pos-
terior to the insertion of the united tendons of $Lms^3$ and $Lms^2$,
apparently on the same bone, ossicle $c$, at its extreme edge, or
possibly on ossicle $b$.    This could not be definitely determined.
The tendon always had much the character of a string of con-
nective tissue, but it was always found well defined through
the greater part of its length.    At its insertion it was always
joined by parts of the posterior portion of tendon $A_3 A\omega''$.

In larvae, $Lms^3$ arose entirely from the upper surface of
the palatine cartilage, and in no instance had it acquired any
attachment to the antorbital process.    In one instance it was
double throughout its length, arising, one part a little in front
of the other, on the upper surface of the palatine, the two
divisions lying side by side and contracting separately into
separate tendons, which united to form a single tendon just
before reaching the large tendon of the adductor.    In several
other specimens there were indications of such a division into
two parts, but there was no actual separation of the fibres.

The second and first divisions of $Lms$ arise, in the adult, as a
single muscle, mainly from the front edge of the hyomandibular
at its extreme upper end, but the line of origin extends forward
from the hyomandibular, across the upper end of the lateral
wing of the parasphenoid, to the front edge of that bone.
In young specimens also, of from 20 mm. to 40 mm. in

length, these two muscles arise as a single muscle, but they have their origin entirely from the front edge of the hyomandibular, the attachment to the parasphenoid being along the upper edge of the muscle and by connective tissue only ; that is, the attachment is from the side of the muscle fibres and not at their ends.   This part of the muscle in these young specimens is a flat band lying close against the inner surface of the levator arcus palatini, and immediately external to the pseudobranch.   It runs almost directly forward from its origin on the hyomandibular to the level of the front edge of the levator arcus palatini, where it turns downward and outward along the outer shelving surface of the metapterygoid, and there separates into two parts, a lower, posterior one, $Lms^2$, and an upper, anterior one, $Lms^1$.   The former, $Lms^2$, contracts near the front edge of $A_3$ to a slender tendon, and joins the large tendon $A_3 A\omega''$ at the point where that tendon is joined by the tendon of $Lms^3$, with which it usually fuses and has the course and insertion already described.   A secondary semi-tendinous connection of this tendon, similar to that of tendon $Lms^3$, was sometimes found along the inner surface of $A_3 A\omega''$.

$Lms^1$, the upper, anterior division of the levator maxillae superioris, contracts near the front edge of $A_2$ to a flat, terminal edge and is inserted by a short, thin, fascia-like tendon on the inner surface of the tendon $A_2 A\omega'$ at its upper, anterior edge, internal to the coronoid process of the mandible and posterior to the hind edge of the splenial.   Near its insertion the muscle is moulded against the front, inner edge of $A_2'''$, the fibres of the two muscles ending at the same level, and the short, fascia-like tendon of $Lms^1$ appearing simply as a branch of, or slip from, the main tendon $A_2 A\omega'$.   It cannot be traced across this tendon as the tendons of $Lms^2$ and $Lms^3$ were across the tendon $A_3 A\omega''$.   At its outer, anterior edge it is strongly attached by connective tissue to the dermis at the outer corner of the mouth, and at its inner, posterior edge it is connected, as already described, by a tendinous or connective tissue band with tendon $A_3 A\omega''$, the point of attachment to that tendon being at the angle formed by the crossing of the two tendons $Lms^3$ and $Lms^2$, where they join $A_3 A\omega''$.

In specimens of from 10 mm. to 12 mm. in length the four divisions of the levator maxillae superioris are directly continuous with the adductor mandibulae, their tendons, found in the adult, not yet having been formed, or at least not being distinguishable. The first and second divisions appear as a single muscle, connected with both the inner and outer divisions of the adductor, and arising from the front edge of the median process of the metapterygoid or from the upper surface of that bone near the process, the attachment to the hyomandibular and the position internal to the levator arcus palatini not yet having been acquired. The third division of the levator is well developed and arises in connection with the first and second divisions, but from the inner division only of the adductor. It extends forward under the eye and is either attached to the upper surface of the palatine cartilage or vanishes in the general tissues above it, having no visible attachment. No trace was found at this age of the fourth division as a separate muscle.

### c. *Levator Arcus Palatini.*

This muscle (*Lap*, Figs. 30, 31, and 36, Pls. XXVI, XXVII, and XXIX), called by McMurrich the first division only of the levator arcus palatini, is a stout muscle lying in origin and insertion internal to the plane of the median, dorsal process of the metapterygoid. It has in its upper part a strong, median, longitudinal aponeurosis, which extends into the muscle from its origin and front edge for about two thirds the length and breadth of the muscle. From this aponeurosis all the fibres of the inner portion of the muscle arise, and those of the outer in great part.

The most anterior fibres of the muscle belong entirely to the outer of these two portions. They are distinctly marked off on the surface of the muscle (Fig. 36) from the remaining fibres of the outer portion, and have their origin from the lateral and front edge of the postorbital process below the dermal postfrontal bone and anterior to the lateral wing of the parasphenoid. They run almost vertically downward, bellying

forward a little, and are inserted along the front edge of the median, dorsal process of the metapterygoid, and on the upper surface of the bone in front of that process. The fibres of the outer portion of the muscle immediately behind this anterior part arise partly from the postorbital process, but mostly from the outer surface of the median aponeurosis of the muscle.

The inner portion of the muscle begins about on a level with the front edge of the metapterygoid process; it arises entirely from the inner surface of the median aponeurosis which, in front of this point, has been merely a strong membrane lining the inner surface of the outer portion of the muscle. Its fibres arise at a considerable angle to the aponeurosis, curving inward and downward and then a little outward, thus forming a strong belly on the inner side of the muscle. The muscle has its insertion on the entire inner surface of the median process of metapterygoid, on the entire inner surface of the strong metapterygoid membrane, and on the outer surface of that small portion of the hyomandibular that lies internal to that membrane, and is marked off from the rest of the bone by the line of attachment of the membrane to it. This small portion of the hyomandibular is much thinner than the rest of the bone and lies depressed below the level of the rest of its outer surface. It extends from the front, upper corner of the bone down along its front edge to the point where the metapterygoid joins or overlaps it, at about two thirds its length.

### d. *Dilatator Operculi.*

The dilatator operculi (*Do*, Figs. 30, 31, and 36, Pls. XXVI, XXVII, and XXIX) is described by McMurrich as a part of the first division of the levator arcus palatini, his description of it being : "Those [fibres] lying most posteriorly are directed backwards, passing under the upper extremity of the preoperculum, thus fulfilling the function of the separate dilatator operculi of the Teleostei."

In all the adult and larval specimens that I have examined, without exception, the dilatator has invariably been found as a

wholly separate muscle, lying immediately above and in close contact with the levator, but having no connection whatever with it except at its origin. It arises from the entire lateral edge of the squamosal back as far as the upper end of the pre-operculum, and from the postorbital process behind and above the origin of the levator. The surface of origin of the two muscles at this place is continuous, but the muscles themselves are, from the beginning, markedly distinct and separate. The dilatator runs backward across the upper end of the hyomandibular, along its outer surface. It passes through the narrow opening between the hyomandibular and the upper end of the preoperculum, and is inserted by a tendon, which forms on its outer surface, on the inner surface of the operculum above and in front of the facet forming the articulation of that bone with the hyomandibular.

In specimens of 12 mm. in length and under, the dilatator is, at its origin, united to and a part of the levator, the two muscles arising, as a single muscle, from the upper edge of the cartilaginous postorbital process immediately in front of the blind, upper end of the spiracular canal, which, at this age, lies entirely outside the cartilage of the cranium. The united muscles pass backward, external to the spiracular canal, and then separate. The levator is inserted on the inner surface of the vertical process of the metapterygoid and on the inner surface of the metapterygoid membrane behind it. Both membrane and muscle extend backward beyond the front edge of the hyomandibular, lying external to that bone and having no apparent connection with it. The muscle here is inserted entirely on the membrane, and the membrane shows no trace of attachment to the hyomandibular, but vanishes, as the muscle does, in the general tissues superficial to it. The outer division of the adductor mandibulae, $A_2$, has its origin, at this age, from the outer surface of this same membrane, there being no attachment whatever to the cranium. The dilatator extends further backward than either the levator or the adductor, and vanishes apparently closely attached to the under surface of the dermis.

**2.  Sub-Group 2.  Muscles innervated by both the Trigeminus and Facialis.**

### a.  *Intermandibularis.*

The intermandibularis (*Im*, Figs. 43 and 44, Pl. XXXI) is a short, stout muscle extending transversely from one ramus of the mandible to the other, immediately internal to the front end of the gular plate.  Its fibres arise from the dentary of one side and are inserted on that of the other, the lines of insertion extending close up to the symphysis of the bones.  Although to all appearance a single muscle, it can be easily separated along its front edge into two parts, each of which extends across the inter-ramal space and is thicker at its origin from the dentary of its own side than at its insertion on that of the other.  The two portions are completely united posteriorly and overlap as the hyohyoidei do, the muscle of the left side lying superficial to or below that of the right.

The superficial or inferior portion of the geniohyoideus, in passing from its origin on the mandible backward and inward toward the middle line of the head, lies partly superficial to the posterior portion of the intermandibularis; and the tendons of the deeper superior portion of the same muscle pass immediately dorsal to the intermandibularis closely attached to it. The branchio-mandibularis is either firmly attached to the hind margin of the intermandibularis or lies immediately dorsal to it between it and the integument of the floor of the mouth.

### b.  *Geniohyoideus.*

The larger part of the geniohyoideus (*Ghi* and *Ghs*, Figs. 43 and 44, Pl. XXXI) lies immediately internal to the gular plate, which plate covers also the intermandibularis and the anterior portions of the sternohyoideus and hyohyoideus.

The gular plate is a large and slightly convex dermal ossification about three quarters as long as the mandible (No. 3, G. Fig. 47, Pl. XLI).  It is pointed in front, where it is firmly attached by ligament to the symphysis of the mandible, and

rounded behind, where, lying in a dermal fold, it overlaps the first branchiostegal rays and covers and protects the isthmus. It nearly fills the space between the rami of the mandible, but is small enough to pass freely up and down between them, when the motions of the mouth require it. The external surface of the bone has the sculptured markings characteristic of the dermal bones in Amia, the ridges proceeding ray-like from a higher central point near the middle of the bone. Immediately internal to this point there is a dense connective tissue, almost cartilaginous in character, in one of the specimens examined; and on a level with it, along the lateral edges of the bone, there is a thick, rigid dermis forming a rim projecting upward and laterally. The gular bone itself does not give direct insertion to any muscle fibres.

The geniohyoideus has two well-marked portions, a superior, deeper one (*Ghs*) and an inferior, superficial one (*Ghi*). The deeper portion is the larger, and is a broad, flat, thick muscle, thinning out anteriorly, where it lies immediately below the integument of the floor of the mouth. It arises from the outer under surface of the ceratohyal, behind the origin of the hyo-hyoideus. It is, at its origin, V-shaped in outline, the two edges of the muscle extending much further back than the central portion. The median edge extends as far as the eighth or ninth branchiostegal ray, the muscle in this part of its course lying close along the bases of the rays. It is directed forward and inward, its median fibres meeting those of the muscle of the opposite side of the head in a vertical aponeurosis which lies in the middle line of the body and extends forward to the hind edge of the intermandibularis. The dense connective tissue mass under the central point of the gular plate lies in or is a part of this aponeurosis, lying near its hind end. It gives insertion posteriorly to a part of the most posterior or median fibres of the muscle, which fibres form a somewhat separate bundle, rising above the rest of the deeper muscle to the level of the superficial one, the posterior fibres of which also have their insertion here. Posterior to this point the fibres of the deeper muscle are more or less continuous with those of the opposite side, and sometimes entirely so, as shown

in Fig. 43. The fibres of the deeper muscle immediately lateral to the median bundle pass forward beyond the hind edge of the superficial muscle, dorsal to it, and have their insertion on the median aponeurosis, or on the hind edge of the intermandibularis. The fibres lying still more toward the lateral edge of the deeper muscle become tendinous, after passing dorsal to the superficial muscle, and are continued beyond that muscle, dorsal to the intermandibularis, as a sheet or series of flat tendons which lie immediately beneath the integumental lining of the floor of the mouth. They are closely attached to that integument, and have their insertion partly in it, and partly on the inner surface of the mandible near the symphysis. The extreme lateral fibres of the muscle run almost directly forward, from their origin on the ceratohyal, parallel to the ramus of the mandible, and have their insertion in the integument of the floor of the mouth along the median edge of the tough layer or fold of dermal tissue that extends from the lateral edge of the muscle downward and medianward to the lower, inner edge of the mandible. Some of the fibres of the muscle are also often inserted along the dermal rim formed at the middle of the lateral edge of the gular plate.

The superficial, inferior portion of the geniohyoideus arises, in young specimens, in a narrow horizontal line from an exposed portion of Meckel's cartilage on the inner surface of the mandible immediately below the lower edge of the splenial and between that bone and the dentary (Fig. 6, Pl. XX). In fullgrown fishes the surface of origin includes also the edge of the dentary immediately below this cartilage. The line of origin extends approximately from the hind edge of the intermandibularis to the level of the front end of the mandibular portion of the adductor mandibulae ; that is, through about one quarter of the length of the mandible. The muscle is flat and broad and runs backward and inward from its origin to the middle line of the head, where it is inserted, with its fellow of the opposite side, on the median, vertical aponeurosis common to it and to the deeper portion of the muscle. Its insertion is below, or superficial to, that of the deeper portion, and only extends back to the point under the middle of the gular plate where, as

already described, it meets the posterior bundle of fibres of
the deeper muscle.

The two divisions of the geniohyoideus are intimately con-
nected at and near their insertion on the median aponeurosis,
but the fibres of the two portions lie at a considerable angle to
each other, and the continuity described by McMurrich was not
found in any specimen, old or young.

In larvae 10 mm. in length some of the fibres of the super-
ficial portion of the muscle are continuous with those of the
intermandibularis, the fibres of the left side being continuous
with the ventral layer of the intermandibularis, and those on
the right side with the dorsal one. In these young larvae the
geniohyoideus is also apparently continuous with the hyohyoi-
deus.

The geniohyoideus acts either as an adductor of the hyoid
arch or as a retractor of the mandible according as the one or
the other of those structures is stationary, or is so considered.
The hyoid arch is loosely connected, in front, with the inner
surface of the mandible by a fold of the tough integument of
the floor of the mouth, which, when the parts are at rest, is
folded under the tip of the hyoid, thus forming the covering of
its under surface and the floor of the anterior part of the mouth.
Back of this, along each side, the hyoid is connected with the
lower, inner edge of the mandible by a strong fold formed by
the closely united external dermis and internal lining membrane
of the mouth, the two membranes being so closely united that
they are with difficulty separated. A strong membranous fold
is thus formed, which, when the mouth parts are at rest, lies
directed upward, from the lower edge of the mandible, parallel
to and against its inner surface. By this arrangement the
hyoid arch, lying between the rami of the mandible, is con-
nected with them, on either side, by a long and flexible fold, so
that its anterior end, which is smaller than the space between
the rami, can be freely elevated and depressed between those
rami to the full extent permitted by the fold. A deep groove
(No. 3, Fig. 16, Pl. XXXV and Fig. 22, Pl. XXXVI) on the
under surface of the head marks the position of this fold,
the groove extending from near the symphysis backward along

the inner edge of the mandible to the preopercular articulation, and then upward and backward between the two upper posterior branchiostegal rays, onto the external surface of the gill-cover behind and beyond them, where it disappears.    The fold may be called the hyoideo-mandibular fold.

In its action as a retractor of the mandible the geniohyoideus is, as Vetter and others have stated, accessory to the sternohyoideus, which is the main retractor of the hyoid and through it of the mandible.    The sternohyoideus is inserted on the under surface of the extreme anterior end of the hyoid arch.    In its action it pulls this end downward and backward in a curved line, so placed, relatively to the curve described by the tip of the mandible in opening, that the hyoid not only pushes the parts below it downward between the rami of the mandible, but, having slipped backward over those parts to the full extent allowed by its integumental connection with them and with the mandible, has its motion transferred to the mandible, and thus acts as its retractor.    The motion so given by the sternohyoideus is accelerated and increased by the action of the geniohyoideus, both divisions of which muscle, by their contraction, tend to hold the mandible down over the anterior end of the hyoid so that it shall follow more promptly the motion given to the latter.    The geniohyoideus also draws the hyoid forward, this being its simplest and most direct action, but it can do this, and so act as an adductor of the hyoid, only when the mandible is fixed by the action of its adductor.

### c. *Hyohyoideus.*

The hyohyoideus (*Hhi* and *Hhs*, Figs. 43–46, Pls. XXXI and XXXII) arises from the median edge of the flat, spreading blade of the ceratohyal, from a surface of depression beginning at the base of the first or second branchiostegal rays and extending forward along the ventral side or edge of the shank of the ceratohyal, and from the inner surfaces of the branchiostegal rays, mainly from the inner surfaces or front edges of the first and second rays.    These first two rays do not generally come into contact, at their bases, with the ceratohyal, the first ray being

often separated from that bone by a considerable interval. The remaining rays are firmly bound by their bases to the under surface of the blade of the ceratohyal, a little beyond its median edge, so that this edge is left free throughout its entire length. From this free edge the hyohyoideus in part arises.

Anterior to the branchiostegal rays, the hyohyoideus runs forward and inward, a broad, continuous band, thick at the lateral edge, but very thin at the median one, where, in its anterior portion, it passes beyond the middle line of the body, and overlaps the muscle of the opposite side to such an extent that at their insertions the two muscles are often nearly but not entirely superimposed. The muscle of the left side lies superficial to that of the right side, and the two end in front in a common, curved, tendinous line, attached at each end by strong tendons to the ventral surface of the hypohyals. Each of the two muscles has its own tendon at either end of the common anterior margin, but the tendons at each end unite more or less completely as they approach their insertion, so that a single tendon is formed, which lies in the extended line of the curved front edge of the muscles. This single tendon then separates into two smaller tendons, one of which continues in the direction of the main tendon, and is inserted on the under anterior surface of the hypohyal. The other turns sharply forward and medianward, and splits up into numerous, smaller, slender tendons, which spread out fingerlike over the under surface of the hypohyal, and are inserted on it, and in the tough tissue in front of it toward the tip of the tongue, the tendons of the one side overlapping and crossing beyond those of the other. No muscle fibres extend onto or into the tongue ; but the presence of such a network of intercrossing tendons, onto which muscle fibres or their tendons are inserted, seems to indicate an earlier stage in, or perhaps a different manner for, the "muscularization" of the tongue than that given by Gegenbaur (No. 45). The tongue in Amia can certainly not be considered as wanting a direct and definite connection with muscle fibres which are not in any way associated with a glandular system.

The two hyohyoidei are not continuous anteriorly; they merely end in curved, tendinous edges, which are superimposed

and bound together.   The curved line of these tendinous edges lies a little behind the symphysis of the hypohyals, and is concave forward, leaving a space in front of it, between it and the hypo-hyals, through which the branchiomandibularis passes.

The anterior or inferior part of the hyohyoideus is somewhat divided into two portions or bundles, one arising from the bran-chiostegal rays, and the other from the shank of the ceratohyal, the division between the two portions being faintly indicated by surface lines.   The fibres arising from the branchiostegal rays are the ones that pass across the middle line of the body, and enter into the tendon that lies on the opposite side of the head, while those arising from the ceratohyal usually pass directly forward into the tendon of their own side.   The fibres arising from the branchiostegal rays are more or less separated into parallel bundles, closely held together by connective tissue. This condition is most apparent near the front edge of the first ray, and there gives to the muscle a stringy or striped appear-ance.   These bundles have their origin in part from the front edge and under surface of the first ray, and in part they are continuations of more or less continuous cord-like muscles, which have their origin from parts beyond this ray, and form what Vetter has called in other fishes the superior part of the muscle.   These muscular cords are wholly, and in places some-what widely, separated ; they branch somewhat, and are alter-nately tendinous and muscular, the muscular portions lying, in every case, across the interspaces between successive rays. Throughout their course they are firmly held in connective tissue, which is closely attached to the inner or dorsal surfaces of the rays.   The little muscular swellings, or bellies, lie most frequently directly in the course of the more or less continuous cord, but they may extend from one cord to another, or arise from the side of a cord in front, and be inserted on the inner surface of the next ray behind.   Along the median or free edges of the rays, each cord forms a continuous line, but toward the edge of the ceratohyal the other arrangements prevail, the cords becoming somewhat crossed and interlaced ; while from the edge of the ceratohyal larger muscle bundles arise, which, running forward and outward along the inner,

dorsal surface of the rays, unite with one or more of the cords encountered there.    Some of these cords, alternately muscular and tendinous, extend as far upward and backward as the inner surface of the interoperculum ; and other small, thread-like cords, found in the integument beyond the free edges of the rays, can be traced nearly, if not quite, to the upper edge of the operculum.    In young fishes the fibres of the hyohyoideus extended up into the region of the levator operculi, but the continuity of the fibres, and hence of the two muscles, could not be established.

### 3. Sub-Group 3.   Muscles innervated by the Facialis alone.

#### a.   *Adductor Hyomandibularis.*

This muscle (*Ah*, Figs. 25 and 37, Pls. XXV and XXIX) is broad and short, extending from the auditory region of the lateral wall of the skull to the inner surface of the hyomandibular. Its line of origin begins on the petrosal, at the hind margin of the facial foramen, and extends backward and upward across that bone, and across the intercalar, nearly to the end of its posterior process.    It is inserted on the inner surface of the hyomandibular, the line of insertion extending from the front edge of the bone, backward and upward, nearly to the hind end of the opercular process.    It lies mostly below the internal opening of the facial canal, through the hyomandibular, but overlaps that opening somewhat, so that the facial nerve, in its passage from its cranial foramen to the internal opening of the canal, lies partly imbedded in the fibres of the muscle. The muscle runs outward, backward, and downward, lying, in its anterior portion, more nearly horizontal, and being there much shorter than in its posterior part.

#### b.   *Adductor Operculi and Levator Operculi.*

These two muscles (*Ao* and *Lo*, Figs. 20–30, Pls. XXIII– XXVI) are continuous at their insertion, which is on the upper portion of the inner surface of the operculum, behind the articulation with the hyomandibular, the surface of insertion occu-

pying a little more than one third of the inner surface of the bone.

The levator has its insertion posterior to that of the adductor, and is double at its origin, arising behind directly from the under surface of the suprascapular, and in front by tendinous attachment to a tendinous formation, which extends backward under the extrascapular, from the hind edges of the squamosal and parietal. A part of the tendon of the muscle can usually be traced through the tendinous formation to the under surface of the parietal, near its hind edge, and at about the middle line of the temporal groove. This long and slender part of the tendon lies immediately above the anterior extension of the trunk muscles that fills the temporal groove.

The adductor lies anterior to the levator. Its superficial fibres form a thin, narrow band, which crosses the hindermost fibres of the adductor hyomandibularis at a considerable angle to them, lying on their upper, outer surface, and arising, just above their origin, from the lateral wall of the skull, the surfaces of origin of the two muscles being continuous. The deeper fibres arise, partly by numerous delicate tendons, partly by direct attachment, from the posterior process of the intercalar, from the postero-lateral angle or edge of the cranium immediately above that process, and from the hindermost lateral extremity of the squamosal, this last attachment not being an important one. The muscle in its deeper part is continuous at its insertion with the levator operculi behind, and with the adductor hyomandibularis in front.

### 4. Review and Comparison of Muscles of Group II.

The muscles described under this group belong to the hyoid and mandibular arches, or perhaps also, in part, to one or more preoral arches (No. 124, p. 448). They form a large, complicated, and very variable group, and their definite innervation is but little known. Any attempt to compare the muscles in Amia with those described in other fishes can, therefore, be of but little value in so far as the possible homologies established for the several muscles or the different parts of those muscles

are concerned.   It seems, however, proper to attempt to bring
my results into some sort of accord with, or relation to, those
of others.   In doing this, I shall frequently refer to dissections
made of several fishes, more particularly of Carcharinus littor-
alis and Galeus canis, in the earlier stages of this investigation.
They were made most superficially, and simply to determine,
approximately, certain points that I thought obscure but of
importance in Vetter's descriptions.   Notes and sketches were
made at the time, and, as I have only them to refer to, the
statements now made regarding the several fishes may not be
entirely reliable.

### a. *Levator Arcus Palatini, Dilatator Operculi, and Muscle Add*γ.

The levator arcus palatini and dilatator operculi are certainly
parts of a single muscle.   They are considered by Vetter (No.
125, pp. 484 and 531) as the homologue of the protractor hyo-
mandibularis in Acipenser, and of the levator maxillae superioris
in selachians.   He derives them, as he does those muscles, from
the dorsal half of the superficial layer of the general constrictor
of the mandibular arch (No. 124, p. 407).   McMurrich, on the
contrary (No. 76, p. 127), derives them from some part of the
adductor mandibulae of selachians, that is, from a muscle that
is developed, according to Vetter and Gegenbaur (No. 124, p.
446), from the deeper layer of the general constrictor of the
mandibular arch.   Whatever their derivation may be, they seem
to me to be the homologue of the muscle called by Vetter in
selachians *Add*γ.   It seems impossible that they should be the
homologue of the levator maxillae superioris, as Vetter sug-
gests, or be derived from that muscle if, as I shall attempt to
show, the muscle $Lms^1$, in Amia, is the homologue of one or
more of the spiracle muscles of selachians, the muscles called
by Tiesing (No. 123, p. 92) the levator palpebrae nictitantis,
retractor palpebrae superioris, and constrictor superficialis dor-
salis *I*γ.   These muscles in selachians cross the outer surface
of the levator maxillae superioris, while $Lms^1$, in Amia, crosses
the inner surface of the levator arcus palatini.

The muscle *Addγ*, in Scymnus and Acanthias (No. 124, p. 448), arises partly tendinous from the under surface of the skin, immediately behind the eye, and immediately in front of the postorbital process.   It runs downward and backward, and is inserted on the outer surface of the adductor mandibulae, being there more muscular than in its upper portion, and being in part a direct continuation of the fibres of *Csv*$_2$, which Vetter considers as the ventral half of the superficial layer of the constrictor of the hyoid arch.   In Heptanchus *Addγ* seems, from Vetter's figure, to arise from the lower rather than the upper corner of the orbit, as in Scymnus ; it runs more nearly backward, its hindermost fibres are inserted on the cartilage at the hind corner of the upper jaw, and it has no connection with the muscle fibres of the ventral half of the constrictor.   The innervation of the muscle is not given in either fish described.   In Mustelus and the rays, Tiesing does not describe this muscle. That it is not peculiar to the fishes described by Vetter is shown by Carcharinus, where a muscle is found closely resembling that of Heptanchus.   It arises near the upper corner of the eye, is inserted in part on the lower end of the hyomandibular, and its posterior portion is crossed by many branches of the facial, which go entirely or in large part to cutaneous or subcutaneous tissues.   The muscle is apparently innervated by branches of a nerve which arises inside the cranial cavity, from the main truncus or ganglion of the trigeminus, the nerve issuing immediately behind the eye, around the front edge of the levator maxillae superioris, and then turning backward along the outer surface of *Addγ*.

*Addγ* is considered by Vetter as the last remnant of the superficial constrictor of some one or more preoral arches (No. 124, p. 448).   Its position, however, in Scymnus and Acanthias, its connection with the adductor, and its continuity with the ventral half of the constrictor, seem to indicate that it belongs to the mandibular arch, and that it is derived from the dorsal half of the superficial constrictor of that arch.   Its innervation, if the innervation found in Carcharinus be correct, agrees closely with that of the levator arcus palatini in Amia, and such slight changes only would be needed in its origin and insertion,

to derive that muscle from it, that it may safely be considered as its homologue. The strong aponeurosis in the upper part of the muscle in Amia corresponds to the tendinous, upper part of the muscle in Scymnus, and in the young of Amia the muscle is connected or associated with the adductor mandibulae much more than with the palatine arch, as it is in the adult. In Galeus canis *Addγ* was not found. It is, however, probably represented in one of the two spiracle muscles.

In Chimaera and Acipenser *Addγ* cannot with certainty be identified in the descriptions given by Vetter, but it may be represented in Chimaera by $Cs_4$, and in Acipenser by the upper tendinous part of $Cs_1$ and $Cs_2$. In the one specimen of Acipenser that I examined, a young Acipenser ruthenus about one foot in length, this upper part of $Cs_1$ and $Cs_2$ seemed to be a tendinous formation into which the muscle fibres of the lower portion were inserted, rather than a tendon of that lower portion. Its position does not differ greatly from that of *Addγ* in Heptanchus, and if it be that muscle, it would account for the unusual insertion, below and in front of the eye, of $Cs_1$ and $Cs_2$, the lower portions of which represent, in Acipenser, that part of the superficial constrictor from which the geniohyoideus in other fishes arises. This part of the constrictor is, in Acanthias (No. 124, Fig. 3, Pl. XIV), inserted at the hind edge of the lower jaw, mainly into a fascia that covers and arises from the lower portion of the outer surface of the adductor mandibulae, just as *Addγ* arises, in the same fish, from the upper portion of that surface. Slight changes only in the insertions of these muscles in Acanthias would bring about the arrangement found in Acipenser.

### b. *Levator Maxillae Superioris, $Csd_1$, and Addβ.*

The levator maxillae superioris, in Heptanchus, arises from the occipital part of the skull under the projecting and overhanging postorbital process. Immediately behind it, and partly continuous with it, is another muscle, called by Vetter $Csd_1$. Both muscles run downward and forward, and are inserted on the inner side of the upper jaw, the hind muscle covering with its

inner, posterior surface the front wall of the spiracular canal.
The two muscles represent, according to Vetter, the dorsal half
of the superficial constrictor of the mandibular arch.

In Carcharinus and in Acanthias much the same relations
exist, but in Scymnus the hind muscle, $Csd_1$, has become
entirely independent. It arises from the hind, upper corner of
the skull, and running forward and downward, unquestionably
along the outer surface of the levator although it is not so
stated, it encircles the front side of the outer ends of the two
spiracular cartilages and is inserted on the inner side of the
quadrate part of the upper jaw. It is called by Vetter the
muscle of the spiracular cartilage.

In Galeus a still further differentiation of this muscle has
taken place, for it is here inserted near the hind edge of the
lower eyelid, some few fibres only, from its inner surface, leav-
ing the main muscle to be inserted on the inner surface of the
upper jaw. A second spiracular muscle is also found. It arises
immediately behind the spiracular canal from the outer surface
of, or from tissues superficial to, $Csd_2$. Its lower fibres run
upward and then forward, partly encircling the spiracle, its
upper fibres directly forward. Beyond the spiracle the muscle
divides, allows the larger spiracle muscle to pass through it,
and then, reuniting, is inserted, according to my notes, near
the edge of the lower eyelid, at the corner of the eye, above
and behind the insertion of the larger muscle. In Mustelus,
also, there are two spiracle muscles. The larger of the two
is described by Tiesing as the levator palpebrae nictitantes, and
is unquestionably derived directly from the muscle called by
Vetter $Csd_1$. The other, smaller, muscle lies internal to the
larger one, and is inserted on the hind end of the upper eyelid.
Its insertion is thus higher than that I have noted for the
corresponding muscle in Galeus. It is called by Tiesing the
retractor palpebrae superioris, and is undoubtedly, as he con-
cludes, also derived from $Csd_1$, or perhaps from that muscle
and $Add\gamma$ also.

In Heptanchus the anterior part of the adductor mandibulae
extends almost to the "palatobasal" articular process of the
upper jaw. In Carcharinus a part of this anterior portion

forms a distinctly separate muscle lying in front of and over-lapping externally the main muscle. It is tendinous above, the long, somewhat slender tendon running forward and becoming muscular again before its insertion, as a short, stout muscle body, on the anterior wall of the orbit immediately below the inser-tion of the obliqui muscles. In Galeus the tendon connecting these two portions of the muscle is short, and the stout, orbital muscle-body lies imbedded in the remaining lower portion of the muscle, with which its fibres are in part directly contin-uous. That part of the lower portion of the muscle that lies immediately posterior to the tendon of the upper portion, has its origin from the upper edge of the cartilage of the upper jaw ; that part that lies in front of the tendon has its origin, in part, from the front wall of the orbit and, in part, it extends for-ward on the under side of the head towards the middle line of the body, having its origin there. The outer fibres of the lower portion of the muscle become tendinous posteriorly, the tendon being in part continuous with a fascia covering the outer sur-face of the adductor and extending to the hind edge of the lower jaw, and in part continuous with a tendinous formation extending into the adductor from its outer surface. The deeper fibres of the muscle are continuous with the fibres of the deeper part of the adductor. A somewhat similar arrangement is found in Acanthias and Scymnus, but the separation of the muscle from the main adductor seems to be more complete than in Galeus, and the tendon in its lower posterior portion more developed. The muscle is, however, considered by Vetter a part of the adductor and is called by him $Add\beta$. The corre-sponding muscle in Mustelus is called by Tiesing the levator labii superioris.

In Heptanchus the special innervation of that part of the adductor that corresponds to $Add\beta$ is not given by Vetter. In Acanthias and Scymnus, he says, that it is innervated by a branch of the r. maxillaris superior trigemini, but Stannius (No. 116, p. 46) finds the corresponding muscle in Spinax, and Tiesing (No. 123, pp. 86 and 96) the corresponding muscle in Mustelus innervated by a branch of the r. maxillaris inferior trigemini. In the three fishes described by Vetter the levator

maxillae superioris, $Csd_1$, and the spiracle muscle are all said by
him to be innervated by a single branch of the r. maxillaris
inferior trigemini, the branch being given off immediately after
the main nerve issues from its foramen. The branch runs
outward and backward under the postorbital process along
the front or outer surface of the levator, and then apparently
backward along the lateral surface of the levator and the outer
surface of $Csd_1$. In Mustelus (Tiesing) the corresponding
muscles have a similar innervation, the nerve innervating them
being sometimes double.

In Galeus the larger spiracle muscle and the levator maxillae
superioris are innervated by a branch of a small double nerve,
which arises inside the cranial cavity, to all appearance from
the upper surface of the truncus trigeminus, or of its ganglion.
As it issues through the main trigeminal foramen this double
nerve lies along the upper surface of the inferior maxillary
nerve, crossing that nerve from its inner to its outer edge.
It then turns under the nerve, to its lower surface, and con-
tinues forward in this position to the upper edge of the upper
jaw. Here, as the inferior maxillary turns downward and back-
ward, the double nerve issues from beneath it, at its inner edge,
and, running forward, supplies the muscle $Add\beta$. The nerve
is so closely applied to the inferior maxillary, and, where it
issues from beneath it, appears so exactly like a branch of that
nerve, that it would not have been recognized as a separate
nerve if the arrangement of the nerves and muscles in Amia had
not indicated that it must be there as such. It is unquestion-
ably the r. ad musc. levator maxillae superioris of Amia, but the
nerve in Galeus is always double, as it was found to be in one
specimen only of Amia. From the outer strand of the nerve
in Galeus, soon after it leaves the cranium, a branch is sent to
the levator maxillae superioris and the larger spiracle muscle,
which two muscles therefore unquestionably correspond respec-
tively to the second and first divisions of the levator maxillae
superioris in Amia. The same strand then innervates the
lower and interior portions of $Add\beta$, which accordingly corre-
spond to the third division of the levator in Amia, while the
orbital part of the muscle, which is innervated entirely by the

inner strand of the nerve, corresponds to the fourth division. This nerve was traced in Galeus on one side only of a single specimen. Its being double suggests an explanation of an apparently abnormal innervation of $Lms^4$ by the superior maxillary nerve, found in one specimen of Amia. The strand destined for the innervation of this muscle in this particular specimen had doubtless become separated from the rest of the nerve and attached secondarily to the superior maxillary which it so closely accompanies. It arises in Galeus from the upper surface of the truncus trigeminus, while in Amia it arises from the under surface of that truncus, an important difference. In Galeus it partly encircles the inferior maxillary, holding that nerve as in a loop. If this position of the nerves represents an earlier condition than that found in Amia, the inferior maxillary, as it moved backward to the position found in Amia, must have caused an apparent shifting of the origin of the nerve from the upper to the under surface of the truncus.

The innervation of the smaller spiracle muscle in Galeus was not determined. The muscle may, from its position, be derived either from $Csd_1$ or from $Add\gamma$. The innervation of the spiracle muscles in Mustelus by two nerves suggests that they are of similar, but perhaps not of identical, origin. The same is true of the muscles innervated by the double nerve in Galeus. It therefore seems probable that $Csd_1$, $Lms$, $Add\beta$ and $Add\gamma$ in Heptanchus, Carcharinus, and other selachians, represent parts of the constrictor superficialis dorsalis of the mandibular arch, or that they represent parts of that constrictor and parts also of the corresponding portion of the constrictor of one or more preoral arches. From $Add\gamma$ the levator arcus palatini and dilatator operculi of Amia are derived, and possibly also one of the spiracle muscles in Galeus and Mustelus ; from $Csd_1$ is derived the larger spiracle muscle of Galeus, or perhaps both the spiracle muscles; from the larger spiracle muscle of Galeus is derived the muscle $Lms^1$ of Amia ; from the levator maxillae superioris of selachians is derived the muscle $Lms^2$ of Amia ; and from $Add\beta$ (the levator labii superioris of Tiesing) the muscles $Lms^3$ and $Lms^4$. In rays this last muscle splits up, according to Tiesing, into several more or less independent portions.

In Chimaera and in Callorynchus there is a muscle, called by Vetter the levator anguli oris, which lies below and in front of the eye, immediately superficial to the adductor mandibulae. The anterior part of this muscle arises as a thin, broad tendon above and in front of the eye.   It contracts immediately to a smaller tendon, and then, becoming muscular, separates into two portions and is inserted by round tendons on the adjoining ends of the maxillary and mandibular cartilages (No. 125, p. 441).   The posterior part of the muscle arises from the lower edge of the orbit, and is inserted by a very slender tendon on the mandibular cartilage and in the skin at the corner of the mouth.   Vetter gives the innervation of both divisions in Chimaera by branches of the inferior maxillary, but his figure seems to show that the anterior division of the muscle is innervated by a branch of the superior maxillary, which nerve supplies also the labialis anterior.   In Callorynchus both divisions of the muscle, and the labialis anterior also, are said by Stannius to be innervated by the superior maxillary (No. 116, p. 46 and Fig. 1, Pl. 1).   This want of agreement in the two descriptions strongly suggests the error to which Vetter has himself called especial attention (No. 125, p. 495, Note 1 ): that of not tracing a nerve or the branch of a nerve to its origin, and leads one to infer that the three muscles are innervated by a nerve corresponding to the r. ad musc. levator maxillae superioris in Amia, and hence that they correspond to the four divisions of the musculus levator maxillae superioris of Amia ; the labialis anterior, probably, to the fourth division of that muscle ; the anterior division of the levator anguli oris, with its double insertion, to the third, which is frequently double through part of its length in Amia ; and the posterior division, which is inserted in part in the skin at the corner of the mouth, to the second and first divisions in Amia, the former of which in Amia has in part this same attachment. The position of the levator anguli oris superficial to the adductor mandibulae is naturally suggested by the arrangement found in Galeus, where *Addβ* lies in part superficial to *Add*.

In Acipenser the large and powerful protractor hyomandibularis is considered by Vetter the homologue of the levator max-

illae superioris of selachians (No. 125, p. 484). He finds the
muscle, in Acipenser, innervated by a branch of the r. maxil-
laris inferior trigemini arising from the still undivided trunk of
the nerve (No. 125, p. 474). Stannius gives the same innerva-
tion, but adds that the muscle contracts when the second root
of the trigeminus is irritated, thus indicating that the nerve is
a branch of the superior maxillary. In the one specimen that
I examined the nerve arose from the upper surface of the
truncus or ganglion of the trigeminus, and as there was no
branch to other muscles or tissues, the muscle it innervated
may correspond either to the first and second divisions of the
levator maxillae superioris of Amia, or to the levator arcus
palatini. The third and fourth divisions of the former muscle
were represented by a tendinous, fatty, and degenerate muscle
tissue extending from the cartilage below and in front of the
eye to the under surface of the skin near the lower end of
the hyomandibular. The r. maxillaris superior trigemini and
the r. buccalis facialis of van Wijhe ran forward across the
upper end of this tissue ; the r. palatinus anterior facialis
internal to it.

In teleosts the levator arcus palatini and dilatator operculi
do not differ greatly from the corresponding muscles in Amia.
The levator maxillae superioris, on the contrary, has undergone
great changes. In most teleosts all four divisions of it have
been largely absorbed by the adductor mandibulae, but in
being absorbed they have determined in many ways the
arrangement and disposition of that muscle. In Esox part
of the levator muscle is undoubtedly represented in the
aberrant bundle $A_3\beta$, of Vetter. The tendon of another part
has undoubtedly determined in Esox, and in Perca, Cyprinus,
and Barbus as well, the insertion of $A_3$ at the hind end of
Meckel's cartilage, and in Perca another tendon persists as
tendon $A_2{}^t$ of Vetter.

In Amiurus there are two tentacle muscles, the adductor
and the abductor tentaculi (No. 75, p. 314). The adductor is
either simply the first and second divisions of the levator max-
illae superioris of Amia, or some or all of the divisions of that
muscle and the muscle $A_3$ combined. The abductor is described

by McMurrich as the anterior portion of the adductor arcus
palatini, and is said by him to be innervated, as the posterior
portion of that muscle is, by a branch of the facialis. But for
this innervation this muscle would certainly seem to be the
third or fourth division of the levator maxillae superioris of
Amia, for both in its origin and insertion it differs but little
from and could easily be derived from that of those muscles.
Pollard says (No. 97, p. 390) that the motor nerves to the cor-
responding muscle in Auchenapsis " run along with the pala-
tine nerve," the palatine nerve referred to being described as a
branch of the trigeminus. Pollard further states, as a general
principle, that the motor supply of all the tentacle muscles in
siluroids must be by nerves that " proceed out with the sensory
nerves " of the tentacles (No. 97, p. 382). This general state-
ment shows, what Pollard does not otherwise definitely state,
that he found all the tentacle muscles in all the fishes he exam-
ined supplied, as the tentacles themselves are said to be, by
branches of the trigeminus. The innervation of the muscle
in Amiurus may therefore be by the trigeminus instead of by
the facialis, as McMurrich states, in which case it is certainly
the homologue of the third and fourth divisions of the levator
maxillae superioris.

In the Marsipobranchii *Addβ* is represented probably in the
group of muscles that in Mixine are innervated by the so-called
r. ophthalmicus trigemini, and to the muscles that in Petromy-
zon are associated with the " Vorknorpel " and innervated by
the r. maxillaris trigemini (No. 37, pp. 34 and 40). The r. oph-
thalmicus trigemini in Mixine, according to Fürbringer (No. 37,
p. 71), gives off a sensory branch which runs forward above the
optic nerve and then itself runs under that nerve, giving off
both sensory and motor branches, the motor branches going to
the muscles of the tentacular circle and the nasal tube. In
Petromyzon the r. ophthalmicus trigemini is said to be entirely
sensory, and runs over the optic nerve, as does the first sensory
branch of the nerve in Mixine (No. 37, p. 62). In Bdello-
stoma also the main trunk of the nerve runs over the opticus,
but whether it contains motor fibres or not Fürbringer does
not state (No. 37, p. 31). The ophthalmic nerve in Mixine

thus finds no homologue in Petromyzon, Bdellostoma, or other fishes, and it seems safe to assume that that part of it that lies under the opticus corresponds to the ramus ad musc. levator maxillae superioris and parts of the ramus maxillaris superior trigemini combined of Amia, while the branch above the opticus is the true ramus ophthalmicus. Whether these deductions be correct or not, I certainly should not accept existing descriptions of these nerves as supporting Pollard's dictum, based in part upon them, that "the topographical position and course of nerves is not of great importance" (No. 97, p. 397).

### c. *Adductor Mandibulae.*

The adductor mandibulae proper in Heptanchus, Acanthias, and Scymnus, as described by Vetter, is an extremely simple muscle ; practically an uninterrupted and undivided layer of muscle fibres extending from near the upper edge of the palatine arch to the lower edge of the mandible. In Carcharinus and Galeus it is not so simple. In both these fishes the r. maxillaris inferior trigemini penetrates the muscle between a superficial and a deeper portion, which are entirely separate at and near the surface. A surface line extending upward and forward across the muscle, from, or from near, the corner of the mouth, marks the front edge of the superficial portion. The fibres of this superficial portion are inserted into a transverse tendinous formation, or aponeurosis, which extends inward and upward into the muscle from a curved surface line which extends from the corner of the mouth backward, outward, and upward toward the hind corner of the upper jaw. This tendon does not extend entirely through the muscle. It is inserted behind and above on the hind corner of the upper jaw, and in front it is attached to the dermis at the corner of the mouth. It is pierced by the r. maxillaris inferior trigemini, in Carcharinus at a considerable depth below the outer surface, in Galeus near that surface, the nerve in the latter fish coming outward toward the surface along the front edge and outer surface of the tendon. In Carcharinus the nerve, having pierced the tendon, or having even passed entirely beneath it near its front edge, continues for some dis-

tance below the surface of the muscle, and then comes to its outer surface ; in Galeus it comes immediately to the outer surface of the muscle and in its further course lies on that surface. In both fishes it is joined near the front end of the muscle by a branch of the facialis, apparently the r. mandibularis externus facialis of Amia.

The tendon above described gives insertion at the corner of the mouth to the tendon of $Add\beta$. From its lower, inner surface the fibres of the superficial portion of the mandibular part of the adductor arise. It seems, therefore, to represent the tendon $A_2 A\omega'$ of Amia, and the muscle fibres arising from it to represent $A_2$ and $A\omega'$, the deeper portions of the adductor muscle corresponding to $A_3$ and $A\omega''$. At the point where the r. maxillaris inferior trigemini pierces or meets the tendon several branches are sent into the adductor muscle, supplying its deeper as well as its outer portions. These branches run backward between the two portions of the muscle, thus corresponding in position to the branches that, in Amia, run downward and backward between $A_2$ and $A_3$, innervating those muscles. That part of the muscle that arises from the upper surface of the tendon, and hence lies directly superficial to the main inferior maxillary nerve, therefore, corresponds to the superficial layer $A_2'$ of Amia. The rest of the superficial portion, or possibly the tendon alone, represents the deeper layers $A_2''$ and $A_2'''$ of Amia, which, if they are represented by the tendon alone, must have acquired their relative importance later.

With the several portions of the adductor found in rays and described by Tiesing I am unable to make any satisfactory comparison whatever.

In teleosts the adductor presents markedly different arrangements in different species. The innervation of the different parts of the muscle not being definitely known, a definite comparison with the muscles found in Amia is not possible. Certain probabilities can, however, be arrived at.

The third and fourth divisions of the levator maxillae superioris of Amia are, as already stated, probably found as special insertions of the adductor in teleosts, or as aberrant bundles

of that muscle.  Their further consideration is not necessary. The first and second divisions of the levator either disappear entirely or fuse with the adductor proper of Amia to form the main portion of that muscle in teleosts.  This McMurrich (No. 76, p. 127) has already suggested, the adductor in teleosts being, in his opinion, equivalent to that of Amia plus the levator maxillae superioris of selachians, that is, plus the second, or the first and second, divisions of the muscle of Amia.  This is indicated by certain features in the innervation of the muscle, such as the innervation of the superficial portion of the adductor in Amiurus by a special branch of the trigeminus, the branch arising proximal to the branch that supplies the deeper portion of the muscle (No. 75, p. 313), and the innervation of a part of the adductor in Silurus, Salmo, and Gadus by branches arising directly from the ganglion of the nervus trigeminus (No. 116, p. 45).  The innervation, in Amiurus (No. 132, p. 368), of the levator arcus palatini and dilatator operculi by certain fibres arising from a superficial branch of the r. ad musc. adductorem mandibulae, has no parallel in Amia.

The deeper part, $A_3$, of the adductor in Cyprinus is inserted mainly by tendon on the inner side of the dentary, but in part it passes into a short, flat muscle $A\omega$, and is inserted at the hind end of Meckel's cartilage and on the adjoining parts of the dentary.  In Barbus, $A_3$ is inserted mainly by tendon at the hind end of Meckel's cartilage, but in part it becomes muscular again and is inserted farther forward on the inner surface of the dentary.  In Perca and in Esox the muscle $A\omega''$ has entirely disappeared, and $A_3$ is inserted wholly by tendon at the hind end of Meckel's cartilage, this manner of insertion being doubtless determined by the preëxisting tendons of *Lsm²* and *Lsm³*.  In Amiurus also the fibres of $A_3$ have followed apparently the tendons of *Lsm³*, but in the opposite direction, that is, toward the origin of the muscle, $A_3$ being inserted by tendon at the base of the maxilla.  The tendon is split near its insertion and the rr. maxillaris sup. trigemini and buccalis facialis run forward between the two ends (No. 75, p. 314 and No. 132, p. 368), as they do between the two heads of *Lms³*, or between *Lms³* and *Lms⁴* in Amia.

The superficial muscle $A_2$, of Amia, has in teleosts undergone much modification, due mainly to the marked development of the superficial portion of the muscle, $A_2'$, and its differentiation in some fishes as a wholly separate muscle, the deeper parts of the muscle $A_2''$ and $A_2'''$ undergoing at the same time an actual or relative reduction.   In Amia the outer fibres of $A_2'$ extend at their insertion almost onto a strong ligament, which extends from the coronoid process of the mandible to the inner surface of the maxilla.   A slight change in the insertion of these fibres would give rise to the condition found in Perca, where the muscle $A_1$, of Vetter, is inserted in part by tendon on the inner surface of the maxilla, and in part has retained its connection with the deeper fibres of the adductor, and is inserted with them on the anterior, lower corner of the articular.   In Barbus this last connection has disappeared, and the muscle is inserted entirely on the maxilla along its lower edge.   In Cyprinus the muscle has separated into two portions, wholly distinct at their insertions on the maxilla, but still united at their origins, while in Moxostoma, which I examined, the separation at the origin has become complete, and each of the two muscles is again partly separated into two portions.   In Moxostoma the deeper muscle $A_2$ of Vetter, which is equivalent to $A_2''$ and $A_2'''$ in Amia, is greatly reduced, and is simply a thick muscle band lying along the upper edge of $A_3$.   That this band is the muscle $A_2$ of Vetter, or a part of it, and not a part of $A_3$, which it has every appearance of being, is evident from the position of the r. ad musc. adductor mandibulae, which runs under the band and issues between it and $A_3$, the remaining lower portion of the muscle ; for it is not probable that this nerve, which lies so constantly between $A_2$ and $A_3$ in other fishes, should have so radically shifted its position in this one as to penetrate $A_3$ from its under surface.

In Esox and Amiurus more primitive arrangements are presented than that found in Amia, for in neither of them is there any separation of a superficial layer, although there are in both indications either of the absorption, or of the preëxistence, of such a layer.   In determining the existence of this layer, the course and position of the inferior maxillary nerve is of first

importance, for the nerve seems to lie always between $A_1$ and $A_2$, a position derived directly from that found in selachians. Apparent departures from this position are often given, but in the three cases that I have examined, namely, Esox, Amiurus, and Moxostoma, it seems due to a misconception of the different parts or layers of the muscle, and not to a variation in the position of the nerve. In Esox no part of the muscle $A_2$ of Vetter is inserted on the hind edge or outer surface of the mandible, the entire muscle joining, or passing into, the well-developed mandibular muscle $A\omega$, and having its insertion with that muscle inside the ramus of the mandible. A superficial portion is, however, indicated, and it is along and slightly under the front edge of this portion, between it and the bundle $A_3\beta$, and then on the outer surface of $A\omega$, that the inferior maxillary nerve enters the mandible. Vetter's description, which leads one to infer that the nerve enters the mandible between $A_2$ and $A_3$, that is, internal to the tendon $A_2\,A\omega$, which it must therefore first pierce to reach its later position on the outer surface of $A\omega$ (No. 125, p. 495), is an error. In Amiurus some of the superficial fibres of $A_2$, the AM of McMurrich, are inserted on the hind edge of the mandible, and as the inferior maxillary nerve enters the mandible along their front edge, on the outer surface of the remaining, deeper portion of the muscle, these fibres undoubtedly represent $A_1$. In Moxostoma, where $A_1$ is found well developed, the inferior maxillary nerve lies along the front or upper edge of $A_2$ and not internal to it, that is, between it and $A_3$, as Vetter describes it in Cyprinus and Barbus.

### d. *Intermandibularis, Geniohyoideus, and Hyohyoideus.*

These three muscles are derived by Vetter from the ventral portion of that part of the general constrictor that, in selachians, lies in front of the first gill slit. In the earlier part of his work Vetter states (No. 124, pp. 411, 417, and 426) that this part of the constrictor is innervated by the facial alone, and that it accordingly belongs entirely to the hyoid arch. Tiesing's results agree with this earlier work of Vetter's. Vetter, how-

ever, in his later publication (No. 125, p. 471), questions the correctness of his earlier determinations, and states positively that, in Sphyrna malleus, Prionodon glaucus, Scyllium canicula, and Galeus canis, that part of the muscle that lies immediately behind the angle of the chin is innervated by the trigeminus, and hence belongs to the mandibular arch. The nerve by which this small anterior portion of the muscle is innervated is said by Vetter to be a branch of the r. maxillaris inferior trigemini, given off close to the symphysis of the mandible. It runs backward and inward over the cartilage into the muscle, and there forms numerous anastomoses with the delicate terminal branches of the r. hyoideus facialis. In the American Galeus canis I could not with certainty identify this nerve. In the specimen examined of that fish a branch was given off by the r. maxillaris inferior trigemini while that nerve still lay on the outer surface of the adductor mandibulae, at some little distance from the end of the muscle, and hence at a still greater distance from the symphysis of the mandible. The branch ran medianward along the outer surface of the adductor, and, crossing the narrow line of cartilage which alone separates the adductor from the ventral constrictor, entered the latter muscle at some distance from its front end. It seems hardly possible that this can be the branch described by Vetter, but it seems still less possible that in this careful work he could have failed entirely to find so large a nerve. In Carcharinus the corresponding branch was a large and important one given off before the main nerve reached the outer surface of the adductor. It separated into two fairly equal portions before reaching the constrictor, one of which ran forward and the other backward in that muscle. A small branch was sent backward and medianward from, or from near, the base of the nerve. In both Galeus and Carcharinus the main inferior maxillary, after leaving the adductor at its front end, continued forward along the outer surface of the cartilage of the lower jaw toward the symphysis, giving off several branches. One of these branches may be the one described by Vetter as going to the extreme front end of the constrictor. I did not so find it, but I did not make careful search, and cannot say that it does not exist. If

there be such a branch, it must correspond to the nerve that I
shall describe in Amia as r. ghi, and the important branch in
Galeus and Carcharinus to the nerve r. ghs ; if there be no such
branch, and if there be no other branch sent to the constrictor
proximal to the one that I have described in Galeus and Car-
charinus, then this one branch alone must give rise to the two
branches in Amia, one of its branches splitting off from the
main nerve as the regions innervated by the branches became
distinct and separate.

Whatever the homologies of these several branches of the
trigeminus may be, their distribution in selachians shows
that a large and important part of the ventral constrictor in
those fishes may belong to the mandibular arch. Vetter's
muscle $Csv_2$, therefore corresponds, in its innervation partly by
the trigeminus and partly by the facialis, to the three muscles
described under this subheading in Amia. As the muscle in
selachians, in its superficial portion, forms an unbroken layer
extending from near the symphysis to and beyond the hind end
of the lower jaw, the determination, even in a general way, of
which part of it gives rise to each of the three muscles in Amia
is largely a matter of supposition.

In Galeus and Carcharinus there is no superficial bundle
of $Csv_2$ inserted on the outer surface of the adductor mandi-
bulae, as described by Vetter in Scymnus and Acanthias. The
hindermost fibres, however, of that part of the muscle that is
inserted along the lower edge of the mandible, and which may
be called the anterior portion of the muscle, form a somewhat
separate bundle, continuous with the rest of the muscle in its
median ventral portion, but distinctly separate from it at its
insertion. This bundle is also distinctly separate at its inser-
tion from that part of the constrictor that lies immediately
posterior to it. When its fibres are separated with the scalpel
from the rest of the muscle, an inner, deeper layer is disclosed
lying immediately internal to it. This deeper layer has its
origin entirely from the hyoid arch, and is continuous behind
with the posterior portion of the superficial constrictor. It ex-
tends forward about one half the length of the mandible, runs
almost directly medianward to the lateral edge of the m. coraco-

mandibularis, which it overlaps slightly ventrally, and is inserted in a fascia or aponeurosis which seems to extend across the ventral as well as the dorsal surface of that muscle.

The facial nerves in both Galeus and Carcharinus come to the outer surface of the head along the front edge of $Csd_2$. Several branches are sent backward to that muscle as the nerve reaches the outer surface, or even before it reaches the surface. A large branch, corresponding apparently to the ramus mandibularis facialis in Amia, is then sent downward in front of the main truncus. After giving off this important mandibular branch, the remaining, larger part of the facial nerve continues downward and backward, immediately behind the upper or hind edge of the upper jaw, to the point where the hindmost bundle of the anterior part of the ventral constrictor is attached to the hind edge of the mandible. There a branch, apparently sensory, is sent medianward and forward across that bundle. After giving off this branch, and after having sent several branches to the posterior portion of the constrictor, the remainder of the facial nerve passes internal to the hindermost bundle of the anterior portion of the muscle, and, lying along the outer surface of the inner, deeper layer of the muscle near its lateral edge, runs medianward and forward, sending branches to the deeper muscle and also to the superficial one. A large and important branch, if not the main nerve itself, enters the superficial muscle near the front edge of the inner, deeper one, and not far from the point where the superficial muscle is entered by the branch already described of the r. maxillaris inferior trigemini. This part of the facial nerve is unquestionably the r. hyoideus of Amia, and the inner, deeper muscle, along the outer surface of which it lies, the only part of the ventral constrictor of the hyoid and mandibular arches that has become an even fairly independent muscle, is the hyohyoideus either wholly or in part.

This muscle, the hyohyoideus, is apparently much more differentiated in Galeus and Carcharinus than in either of the three selachians described by Vetter, for in them the muscle is said to arise from the inner surface of a tendinous streak or aponeurosis that forms the median, ventral portion of the superficial

muscle. It is, in all three, continuous behind with the posterior
portion of the superficial muscle, and the facial nerve in all pen-
etrates the muscle at this point ; the point of penetration in
Heptanchus being at about one third the length of the upper
jaw, and in Scymnus and Acanthias at its hind end, as in Galeus
and Carcharinus. In all three fishes the nerve separates at
once into two parts : a r. hyoideus which runs forward along the
outer surface of the hyohyoideus, supplying it, mainly (No. 124,
p. 418), but sending branches also to the superficial muscle, and
a branch which runs forward along the inner surface of the lower
jaw and is said, in Scymnus and Acanthias, to supply in part the
most anterior portion of the constrictor.

That part of the superficial constrictor that lies in front of
the point where the muscle is penetrated by the facial nerve
thus forms an anterior portion, innervated in its anterior part,
probably, by the trigeminus mainly, but by the facialis in part,
and in its hindermost portion by the facialis mainly or alone.
From the anterior part of the muscle the intermandibularis and
the inferior division of the geniohyoideus probably arise ; from
the next following and hindermost portions the superior division
of the geniohyoideus. In the several selachians here consid-
ered and the other fishes described by Vetter a regular transla-
tion of this last portion of the muscle from before backward is
shown. In Heptanchus its hindermost fibres extend only to a
point about two thirds the length of the mandible ; in Scymnus
and Acanthias they have reached the hind end of the lower
jaw ; and in Acipenser they have passed beyond the mandible,
the muscle arising in part ($Cs_1$ and $Cs_2$) from a fascia below
and in front of the eye, an origin most naturally arising from
the insertion of its superficial bundle in Scymnus, and in part
($Cs_3$) from the upper end of the ceratohyal above and behind
the surface of origin of the hyoideus inferior. In Amia this
last attachment only is found. In Acipenser the r. maxillaris
inferior trigemini, after entering the lower jaw, sends two
branches perpendicularly across the outer surface of Meckel's
cartilage (No. 129, p. 233). The anterior of these two branches,
in the specimen I examined, went to the muscle $Cs_6$ of Vetter,
and the posterior one backward along the outer surface of $Cs_1$

and $Cs_2$. Whether or not it extended to $Cs_3$ and $Cs_4$ I did not attempt to determine, nor did I attempt to trace the course of the facial nerve. It was sufficient for my purpose at the time to know that $Cs_1$ and $Cs_2$ represent, in all probability, the superior division of the geniohyoideus of Amia, and that $Cs_3$ either represents also a part of that muscle or that, continuing its backward translation, it has finally lost its attachment to the upper end of the ceratohyal and become associated with $Cs_4$, probably as part of the superior division of the hyohyoideus, $Cs_5$ representing, in part at least, the inferior division of that muscle.

As the lateral ends of that part of the constrictor that gives rise to the superior division of the geniohyoideus thus travelled backward relatively to the mandible, the median ventral portion of the entire muscle seems to have travelled in the same direction, and relatively at a still more rapid rate, thus giving rise to the backward and inward direction of the fibres seen in the entire constrictor in Scymnus and Acanthias, in the mylohyoideus in Acipenser, and in the inferior geniohyoideus in Amia. The backward translation of this median part of the muscle then apparently ceased, and a translation relatively forward of the median end of the superior geniohyoideus alone began, giving rise to the condition in Acipenser, where the median portion of the superior muscle, $Cs_1$ and $Cs_2$, overlaps externally the inferior muscle, $Cs_6$, and to the condition in Amia where the superior division of the muscle extends in part internal to the inferior division and is inserted in the median aponeurosis, on the hind edge of the intermandibularis, on the floor of the mouth, or on the mandible near the symphysis.

In teleosts the inferior geniohyoideus is gradually absorbed by the superior muscle and finally disappears entirely. The first stage in this process is shown in Esox, where the fibres of the inferior division, although retaining their special origin on the mandible, have become at their insertion directly continuous with fibres corresponding to the median, superficial portion of the superior muscle in Amia. In Perca and Cyprinus the origin of the fibres of the inferior muscle on the mandible has disappeared, but its insertion is still in a measure retained, for

the geniohyoidei of opposite sides are still somewhat united at the middle line of the head. In Barbus even this indication of an inferior muscle has disappeared, the two geniohyoidei simply touching each other in the middle line without any connection whatever. In all four fishes the muscle runs forward either below or above the intermandibularis, or in Barbus between the fibres of that muscle, and is inserted, in all, on the mandible, or in the floor of the mouth immediately behind it.

The inferior hyohyoideus of Amia, and probably only the anterior or lateral part of that muscle, is represented in Heptanchus, Scymnus and Acanthias by the inner, deeper layer that lies internal to the posterior part of the anterior division of the ventral constrictor. It is still connected at its ventral end in these fishes with the general median aponeurosis, but in Galeus and Carcharinus this connection has disappeared and the muscle is inserted, along the edge of the coraco-mandibularis, in a fascia which is most strongly developed along the dorsal, inner surface of that muscle. This part of the hyohyoideus is probably represented in Acipenser by $Cs_5$, which is still inserted on the inner surface of $Cs_2$, as it is in Heptanchus and Scymnus. In Chimaera it is represented by the hyoideus inferior of Vetter which shows a separation into two parts. The anterior part is inserted on the lower jaw of its own side, while the other, running internal to the coraco-mandibularis between it and the coraco-hyoideus, passes beyond the middle line of the head and is inserted on the lower jaw of the opposite side of the head, overlapping at its insertion the muscle of that side. In Amia the lateral part of the inferior portion of the hyohyoideus corresponds closely in origin and insertion with this muscle in Chimaera. It arises from the ceratohyal immediately beneath and distal to the geniohyoideus, and is inserted on the under surface of the hypohyal and in the integument of the floor of the mouth beyond it, its tendons passing internal to the branchiomandibularis, between it and the sternohyoideus, and, beyond the middle line of the head, overlapping the tendons of the muscle of the opposite side and extending toward the inner surface of the mandible. If this part only of the muscle in Amia is represented by the entire muscle in selachians, Acipenser and Chi-

maera, the remainder of the muscle in Amia, that is, the superior division and the median part of the inferior division, must be represented by that part of the ventral constrictor which in selachians lies behind the point where the facial nerve penetrates the muscle, with the addition perhaps of some part of the superficial layer immediately in front of it. This muscle is represented in Acipenser by $Cs_4$, with or without the addition of $Cs_3$, and in Chimaera by $Cs_1$ or $Cs_1$ and $Cs_2$; $Cs_5$, although lying internal to the branchiomandibularis, probably representing some part of the geniohyoideus. In both these fishes the muscle is still inserted in a median aponeurosis and lies, as it does in Amia, external to the coraco-mandibularis, the homologue of the branchiomandibularis. In Amia the insertion has shifted to the under surface of the hypohyal, the muscle of either side crossing the middle line of the head and overlapping its fellow of the opposite side.

In the teleosts described by Vetter an apparent reversion or retrogression in the hyohyoideus has taken place. The branchiomandibularis, which in Amia lies between the two parts of the muscle at their insertion, has in the teleosts disappeared, and the two parts of the hyohyoideus, no longer kept apart by it, tend to unite; either that or the anterior and lateral part of the muscle gradually disappears. In Esox the tendinous front edge of the muscle, found in Amia, has disappeared, and the muscle shows at its insertion a distinct separation into two parts. In Perca the anterior of these two parts has been reduced to a few fibres only, and in Cyprinus and Barbus not only have these disappeared, but the attachment to the hypohyal, still retained in Perca, has been lost, and the muscle is inserted entirely in a median aponeurosis with its fellow of the opposite side of the head, or has become continuous with that muscle.

In selachians the hyohyoideus and the hyoid branch of the facial that supplies it both lie external to the cartilaginous gill-rakers, while in Amia, even in young larvae, and in teleosts, both muscle and nerve lie internal to the branchiostegal rays. Vetter suggests, in explanation (No. 125, p. 537), that the muscle has passed from the outer surface of the gill-rakers to the

interspaces between them and then to their inner surfaces, dragging the nerve along with them. It seems much simpler to suppose that the branchiostegal rays and gill-rakers are not homologous structures, and that the former have been developed in their present position as simple dermal bones.

### e. *Adductor Hyomandibularis, Adductor Operculi, and Levator Operculi.*

These three muscles arise from the muscle $Csd_2$ of Vetter, that is, from a muscle which he derives from the dorsal half of the superficial constrictor of the hyoid arch. $Csd_2$ arises in its anterior portion, in Heptanchus, Acanthias, and Scymnus, from the upper, outer corner of the occipital region of the skull. In its posterior portion it arises in Heptanchus mainly from the lateral edge of a superficial dorsal fascia, and in Acanthias and Scymnus from the edge of a corresponding fascia, and from a tendinous streak which marks the place where the long free edge of the first gill-cover or wall in Heptanchus has in part disappeared by fusion with the next following partition (No. 124, Note 1, p. 414). In Acanthias this fusion incloses and marks the position of a part of the lateral canal of the head.

The posterior part of the muscle in all three fishes is either directly continuous with the ventral half of the constrictor, or is inserted with that muscle in an aponeurotic formation which lies between them. The anterior part of the muscle is, in Heptanchus, inserted mainly along the hind, upper edge of the upper jaw, a thin layer only, which separates from the inner surface of the muscle, being inserted on the outer edge of the upper member of the hyoid arch. In Acanthias the muscle is inserted mainly on the front, lateral edge of the hyomandibular, a small portion only, forming the anterior bundle of the muscle, being inserted on the upper, hind corner of the upper jaw. In Scymnus even this slight attachment to the upper jaw is not found, the anterior fibres of the muscle, corresponding to the anterior bundle in Acanthias, being inserted instead on a strong ligamentous band which extends from the upper edge of the hyomandibular to a projecting corner of the hind edge of the upper jaw.

In Galeus $Csd_2$ arises, as it does in Scymnus and Acanthias, from the upper, outer corner of the skull, from the line marking the position of the lateral canal of the head, and from the tendinous streak which extends upward and forward from the upper end of the first gill slit. That part of the muscle that arises from the skull has, however, been differentiated as a wholly separate muscle and lies immediately external to the anterior part of the rest of the muscle, the front edges of the two exactly coinciding. It is a thin, delicate, band-like muscle, nearly as broad as it is long, inserted entirely on the hyomandibular and lying immediately external to the large spiracle muscle. The facialis, as it runs outward around the front edge of the muscle below the spiracle muscle, sends a branch outward and then backward along its outer surface to supply it. It thus corresponds in its innervation to the adductor hyomandibularis in Amia, and but slight changes in origin and insertion would be needed to produce that muscle. The muscle in Galeus has simply to travel backward at its origin beyond the origin of the spiracle muscle and then downward along the side of the skull. If this little muscle in Galeus is the homologue of the adductor hyomandibularis in Amia, the remaining larger portion of $Csd_2$ must either disappear or, more probably, give rise to the opercularis in Acipenser, and to the adductor operculi and levator operculi in Amia, which muscles arise wholly or in part from the dermal bones of the head, as does the muscle $Csd_2$ in Galeus.

In Chimaera the adductor operculi and the levator operculi are probably represented by $Cs_2$; the retractor hyomandibularis of Acipenser and the adductor hyomandibularis of Amia being represented by the muscle which Vetter has called the hyoideus superior, and which he considers the homologue of the superior division of the hyohyoideus in teleosts.

### 5. Nervus Trigeminus and Nervus Facialis.

The several branches of the nervus trigeminus and nervus facialis arise from what is commonly called in fishes the Gasserian ganglion, or, more properly, the trigemino-facial ganglionic complex. This ganglion, in the adult Amia, lies in the

upper, lateral chamber of the eye-muscle canal, and hence, although lying inside the cartilaginous cranium, lies outside the cranial cavity, properly so-called. From the ganglion arise the nine large nerves that form the trigemino-facial complex, as generally described, and several smaller nerves which may be considered as belonging to one or other of the nine principal ones. Of the nine principal nerves, three are commonly considered to belong to the trigeminus, two to the dorsal root of the facialis, and four to the ventral root of the facialis. The three nerves that are assigned to the trigeminus are, the r. ophthalmicus superficialis trigemini plus the portio ophthalmici profundi, or trigeminus I; the r. maxillaris superior trigemini, or trigeminus II; and the r. maxillaris inferior trigemini, or trigeminus III. The two nerves that are assigned to the dorsal root of the facialis are, the r. ophthalmicus superficialis facialis, and the r. buccalis facialis, of which the r. oticus is properly a branch (No. 3, p. 516). The four nerves that are assigned to the ventral root of the facialis are, the r. palatinus facialis, the r. hyoideus facialis, the r. mandibularis internus facialis, and the r. mandibularis externus facialis. As the latter nerve innervates only the canal organs and the surface pit organs of the operculo-mandibular portion of the lateral canal system (No. 3, p. 518), and as it arises in Amia (Allis) and in Rana (Strong) as a branch of the so-called dorsal root of the facialis, and in sharks (Ewart) as a part of that root or as a separate dorsal root, it should be added to the dorsal branches. The rami maxillaris superior and maxillaris inferior trigemini together form the truncus maxillaris trigemini, which is in Amia very short; and the rami hyoideus, mandibularis internus, and mandibularis externus facialis together form the truncus hyoideo-mandibularis facialis, which is somewhat longer. The mandibularis externus and mandibularis internus, although arising from different roots, are at first intimately associated with each other, and after separating from the truncus hyoideo-mandibularis they together form the truncus mandibularis facialis. The ophthalmicus superficialis trigemini and the ophthalmicus superficialis facialis always arise separately from the ganglionic complex and are, the portio minor and the

portio major respectively of the ophthalmicus superficialis tri-
gemini of Schwalbe and Van Wijhe.

### a. *Trigemino-Facial Ganglion.*

The trigemino-facial ganglionic complex, as commonly con-
sidered, usually includes the profundus ganglion. As that
ganglion is found, in Amia, as a wholly separate ganglion, the
complex, in Amia, consists of a profundus ganglion and what
will be called, in these descriptions, the trigemino-facial ganglion
proper. As this last ganglion contains the so-called lateral
elements of the facialis, it should, perhaps, not be assigned
entirely to the trigeminus and facialis.

In 12 mm. larvae the profundus ganglion lies in front
of and internal to the anterior, upper end of the trigemino-
facial ganglion proper, separated from that ganglion by a de-
scending portion of the orbital vein or venous sinus. It has
a long and delicate root running almost directly backward to
the extreme lateral portion of the anterior surface of the
medulla oblongata, where the fibres turn inward and backward
and enter the brain as a distinct bundle having a somewhat
deep origin. The root is entirely separate from the anterior,
or trigeminal, root of the trigemino-facial ganglion, lying in
front of, above, and internal to it, and separated from it by
the orbital vein.

The trigemino-facial ganglion proper consists of two dis-
tinct or nearly distinct parts, the main ganglion and the
ganglion of the ophthalmic and buccal branches of the facialis.
The former, in the earliest larval stages examined, is a large,
irregular mass of cells, separated imperfectly into three parts
or regions, an anterior, a median, and a posterior, the anterior
and posterior portions each being traversed by a large bundle
of fibres. The anterior end of the ganglion has two projections,
an outer one giving origin to the truncus maxillaris trigemini,
and an inner one to the ramus ophthalmicus superficialis trige-
mini. The latter, or inner end lies at a higher level than the
outer one, running upward and forward in front of, and in a
measure internal to the ganglion of the ophthalmic and buccal

branches of the facialis. From its base the anterior root of the ganglion arises. It is a short, stout root, running inward and backward to the anterior and lateral corner of the medulla, where it has its origin. It is here distinctly double, having a dorsal posterior portion and a ventral anterior one. The former arises by fibres that spread and extend backward in the brain, parallel to the outer surface of the brain, almost to the level of the origin of the facialis. The other, or ventral portion, arises in the brain as two bundles, which extend, as bundles, backward and medianward into the brain. The anterior bundle arises, as seen in horizontal sections, in front of the ventricular cavity of the brain, from the anterior, upturned end of the tract from which the ventral motor component of the facialis arises, and which I take to be the posterior longitudinal fasciculus of Osborne in cryptobranchs. This bundle is the motor V, or V minor, of Strong in Rana.

The fibres of the antero-ventral part of the anterior root of the main ganglion form the larger part, if not all, of the anterior of the two bundles of fibres that traverse the ganglion. From this transverse bundle the truncus maxillaris in large part arises, the bundle turning downward in that part of the ganglion that lies external to the orbital vein, and then outward and forward into the truncus. In one 20 mm. larva the transverse bundle, or commissure, was distinctly double, the fibres of both parts arising from the antero-ventral part of the anterior root of the main ganglion. Both parts entered the truncus maxillaris. Whether they represent the two bundles into which their root separates on entering the brain, or only one of those bundles, was not determined. The fibres arising from the postero-dorsal portion of the main root disappeared gradually as they approached the truncus maxillaris. The transverse bundle in the ganglion is called by Strong in Rana (No. 121, p. 135) the motor bundle of the portio minor of the trigeminus.

The orbital vein, which lies at first anterior to, and internal to, the upper, anterior part of the ganglion, runs outward, downward, and backward under that portion and its root, and issues on the dorsal surface of the median and posterior portions of the ganglion. It thus pierces the main ganglion under its

anterior root and between its anterior and posterior portions. While on the dorsal surface of the ganglion it lies between it and the ganglion of the ophthalmic and buccal branches of the facialis. It there receives a large branch from the direction of the ramus buccalis and truncus maxillaris, and, continuing downward and backward along the lateral surface of the ganglion, joins or becomes the jugular vein. Under the vein the anterior, or trigeminal, part of the ganglion becomes continuous with the posterior or facial portion.

The ramus ophthalmicus superficialis trigemini arises from the extreme upper, anterior end or horn of the main ganglion, and the fibres that enter it do not apparently arise at all from the antero-ventral portion of the anterior root, or from the anterior transverse commissure of the ganglion. They arise, in large part, from the superficial portions of the median portion of the main ganglion, and run forward above and below the anterior commissure along the outer surface of the ganglion. A large bundle of these same fibres, arising from the ventral surface of the ganglion, joins the truncus maxillaris.

The ganglion of the ophthalmic and buccal branches of the facialis lies, as already stated, immediately behind the upper, anterior and inner end or horn of the main ganglion. It reaches to a somewhat higher level than that ganglion and is Y-shaped in form, as already described in my earlier work (No. 3, p. 516), the anterior end of the main ganglion lying between and below the arms of the Y. Its ophthalmic branch is, in young larvae, much larger than the ophthalmic branch of the trigeminus, and at these ages the terminal buds on the top of the snout are not yet largely developed. In later stages, when the buds become numerous, the two nerves have about the same size.

No fibres connecting the ganglion of the ophthalmicus and buccalis facialis with the main portion of the trigemino-facial ganglion were found in larvae or in the adult, but I cannot positively say that they do not exist. The ganglion has a large, long root which runs at first inward and backward, and then directly backward along the side of the medulla, passing between it and the inner edge of the anterior wall of the ear

capsule into that capsule. It enters the brain immediately in front of the root of the acusticus, almost as a part of that root, the two roots issuing each by two or more rootlets from the summit of a slight swelling or eminence on the side of the medulla. From its under surface, close to its origin, or possibly as a separate root, a large branch is sent downward, outward, and forward, to the posterior portion of the main trigemino-facial ganglion, where it turns outward and then outward and backward, traversing the ganglion and issuing as part of the truncus hyomandibularis facialis. It forms a large part of the posterior bundle already spoken of as traversing the ganglion. These two roots, or two branches of a single root, together form the sense-organ, or, as it has been called, lateral component of the trigemino-facial ganglionic complex.

Close to and intimately associated with this lateral root arise two other bundles which together form the true posterior or facial root of the main ganglion. The anterior of the two arises, as a bundle, at a high level in the brain, probably from the fasciculus communis of Osborne and Strong, and issues in front of and internal to the sense-organ root. It enters mainly into the median portion of the main ganglion, and from this portion arise the ramus palatinus facialis as well as the fibres already spoken of that go to form a large part, if not all, of the ophthalmicus superficialis trigemini and a considerable part of the truncus maxillaris. The other root arises also as a distinct bundle deep in the brain, but nearly on the same level as its point of exit, which is in front of and below the root of the acusticus and below the lateral root of the complex. It is probably the motor root VII ab of Strong in Rana, although its position is not exactly the same as that given by him. It enters the posterior portion of the main ganglion immediately in front of the hyomandibular branch of the lateral root, and apparently traverses the ganglion with that root, forming with it the posterior transverse bundle or commissure of the ganglion.

The abducens in these larvae arises immediately behind the posterior end of the ganglion of the acusticus, and in my sections is at its origin distinguished with difficulty from that

ganglion.   Whether it arises in part from the ganglion, or wholly from the brain, it certainly has a much more lateral and higher origin than in the adult, agreeing in this with the origin of the nerve in the adult of Petromyzon (No. 1, p. 21), but lying behind instead of in front of the so-called motor root of the trigeminus.

In the adult, as in the young, the trigemino-facial ganglion (*gg*, Figs. 38, 39, and 64, Pls. XXX and XXXVIII) has two apparent roots, an anterior (*rtfa*) and a posterior one, the latter consisting of two parts, the true posterior root of the ganglion (*rtfp*) and the sense-organ root or roots (*robf*). The anterior root is the smaller of the two. It runs forward and outward in the cranial cavity, from its place of origin, pierces the lining membrane of the eye-muscle canal in front of the utricular fossa, and enters the anterior and upper part of the main ganglion. From its inner, anterior surface, while still inside the cranial cavity, the much smaller root of the ophthalmicus profundus (*rp*) arises.

The two portions of the posterior root arise close together, or as a single root, immediately in front of and above the root of the acusticus. The part destined for the ganglion of the ophthalmicus and buccalis facialis separates at once from the rest of the root, but runs forward and outward with it, closely applied to its upper surface, and escapes with it from the cranial cavity through the inner, membranous edge of the front wall of the utricular fossa, separated by that edge from the anterior root at its exit. The front wall of the fossa, which is also the hind wall of the upper, lateral chamber of the eye-muscle canal, is formed by a process of the petrosal and a cartilaginous ridge continuous with that process immediately above it. The inner membranous edge of this wall and not the wall itself is what is pierced by the root of the facialis as it issues from the cranial cavity. Having entered the upper chamber of the eye-muscle canal, the upper portion of the root enters its own ganglion and the lower portion enters the posterior portion of the main ganglion.

### b. *Ramus Ophthalmicus Superficialis Trigemini and Ramus Ophthalmicus Superficialis Facialis.*

The ophthalmicus superficialis trigemini (*opt* in many figures) arises in the adult, as in larvae, from the median and upper portion of the trigemino-facial ganglion immediately under the ophthalmicus facialis (*opf*). It leaves the upper, lateral chamber of the eye-muscle canal and issues from the cranium with the ophthalmicus facialis by the ophthalmic foramen through the alisphenoid (*opfr*, Figs. 9 and 10, Pl. XXI), immediately above the large trigeminal foramen. The two ophthalmic nerves are, from the very beginning of their extra-cranial course, closely and intimately associated. They run upward and forward along the side of the cranium, and not at some distance from it, as Schneider states, lying at first under the overhanging, cartilaginous roof of the orbit, and then under the overhanging frontal, which, opposite the eye, where the roof of the cnondrocranium is relatively narrow, projects so much beyond that roof that the nerves lie approximately under the middle line of the bone, and hence under that part of the supra-orbital sensory canal that lies in that bone.

About opposite the front edge of the eye, the two ophthalmic nerves leave the orbit, and reach the upper surface of the chondrocranium, passing as they do so through a notch in the edge of the cartilaginous cranium. This notch (*ip*, Figs. 8, 9, and 10, Pl. XXI) lies a little behind the prefrontal ossification, and a little in front of the middle point of the frontal bone, and is the preorbital incisure of Pristiurus, Prionodon, and Zygaena (No. 44, p. 71). It marks, as in those fishes, the posterior limit of the preorbital process (*pr*). The anterior limit of the process is defined, as in those fishes, by an ethmoid incisure (*ie*). Having reached the upper surface of the cranium, the ophthalmic nerves continue directly forward, soon entering a groove on the under side of the frontal bone, in which they pass above the projecting hind end of the premaxillary. Leaving the groove at the front end of the frontal, they pass through the narrow strip of dermal, or subdermal, tissue that lies between that bone and the nasal, and then pass under the nasal, lying, in

this part of their course, in the thin membranous covering of the nasal sack. There is no ethmoid canal in Amia, and as that canal is found in Polypterus (No. 93) it would seem to depend on the presence of a ramus ophthalmicus profundus, and on the partial fusion of the canal for that nerve with the canalis pre-orbitalis. If, then, the canal for the profundus in Polypterus is, in its proximal portion, the orbito-nasal canal of selachians, as seems probable, that canal must sometimes in ganoids fuse with the preorbital canal, as in Polypterus, and sometimes with the olfactory canal, as in Amia. In selachians the distal portion of the canal seems to fuse with or become the ethmoid canal, for Gegenbaur, in all his ventral and lateral views, never gives but one anterior opening, calling it sometimes the distal open-ing of the ethmoid canal and sometimes that of the orbito-nasal. There may even be two canals for the profundus in selachians : one, the ethmoid canal, for the dorsal branch of the nerve, Ewart's nerve *on'* (No. 29, Fig. 2) ; and one, the orbito-nasal, for its orbital branch, Ewart's nerve *or*. Ewart unfortunately does not describe the course of the latter. It would seem, however, from its name to remain in the orbit.

Immediately after issuing from its foramen, the ophthalmi-cus trigemini gives off two branches one of which arises from the outer and the other from the inner surface of the nerve. These branches are flat and broad at their bases, and closely embrace the ophthalmicus facialis. They run upward and backward, one on either side of the facialis, pierce the alisphe-noid, and, entering the cartilage of the cranium by the same or by closely adjoining canals, issue on its upper surface either by the same or by adjoining foramen. They are the first two, or first pair, of frontal branches of the nerve, and the course and position of the canal or canals traversed by them, as also that of the other small canals traversed by other frontal branches of the nerve, vary greatly in different specimens. There are frequently both in the young and in the adult, small branch canals connecting these canals and giving passage to delicate anastomosing branches of the nerve.

One of these first two branches of the ophthalmicus tri-gemini is accompanied by the first branch of the ophthalmicus

facialis. This branch of the facialis (*opfal*) arises inside the cranium from the upper surface of the nerve, near its origin from its ganglion, and issues with it through the ophthalmic foramen, lying along its upper surface. It then turns upward and backward, with the two frontal branches of the trigeminus, and having reached the upper surface of the chondrocranium turns medianward and backward under the frontal and parietal bones and supplies the anterior dorsal line of pit organs (No. 3, p. 513). In Ammocoetes the corresponding branch arises, according to Hatschek, from a small anterior portion of the facial ganglion, and is the nervus temporalis facialis or cutaneus dorsalis facialis (No. 54, p. 156). It is, in Amia, always closely accompanied, after reaching the upper surface of the cranium, by the inner of the first two frontal branches of the ophthalmicus trigemini.

The other or outer nerve of the first pair of branches of the ophthalmicus trigemini usually separates into two parts, both of which run almost directly backward along or near the outer edge of the temporal groove, and either unite again to form a single nerve or are connected by anastomosing branches. From the median one of its two parts an anastomosing branch is usually sent to the inner nerve of the pair. The lateral one is always directly continuous with a nerve formed usually by the fusion of two branches of the vagus. From the compound nerve thus formed several branches are sent to the dermal bones and underlying tissues of this part of the head, but which of these branches belonged to the trigeminus and which to the vagus could not be determined.

Immediately in front of the inner one of the first two branches of the ophthalmicus trigeminus there were, on each side of the head in the specimen used for illustration, two branches arising close together and forming what may be for convenience called the second pair of frontal branches. About on a level with them, or a little in front of them, the branch of the facialis destined to supply organ 7 of the supraorbital sensory canal arises (*opf.so*[7]). These three nerves all run upward and backward through the cranial cartilage and issue on the upper surface of the chondrocranium, but their course and

their relations to each other vary greatly in different specimens. On the left side of the head, in the specimen here described, the facialis branch to organ 7 was accompanied by the anterior of the two trigeminal branches that form the second pair, the other trigeminal branch of the pair issuing by a separate and slightly lateral canal. On the right side the facial branch to organ 7 joined the inner one of the first pair of trigeminal branches, those forming the second pair issuing separately by two somewhat anterior openings. On both sides of the head there were, associated with these first two pairs of frontal nerves, one or two branches of a nerve which arose from the trigemino-facial ganglion under and lateral to the root of the ophthalmicus trigemini and which will be later described as nerve 'a.' On the left side of the head these branches of nerve 'a' pierced the cranial cartilage near its lateral edge and issued on the top of the cranium lateral to and slightly in front of the two pair of trigeminal branches; on the right side, where two pair of frontal nerves issued at some distance apart, the branches of nerve 'a' issued lateral to, but between them.

The next branch of the ophthalmicus facialis is the one to organ 6 supraorbital. It runs upward and backward external to the cranial cartilage, lying in or just in front of a strong notch in the cartilaginous roof of the orbit. On a level with this nerve, in the specimen used, four branches arose from the ophthalmicus trigemini on the left side of the head, and three on the other. One of the branches on the left side, and two of those on the right, lay internal to, or above, and the others external to, or below, the ophthalmicus facialis. Those lying internal to the facialis ran upward immediately behind the branch to organ 6, either lying in the notch already described, or passing through special canals in the cartilage. Having reached the upper surface of the cranium, they turned median-ward and were distributed to tissues toward the middle line of the head. The other branches, issuing below the ophthalmicus facialis, ran outward and forward into the fatty tissues above the eye. On the right side of the head there were no branches from the ophthalmicus trigemini between this group of nerves associated with the facialis branch to organ 6 and those asso-

ciated with the branch to organ 7. On the left side there were, however, between these two groups, two pair of trigeminal branches, one branch in each pair lying above, or internal to, and the other below, or external to, the facialis. Some of these nerves pierced the cranial cartilage and issued on the top of the cranium, others were distributed to fatty and other tissues above the eye. One of the nerves in each pair was double at its origin. Intimately associated with an internal branch in the anterior pair there was an external branch which arose toward the base of the ophthalmicus trigemini, ran forward closely applied to the external surface of the ophthalmicus facialis, and, turning inward across the upper surface of that nerve, joined the internal branch referred to. So closely and intimately was this branch associated with the facialis that Mr. Nomura for some time stoutly maintained that it was a branch of that nerve, although repeatedly assured that, there being no supraorbital organs between organs 6 and 7, there could be no branches of the ophthalmicus facialis between the branches to those organs. This marked instance of misinterpretation by an exceedingly careful workman is given simply to enforce the statement already made that many so-called anastomoses may be simply instances of intimate juxtaposition, and that similar misinterpretations made by others have undoubtedly given rise to much of the confusion in the literature of the subject.

The branch of the ophthalmicus facialis to organ 5 supraorbital is given off as the main nerves pass to the upper surface of the chondrocranium, and it had on the left side no trigeminal branch associated with it. On the right side two branches arose from the trigeminus near it, both passing outward and forward below the facialis, and both distributed to fatty and other tissues above and in front of the eye.

The branch of the facialis to organ 4 is given off after the nerve has entered the groove in the frontal, and has passed, or is about to pass, above the hind end of the premaxillary. The branches to organs 3, 2, and 1 are given off after the nerve passes under the nasal, the nerve ending in organ 1.

On a level with the facial branch to organ 4, a branch is

given off by the ophthalmicus trigemini on both sides of the head, and the nerve then breaks up into several terminal branches all of which lie under the nasal bone, in the membrane covering the nasal sack, and run forward on both sides of the facialis toward the end of the snout.

The portio ophthalmici profundi (*popp*) joins the ophthalmicus superficialis trigemini a little proximal to the branches of that nerve that accompany the branch of the facialis to organ 6 supraorbital. On the right side of the head, in the one specimen, the superficialis had separated, opposite the point of union with the portio profundi, into two nearly equal strands. No other instance of such a separation was found.

### c. *Ramus Buccalis Facialis.*

The buccalis facialis (*bf*) arises from the outer side of the ganglion common to it and the ophthalmicus facialis. It issues from the upper, lateral chamber of the eye-muscle canal, through the large trigeminal foramen, with the superior and inferior maxillary nerves, lying immediately above them. It then turns downward and runs downward, outward, and forward behind and under the eye, and then forward and inward toward the end of the snout, lying immediately external to and above the maxillaris superior. It is distributed entirely to the organs of the infraorbital sensory canal (No. 3, p. 514). On the left side of the head there were, in the specimen used for illustration, fifteen of these organs, on the right side the normal number, fourteen.

The oticus facialis (*of*), which is simply a branch of the buccalis, arose on both sides close to the base of that nerve. It ran upward and outward through the cranial cartilage and, as in the embryo, supplied organs 15 and 16 infraorbital and the organ of the spiracular canal. Close to its base the branch to organ 14 infraorbital arose, a little further forward the branch to organ 13, and close to this last branch, on the left side of the head, two branches, and on the right, a single one, double at the end. There were beyond this group of nerves eleven buccal branches on each side of the head, the first

branch arising some little distance beyond the most distal one of the group. There must accordingly have been fifteen organs or groups of organs in the infraorbital canal on the left side of the head, and either fifteen organs or fourteen organs, one of them double, on the right. Unfortunately no attention had been given at the beginning of the dissection, which was begun on the left side, to determine, either the number of dendritic systems or the number of sense organs in the canals, it being assumed, from earlier embryological work, that the branches of the nerves concerned would everywhere fully indicate the number of sense organs and the arrangement of the dendritic systems. After the dissection of the left side had been nearly completed and fifteen branches of the buccal instead of fourteen had been found, the advantage of knowing the number and position of the sense organs in the different canals became apparent, and on the right side the outer surface of all the bones concerned was carefully removed, so as to lay open the canals that traversed them and thus expose, *in situ*, the sense organs, and the ends of the nerves that supplied them. The number and position of the organs, as indicated by the projecting nerve ends, being everywhere normal on this side, no further thought or attention was given to the matter. Later it was found that one of the branches of the buccalis was double at the end, but as the dermal bones had all been removed it was too late to determine which organ or organs had been supplied by it. In my earlier work on larvae, groups 11 and 12 infraorbital were always supplied, either by branches of a single nerve or by nerves arising close together from the buccalis, and there should therefore normally be, both in the adult and in the embryo, but ten groups of organs beyond group 11, and but ten nerves beyond the double nerve supplying groups 11 and 12. In the present instance, in this specimen used for illustration, there were, however, eleven such nerves on each side of the head. There must, therefore, have been an extra system, 10′, on both sides, or, assuming no error in observation, the nerve to group 11 must have become somewhat widely separated at its origin from the nerve to group 12, and that group must have become entirely double on the left side, and partly

so on the right, as indicated by the projecting nerve end in the figure (*bf.io*[12], Fig. 19, Pl. XXIII).

The branches to organs 10 to 7 infraorbital, inclusive, arose successively from the buccalis at fairly equal distances apart. The branches to organs 6 and 5 arose close together, that to organ 6 passing outward above, that is, behind and lateral to the fourth division of the levator maxillae superioris as in 40 mm. specimens, but that to organ 5 passing through that muscle at its origin instead of under it, that is, in front of it, as in the young. The buccalis itself also passed through this fourth division of the muscle instead of between it and the third division as in the young. Beyond this point the branches to organs 4, 3, 2, and 1 were given off regularly in succession, the nerve ending in the last-named organ.

### d. *Truncus Maxillaris Trigemini.*

The truncus maxillaris separates immediately, in the adult, into its two main portions, the r. maxillaris superior (*mst*) and the r. maxillaris inferior (*mit*). From these two nerves close to their origin, from the truncus maxillaris itself, from the upper surface of the main trigemino-facial ganglion, and perhaps also in part from the r. ophthalmicus superficialis, several small nerves arise. They vary in number and arrangement in every specimen and are the homologues of the three accessory trigeminal branches described by Strong in Rana (No. 121, p. 111). In the specimen used for illustration one of these nerves issued from the cranium with the ophthalmic nerves through the ophthalmic foramen ; the others all issued with the superior and inferior maxillary nerves through the main trigeminal foramen. They formed numerous anastomoses with each other and were distributed almost entirely to the fatty and other tissues above and behind the eye and to the dermal and subdermal tissues of the cheek. On the left side of the head there were five of these nerves, *a*, *b*, *c*, *d*, and *e*. They are shown much enlarged in Figs. 38 and 39, Pl. XXX. In these figures the nerves are all pulled somewhat apart and hence somewhat displaced from their natural positions, and in

Fig. 39 the ophthalmicus facialis and the buccalis facialis are turned back for better illustration. The mandibularis facialis is not shown.

Nerve 'a' (*r.a*) arose under the bundle of fibres that go from the posterior root of the trigeminus to the r. ophthalmicus trigemini. It lay at first under the ophthalmic nerve, closely applied to it, issued with it through its foramen, and had as it issued every appearance of being a branch of it. One of its branches, running upward between the ophthalmicus and buccalis facialis, pierced the cartilaginous roof of the orbit and, reaching the top of the chondrocranium, was distributed with those branches of the ophthalmicus trigemini that are associated with the first, or first two branches of the ophthalmicus facialis, as already described. Other shorter branches entered the tissues above and behind the eye. One long branch joined a branch of nerve 'c,' and ran outward with it (*r.ac*), in front of, dorsal to, and external to the buccalis, around the front edge and then backward along the outer surface of the adductor mandibulae, lying near the upper edge of that muscle. It lay dorsal to and external to nerve 13 buccalis facialis, between it and nerve 14. Nerve 'b' (*r.b*) arose near nerve 'a,' ran forward below it, formed no anastomoses with the other nerves, and was distributed entirely to tissues above the eye. Nerve 'c' (*r.c*) arose immediately lateral to nerve 'b.' It separated into two parts, one of which ran outward in front of the buccalis below or internal to nerve 12 buccalis facialis, between it and nerve 11, and then, after being joined by a branch from nerve 'e,' ran (*r.ce*) outward and backward along the outer surface of the adductor mandibulae. It lay in this part of its course immediately dorsal to nerve 'ac.' The remainder of nerve 'c' separated into two parts, one of which joined the long branch of nerve 'a,' as already described, while the other ran downward and joining a branch of nerve 'd' was distributed to tissues behind and below the eye (*r.cd*). Nerve 'e' (*r.e*) arose immediately lateral to nerve 'c.' It separated into two parts one of which issued behind, that is, internal to the buccalis, while the other ran outward and downward in front of and external to that nerve. The ramus buccalis thus lay between the two main branches of nerve 'e,' and as

terminal portions of those branches fuse, the buccalis, in a way, can be said to perforate the nerve so formed, as it does one of the corresponding nerves in Rana (No. 121, p. 117).  The internal branch of the nerve followed closely the maxillaris inferior and was distributed to dermal and subdermal tissues along its course, one long branch, 'r.e,' being sent downward behind the eye and another backward to join a branch of nerve 'c,' as already stated.  The external portion of the nerve fused at once with nerve 'd' and the nerve so formed having crossed the buccalis between nerves 10 and 11, followed the course of the internal part of nerve 'e' lying immediately above it and fusing in part with it.  It also sent an anastomosing branch to that branch of nerve 'e' that was distributed to tissues behind and below the eye.

The four nerves *a*, *b*, *c*, and *e* arose apparently from that part of the trigemino-facial ganglion that gives origin to the maxillaris inferior.  Nerve 'd' (*r.d*) on the contrary arose by two roots from that portion of the ganglion that gives origin to the maxillaris superior.  Both roots joined the external portion of nerve 'e.'

### e. *Ramus Maxillaris Superior Trigemini.*

This nerve (*mst*) accompanies the buccalis in its course through the orbit, lying at first immediately under it and then immediately internal to it.  The two nerves run downward and forward internal to, above, and in front of the first and second divisions of the levator maxillae superioris, and then dorsal to the third and fourth divisions of that muscle.  They lie while in the orbit immediately external to, that is, behind or below, the periorbital lymph space.  The membranes lining that space form a sort of sack which encloses the eye and eye muscles, as shown in Fig. 20, Pl. XXIII.  In this figure the eyeball, which lies within the sack, and the fatty tissues which lie above and behind it, have been removed and the ventral nerves are seen through the membrane.

As the maxillaris passes through the orbit it gives off several branches.  In the specimen used for illustration there were on each side of the head eight of these branches, seven of them

running outward and forward above the buccalis and one run-
ning outward and forward below it. The seven branches that
lie above the buccalis form several anastomoses with each other
and accompany approximately the branches of the buccalis to
organs 5 to 10, inclusive. In this relation to the buccalis they
agree with the branches of nerves *a, c, d,* and *e,* which accompany
approximately the branches of the buccalis to organs 11 to 14.
The one branch that lies below the buccalis (*mst.mx*) leaves the
maxillaris about opposite the buccal branch to organ 8, and is
by far the largest branch of the nerve. It runs outward and
downward and then forward across the upper surface of the
third and fourth divisions of the levator maxillae superioris and,
near the front edge of the fourth division of that muscle, breaks
up into several branches. In the specimen used for illustration
there were four of these terminal branches on each side of the
head. Three of them turned outward and backward to supply
the inner and outer surfaces of the maxilla, the other continuing
forward under the front articular end of the maxillary bone
toward the front end of the head, and breaking up into several
branches all of which lay above the palatine and vomer and
below the premaxillary. At the point where the main nerve
breaks up into its four terminal branches it lies external to and
close to the r. palatinus posterior facialis with which there is an
interchange of fibres or partial anastomosis. This anastomosis
is undoubtedly the one which in Cyprinidae, according to Sage-
mehl, represents unquestionably the ganglion sphenopalatinum
of higher animals (No. 107, p. 558).

The main maxillaris superior leaves the orbit under the pre-
orbital process and between the third and fourth divisions of
the levator maxillae superioris close to their origins. On the
left side of the head it passed with the buccalis through *Lms4*
and a branch was sent outward and forward between the two
heads of that muscle. The main nerve then runs forward
above the articular head of the maxillary and separates into two
main branches and other smaller ones. One main branch runs
forward and downward, sending one branch backward and down-
ward along the anterior part of the lower edge of the maxilla,
and others forward, and forward and inward to the tissues

between the premaxillary and the external dermal bones, and along the edge of the upper lip. The other main branch of the nerve turns inward and forward above the premaxillary and crosses the depression in that bone that forms the bottom of the front end of the nasal pit. The nerve here lies on a level with the outer edges of the bone and hence somewhat above the bottom of the pit, extending across it immediately under the front end of the olfactory tissue. In this part of its course several branches are given off, all of them running forward or forward and inward under the buccalis and above the premaxillary to the dermal and subdermal tissues toward the end of the snout. The branches form numerous anastomoses with each other and with other terminal branches of the nerve and also with a terminal branch of the palatinus anterior facialis which, having traversed the cartilaginous base of the skull, issues by a foramen between the inner edge of the septomaxillary and the inner edge of the olfactory perforation of the premaxillary, and reaches the upper surface of that last bone (*paf*, Figs. 21 and 25, Pls. XXIV and XXV, and *paf.fr*, Figs. 8 and 10, Pl. XXI).

One small branch of this branch of the palatinus facialis was traced into one of the teeth of the premaxillary.

### f. *Ramus Maxillaris Inferior Trigemini.*

The maxillaris inferior (*mit*) is the largest of the three main divisions of the trigeminus. It runs at first downward, outward, and forward, and then downward, outward, and backward around the front edges of the muscles $Lms^1$, $Lms^2$, $Do$, and $Am$. It then runs downward and backward along the outer surfaces of $A_2'''$ and $A_2''$ to the upper edge of $A_2'$, at about one third the distance between the insertion of the muscle and its origin. Here it turns downward and then downward and forward between $A_2'$ and $A_2''$, and entering the hollow of the mandible reaches the inner surface of the coronoid process of Meckel's cartilage near its anterior edge and at the posterior end of the surface of insertion of $A\omega'$. In its course up to this point it has been a broad, thin, ribbon-like nerve, the flat surface of the

nerve lying on the outer surface of $A_2''$ and $A\omega'$. Here, however, it is twisted inward and downward and, although still broad and thin, lies imbedded in and between the fibres of $A\omega'$ at their insertion, so that the thin edge of the nerve is presented superficially. Continuing downward and forward in this position, along the inner surface of the articular and dentary, it gradually becomes rounded in outline and issues from the hollow of the mandible, at its extreme front end, through a long longitudinal opening (*mitfr.*, Figs. 6 and 7, Pl. XX) between the dentary, below, and Meckel's cartilage and the lower edge of the splenial, above. Beyond this point it extends to the tip of the mandible lying along the inner surface of the dentary median to Meckel's cartilage and immediately below the lower edge of the splenial. It is in this part of its course closely associated with the r. buccalis facialis which lies immediately median to it, the two often appearing as a single nerve. Between them there is, however, no interchange of fibres, and they are easily separated in dissection.

The first branch given off by the maxillaris inferior, *r.lap.do*, leaves the nerve from its outer surface before it has separated from the maxillaris superior, that is, it arises from the truncus trigeminus, but from that portion of the truncus that contains the fibres destined for the inferior division of the nerve. It is given off immediately after the truncus enters the orbit and runs outward and forward to the inner surface of the levator arcus palatini near its front edge. There it separates into two parts, an inner, posterior portion and an outer, anterior one. The inner, posterior portion runs backward and slightly upward along the inner surface of the muscle, sending branches into it and lying between it and the lateral wall of the cranium immediately above the united first and second divisions of the levator maxillae superioris. It reaches the front edge of the hyomandibular near its upper end, and, running along the outer surface of that bone, along the line separating the dilatator operculi and the posterior portion of the levator arcus palatini, sends branches to those two muscles. The other or outer portion of the nerve runs outward around the front edge of the levator arcus palatini, and then backward along the outer sur-

face of that muscle, between it and the adductor mandibulae. On the left side of the head it here separated into two parts, one of which sent branches into the levator arcus palatini and finally entered that muscle, while the other continued backward along the outer surface of the dilatator operculi. Whether this second branch sent branches into either of the two muscles or not was not determined. On the right side of the head the outer portion of the nerve was found as a single branch extending across the outer surface of the levator arcus palatini and backward onto the outer surface of the dilatator operculi.

Not far from the origin of this first branch of the maxillaris inferior, but from the under surface of the main nerve, a second branch, *r.lms,* is given off. In young specimens it arises at almost the same level as the first branch, close to the root of the truncus trigeminus if not from the ganglion itself, for ganglionic cells are found beyond both nerves, extending even as far as the point where the truncus separates into its superior and inferior portions. This second branch is often double through a large part of its course, the two portions being closely applied to each other. It runs at first downward and forward immediately internal to and in front of the first and second divisions of the levator maxillae superioris, and then forward under the eye, lying at first a little below and lateral to the united buccalis facialis and maxillaris trigemini, and then immediately beneath them. At the front end of the orbit it runs diagonally across the upper surface of the third division of the levator maxillae superioris, sending branches into that muscle, and then forward between the third and fourth divisions of the muscle, sending one branch to the outer surface of the latter muscle, and itself entering it on its inner surface. As the nerve ran downward and outward internal to the first and second divisions of the levator maxillae superioris it sent, in different dissections that were made, one, two, or three branches into those muscles; and a little further forward, in one specimen, a branch, unfortunately cut in dissection, was found entering the tissue near the corner of the mouth, and in another a loop was found in the main nerve, as if its two portions had become partially separated.

The third branch, *r.am*, of the maxillaris inferior arises some distance beyond the points of origin of the first two branches. It is given off from the inner surface of the main nerve as it turns outward, downward, and backward around the front edges of the levator arcus palatini and adductor mandibulae, and is destined to supply the two divisions of the latter muscle. It separates at once into two main portions, both of which run downward and backward, one entering at once the deeper portion of $A_2$, and the other lying along the outer surface of $A_3$, and sending branches backward and downward into it.

The next or fourth branch of the maxillaris inferior is given off from the posterior margin of the ribbon-like nerve just before it passes under the upper edge of $A_2'$. It runs backward and downward and then downward along the outer surface of $A_2'$, and is, apparently, destined entirely to the supply of cutaneous and subcutaneous tissues. It separates into three or more portions, all of which continue downward along the outer surface of $A_2'$, to the extreme lower edge, or corner, of that muscle. Here one of the three main branches of the nerve turns forward and sends branches upward and downward along the outer surface of the hind edge of the mandible; the second and largest branch turns inward and forward around the lower edges of $A_2$ and $A_3$, and then runs forward along the inner surface of $A\omega$, lying a little above the upper edge of Meckel's cartilage; the third or posterior branch turns inward and backward between the lower end of the preoperculum and the symplectic, and then runs backward and upward along the inner surface of the preoperculum, following approximately the line of the hind edge of that bone. In larvae branches of this third branch were traced directly to the terminal buds on the inner surface of the mouth cavity near the upper end of the hyoid.

Near the point of origin of this fourth branch of the maxillaris inferior a small branch is always found arising from the outer surface of the nerve and running forward along the tendon that extends from the origin of $A_2'$ to the inner surface of the maxilla. It is usually imbedded between $A_2'$ and $A_2''$, and is not seen without separating slightly those muscles.

The fifth branch, *r.ghs*, of the maxillaris inferior is always

given off at the point where the main nerve reaches the inner surface of the coronoid process of Meckel's cartilage and is twisted inward and downward. It runs downward along the inner surface of the cartilage and along the inner surface of the articular in front of the cartilage, across the hind end of the surface of insertion of Aω′, to the lower edge of that muscle. There it crosses the anterior edge of ossicle 'c,' then turns inward between the articular and the lower horizontal part of Meckel's cartilage, and, having reached the inner ventral angle of the cartilage, issues from the mandible between the articular and the lower edge of the splenial, lying immediately dorsal to and in contact with the r. mandibularis facialis externus. It then turns upward and backward and enters the thin fold of skin that extends from the inner, lower edge of the mandible to the outer edge of the geniohyoideus muscle, and which I have already described as the hyoideo-mandibular fold. Traversing this fold upward and backward it reaches the outer edge of the geniohyoideus near its origin from the ceratohyal, and there turns downward and forward along the outer surface of that muscle. It crosses the muscle, reaching its median edge opposite the base of the third or fourth branchiostegal ray, and passes under that ray onto the outer, ventral surface of the inferior division of the hyohyoideus. There it anastomoses completely with the r. hyoideus facialis, the two nerves running directly into each other and forming a complete circuit, so that it is impossible to tell where one ends and the other begins. One long branch, apparently belonging to the nerve *r.ghs*, is sent forward along the outer surface of the superior division of the geniohyoideus; it sends branches into that muscle, and runs directly into a terminal branch of nerve *r.ghi*, described below, forming with it a complete circuit, similar to that formed by the main nerve and the r. hyoideus facialis. Numerous other smaller branches are sent from the united main nerves forward onto the outer surface of the superior division of the geniohyoideus, and forward, medianward, and backward, internal to the branchiostegal rays onto the outer surface of the hyohyoideus, being distributed mainly to the inferior portion of that muscle.

Beyond its fifth branch, *r.ghs*, and while still inside the hollow of the mandible, the maxillaris inferior gives off several branches, the arrangement of which differs in almost every dissection. There is always one important branch, and often two or more smaller ones, sent upward and forward along the inner surface of the dentary, imbedded in the fibres of *Aω'* at their insertion, toward the upper edge of the mandible; and one important branch sent downward and forward. This last branch may reunite with the main nerve just before the latter issues from the hollow of the mandible, as was the case on both sides of the head in the fish used for the drawings of the under surface of the head.

Immediately after issuing from the hollow of the mandible, the maxillaris inferior often sends one or more small anastomosing branches (Fig. 44, Pl. XXXI) upward along the inner surface of the splenial to the r. mandibularis facialis internus, and then gives off an important branch, *r.ghi*, which, like *r.ghs*, runs downward and then medianward and forward ventral to the horizontal part of Meckel's cartilage, between it and the dentary, and dorsal to and in immediate contact with the r. mandibularis facialis externus. It then turns upward and forward, and having traversed the anterior end of the hyoideo-mandibular fold reaches the outer surface of the inferior division of the geniohyoideus, where it continues its course medianward and forward, sending numerous branches forward and backward along the outer surface of that muscle. One large branch directed backward passes the hind edge of the inferior division of the muscle, and, continuing along the outer surface of the superior division of the muscle, anastomoses with a branch of *r.ghs* as already described. A branch, *r.im*, is always sent forward and medianward to the outer, ventral surface of the intermandibularis, which it apparently innervates. McMurrich concluded (No. 76, p. 131) that both the intermandibularis and the anterior division of the geniohyoideus were innervated by the trigeminus.

Beyond the branch *r.ghi* the maxillaris inferior, as it continues forward to the symphysis, sends many branches upward and forward in the dentary toward the upper edge of the mandible.

### g. *Truncus Hyoideo-Mandibularis Facialis.*

The large truncus hyoideo-mandibularis facialis (*hmf*) arises, in 35 mm. specimens, from the ventral, outer, and hinder part of the trigemino-facial ganglion.   It runs forward and outward, then outward, and finally outward and backward through the large facial foramen which lies in the petrosal near the front edge of that bone and a little behind the lateral wing of the parasphenoid.

In the adult the truncus arises from the lateral surface of its ganglion, nearly at right angles to that ganglion and to the line of its posterior root.   It runs directly outward, or outward and backward, through its foramen, and then slightly upward along the upper surface of the adductor hyomandibularis, where it sends a large branch, the r. opercularis facialis (*oprf*) backward and upward along the upper surface of the adductor hyoman-dibularis, and beyond it onto the outer surfaces of the adductor and levator operculi, all three muscles being supplied by the nerve.   After the nerve reaches the outer surface of the adductor operculi it breaks up into numerous branches, which spread out over the surfaces of that muscle and the levator operculi, lying immediately internal to a similar network of branches belonging to the glossopharyngeus and first vagus nervus and destined to supply the cutaneous and subcutaneous tissues of the operculum.

The r. opercularis facialis, on the left side of the fish used for this dissection (Fig. 25, Pl. XXV), was easily separated from the truncus facialis as a thin, flat layer closely applied to the posterior surface of that nerve, the layer separating even beyond the ganglion onto the main root of the nerve, and being connected distally by fibres with the main truncus.

After giving off the r. opercularis the truncus turns down-ward, and running downward, outward, and backward, somewhat imbedded in the fibres of the adductor hyomandibularis, enters and passes through the facial canal in the hyomandibular (*fc*, Figs. 1, 2, and 5, Pl. XX).   After issuing from that canal the truncus facialis continues downward, outward, and backward, lying in the depression described on page ooo on the outer

surface of the hyomandibular, and separates almost immediately into its two main portions, the truncus mandibularis facialis (*mf*) and the r. hyoideus facialis (*hf*). The former lies anterior to the latter, turns downward, and soon separates into its two portions the r. mandibularis externus facialis (*mef*) and the r. mandibularis internus facialis (*mif*).

The r. mandibularis externus facialis supplies only the sense organs of the operculo-mandibular canal and those of the pit lines of the cheek and mandible (No. 3, p. 518). The first branch of the nerve is given off before the main truncus has separated into its mandibular and hyoidean portions. It arises from the mandibular part of the truncus, while this truncus is still inside the canal of the hyomandibular, or immediately after it issues from that canal. It runs upward and backward, enters the preoperculum, and then separates into two parts, one going to organ 15 of the operculo-mandibular line, and the other to organ 16. This branch and the others also that go to organs in the preoperculum enter the bone along its inner, anterior edge.

The second branch of the main nerve, in 35 mm. specimens, goes to the horizontal cheek-line of pit organs, and the third one to organ 14 operculo-mandibular. In the adult these two branches arise together, after the truncus has separated into its hyoidean and mandibular portion, but before the latter has separated into an externus and internus. The branch to the cheek-line (*mef.hl*) passes outward through the adductor mandibulae, near its origin from the preoperculum, and then runs forward along the outer surface of that muscle ; the branch to organ 14 runs directly backward to that organ. In the adult specimen used for illustration a small communicating branch was sent from the branch to organs 15 and 16, to the one for organ 14 ; and in one 35 mm. specimen a similar branch was sent from the branch to the cheek-line to that for organ 14. Whether these communicating branches belonged to the externus or were simply fibres of the internus, accompanying the branches of the externus, it was not possible to determine. In no other part of the lateral-line system were connecting branches of the kind found, and it is to be noted that in both dissections the connecting branch ran to organ 14 operculo-mandibular.

The next or third branch of the mandibularis externus, in the adult used for illustration, was given off just before the internus separated from that nerve. It ran almost directly downward and entered the preoperculum opposite organ 13, sending a branch to that organ and then continuing downward in the bone to organs 12 and 11. Before entering the bone a branch was sent outward and downward through the adductor mandibulae and then outward and downward along the outer surface of that muscle. It separated into two portions and supplied the pit organs of the vertical cheek-line and mandibular line, the branch to the latter crossing superficially the branches of the fourth branch of the trigeminus, where they turn inward at the lower corner of the adductor. In 35 mm. specimens, and in most adult specimens also, there were two branches arising from the externus after the internus had separated from it, one branch going to organ 13 and one to organs 12 and 11, the branch to the cheek-line arising between the other two, or as a branch of the one to organs 12 and 11.

After giving off the branch to organs 12 and 11 the externus passes under the cartilaginous posterior corner of the palato-quadrate arch, and then under the preoperculum into the canal between that bone and the symplectic. From that canal it issues at about the middle of the hind edge of the symplectic, between that bone and the preoperculum, and, turning slightly backward, passes external to the ligamentum mandibulo-hyoideum, then downward and forward around the ventral surface of that ligament into a groove on the median surface of ossicle 'a.' It then continues forward in that groove, and in a prolongation of the groove on the median surface of the ventral thickened part of the articular, the nerve being here covered internally by the lower edge of the splenial.

While passing downward along the external surface of the ligamentum mandibulo-hyoideum a branch is sent forward to organ 10 operculo-mandibular, the branch entering the articular along its hind edge lateral to the articular process for the symplectic. This branch seems to have been mistaken by van Wijhe for the main r. mandibularis internus (No. 129, p. 292). The next branch of the externus, that to organ No. 9, is given

off while the nerve lies in the groove on the median surface of the articular. The nerve then comes to the upper surface of the thickened lower edge of the articular at the point where the branch *r.ghs* of the maxillaris inferior trigemini issues from the hollow of the mandible. In that position, lying immediately ventral to the median edge of Meckel's cartilage, it runs forward, sending branches to organs 8, 7, 6, 5, and 4. Beyond this last branch, at the point where the maxillaris inferior trigemini issues from the hollow of the mandible, the externus issues from beneath Meckel's cartilage, and, passing immediately ventral to r. ghi trigemini, continues forward along the inner surface of the dentary, sending branches to organs 3, 2, and 1.

The r. mandibularis facialis internus enters the canal between the preoperculum and symplectic with the externus, lying in front of that nerve, but it issues along the anterior instead of along the posterior edge of the latter bone, between it and the posterior edge of the quadrate, and reaches the inner surface of the palato-quadrate arch. It then turns forward, crosses internally the lower end of the quadrate, enters the mandible opposite the articular cup of the quadrate, and, lying between the splenial and the vertical part of Meckel's cartilage, near the upper edge of the latter, continues forward to the tip of the mandible. It lies dorsal to the origins of the geniohyoideus inferior and intermandibularis, and may receive, near the hind edge of the former, an anastomosing branch or branches from the maxillaris inferior trigemini, as already described.

The r. hyoideus facialis, after leaving the main truncus facialis, runs downward and backward along the outer surface of the hyomandibularis, passes through a canal between that bone and the preoperculum, and issues on the inner surface of the gill-cover beyond the hind edge of the cartilaginous interspace that lies between the ventral edge of the hyomandibular and the dorsal edge of the symplectic. The opening of the canal lies immediately ventral to the ventro-posterior process, or corner, of the hyomandibular, and immediately dorsal to the articular cup for the hyoid, and the nerve, continuing downward and backward internal to the suboperculum and interoperculum,

passes dorsal to and behind the interhyal, dorsal to or across the upper end of the ceratohyal, and then downward and forward along the inner surface of the branchiostegal rays, lying immediately behind the posterior edge of the ceratohyal. On reaching the fourth or third ray from in front, it anastomoses completely with the r. ghs trigemini as already described. During its course along the inner surface of the gill-cover a large branch is sent backward along the lower edge of the operculum, another along the lower edge of the sub-operculum, and others, irregularly distributed, along the inner surface of the interoperculum, the inner surface of the branchiostegal rays, and the outer surface of both divisions of the hyohyoideus, which muscle they supply.

The r. palatinus facialis ($pf$) arises from the ventral surface of the trigemino-facial ganglion toward its median edge. It runs downward and forward in the upper, lateral chamber of the eye-muscle canal, and, passing through the palatine foramen, enters the palatine canal between the parasphenoid and the ventral surface of the cartilaginous cranium. There it turns forward and separates into two parts, the r. palatinus anterior facialis ($paf$) and the r. palatinus posterior facialis ($ppf$). The latter turns outward and forward through the foramen palatini posterioris facialis, immediately in front of the anterior edge of the lateral wing of the parasphenoid, passes immediately ventral to the efferent pseudobranchial artery, crosses the space between the lateral edge of the parasphenoid and the median, dorsal edge of the palato-quadrate arch and reaches the dorsal surface of the entopterygoid ($ENP$, Fig. 2, Pl. XX). Along the outer, dorsal surface of that bone it runs downward, outward, and forward to the edge of the cartilaginous palato-quadrate ($pq$), under which it passes, then under the posterior edge of what van Wijhe calls the autopalatinum ($AUP$), and onward to the lateral edge of that bone, either coming to the upper surface of the bone through a foramen near its hind edge and then lying on that upper surface, or continuing downward to the edge of the bone between it and the dermopalatinum ($DP$). The nerve then passes beyond the lateral edge of the palatine, and lying in the thick upper lip, turns forward, sends anasto-

mosing branches to the r. maxillaris superior trigemini, passes
under the articular head of the maxillary, and is distributed to
the lip and to tissues ventral to the premaxillary. During its
course several branches are sent forward and backward along
the inner surface of the palato-quadrate arch, the branches,
like the main nerve, lying dorsal and external to the entoptery-
goid and the other dermal bones of the inner surface of the
arch, but ventral and median to the cartilage of the arch and
the bones formed in that cartilage. The main nerve may
come to the upper surface of the autopalatinum, and hence to
the upper or external surface of the arch, near the lateral edge
of the autopalatinum, and hence of the arch, but it is for a
very short distance only, and the branch or branches given
off during this part of its course reënter the autopalatinum
from its upper surface. In a 40 mm. specimen in which the
nerve was traced it lay between the cartilage of the arch and
the forming dermopalatinum ; in a 44 mm. specimen it pierced
the cartilage near its edge. In one specimen it was double on
both sides of the head as it issued from its foramen, the two
branches or parts soon uniting again.

The r. palatinus anterior facialis (*paf*) after its separation
from the posterior nerve continues forward in the palatine canal
accompanied by the r. pharyngeus glossopharyngei and a branch
of the internal carotid artery. It soon gives off a large branch,
which comes to the outer surface of the base of the skull and
runs forward along the edge of the skull, ventral to the vomer,
toward the end of the head. Somewhat further forward the
glossopharyngeus leaves the palatine canal, and with or with-
out a branch of the facialis, comes to the ventral surface of the
vomer and continues forward in that position toward the end
of that bone, lying median to the first branch of the facialis.
The remaining main portion of the palatinus continues forward
in the vomer, or between it and the chondrocranium, sending
one important branch, if it be not the main nerve itself, upward
through the base of the skull in a relatively long canal which
issues at the median edge of the septomaxillary, between it
and the median edge of the olfactory perforation of the pre-
maxillary, as already described. This last branch anastomoses

with the terminal branches of the r. maxillaris superior trige-
mini, and sends branches along the ventral and along the
dorsal surface of the premaxillary, one branch of the nerve
being traced into one of the large teeth of that bone.

### 6.  Ear, Nervus Acusticus, and Nervus Lineae Lateralis Vagi.

#### a. *Ear.*

In 14 mm. larvae of Amia the ear (Fig. 57, Pl. XXXV, of
adult ear) already shows the well-known divisions of the teleos-
tean ear.   The utricular chamber is large, and has a central, an
anterior, a posterior, and a vertical portion.   The two former,
together, are the utriculus proper, the two latter the sinus
utriculi posterior and sinus utriculi superior respectively of
Wiedersheim's figures (No. 128, p. 351).   The utriculus and
the sinus posterior lie in vertical planes approximately at right
angles to each other, and at 45° to a vertical longitudinal plane
of the body.   From the anterior, outer end of the utriculus the
canalis anterior (*csa*) arises, the lower end of the canal being
already slightly differentiated as the ampulla anterior (*aa*).
The crista acustica amp. ant. extends transversely across the
lower anterior wall of this ampulla.

Immediately proximal to the ampulla anterior, from the outer
side of the utriculus, the canalis externus (*cse*) arises, running
at first almost directly backward, and then turning sharply
inward and forward, against the anterior surface of the sinus
posterior, to join the lateral surface of the central portion of
the utricular chamber, that is, the posterior portion of the
utriculus proper.   The ampulla externa (*ae*) is slightly indi-
cated, the crista acustica amp. ext. forming a transverse, ver-
tical, ridge-like projection at the boundary line between it and
the canal.   The crista lies nearly in the middle of the lateral
portion of the canal.

The sinus posterior runs outward and backward at a little
more than 45° to the axis of the body, and lies close against
the inner and posterior wall of the posterior portion of the
canalis externus.   From its outer end the canalis posterior
(*csp*) arises, the ampulla posterior (*ap*) being slightly indicated

at its lower end, and containing, as a transverse ridge across its lower surface, the crista acustica amp. post.

The sinus superior arises from the central portion of the utricular chamber, median to the opening of the canalis externus, runs directly upward, and receives at its upper end the median, upper ends of the anterior and posterior canals.

The sacculus (*sc*) begins immediately under the central portion of the utricular chamber, connected directly with the latter by a large opening without the intervention of what could be properly called a canalis utriculo-saccularis. The front wall of the sacculus is nearly vertical, and lies on the level of the anterior edge of the opening connecting it with the utriculus. Posteriorly, on the contrary, the sacculus extends directly backward a considerable distance, at first immediately under the sinus posterior, and then immediately below but median to that sinus. Its upper wall lies close to the ventral wall of the utriculus, and there is, as yet, no marked constriction indicating that this portion is the future lagena. On the inner and upper wall of this portion, immediately behind the posterior edge of the opening connecting the sacculus with the utriculus, is the large papilla acustica lagenae, and immediately above it, in the ventral and median wall of the sinus utriculi posterior, the smaller macula fundi utriculi, or macula acustica neglecta, whichever it may be. From Wiedersheim's description and figures these two maculae seem to occupy exactly the same position in fishes and reptiles, and to be apparently identical (No. 128, p. 347). In Amia the organ is connected by a line of thickened epithelium with the papilla lagenae.

The macula acustica sacculi lies in the inner and anterior wall of the sacculus, and the macula acustica utriculi in the ventral and slightly lateral wall of the utriculus in front of the opening between the utriculus and sacculus.

In the adult, as the figure shows, the main divisions of the ear differ but little from those of larvae. The cristae and maculae acusticae have, however, acquired somewhat different positions, but no special description of them seems necessary.

Neither in the adult nor in any of the larvae did I find any satisfactory indication of a ductus or saccus endolymphaticus.

Why these structures, found so generally in fishes, should be wanting in Amia I cannot imagine.   I can only suppose that my sections were imperfect, so far as the ear is concerned, or that the ear not belonging especially to my subject, and but little attention having been given to it, I failed to recognize the endolymphatic parts of it.   The membranous parts of the ear were often much collapsed and distorted in my sections, but in the adult they seemed perfect.

Along the median surface of the sinus superior, in the adult, there was always a broad band of delicate, strongly pigmented tissue, running upward from about where the ductus endolymphaticus would naturally begin.   It was always closely attached by its edges to the wall of the sinus, and in that wall there was a white line, due apparently to a thickening of the tissue of the wall.

### b. *Nervus Acusticus.*

The nervus acusticus, in young larvae, arises from the summit of the lobus acusticus by three distinct roots, two of them often so fused that it is difficult to distinguish them in sections.   In different specimens they seem, as they issue, to have slightly different positions relative to each other.   In one 14 mm. specimen, cut in horizontal sections, they were particularly distinct, one root lying slightly above the other two, which two were, however, cut in the same section.   They all had their apparent origins immediately below the utriculus and immediately in front of the sacculus, and each of them entered a definite part of the large irregular acoustic ganglion. The upper root, which is the main posterior root of the nervus, gave origin to the ramus cochlearis, which turned directly backward internal to the sacculus, supplied at once the macula sacculi, then the papilla lagenae and macula fundi utriculi by branches arising close together, and then, turning outward and backward above the lagena, between it and the ventral surface of the sinus utriculi posterior, and always separating into two terminal branches, supplied the crista ampullae posterioris.

The other two roots together formed the main anterior root of the nervus and, together, gave origin to the ramus vestibu-

laris. The fibres of the anterior of the two roots ran forward and outward, and supplied first the macula utriculi, which lay immediately above the ganglion of the root, and then the crista ampullae anterioris. The fibres of the posterior of the two roots ran outward and slightly forward and supplied the crista ampullae externae.

The main posterior root, or ramus cochlearis, and its ganglion were always much more distinctly separated from the other two roots and their ganglia than those two roots and ganglia were from each other.

As the ramus ampullae posterioris passes outward and backward between the lagena and the sinus posterior it passes immediately above the root of the nervus glossopharyngeus, which runs at this point almost directly outward. The two nerves here come into intimate relations and there is always an interchange of fibres, the exact nature of which I have been unable to satisfactorily determine. To the best of my observation some of the fibres of the acusticus join the dorsal or sense-organ root of the glossopharyngeus, but whether they run distally or proximally in that root I am unable to say. Distal to this point the dorsal root of the glossopharyngeus is separate and distinct from the remaining much larger portion of the root of that nerve, lying immediately behind it and having an entirely separate ganglion, as already described in my earlier work (No. 3, p. 516). While passing inward under the acusticus, through the narrow space between the lagena and utriculus, the two roots of the glossopharyngeus are much flattened, and the large fibres of the dorsal root were always lost in my sections. Median to the acusticus, after passing under it, they, however, reappear in the sections and receive an important bundle of fibres from the acusticus, the bundle being apparently as large as the dorsal root of the glossopharyngeus itself. Proximal to this anastomosis the dorsal root still persists, lying in its accustomed position immediately behind and in contact with the main root of the glossopharyngeus. This latter root, in a 14 mm. and 20 mm. specimen of which I had excellent sections, then passed through the root of the nervus lineae lateralis, near its ventral edge, and there was again an interchange of

sense-organ fibres.  In the 35 mm. and 40 mm. specimens examined in my earlier work the ventral portion of the root of the nervus lineae lateralis seemed to pass through the dorsal part of the main root of the glossopharyngeus, receiving there an important addition to its fibres (No. 3, p. 517).  In all the adult specimens that I have examined such is also the apparent relation and arrangement of the nerves, for I have never found a ventral bundle of the nervus lateralis lying ventral to the root of the glossopharyngeus.  I therefore conclude from my present work that the relations of the nerves must vary in different specimens, or at different ages.  I am also convinced that it is the so-called dorsal root of the glossopharyngeus that receives fibres from the nerve of the lateral line, and not the reverse, as I formerly supposed.  Whether that dorsal root is formed entirely of fibres derived first from the root of the nerve of the lateral line and later from the ramulus ampullae posterioris acustici or not, I have been unable to positively determine. Such seems to me, however, to be the case.  As the communicating branch between the acusticus and the dorsal root of the glossopharyngeus lies proximal to the crista amp. post., there is thus something in Amia to support the view that the nerve to the posterior ampullae is sometimes derived from the glossopharyngeal nerve (No. 5, p. 232).

In the adult, in the four specimens examined after my attention was called to the matter, two small anastomosing branches were always found, one running proximally from the posterior surface of the glossopharyngeus distally into the outer and lower surface of the nervus lineae lateralis, and the other distally from the anterior surface of the glossopharyngeus proximally into the inner surface of the ramulus amp. post. acustici.  The nature of these branches could not be determined from macroscopic examination.

c.  *Nervus Lineae Lateralis Vagi.*

This nerve in 14 mm. specimens arises close to the root of the glossopharyngeus, slightly above and in front of that root and slightly behind the root of the acusticus.  It seems to

arise, however, immediately behind the slight swelling of the tuberculum acusticum, and not from that swelling, as Strong states to be the case in all fishes (No. 121, p. 179). It runs almost directly backward, as described in my earlier work, anastomoses with the root of the glossopharyngeus, as described above, and then continues backward, lying at a higher level than the ganglion on the posterior root of the acusticus, and at a lower level than the issuing roots of the vagus. On these latter roots an intracranial ganglion is formed, the root of the lateralis lying along its lower, outer surface. The lateralis then issues with the vagus through the vagus foramen, enters its ganglion and has the distribution already given (No. 3, p. 517). In the 14 mm. and 20 mm. larvae in which a ventral bundle of the root was found passing ventral to the root of the glossopharyngeus it could be distinguished as a separate bundle almost to the exit of the nerve through its foramen.

### 7. Review and Comparison of Nerves.

There are, as is well known, several kinds of more or less specialized sense-organs of ectodermal origin, found in greater or less number in all vertebrates. In the Ichthyopsida, where these organs are found in the greatest number and variety, the innervation of certain of them in certain forms is now definitely known, that of others is still largely unknown, as is also the development and interrelationships of all the organs. Some intimation of the relationships of certain of them can, however, be deduced from their innervation as already known, and the innervation, and hence relationship, of others is in a measure indicated by the relative size and extension of certain nerves, or their branches, in forms where certain of the organs undergo unusual development.

In Amia three kinds or varieties of these organs are found, namely, canal organs, pit organs, and terminal buds,—the organs of the eye, the ear, and the nose being left out of consideration. The canal organs are always found, in the adult, inclosed in the canals of the lateral line system, and the pit organs at the bottom of slight pit-like depressions that are arranged in lines

having definite and constant relations to those canals or canal organs. The terminal buds are, on the contrary, found scattered irregularly over the external surface, mainly of the head and fins, and on the inner surface of the mouth and branchial cavities (No. 3).

The canal organs and pit organs in Amia are all innervated by what are called the dorsal, suprabranchial, or lateral branches of the facialis, glossopharyngeus, and vagus nerves, and they all belong to what are called by Merkel nerve hillocks. In early stages of development they closely resemble each other (No. 3, p. 502), but in later stages they differ greatly, the pit organ retaining its embryological form and individuality, while the canal organ first increases greatly in size, and then, by the independent growth of other similar organs immediately adjoining it, gives rise to a large sensory patch, or nerve ridge (Merkel), in which the separate organs lose to a greater or less extent their individuality.

Portions of the canal lines in Amia are represented in certain teleosts by lines of surface organs, and certain of the pit lines in Amia are apparently represented in certain teleosts and bony ganoids by canals containing organs. To the examples already given of this in my earlier work (No. 3, pp. 471 and 521) must probably be added the mucous canal at the base of the dorsal fin in Polypterus, mentioned, but not described, by Pollard in his work on the lateral line system in siluroids. He there states (No. 94, p. 545) that this canal is represented in Amia by the dorsal body line of pit organs. As I do not find a description of the canal and its innervation, I am unable to judge whether this be so or not. The canal may, however, be of the kind described by Emery as the accessory lateral lines of Fierasfer (No. 25, p. 209), and hence not comparable at all with the canals of the lateral line system as found in Amia and teleosts. As there is in Polypterus a supratemporal crosscommissure, the canal in question cannot be that canal, as is probably the case with the canal described by Pollard in the same place, that is along the base of the dorsal fin, in Clarias and Auchenapsis (No. 94, pp, 530 and 533). In these two fishes the innervation of the canal is given as by a great recur-

rent dorsal branch of the facialis; but that recurrent branch is joined, in both fishes, by an important branch from the first branch of the lateralis vagi, and as this branch of the lateralis vagi supplies organ No. 8 of the main lateral canal, in both fishes, it is in all probability this branch, and not the recurrent facialis, that supplies the organs of the canal in question. Organ No. 8 lies in the squamosal near its hind edge, and as there is, according to Pollard, no extrascapular and no supratemporal crosscommissure in siluroids, the organ No. 8 of Clarias and Auchenapsis probably corresponds to organs 18 and 19 of the main canal in Amia. The commissural or recurrent branch that connects the nerve supplying it with the recurrent dorsal branch of the facialis would, therefore, correspond to the supratemporal extension of the nerve supplying organs 18 and 19 in Amia. The canal along the base of the dorsal fin in Clarias and Auchenapsis would then be the supratemporal crosscommissure of Amia, somewhat changed in position and direction, a change that could easily arise since the bone traversed by the canal in the one form is wanting in the other.

The great recurrent branch of the facialis described by Pollard seems to be the ramus lateralis trigemini of other authors. In Silurus glanis this nerve is distributed to the dorsal fin, as in Clarias and Auchenapsis. In Trichomycterus and Chaetostomus a large branch is sent from it to the pectoral fin (No. 94, pp. 536 and 539), and in Gadus morrhua I find this branch distributed to the breast fin also. In elasmobranchs and Amphibia the nerve is wanting, so far as I can find. Its distribution indicates that it is destined largely or entirely to the supply of terminal buds, for these buds are not found on the body in elasmobranchs and Amphibia, and are found in great quantity, but with a greatly varied distribution, on the body, and more especially on the fins, in teleosts. The nerve in Gadus lies immediately beneath the skin, but crosses the lateralis vagi internal to that nerve. It arises as two nerves or bundles from the deeper portions of the trigemino-facial ganglion, the two bundles embracing the root of the buccal and ophthalmic branches of the facialis exactly as the first pair of

branches of the ophthalmicus superficialis trigemini in Amia embrace the ophthalmicus facialis.    The nerve in Gadus has, contrary to the arrangement of the branches found in Amia, an intracranial course, as it has in siluroids, issuing on the top of the skull near its hind end.    As the trigemino-facial ganglionic mass lies, in Clarias at least (No. 94, p. 529), inside the skull beside the brain, this difference of course is probably of no importance.

In Clarias Pollard states that tube 5 of the infraorbital or main canal "must be regarded as the last rudiment of a canal represented in Amia by the vertical line of pit organs"; that tube 6 "appears also to be the rudiment of a canal represented in Amia by the horizontal line of pit organs"; and that tube 8 "corresponds to the rudimentary canal represented in Amia by the dorsal line of pit organs."    These conclusions are certainly wrong, for the canals of all the lateral lines are always formed in sections, each section inclosing an organ, and the tubes leading to the surface from the developed canals always lie between two adjacent organs, and represent simply the fused open ends of two such sections (No. 3, p. 523).    A sense organ is primarily never found in such a tube, and the tube can in no way represent a line of surface organs.    The terminal tubes of a canal line may lie in the direction of such a line of surface organs, and may even inclose secondarily one of those organs, as is the case with tube 8 supraorbital in Amia (No. 3, p. 506). The tube has, however, in its development nothing whatever to do with the line and cannot represent it.    A line of pit organs represents apparently the possibility of a canal; with the disappearance of the organs the possibility of the canal, even in rudiment, would certainly disappear.

Pollard did not find any lines of pit organs in siluroids, and Collinge (No. 20) does not mention any.    I, however, find them both in Amiurus catus and in Silurus glanis.    In both these fishes there is an anterior crosscommissural line over the top of the end of the snout, and two or possibly three lines on the top of the head; and in Silurus there are three lines on the side of the head.    One of the lines on the top of the head is a direct continuation of the supraorbital canal, and in larvae

of Amiurus, as in Amia, it is a direct continuation of the line of the supraorbital canal organs. Those on the side of the head in Silurus are one vertical, one horizontal, and one on the hind end of the mandible; the lines do not, however, have the same positions relatively to each other that they have in Amia.

The arrangement of the canals in larvae of Amiurus shows beyond question that Pollard's pore 6 infraorbital in Clarias is formed by the fusion of pores 6 infraorbital and 6 supraorbital, his pore 6 supraorbital being therefore in reality pore 7 of that line, and the terminal pore of the line. That it is the terminal pore of the line is shown sufficiently by the presence of a sense organ in what Pollard considers as its tube, and by the absence of such an organ in what he considers as the continuation of the main canal. Pore 7 of the main canal in Clarias has fused with the terminal pore of the operculo-mandibular canal and is found on that line, but not shown in Pollard's figure, and pore 7 in his figure is in reality pore 8. The same is true of Auchenapsis and Chaetostomus. In each of these fishes the last pore and tube of the ascending portion of the suborbital canal, behind the eye, is a double system, formed by the fusion of a suborbital and supraorbital pore and tube; and the last pore and tube of the operculo-mandibular canal is also a double system, formed by the fusion of a tube and pore of the main line with the terminal pore and tube of the operculo-mandibular line.

The sense organs of all the canals in all the fishes described by Pollard are, therefore, with a single exception, regularly placed one between each two consecutive tubes of the line to which it belongs, instead of being irregularly placed, as the figures and numbering used by Pollard would indicate. The single exception, or apparent exception, is in the operculo-mandibular line in Chaetostomus, where one organ seems to be wanting, but the development, if it were known, would undoubtedly show that the irregularity even here is only apparent. In Chaetostomus the operculo-mandibular canal turns backward and downward into the interoperculum and contains an organ in that bone. This may be another instance of a pit line in one form becoming a canal in another, for in Gadus

there is a line of surface organs on the outer surface of the opercular bones immediately behind the preoperculum. They are all innervated by a special branch of the mandibularis externus facialis, and are therefore of the character of pit organs, and unquestionably represent in Gadus one of the cheek-lines in Amia, or a similar line not found in Amia. One of these organs has possibly become inclosed in a canal or tube in Chaetostomus. The organ may, however, belong to the main canal line, for Collinge states (No. 20, p. 277) that the operculo-mandibular canal in Clarias nienhofii traverses the inter-operculum. Pollard, unfortunately, does not describe the operculo-mandibular canal in Clarias. Collinge's statement cannot therefore be controlled, and his statements seem sometimes to need considerable control or confirmation.

In Esox lucius, for example, Collinge says (No. 20, p. 288), "there is a distinct and very large lateral canal" extending the full length of the body. I find only a lymph canal lying under the dermis. Other investigators have doubtless only found this same canal, as, according to Collinge's own statement, they find no lateral canal. In Salmo salar he describes (No. 20, p. 296) "a series of functionless canals passing through the substance of the bone" and not communicating with the surface ; and a series "of small, drainpipe-like ossifications" from which have arisen a "more superficial series of canals," "which gradually replaced altogether those traversing the deeply seated cranial elements." In Salmo namaycush I find the canals traversing the same bones they do in Amia, excepting only the antorbital and ethmoid. The extrascapular of Amia is, however, represented in Salmo namaycush by a series of small, tube-like bones, such as Collinge describes in Salmo salar, and there is a small bone of a similar character between the upper end of the preoperculum and the lateral edge of the squamosal. As the canals in Coregonus (white fish) also lie in the main cranial bones, Collinge's statement regarding Salmo salar seems most exceptional, the process of development he assumes still more so. Why organs once found in canals resembling those of other fishes should afterwards migrate into a secondary, superficial series of canals, certainly

needs some explanation. Collinge further repeatedly states that the rami buccalis, oticus, and ophthalmicus superficialis are branches of the trigeminus, and then, based on this assertion, arrives at the conclusion (No. 19, p. 515) that, "The fact of the trigeminal actually innervating a part of the sensory canal system is of special interest." Had he assigned the nerves to the facialis, or to the lateralis, as is usually done, the recorded facts would lose their special interest.

Collinge, in referring to my earlier work, corrects a statement made by me regarding the operculo-mandibular canal in Amiurus catus. As I find the canal in Silurus glanis as he gives it in Amiurus, he is undoubtedly correct. In further reference to my work, he says (No. 19, p. 504) that I gave the name "peripheral organs" to "a system of fine dermal canals" "opening by a series of fine branches to the surface by isolated pores." This is as evidently an error. For these canals and their openings in Polyodon, Collinge proposes the name " cluster pores." Later in the text (No. 19, p. 513), cluster pores are said to be a "series of organs" agreeing "almost in every detail with the Spaltpapillen of Fritsch," and containing, like them, sense cells, into which fine terminal nerve fibres pass. " Primitive pores " in Polyodon are said to be "fine pore-like openings, spoken of as pin-hole pores by many authors." These primitive pores connect with the small branches of the main canals (No. 19, p. 509), apparently as their external openings, but each pore contains a sensory organ at its base (No. 19, p. 514), and in section (No. 19, Fig. 5) closely resembles a pit organ of Amia. As the branches of the dendritic canal systems open on the external surface by these "pores," each of which contains an organ, and as no organs of any kind are definitely described at any place in the canals themselves, it is evident that Polyodon, as the author states, "exhibits a number of interesting features at present not known to occur in any other ganoid."

In Gadus a line of surface organs is found along the lower edge of the mandible, parallel to the mandibular canal, and it is innervated by a long branch of the externus facialis, which first runs forward through the adductor mandibulae, to

the hind edge of the infraorbital canal behind the eye, and there turns downward and reaches the mandible. A nerve in Esox corresponding in position to this nerve in Gadus innervates a line of surface organs lying on the upper jaw immediately below the infraorbital canal. In Polyodon the maxillary branch of the hyomandibular canal (No. 19, p. 512) may represent this line.

Although certain portions of the canal lines of Amia are thus represented in teleosts by lines of surface organs, and vice versa, I find nothing in the literature at my disposal that tells definitely whether the organs in such cases change in character; that is, whether the organs found on the external surface in teleosts, in lines that are represented in Amia by canals, have the character of canal organs, or of pit organs, or of some intermediate or different form of organ. Indirectly one is led to conclude that they retain or acquire an intermediate character, for Leydig includes them with pit organs and terminal buds, under the general term "Becherorgane." He also states, definitely (No. 74, p. 125), that "freie Sinneshügel zu solchen werden können welche in Schuppencanälen liegen" (No. 74, p. 90), that the "Becherorgane" tend to differentiate into several distinct groups, and that this differentiation, or tendency to differentiate, is more strongly marked in Amphibia and in reptiles than in fishes. He, however, places together, under the general term "lateral organs," the canal organs, and the canal organs only, in fishes, and the surface or exposed nerve hillocks of the lateral lines in Amphibia (No. 74, p. 106), thus making a distinction between these exposed lateral organs in Amphibia and similar ones found in fishes. He says, however, as does Wiedersheim (No. 128, p. 298), that between the several kinds of organs, canal organs included, there are so many intermediate forms that it is impossible to draw a sharp limiting line between any of them.

Wiedersheim says (No. 113, p. 298) that nerve hillocks, in their development, pass through the stage represented by the terminal bud, and that the latter is, therefore, the oldest phylogenetically of all these organs. That being so, what has been established for the development of nerve hillocks and the nerves

innervating them, must be, in general, true for the terminal buds and the nerves that innervate them. The nerve hillocks, or sense organs of the lateral line, are said by Beard (No. 10, p. 209) and Wilson (No. 131, p. 244) to arise separately and independently along lines of sensory epithelium that either differentiate in one or more directions from certain central points, or grow directly from such points by cell division. From the deeper layers of this sensory epithelium the nerve supplying the organs of the line arises. Terminal buds and the nerves innervating them should therefore arise in this same way.

The only nerves in Amia from which I have been able to trace branches definitely to terminal buds are the ophthalmicus superficialis trigemini and the maxillaris inferior trigemini. The former of these two nerves in 14 mm. specimens derives the larger part, if not all, of its fibres from the median part of the trigemino-facial ganglion, that is, from that part of the ganglion that is formed on, or in connection with, the fasciculus communis root. From this same part of the ganglion a large bundle of fibres is sent to the truncus maxillaris trigemini; from it arises also the ramus palatinus facialis, which is distributed in Amia to a region covered with terminal buds, and which in Rana innervates such buds (No. 121, pp. 121 and 123); and from it also a bundle of fibres is possibly sent to the truncus hyoideo-mandibularis facialis, as is said to be the case in Rana and Amblystoma, in which animals it gives origin, according to Strong, to the ramus mandibularis internus facialis, which nerve in Rana innervates terminal buds (No. 121, pp. 130, 132, and 195). I was unable to definitely trace this bundle of fibres in Amia into the truncus hyoideo-mandibularis, and the mandibularis internus in larvae of Amia, through the full length of its course, never passes near a region where terminal buds are numerous. In the lining membrane of the mouth cavity, internal to the hyomandibular, and hence not far from the truncus hyoideo-mandibularis, there are many buds, but the pretrematic branch of the glossopharyngeus runs near them, and branches of that nerve are found extending toward them. It is therefore, in all probability, that nerve and not the mandibularis internus that innervates them.

Terminal buds in Amia are just beginning to appear on the outer surface of the head in 10 mm. larvae. Their distribution is shown in Figs. 4 and 5 of my earlier work; Figs. 6 and 7 showing a slightly more advanced condition. It will be noticed that the organs are, in many places, arranged in lines, and that well-marked lines are found on each side of the line of the supra-orbital canal line, and on one side only of the infraorbital line, that is, on its dorsal side, between it and the eye. Other lines of organs are found on the maxilla and at its base, below and in front of the line of the infraorbital canal. If, now, the rami ophthalmicus superficialis and maxillaris superior trigemini of the adult be considered, it is seen that branches arise from the ophthalmicus trigemini in pairs, one branch issuing on the median side and one on the lateral side of the ophthalmicus facialis ; that on the maxillaris superior all the small branches below and behind the eye, excepting only the accessory branch "r.c," issue on the dorsal or median side of the buccalis, and that the one large branch, the one destined to supply the maxilla, issues on the ventral side of that nerve. The branches of these two trigeminal nerves in the adult thus have the same relations to the nerves innervating the canal lines that the lines of termi-nal buds in 10 mm. larvae have to the lines of the canals. As the trigeminal nerves both lie deeper than the corresponding facial nerves, and as their branches issue on both sides of the latter, it is evident that they must first have been split off from the ectoderm before the facial nerves, split off apparently from exactly the same lines, could have arisen. This is exactly what Platt finds for the ophthalmic nerves in Necturnus (No. 91, p. 949).

The branch of the trigeminus that goes to the maxilla in Amia is the branch which, according to Pollard, furnishes the sensory supply of the maxillary tentacle in siluroids, and a part of that of the coronoid tentacle. Where there is but a single tentacle at the angle of the mouth, the maxillo-coronoid tentacle, the larger part of its sensory supply is from this same nerve. The nerve is called by him the ramus maxillaris. His ramus premaxillaris is represented in Amia by those branches of the maxillary nerve of Amia that continue forward toward the pre-

maxillary, instead of turning backward into the maxilla ; and his ramus palatinus trigemini (No. 97, p. 400) is either what I have considered as the main terminal portion of the maxillaris superior trigemini, or that nerve plus the palatinus posterior facialis. The name and distribution given by Pollard seem to indicate that it is the two nerves fused, but as he does not describe the branches of the nervus facialis one cannot be sure. In the fishes described by Pollard the anterior end of the palato-quadrate is found as a distinct and separate piece, which is called by him the prepalatine, and is said to be the homologue of the autopalatine of van Wijhe in Polypterus (No. 97, p. 403). The autopalatine is found in Amia also (*AUP*, Fig. 2, Pl. XX), and the ramus palatinus posterior facialis runs under it, as Pollard says his palatinus trigemini does in Misgurnus. This nerve in Misgurnus is thus certainly the r. palatinus posterior facialis of Amia. As this nerve and the r. maxillaris superior trigemini, in Amia, are both sensory nerves distributed to the same or to adjoining regions, and as they partially anastomose in Amia at the proximal end of the maxillary bone, there seems no reason why they might not fuse entirely if the bone or cartilage that normally separates them should disappear, or become reduced and displaced, as it has in siluroids. That the palatinus of Misgurnus, lying below the palato-quadrate cartilage should be the homologue of a nerve lying above that cartilage seems wholly inadmissible.

In addition to the terminal buds described above there are, in 10 mm. larvae, indications of the beginning of similar organs above organ 16 infraorbital and on the side of the cheek above the horizontal line of pit organs (No. 3, Fig. 4). In a 11 mm. larva (No. 3, Fig. 6) the organs on the cheek have increased, and those above the horizontal pit line are seen to be arranged in horizontal lines. Buds have also appeared on the mandible on both sides of the mandibular canal line, and a spot on the operculum at its upper anterior end indicates the beginning of similar organs there. In 14 mm. specimens (No. 3, Fig. 8) the buds have increased in number, those on the cheek below the horizontal pit line being arranged in lines that run backward, or downward, or downward and forward. In 18 mm.

larvae (No. 3, Fig. 12) the buds have increased at the upper end of the operculum, are beginning to appear along the branchio-stegal rays, spreading backward from the mandible, and are also found above organ 17 infraorbital.

The buds on the cheek as they first appear in larvae have approximately the position and direction of the accessory trige-minal nerves in the adult, and these accessory nerves have the same relation to the branches of the buccalis, behind the eye, that those branches of the maxillaris superior, that arise below the eye, have to the buccal branches there. These branches seem, therefore, to innervate the terminal buds, and in that case they would belong to the fasciculus communis root, and not to the general cutaneous root of the trigeminus, as Strong finds in Rana. The only other supposition is that the buds on the cheek are innervated by the mandibularis internus facialis, which seems improbable.

The buds on the mandible lie in the region supplied by the anterior branches of branch 4 of the maxillaris inferior trigemini. These branches have the course and position of the ramus coronoideus of Pollard, a nerve which, in siluroids, innervates part of the sense organs of the coronoid tentacle. Accompanying this nerve in Silurus glanis I find a large branch of the mandibularis facialis. This nerve in Silurus may be a branch corresponding to the one which in Amia innervates the mandibular line of pit organs, and in Gadus innervates a man-dibular line of the slit-like organs peculiar to that fish. If this be the case, pit organs or other organs of a similar character should be found in Silurus in the region innervated by the nerve. Of this I have not yet attempted any investigation. The nerve corresponds almost exactly to a branch of the facialis in Carcharinus.

The buds above organ 17 infraorbital, and hence posterior to the middle pit line of the head, are innervated either by the first pair of branches from the ophthalmicus superficialis trige-mini, or by the branches of the first vagus that anastomose with that nerve. The branch of the glossopharyngeus that innervates the pit organs of the middle head line lies super-ficial to the united trigeminal and vagus nerves, as it should if

nerves to terminal buds in any region are split off from the ectoderm before the similar formation, in the same region, of nerves that supply the sense organs of the canal and pit lines. The branch of the glossopharyngeus to reach its destination runs first outward below the branches of the trigeminus and vagus and then medianward above them.

The buds on the upper end of the operculum must be inner-vated either by the first dorsal branch of the vagus, by the hyoideus facialis, or by terminal extensions of the accessory branches of the trigeminus. Those on the gular plate and on the lower end of the gill cover are almost certainly innervated by those branches of the trigeminus that I have called *r.ghs* and *r.ghi*. The growth of the buds upward and backward from the lower part of the gill cover, corresponding to the direction of the branches of these two nerves, strongly indicates their innervation by them. If their innervation be by these nerves, the mandibularis internus facialis would probably take no part in the innervation of any of the buds on the outer surface of the head, and the nerve must be, in Amia, either a general cuta-neous nerve or a nerve like the palatinus facialis destined to supply terminal buds in the mouth cavity, as Strong supposes it to be. The almost total absence of buds along its course in Amia is, however, against this last supposition, and its course and position indicate strongly a nerve comparable to the one called by Pinkus branch 4 of the hyomandibularis in Protop-terus (No. 89, p. 304). As the nerve, in Amia, lies behind the spiracular canal it is a posttrematic branch of the facialis, and cannot, therefore, be the chorda tympani, for the course of that nerve in man through the upper portion of the tympanic cavity and then downward anterior to that cavity certainly indicates that it is a prespiracular nerve. That this nerve, in Amia, is the homologue of the nerve of the same name described by Ewart, Pollard, and Strong in other Ichthyopsida, and consid-ered by them as the homologue of the chorda tympani, is hardly open to question. The nerve in Amia is probably to be compared to the branch which, on each of the branchial arches, runs downward over the anterior face of the arch onto the inner surface of its ventral portion. Its position in Amia,

along the inner surface of the mandible, could be easily derived
from that in selachians as given by Vetter. In Heptanchus
what seems to be the nerve is shown lying along the posterior
edge of the mandible ; from this position, as the hyoideo-man-
dibular fold of Amia was formed, the nerve could as naturally
come to lie along the inner surface of the mandible as along
the lateral surface of the hyoid. The more superficial nerves
*r.ghs* and *r.ghi* of Amia would at the same time acquire their
somewhat peculiar course.

The nerves *r.ghs* and *r.ghi* vary greatly in importance and
in position in different fishes. In Gadus they, and part also of
branch 4 of the maxillaris inferior of Amia, seem to be repre-
sented by a single nerve, the larger portion of which lies along
the lower edge of the mandible, small branches only passing
into or toward the hyoideo-mandibular fold. In siluroids they
are in all probability the nerves of the submandibular and
mental tentacles, the nerves called by Pollard the ramus sub-
mandibularis and ramus mentalis trigemini. Pollard states
(No. 97, p. 411) that these nerves in siluroids either contain,
or are accompanied by, motor fibres, destined to supply the
muscles that move the tentacles. The muscles themselves
he does not describe. As all tentacle muscles in siluroids are
said to belong (No. 97, p. 382) to a special system, not homolo-
gous with the metameric body muscles, they must either have
entirely disappeared in Amia or have become absorbed in other
muscles.

In Auchenapsis and Callichthys Pollard describes a ramus
mandibularis externus trigemini, which he says (No. 97, p. 410)
may be a dissociated branch of his ramus mentalis trigemini.
The mentalis lies internal to the coronoid process of Meckel's
cartilage, the externus external to that cartilage and external
to the mentalis (No. 97, p. 391). In Callichthys the externus
supplies the anterior face of the mental tentacle. Whether
this nerve finds its homologue in one of the branches of branch
4 of the maxillaris inferior trigemini of Amia, in some part of
nerves *r.ghs* and *r.ghi*, or in a branch of the facialis, seems
open to question. It is to be noted that in Callichthys there is
no lateral line canal in the mandible (No. 94, p. 534), and that

in Auchenapsis the mandibularis facialis does not divide into
an externus and an internus (No. 94, p. 533).

If these several nerves are destined largely or entirely to the
supply of terminal buds, as seems probable, the variation in
their importance and position in different fishes is easily ex-
plained, for the distribution of terminal buds and their number
in any particular region varies continually. The nerves in
Amia strongly indicate that in this variation certain of the
terminal buds have wandered from the outer surface of the
head into the mouth cavity. No other explanation can be
given of the course and terminal distribution of the two inter-
nal branches of the fourth branch of the maxillaris inferior
trigemini. That sense organs can pass from the outer surface
of the head to its inner surface is seen in the organ of the
spiracular canal in Amia (No. 3, p. 501). Furthermore, Beard
states (No. 10, p. 191, footnote 1) that he has evidence indicat-
ing or proving that the end organs of taste arise from epi-
blastic thickenings which have wandered through certain gill
clefts into the buccal cavity.

The two internal branches of the fourth branch of the maxil-
laris inferior trigemini, in Amia, with or without the branches
*r.ghs* and *r.ghi*, seem to be represented in Protopterus by the
inferior branch of the palatinus facialis, which Pinkus consid-
ers the homologue of the chorda tympani (No. 89, p. 310).
This inferior branch of the palatinus as a separate nerve is not
found in Amia. In teleosts also it is not given by Stan-
nius, but it is described by him in selachians. Van Wijhe
does not give it in Acipenser or in the other ganoids described
by him, but Collinge probably describes it, in Polyodon, as the
mandibularis internus facialis, for that nerve, as shown in his
Fig. 12, lies in front of the spiracle, and he says that it inner-
vates "the primitive pores of the sides of the head and mouth"
(No. 19, p. 518). It lies immediately in front of and closely
accompanies the mandibular branch of the trigeminus which
innervates "primitive pores" in the region of the maxilla.
These two nerves if fused would seem to represent the maxil-
laris inferior trigemini of Amia, provided the "primitive pores"
described by Collinge are terminal buds or their derivatives.

In Protopterus the inferior branch of the palatinus facialis sends a branch to the pharyngeal branch of the glossopharyngeus, and another to the mucous membrane in the hind part of the mouth cavity (No. 89, p. 309). In Rana bundles of fibres from the fasciculus communis tract join and issue with the united glossopharyngeal and vagus roots, and form in part the pretrematic and pharyngeal branches of those nerves.

The fibres of the fasciculus communis tract thus seem destined to form in part or in whole the pretrematic and pharyngeal branches of the nerves with which they are associated, and it is always in the regions which, whether on the outside or the inside of the body, are, from their relation to these nerves, presumably innervated by them, that terminal buds are found. The fibres of the tract that join and form part of the facialis enter into, or form entirely, the inferior and superior branches of the palatinus facialis, and those two branches are respectively the pretrematic and pharyngeal branches of the nervus. The fibres that join the glossopharyngeus enter, in Rana (Strong), into the ramus lingualis and the ramus pharyngeus, which are, respectively, the pretrematic and pharyngeal branches of that nerve. As there are no communicating branches in Amia from the palatinus facialis to the glossopharyngeus, the fasciculus communis component of the latter nerve must be a postspiracular nerve, as must, therefore, probably also be both parts of Pinkus' branch of the palatinus facialis to the nerve in Protopterus.

Whether there is or is not a prefacial component of the fasciculus communis tract seems problematical. Brandis (No. 14, p. 539) says there is none in birds. The ophthalmicus superficialis trigemini may, however, be in part such a nerve, as may also be the ciliaris brevis. The ophthalmicus trigemini innervates terminal buds, and is therefore functionally to be classed with the palatinus facialis and the ramus anterior of the glossopharyngeus, both of which are pretrematic or pharyngeal nerves. The pretrematic and posttrematic nerves in Petromyzon all arise, according to Kupffer (No. 67, p. 45), in connection with epibranchial ectodermal thickenings, and the pretrematic nerves, all, from and including the trigeminus

backward, become connected with a second, pretrematic, series of ectodermal thickenings. From both these thickenings ganglia arise, the first or epibranchial ganglion being common to the post- and pretrematic branches of the nerve, while the second or pretrematic ganglion is connected with the pretrematic nerve alone. In Petromyzon no ectodermal sense organs of any kind arise in connection with the epibranchial ganglia (No. 67, p. 30). If this be true for other fishes, terminal buds must arise in connection with the pretrematic ganglia and ectodermal thickenings, if their origin and development is similar to that generally accepted for the organs of the lateral line. They would, therefore, seem to be the rudimentary sense organs found by Froriep on the facialis, glossopharyngeus, and vagus in calf embryos, and as the eye belongs, according to Kupffer (No. 67, p. 50), to the line of epibranchial ganglia, the lens and its nerve, the ciliaris brevis, may belong to the pretrematic series. Sedgwick says (No. 114, p. 97) that the profundus ganglion, from which the ciliaris brevis and other ciliary nerves arise, is, when it is first laid down, in contact with the ectoderm.

In short, the nerve fibres arising from the fasciculus communis tract seem destined in large part, if not in whole, to the supply of terminal buds, as Strong (No. 121) has suggested might be the case. The fibres so arising may issue from the brain as a separate and distinct root, on which a separate and distinct ganglion is found ; they may issue in part as components of certain nerves with the roots of those nerves, and in part as a separate root which becomes immediately more or less fused, it and its ganglion, with other roots and ganglia ; or they may apparently issue entirely as components of certain nerves.

To the first category belongs, apparently, Protopterus ; to the second, Amia, Rana, and many other fishes and amphibia ; to the third, birds, judging from Brandis' descriptions (No. 12, p. 539, and No. 13, p. 647), for he finds the fibres of the funiculus solitarius issuing with the facialis and glossopharyngeus, and possibly also with the vagus, and the funiculus solitarius of higher vertebrates corresponds, according to Strong

(No. 121, p. 186), in every detail with the fasciculus communis of fishes and Amphibia.

### III. MUSCLES INNERVATED BY THE GLOSSOPHARYNGEUS AND VAGUS, AND THE NERVI GLOSSO-PHARYNGEUS AND VAGUS.

#### 1. The Visceral Arches.

The visceral arches in Amia have been described by Bridge (No. 15) and by van Wijhe (No. 129). There are seven of them in all, the mandibular, or perhaps more properly palato-mandibular, arch, the hyoid arch, and five branchial arches. Of the latter, four are complete arches bearing each a double row of gill filaments, while the fifth is a half arch without gill filaments.

Between the fifth arch and the next preceding, or fourth arch, the gill opening lies entirely on the ventral aspect of the arches, and does not extend to their hinder, outer angle ; that is, it lies between the ceratobranchials only, of the two arches, and does not extend upward onto the dorsal aspect of the arches between the epibranchials, as is the case with the other openings. Between the fifth arch and the next following, or pectoral arch, there is a relatively large space, closed externally by a thin layer of integument which extends upward and forward, beyond the upper end of the fifth arch, along the posterior edge of the dorsal portion of the fourth arch, and then forward, across the dorsal ends of that arch and the other anterior arches, median or proximal to the gill filaments, to the inner upper angle of the operculum.

Immediately internal to the dorsal portion of this thin membrane, and attached to it, there is, in the adult, a much degenerated glandular formation, extending from near the upper anterior corner of the opercular opening back to the front edge of the supraclavicular. It is evidently the thymus, but apparently so degenerated that it disintegrates and floats away when stirred with a scalpel under water, or even when washed with a pipette. In embryos it is found as a well-defined, relatively large mass, not at all degenerated in appearance, and not

apparently attached to, or in any way directly connected with, the epidermis. It extends, in embryos, approximately from the level of the dorsal end of the second arch backward, above and beyond the dorsal ends of the other arches, onto the outer surface of the front edge of the supraclavicular. A large venous vessel lies at first immediately dorsal to it, and then immediately internal to it, but in the purely superficial examination that was made no branches were found leading into it.

There is no hyoidean demibranch or opercular gill, but there is a well-developed pseudobranch, lying in what Wright has called the pseudobranchial canal (No. 133, p. 493).

### a. *Basal Line.*

Between the distal ends of the branchial arches there is a median line of basal elements, three in number in the adult ($BB^{1-3}$, Figs. 49–51, Pl. XXXIII).

The first of these elements is as long as, or a little longer than, the other two together, and is, in its middle portion, ossified, the ossified portion lying between the ventral ends of the second and third arches. The other two elements are entirely cartilaginous, and it is perhaps worthy of note that the single piece of bone in the entire line gives no direct attachment either to the arches themselves or to the muscles of the arches. In Lepidosteus the corresponding portion of the basal line is also osseus (No. 129, p. 272).

The anterior cartilaginous portion of the first element is, roughly speaking, square in section. It is longer than either of the other two portions, and has a deep transverse fissure across its ventral surface near its front end. The part in front of this fissure is considered by van Wijhe as the basihyal partly fused with the first basibranchial. On its anterior face, extending close to the median line, it has two deep depressions in which it receives the articular ends of the hypohyals. Immediately behind the fissure, beginning in it and extending upward and backward transversely across the element, there is on either side a deep articular cup for the articular end of the first hypobranchial ; and immediately in front of the

middle bony portion of the element there is a similar articular cup for the articular end of the second hypobranchial. The bony portion of the element is much constricted in its middle portion, both dorso-ventrally and laterally, the dorsal surface of the piece being, however, left nearly its full and even width, so that the piece in section is V-shaped, or even T-shaped. At the hind end of its ventral edge there is, on either side, a roll-like projection directed backward, outward, and downward. The posterior cartilaginous portion of the element is shorter than either of the other portions, is V-shaped or T-shaped in section, the broad surface above, and on either side, at the extreme front end of its ventral edge, it projects outward and forward, and caps the ends of the roll-like projections on the hind end of the bony portion of the element. From each of these processes arises a short, tough, conical ligament which is inserted on the end of a cylindrical process of the third hypo-branchial, the body of that hypobranchial articulating with the basibranchial in a very slight depression at the anterior end of its dorsal edge.

The second basibranchial has somewhat the shape of a short, slightly curved T-rail, the flat surface of the rail presented dorsally on the outside of the curve, and the ventral edge thickened slightly at its front end, where it gives attachment to a short, tough ligament similar to the one found on the pre-ceding element. This ligament connects with a process of the fourth hypobranchial, the body of that hypobranchial articulat-ing with the basibranchial near its dorsal edge, in the same way and manner that the third hypobranchial articulates with the preceding element.

The third basibranchial lies in a continuation downward and backward of the curved line of the second basibranchial. It is strongly T-shaped in section, and ends in a long, prow-like point, directed backward and lying about on a level with the ventral edge of the first basibranchial. This gives to the element a keel-shaped appearance, the edge of the keel below, with a strong angle at about the middle of its length.

The first and second basibranchials are strongly connected by ligament, the ligament continuing backward along the ven-

tral edges of the second and third basibranchials to the ventral angle of the latter piece. The dorsal surface of the entire line has a fairly even width.

In a 20 mm. specimen, examined in sagittal sections, the basal line consisted of three elements, as in the adult; in two 14 mm. specimens, examined in sagittal and horizontal sections, the arrangement was somewhat different. In both these latter specimens the second element was distinctly continuous with the posterior end of the first element, the two pieces being separated ventrally by a deep fissure, but connected dorsally by a thin bridge of cartilage, exactly as the portion called by van Wijhe the basihyal was connected with the anterior end of the element. This latter connection was as distinctly marked as in the adult. The third element seemed to be in process of separation from the second, the outlines of the abutting ends of the two pieces being indicated and evident, but the space between them filled with semi-cartilaginous tissue. These early stages in Amia, and the continuous basal line in embryos of Lepidosteus and Acipenser (Parker), therefore indicate that the basal line is, in ganoids as it is in teleosts (No. 118), originally a continuous mass, which later separates into several members, rather than that it is formed by the fusion of several originally independent members, Amia representing an intermediate condition in the process, as van Wijhe's statement that the basihyal is partly fused with the first basibranchial would seem to indicate (No. 129, p. 284).

No indication of a separate basihyal was found in Amia, either in the adult or in larvae.


### b. *Branchial Arches.*

The first and second branchial arches (Figs. 49–55, Pls. XXXIII–XXXV) consist each of a hypobranchial, a cerato-branchial, an epibranchial, and one or two pharyngobranchials; the third and fourth each of a hypobranchial, a ceratobranchial, and an epibranchial, and a single large piece which serves as a pharyngobranchial for the two arches, but is considered by van Wijhe as the pharyngobranchial of the third arch only. The

fifth arch consists of a single large piece generally considered as a ceratobranchial, and a very small piece of cartilage, usually but perhaps not always found attached to its outer dorsal end. The piece that I consider as the epibranchial of the fourth arch is considered by van Wijhe as the infrapharyngobranchial of that arch. Bridge states that there are both an epibranchial and a pharyngobranchial in the fourth arch, but he does not describe them.

The hypobranchials ($HB.I–IV$) of the first four arches diminish regularly in length from the first to the fourth, the first being nearly as long as its ceratobranchial, the fourth only about one half as long. The first two hypobranchials closely resemble each other in shape, as do also the second two. The first two bend sharply inward at their lower ends, and are compressed laterally, much as if the end of the bone had been flattened dorso-ventrally, and then, after being twisted through somewhat more than a right angle, had been sharply bent backward through about forty-five degrees. This flattened end of the element is triangular or wedge-like in shape, and is strongly capped with cartilage. The shank or upper portion of the element is semi-cylindrical in shape, with its ventral surface strongly grooved, the lower end or opening of the groove lying in front of the bent and flattened end of the piece. Between the shank and the bent end there is, on the anterior and ventral edge of each element, a slight process, and on the posterior side a slight ridge for the attachment of the strong interarcual ligaments that connect each arch with the arch on either side of it.

The most anterior of these ligamenta interarcualia ventralia ($liv.h$) arises from the dorsal surface of the hypohyal, and forms with the others ($liv.I–IV$) a nearly straight, though disconnected line, running backward and inward from the hypohyal to the distal end of the fifth ceratobranchial. The anterior ligament is described by van Wijhe in Amia, and by Vetter in Esox, but in both cases it is described as passing from the first hypobranchial to the ceratohyal, instead of to the hypohyal as I have found it. It is considered by Vetter as a part of the obliquus ventralis of the first arch. A similar ligament is found between

the first and second arches in Esox, and is considered by Vetter as part of the obliquus of the second arch.

The hypobranchials of the third and fourth arches are much flatter than those of the first and second. Their lower ends are bent backward and upward slightly, but there is no twist or lateral compression to the bone, and at the bend or angle on the front edge of each there is a small process directed downward and forward, approximately in the line of the anterior edge of the piece, to form a second articulation or connection with the basibranchial. Both distal ends of the element are capped with cartilage, as is also the proximal end, the proximal cap of the fourth hypobranchial and that on the larger of its distal ends being often continuous along the hind edge of the element, the bony portion of the element having in such cases a semicircular form, the arc of the circle directed backward. The two hypobranchials are appreciably grooved, on their ventral surfaces, only at their outer ends and mainly in the cartilaginous cap of the piece. The lower end of the groove lies or opens between the two articular ends of the element. At the base of the anterior articular end of each element, on its anterior edge, there is a slight process for the attachment of the interarcual ligament, and there is a similar process, or more properly eminence, on the posterior edge of the third hypobranchial, where the proximal cartilaginous cap joins the bony part of the element, and on the fourth hypobranchial in the proximal cartilaginous cap itself, the process on this last piece being sometimes so pronounced that it forms an angular projection overlapping ventrally the end of the fifth ceratobranchial. Similar processes are indicated in the proximal cartilaginous caps of the third and second hypobranchials. The bony portion of the fourth hypobranchial is formed concentrically around the point of attachment of the interarcual ligament between that arch and the third arch, and in the bone of the third hypobranchial is also indicated a similar concentrical arrangement around the point of attachment of the ligament between that arch and the second. The ligament between the fourth and fifth arches is inserted along the entire anterior edge of the distal cartilaginous cap of the fifth ceratobranchial, and extends

also slightly onto the edge of the bone immediately beyond the cap. This interarcual attachment, on this arch, is stronger even than that of the ceratobranchial to the basal line.

The ceratobranchials (*CB.I–IV*) of the first four arches are semi-cylindrical pieces, slightly curved upward and deeply grooved on their ventral surfaces. They are thicker at their inner, or distal, than at their outer or proximal ends, this being especially marked in the third and fourth arches, and they are capped at both ends with cartilage. The distal cartilaginous caps have each a prominent swelling on the anterior half of their ventral surface, and beyond, that is distal to, the swelling, the ventral edge of the cartilage is bevelled, so that the articulation with the hypobranchial lies at a higher level than the ventral surface of the piece, thus leaving a hollow for the reception of the outer ends of the muscle of the arch. The proximal cartilaginous caps bend slightly upward, and that of the fourth arch has on its posterior edge a large, flat, thin projection, giving to the cap a triangular shape. The ceratobranchial of each arch is bound to its hypobranchial by strong ligamentous or connective tissue, which passes in between the two pieces and forms a tough, relatively thick, bi-concave pad between them.

The ceratobranchial of the fifth arch (*CB.V*) is shorter and more slender than those of the other arches, is only faintly, or not at all grooved on its ventral surface, and is triangular in section. The cartilaginous cap at its distal end is relatively large and flat, that at its proximal end much like those on the other arches. Attached to the extreme posterior and outer corner of the proximal cap is a small piece of cartilage (*EB.V*), already referred to, which represents either the epibranchial or one of the pharyngobranchials of the arch. Because of its relation to the ceratobranchial I have considered it as an epibranchial, although in its relation to the muscle of the arch it strikingly resembles the suprapharyngobranchial of the second arch.

The epibranchial of the first arch (*EB.I*) is triangular in shape, the base of the triangle directed inward and forward, that is, proximally, with its posterior corner lying at a much

higher level than the anterior one, and its ventral surface deeply grooved through its entire length. Both ends of the piece are capped with cartilage, the cap on its outer, distal end curving downward and diminishing gradually in thickness toward the end, where it meets and is bound to the upturned cartilaginous end of the ceratobranchial of its arch, a space being thus left in the angle between the two pieces. The cap on the inner, proximal end of the piece extends backward a little along the posterior edge of the piece, and has on the distal end of this part, just before it unites with the bone, a slight eminence, which in one specimen had on its summit a small tit-like process. The cartilage along the middle portion of the cap seems sometimes to be wanting, thus leaving two separate caps on the proximal end of the piece.

The posterior, upper corner of the proximal end of the first epibranchial articulates with the infrapharyngobranchial (*IPB.II*) of the next following, or second arch, at about the middle of its anterior, upper edge, the articulation being with a prolongation of the cartilaginous proximal cap of that element. The ligamentous articular attachment of the two elements, however, extends outward, that is, distally, for a short distance along the anterior edge of the bony portion of the infrapharyngobranchial, and is continuous both at its origin and insertion with the ligamentum interarculae dorsale of the first arch. This ligament (*lid.I*) arises from the posterior edge of the proximal cartilaginous cap of the first epibranchial, and is inserted along the anterior edge of the second infrapharyngobranchial, from the middle of its length to the outer end of the piece, the insertion even passing slightly onto the adjacent proximal end of the second epibranchial. Between the two ligaments, or more properly through the continuous united ligaments, there is a sharply defined circular aperture for the passage of the pharyngeal branch of the first vagus nerve.

The anterior and lower corner of the proximal end of the first epibranchial articulates with the infrapharyngobranchial of its arch. This latter element (*IPB.I*) is a long slender bone capped at each end with cartilage and slightly grooved on its posterior or postero-dorsal surface, the groove turning forward

under the proximal end of the piece onto and across its ven-
tral surface.   Nothing at all corresponding to the supra-
pharyngobranchial of van Wijhe was found, unless it be the
little tit-like process found on the posterior edge of the proxi-
mal cartilaginous cap of the first epibranchial, and already
described.   In Scomber, however, such a piece is always
found lying between, and forming the connection between, the
first epibranchial and the second pharyngobranchial.

   The epibranchial of the second arch (*EB.II*) is shorter than
that of the first.   Like the latter it is somewhat triangular in
shape, the base of the triangle directed proximally, and so
placed that its posterior edge is at a much higher level than
the anterior one.   Both ends of the element are capped with
cartilage, and both resemble the corresponding ends of the first
epibranchial ; the proximal cap on this element is, however, much
more developed than that of the first epibranchial, extends
further outward along the posterior edge of the element, and
has a well developed posterior process at its outer distal end.
On this process there was, in the specimen used for illustration,
a small piece of cartilage which lay against the outer edge of
the levator of the arch, and is undoubtedly the suprapharyngo-
branchial of the arch (*SPB.II*).   Whether this element is
always present in all specimens or not I cannot say, as it was
found only when especial attention was directed to it in the
last of several dissections.   The posterior angle of the proxi-
mal end of the epibranchial articulates with the outer end of
the anterior portion of the cartilaginous rim of the third infra-
pharyngobranchial, the articular ligament connecting the two
pieces extending slightly onto the adjoining bony portion of
that piece.   The posterior process of the element is connected
by ligament, the ligamentum interarcuale dorsale II (*lid.II*),
with the outer edge of the bony portion of the third infra-
pharyngobranchial, with the anterior end of the posterior, outer
cartilaginous edge of that piece beyond the bone, and with the
cartilaginous cap on the proximal end of the third epibranchial.
Between this ligament and the articular ligament there is a
large oval opening through which descends the pharyngeal
branch of the second vagus nerve.

With the anterior and larger portion of the proximal end of
the second epibranchial the infrapharyngobranchial (*IPB.II.*)
of the arch articulates.   It is a large, somewhat rectangular
piece, as long as, or slightly longer than, the epibranchial of the
arch, and lies inclined in the opposite direction to that piece ;
that is, with its anterior edge at a much higher level than
the posterior one.   It is capped on both ends with cartilage,
the cartilage on the proximal end extending outward along the
anterior edge of the piece for about one half its length.   At
this outer or distal end it articulates with the first epibranchial,
its edge overlapping dorsally the edge of that piece.

The third epibranchial (*EB.III*) is a short, nearly straight
piece deeply grooved on its dorsal surface and with a rounded
proximal end by which it articulates with the outer, posterior
cartilaginous edge of the third infrapharyngobranchial, and
with the anterior corner of the fourth epibranchial.   Distal to
this last articulation the proximal cartilaginous cap of the
piece extends outward along the posterior edge of the element,
for about one half its length, and ends in a strong, upturned
process which much resembles the corresponding process on
the second epibranchial plus the suprapharyngobranchial of
that arch.   This cartilaginous posterior process, in some speci-
mens, becomes independent of, and not connected by cartilage
with the proximal cartilaginous cap.   From its posterior edge
at its base the ligamentum interarcuale dorsale III (*lid.III*)
arises and is inserted along the anterior edge of the bony por-
tion of the fourth epibranchial.   Between this ligament and
the articular ligament there is, as on the other arches, an
opening through which descends the pharyngeal branch of the
third vagus.

The upper pharyngeal bone (*IPB.III*) is a large piece lying
perpendicular to the direction of the arches, rounded in front
and pointed behind, and may be considered, with van Wijhe, as
the third infrapharyngobranchial alone, or, and perhaps more
properly, as the fused infrapharyngobranchials of the third and
fourth arches, and possibly of the fifth arch also.   It is mainly
cartilaginous, the bony portion of the piece being a circular
ossification lying at its anterior, outer edge and entirely sur-

rounded by cartilage excepting in that portion that lies between the points where the element articulates with the second and third epibranchials. This exposed bony edge thus corresponds to the distal half of the anterior edge of the second infrapharyngobranchial. At about the middle of the postero-lateral edge of the element there is a slight thickening on its dorsal surface marking the point where it articulates with the fourth epibranchial. In front of and behind this thickening, near the edge of the piece, there are one or two perforations for the passage of pharyngeal branches of the vagus.

The fourth epibranchial (*EB.IV*) is a flat piece, not grooved on its dorsal surface, and about as long as the second epibranchial. It is capped at both ends with cartilage, the proximal cap having three strong processes, an anterior, a median, and a posterior, the two latter passing beyond the outer edge of the third infrapharyngobranchial dorsal to that piece. By the anterior process it articulates with the third epibranchial, and by the posterior with the third infrapharyngobranchial, the attachment to the latter piece being very strong, the ligament passing between the two pieces and forming there a tough pad of tissue. The piece is considered by van Wijhe as the fourth infrapharyngobranchial because of the articulation in front with the third epibranchial; but as it articulates also, and much more strongly, with the posterior portion of the large and perhaps compound third infrapharyngobranchial, there is equally good reason for considering it as an epibranchial. Between the posterior articulation, with the third infrapharyngobranchial, behind, the two articulations at the proximal end of the third epibranchial in front, the third infrapharyngobranchial below, and the fourth epibranchial above, there is a large open passage, but no vessel or structure of any apparent importance was found passing through it.

The fifth epibranchial (*EB.V*) has been described with the ceratobranchial of its arch.

### c. *Hyoid Arch.*

The hyoid arch, as ordinarily considered, consists of three parts. These three parts are, in Amia, the hypohyal, the

cerato-epihyal, and the interhyal of Bridge. No pharyngeal element is ordinarily given, as such, but the hyomandibular and symplectic are generally considered to represent the upper end of the arch. I consider them the pharyngeal elements. The cerato-epihyal and interhyal of Bridge then become the cerato-hyal and epihyal, respectively, as van Wijhe has already suggested that they must be (No. 129, p. 308).

The hypohyal $(HH)$, which is more than half cartilage, is the distal member of the arch. To its anterior and ventral portion the large tendon of the sternohyoideus is attached, and around this point as a centre the piece is partly ossified, the ossification extending only about half way through the element so that its dorsal surface is left entirely cartilaginous. From the distal, dorsal, and inner corner of the piece a large cartilaginous process is directed medianward and backward, and articulates with the front end of the first basibranchial. There is no perforation for the passage of an arteria hyoidea as in Scomber, Perca, and Gadus.

The ceratohyal $(CH)$ is the next following member of the arch, and is in larvae a single continuous piece of cartilage. In the adult it contains two ossifications. The lower and much larger one is the bone considered by Bridge and van Wijhe as the ceratal member of the arch. It is a long, strong, curved piece having an upper, or proximal, flattened or blade-like portion, the thin, outer edge of which is deeply grooved for nearly its entire length, and a lower, or distal, rounded portion or shank which is not grooved. The thin, outer edge of the proximal portion is directed ventrally and backward, and corresponds to the ventral surfaces of the ceratobranchials. The nerve of the arch, the ramus hyoideus facialis, lies, as on the other arches, immediately external to this groove, but no associated artery was found.

The ceratohyal is capped with cartilage below, and articulates with the hypohyal, a thick pad of connective tissue lying between and separating the two elements. Above, or proximally, the lower bone ends abruptly in a nearly straight edge at right angles to the longitudinal edges of the element. Beyond this upper edge of the lower ossification there is a

large triangular terminal piece which to all appearances is simply the greatly enlarged cartilaginous proximal cap of the element. In it is found the second, and perhaps secondary, ossification of the piece, the relatively small semicircular bone considered by Bridge as an epihyal. The free straight edge of this ossification forms the larger part of the dorsal side of the triangular cap of the element, but it does not extend quite to the upper tip or quite to the lower edge of the cap. Its semicircular side is thus left entirely surrounded by cartilage, and the bone touches and is connected with the large lower ossification only by means of a small thin splint-like process, which projects downward from its outer surface at its lower corner and dove-tails into a corresponding depression on the outer surface of the dorsal edge of the lower bone. Near the upper end of its straight, free edge, and directed at right angles to that edge, there is a strong condylar process always strongly capped with cartilage, the cap being sometimes connected with the main cap by a narrow line of cartilage along the upper, dorsal edge of the element. On the flat outer surface of the ossification there is a large depression in which the upper posterior end of the ligamentum mandibulo-hyoideum (*lmh*) is inserted, and it is around this point as a center that the ossification is formed. A bone having the shape of the one shown in van Wijhe's Fig. 13 I did not find in any of the specimens examined, but I do not doubt that, in specially developed specimens, it may exist.

The epihyal (*EH*), or next following member of the arch, is the interhyal of Bridge and van Wijhe. It is a small rhomboidal piece of cartilage articulating with the condylar process of the ceratohyal, and directed upward, forward, and slightly inward in the line of the long axis of the hyomandibular, at right angles to the lower edge of that bone, and at right angles to the axis of the upper end of the ceratohyal. Its flat surface is perpendicular to that of the hyomandibular and perpendicular also to that of the blade of the ceratohyal, the edge of the piece being, therefore, presented laterally. Its inner edge is longer than the outer one, and lies in the plane of the inner surfaces of the hyomandibular and the blade of the ceratohyal.

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Its outer edge projects externally beyond those bones.   On
its lower or distal edge, at the inner, lower corner of the piece,
there is a deep facet which receives the condylar end of the
ceratohyal, and at its outer, distal corner there is a slight
enlargement which gives attachment to the articular ligament,
which binds the piece to the cartilaginous cap or to the sec-
ondary ossification of the ceratohyal.   A slight depression on
the upper surface of the piece between these two corners indi-
cates possibly the longitudinal groove found on the dorsal
surfaces of the epibranchials.   At its broader, anterior end the
epihyal articulates with the cartilaginous interspace between
the hyomandibular and symplectic.   A strong double ligament
(*lid.h*), arising from the inner surface of the hyomandibular,
extends along the inner edge of the epihyal, and is inserted,
one half on the posterior end or edge of the cartilaginous cap
of the ceratohyal, and the other on the inner surface of that
cap near its posterior or postero-ventral edge, and beyond the
cap on the inner surface of the upper end of the lower ossifica-
tion, the ligament turning abruptly through a right angle at
about the middle of its course.   These ligaments are not the
articular ligaments of the arch, they resemble much more, one
or both of them, the interarcual ligaments of the branchial
arches, and I consider them as such.

The pharyngohyal, or proximal member of the hyoid arch,
is undoubtedly the single piece formed by the hyomandibular,
the symplectic and the interspace of cartilage connecting those
two bones.   The hyomandibular (*HMD*, Figs. 2 and 5, Pl. XX)
is much the larger of the two bones, and is, roughly speaking,
rectangular in shape with a large process, the opercular process,
capped with cartilage and directed upward and backward from
the middle of its posterior edge.   At the middle of the outer
surface of the bone there is a large opening, the external open-
ing of the canal by which the facial nerve traverses the bone,
and immediately below that opening, marking the further
course of the nerve and its three main branches, there is a
slight depression on the outer surface of the bone extending to
its lower edge and widening or separating into two parts as it
proceeds.   Beyond the lower edge of the bone the two depres-

sions are continued across the cartilaginous interspace, one passing dorsal to and behind the articulation with the epihyal, and the other ventral to and in front of it. The upper end of the bone is capped with cartilage, and articulates with the side of the skull in a deep facet lying immediately under its over-hanging upper, outer edge and extending forward, downward, and inward from its posterior, outer corner to the hind edge of the postorbital process. The facet is deepest at its anterior end, and there lies immediately behind the spiracular canal. The anterior edge of the hyomandibular, in front of the facial canal, is thin, and is, as van Wijhe concluded (No. 129, p. 269), of secondary origin, for even in fishes 40 mm. in length it is found as membrane only. The facial canal, however, lies entirely in that portion of the bone that is of cartilaginous origin, and in the smallest specimens examined, those 12 mm. in length, it apparently lay relatively further from the front edge of the element than in the older ones.

The symplectic (*SY*, Fig. 4, Pl. XX) is an irregularly shaped bone directed downward and forward and ending below in a large facet lined with cartilage, by which it articulates with a large process at the lower end of the hind edge of the coronoid process of the mandible, the process containing and being strengthened by ossicle *d* of Bridge. It lies entirely below the hyomandibular, with its upper, posterior edge on a level with the corresponding edge of the quadrate, and its anterior half covered externally by the posterior and upper portion of that bone. A depressed portion on the inner surface of the quadrate receives the symplectic, and the two bones are firmly bound together by tissue. The rest of the symplec-tic, excepting only the lower end of its articular head, is cov-ered externally by the lower end of the preoperculum, which abuts with its front edge against the thickened hind edge of the quadrate. Between the three bones, the preoperculum and the quadrate externally, and the symplectic internally, a space or canal is left through which the mandibular branches of the facialis pass. The three bones are firmly bound together by tissue, as are also the upper end of the preoperculum and the hyomandibular, the former bone crossing, and being closely

applied externally to the lower posterior portion of the latter and to the base of its opercular process. The thin hind edge of the deeper portion of this part of the preoperculum overlies, and corresponds with, the thin hind edge of the hyomandibular between the opercular process and the lower corner of that bone. A groove on the inner surface of the preoperculum, corresponding with the posterior or hyoidean portion of the facial depression on the outer surface of the hyomandibular, marks where the hyoideus facialis passes downward and backward between the two bones.

The symplectic lies, as van Wijhe has stated, nearly at right angles to the hyomandibular, and the two bones are separated and connected by a triangular interspace of cartilage. The anterior corner of this piece of cartilage is overlapped and covered externally by the posterior, cartilaginous corner of the palato-quadrate arch. The rest of the piece, excepting only a very small portion, is covered externally by the preoperculum, and the hind edge of the piece is turned outward, upward and forward against the inclined hinder and inner surface of the pre-operculum, and firmly attached to that surface by tissue. On this turned-up portion lies the oblong articular facet for the epihyal. The long axis of the facet lies, as does the epihyal, in a plane perpendicular to the lower edge and to the outer sur-face of the hyomandibular, but the axis of the facet is inclined at a considerable angle to the plane of the hyomandibular, instead of being at right angles to it, as might have been ex-pected. The facet therefore lies on the inner surface of the cartilaginous interspace, instead of on its hind edge, and the relatively long front edge of the epihyal is inclined to the axis of that piece, instead of being at right angles to it.

### d. Operculum and Branchiostegal Rays.

The three opercular bones, the operculum ($OP$), suboperculum ($SOP$), and interoperculum ($IOP$), fit with their anterior edges under and against the outer, posterior edge of the preoperculum ($POP$), separated from it on the outer surface of the undissected head by a fold or crease in the dermis, and separated also from the first branchiostegal ray by a similar fold or crease. The

three bones are bound tightly together by connective tissue, extend nearly the entire length of the preoperculum, and form the upper portion or piece of the gill cover. The operculum articulates, by a large facet on its inner surface, with the end of the opercular process or the hyomandibular. The facet is not lined with cartilage, and even in the youngest larvae examined there is not the slightest indication of cartilage at or in any part or portion of the bone. The bone in Amia is strictly of dermal origin.

The suboperculum is attached by ligament to, but does not articulate by facet with, the lower, posterior, cartilaginous corner of the hyomandibular; and the interoperculum is attached by strong connective tissue to the upper end of the upper ossification of the ceratohyal. The interoperculum is also attached by ligament (*lmi*) and by strong, tough, dermal tissue to ossicle *a*, and to the hind corner of the mandible immediately external to the insertion of the ligamentum mandibulo-hyoideum. The operculum overlaps considerably at its lower edge the upper edge of the suboperculum, and the latter overlaps slightly the interoperculum.

The first branchiostegal ray (*BRG*) extends from the lower end of the interoperculum almost to the upper, posterior end of the suboperculum. It is overlapped by both these bones and is strongly attached by connective tissue to their under surface. It is also attached by strong connective tissue to the upper ossification of the ceratohyal, near its lower edge, and by strong, tough, dermal tissue to the hind end of the mandible. The hyoideo-mandibular fold or crease extends upward and backward between it and the second ray, so that on the undissected head the first ray has more the appearance of belonging to the opercular bones than to the branchiostegal rays. The next following rays (indicated in Fig. 43, Pl. XXXI) are loosely attached to the outer surface of the lower ossification of the ceratohyal, near its hind edge, the most anterior or distal rays lying beyond the edge of the ceratohyal upon the outer surface of the hyohyoideus. There are usually ten or eleven rays in all, and in none of the larval stage examined was there the slightest indication of cartilage, either in them or connected with them.

### e. *Mandibular Arch.*

The mandibular arch as generally considered consists of two parts, the palatine arch, or palato-pterygo-quadrate apparatus, and the mandible; but the mandible and pterygo-quadrate represent, quite possibly, the entire mandibular arch, the palatine part of the apparatus representing or belonging to a pre-mandibular arch. To the description of the parts as given by Bridge and by van Wijhe I have but little to add.

At the hind end of Meckel's cartilage (*M*, Figs. 2, 6, and 7, Pl. XX) four ossifications, ossicles *a*, *b*, *c*, and *d* of Bridge, were sometimes found, and sometimes but three, ossicles *b* and *c* being fused, a faint line almost always indicating the line of fusion. The outer, lower corner of ossicle *a* had usually a slightly different color from the rest of the bone, and there was sometimes a faint line between the two portions; but, like van Wijhe, I never found the separate dermal scale described by Bridge. The canal (*mefc*) for the ramus mandibularis externus facialis, on the inner surface of the ossicle, was always continued forward some little distance on the inner surface of the articular, and opened underneath the horizontal part of Meckel's cartilage, beyond ossicle *b*. Ossicle *d* was always capped with cartilage, and between the mento-meckelian ossicles (*MM*) of the two sides of the head there was always a median, tough, semi-cartilaginous piece, intimately connected with the ossicles, as if it were the fused, cartilaginous tips of those bones. It may, however, be in part a basimandibular, such as White describes in Hexanchus and Laemargus (No. 127, p. 60). The hind edge of the cartilage of the coronoid process was always seen as a line behind the hind edge of the supra-angular, and the process itself was always found well developed as a process and not as a separate piece, even in specimens 12 mm. in length. In the palatine arch the anterior process of the metapterygoid was always capped with cartilage, as van Wijhe found it, and there was always on the hind edge of the quadrate, between it and the symplectic, a small piece of cartilage that escaped his notice.

### f. *Review and Comparison of the Visceral Arches.*

A complete visceral arch in fishes consists normally, according to generally accepted views, of four pieces or elements : a pharyngeal element above, then an epal, a ceratal, and a hypal element, in the order named. The four elements or pieces in the adult articulate one with the other, and the hypal element articulates also at its distal end with a median, ventral line of one or more basal elements.

The arch is always preformed in cartilage and it may persist as such in the adult, or one or more of its elements may become more or less ossified, the ends of the elements always remaining cartilaginous even in fishes where the ossification is most complete. The bones so preformed in cartilage may receive membraneous additions, as the hyomandibular in Amia, and the ossification of the cartilage may take place from two or more different and apparently independent points or centers, as in the ceratohyal of Amia and the hypohyal of Perca and of Scomber, the element in such cases presenting, in the adult, two or more osseous portions, usually, but perhaps not always, separating by an interspace of cartilage.

The anterior branchial arches always present the most complete and normal development. In the hyoid and mandibular, or palato-mandibular, arches the development is irregular or exaggerated, and in the posterior branchial arch it is always abbreviated, one or more of the normal elements of the arch not being found. In this last arch, and also in the other branchial arches that may be incompletely or irregularly developed, the ceratobranchial is always found, and the outer, lateral angle of the arch, in the adult, is always at the outer, upper end of that element, between it and the next preceding element, whatever that element may be. The pharyngobranchial is probably also always found in all the arches, but it is subject to much variation in different species, and also in the different arches of the same individual. It may be found as a single piece ; as a small and unimportant piece detached from the other pieces, as is probably the case in the fifth arch of Amia and of Scomber ; as a piece with two processes or portions, as

in the second and third arches of Polypterus (van Wijhe); or as two independent pieces, as in Acipenser and in the first arch of Polypterus (van Wijhe), the two pieces, or two portions, where they exist, being always the one anterior and inferior and the other posterior and superior. Van Wijhe says (No. 129, p. 225) that where but one pharyngobranchial is found it always lies below the "Kiemenvene," by which must be meant the efferent artery of the arch, and that where two are found the anterior of the two always lies below that artery and the posterior always above it. He has, accordingly, called them the infra- and supra-pharyngobranchials, respectively, and has suggested that the latter should be considered as a fifth element of a complete and normal arch.[1] He also states, as a general rule, that the epibranchial of an arch always touches or articulates with the infrapharyngobranchial of the next following arch, a relation that would naturally arise if the arches are primarily Y-shaped at their dorsal ends, and continuous one with the other, as Stöhr (No. 117, p. 9) gives them in early stages of Triton.

At the point where the epibranchial touches the next following infrapharyngobranchial, in Amia, the two elements are always connected by an articular ligament. They are also always connected, in Amia, by a second series of ligaments, the interarcual ligaments, which connect the epibranchial with the next following infrapharyngobranchial or epibranchial, one or both. This important series of ligaments seems to have escaped van Wijhe's notice.

The first branchial arch may articulate with, or be closely attached by ligament to the cranium by the anterior of its two pharyngeal elements alone, as in Amia, by the posterior element alone, as is apparently the case in Polypterus (van Wijhe), or by both elements, as in Acipenser (van Wijhe). Where the articulation or attachment is by the anterior element it is, in

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[1] Attention should here be called to a mistake in the lettering of the pharyngo-branchials in van Wijhe's Fig. 2 (No. 129) which shows the branchial arches of Acipenser. If the pharyngobranchials on the first two arches in this figure be compared with those in his Fig. No. 3, and with those in Parker's Fig. No. 5 (No. 86, Pl. XVIII), it will be evident that the anterior processes in van Wijhe's figure should be the infra- and not the supra-pharyngobranchials, as they are marked.

the ganoids described by van Wijhe, with or to the parasphe-
noid and not with the cranium proper ; where it is by the
posterior element, it is directly with or to the cartilage of the
cranium.

The second arch also may be attached to the cranium by
ligament or by tissue, but it is always less firmly and less closely
attached than the first arch.   In ganoids this attachment of the
second arch is always to the parasphenoid and always by means
of the anterior pharyngeal element of the arch, the posterior
pharyngeal element, where it exists (and where it does not exist,
the corresponding process of the epibranchial), being as a rule
attached to the anterior pharyngeal element of the next
following arch.

The posterior arches do not reach the cranium.   They are
simply suspended in the tissues beneath it or beneath the front
end of the spinal column.

In Amia the suprapharyngobranchial, on the arches where
it is found, lies behind the efferent artery of its arch rather
than above that artery, as van Wijhe states, although it would
certainly lie above the artery if prolonged.   It lies also behind,
and hence, in the same limiting sense, above the posttrematic
branch of the nerve of its arch, and behind, or more properly
external to, the external levator muscle of its arch, which mus-
cle is innervated by a branch of the posttrematic nerve given
off as that nerve passes outward in front of it.   The supra-
pharyngobranchial and levator muscle of each arch both lie in
front of the pretrematic branch of the nerve of the next follow-
ing arch.

If now, in Amia, the relations to the muscles, nerves, and
artery of the hyoid arch of that part of the hyomandibular
that lies posterior to the facial canal be considered, it is
seen : 1st, that it lies behind an artery which accompanies the
truncus hyoideo-mandibularis facialis, which truncus contains
the posttrematic elements of the nervus facialis ; 2d, that it
lies external to the adductor hyomandibularis ; and 3d, that that
muscle lies behind the posttrematic branch of the facial nerve,
between that branch and the pretrematic nerve of the next
following arch, and that it is innervated by a branch of the post-

trematic nerve sent to it as the nerve passes outward across it.
It thus fulfils all the conditions of a suprapharyngeal element,
and seems certainly to be that element of its arch. If it be
that element, that part of the hyomandibular that lies in front
of the facial canal must either be the infrapharyngeal element
of the arch completely fused with the suprapharyngeal element,
or an outgrowth of this latter element. Under the former
supposition the symplectic would be a special outgrowth of the
infrapharyngeal element ; under the latter it would be that
element itself. In either case the element would lie below
the nerve and artery of the arch, as it should, and be connected
by ligament, or even by fusion, with a part of the next preced-
ing arch. The adductor hyomandibularis then becomes the
levator of the hyoid arch, naturally transformed, by the great
development of the hyomandibular, into an adductor, and the
double ligament on the inner surface of the hyomandibular, con-
necting it with the epihyal and ceratohyal, can be accounted for
as two remnants of the articular and interarcual ligaments that
originally bound the epal element of the hyoid arch to the infra-
pharyngeal element of the next following, or first branchial arch.

The hyomandibular does not always, in the adult of fishes,
lie behind the posttrematic branch of the facialis, as it practi-
cally does in the adult Amia, and as it does in Heptanchus
(No. 124, Fig. 2) and in Silurus (No. 98, p. 19). In Acipenser
it lies entirely in front of the nerve (van Wijhe) ; in Spatularia
either entirely in front of it, according to van Wijhe, or behind
it, according to Collinge ; in Ceratodus behind it (van Wijhe) ;
in Polypterus between its two branches (van Wijhe and Pollard) ;
while in Lepidosteus and many teleosts it is pierced by the
nerve as in Amia. If, then, the hyomandibular be the supra-
pharyngeal element of its arch, or that element and the infra-
pharyngeal element combined, it must, in moving upward to
acquire its articulation at a high level on the lateral aspect of
the skull, have passed in some instances in front of the facialis,
in others behind it, and in others across it or between its two
branches.

In the mandibular arch, in Amia, the large median process of
the metapterygoid fulfils, in its arch, even better than the hyo-

mandibular does in its arch, the conditions required of a supra-pharyngeal element. The anterior process of the metapterygoid also fulfils those required of an infrapharyngeal element, for the truncus maxillaris trigemini, and the artery accompanying that truncus, lie between the two processes, in front of the levator of the arch, and that levator, the levator arcus palatini, is inserted largely on the inner surface of the median process, and lies entirely internal to that process. The anterior process is connected by connective tissue with the skull at a low level, and the median process by ligamentous tissue and by muscle at a high level. If the metapterygoid be the pharyngeal element of the arch, the quadrate becomes the epal element, and it fulfils in a measure the conditions required of such an element. Its distal end lies at the outer angle of the arch, as it should, and it is firmly bound to the symplectic, which is the anterior and inferior process of, or the infrapharyngeal element itself, of the next following or hyoid arch. In the Characinidae (No. 106, p. 65) the quadrate is not firmly and rigidly bound to the hyomandibular as in Amia. It is loosely bound, so that considerable movement is possible between the two pieces, as between the corresponding pieces in the branchial arches.

The metapterygoid being the proximal element of the mandibular arch, the palatine would probably be part of a premandibular arch, and the coronoid process of the mandible possibly another. The four divisions of the levator maxillae superioris would then be the levator, interbranchial, or interarcual muscles of this arch. That there are two or more premandibular arches has been often asserted; that parts of one or more of these arches are represented in the palato-quadrate, or so-called palatine arch is indicated in many ways; by the innervation of the levator maxillae superioris by a special branch of the trigeminus; by the general course of the trigeminus and its branches, the ramus maxillaris inferior lying always internal to the coronoid process where it would not naturally lie if the process belonged to the outer, anterior edge of the mandibular arch; by the fact that the palatine is found as a wholly detached and separate piece in siluroids (Pollard) and possibly also in Scymnus lichia (No. 99, p. 232), and that it is jointed

to the rest of the so-called palatine arch in the Cyprinidae (Sagemehl) ; that the coronoid cartilage is sometimes found as a separate piece, as in Lepidosteus (No. 129, p. 268) ; and that the palatine and pterygoid elements may (No. 118, p. 11), or generally do (No. 97, p. 403), fuse late. Hubrecht's figures (No. 59, Figs. 1 and 2, Pl. XVII) showing the arrangement of the labial cartilages in Chimaera and Callorhynchus also strikingly suggest the probability of parts of two arches being connected with the mandible.

Further evidence in support of this is found in the development of the arches in Salmo, Pristiurus, and Raja, as given by Parker. In Salmo (No. 84, Fig. 1, Pl. III) the hyomandibular is shown as the proximal element of the hyoid arch, the metapterygoid as the proximal or the two proximal elements of the mandibular arch, and the palato-pterygoid as an entirely separate piece lying in front of the upper end of the mandibular arch. In Pristiurus and Raja (No. 85, pp. 214, 216, and 219) the spiracular cartilage, or metapterygoid, and the hyomandibular arise as separate pieces at the distal ends of the mandibular and hyoid arches respectively. The spiracle lies between the two elements. In Raja it is an anterior process of the upper end of the hyoid arch that becomes the hyomandibular, while a posterior process in the mandibular arch becomes the metapterygoid. This seems to indicate that the hyomandibular may be in Raja an infrapharyngeal instead of a suprapharyngeal element. It may possibly be such in Amia, also, or it may be in one line of descent one of these elements, in another the other, and in still another the two combined, thus accounting for its varying and puzzling relations to the facial nerve. That either one or the other of the two pharyngeal elements may acquire an articulation with the skull is seen in the branchial arches.

Pollard's assertion (No. 98, p. 24) that "the supposed hyomandibular of Elasmobranchs does not correspond to the hyomandibular of Teleostei and sturgeons," is based in part on certain errors, for the levator maxillae superioris muscle of elasmobranchs is not, if I am right, the homologue of the levator arcus palatini of teleosts, and there is some question as to its

being the homologue of the protractor hyomandibularis of sturgeons. The spiracle is also probably not inconstant in its relations to the hyomandibular if one does not first accept the conclusion that "the hyomandibular of Teleostei must be sought in the articular portion of the quadrate of Heptanchus."

Regarding Pollard's cirrhostomial theory, in general, I find nothing in Amia, so far as my work has gone, that seems in any way to support it. On the contrary, everything seems to indicate the development of the mouth parts from visceral arches. As to the tentacles of fishes, at least of all gnathostome fishes, I am strongly inclined to think that they are simply special sensory structures developed in connection with terminal buds, or perhaps of such buds and also of other organs more nearly related to the pit organs of Amia. That procartilaginous or even cartilaginous or bony pieces, such as Pollard describes, should develop in connection with such accumulations of these organs is not surprising, if dermal sense organs are, as Klatsch states (No. 65), active centers of "skleroblastic" formation.

It is perhaps worthy of note that in Amia there is a little fold or flap of dermal tissue on the upper edge of the lower jaw, just in front of the coronoid process, that is, about where the coronoid tentacle of siluroids is found. A similar fold is found in Esox and Scomber also, and doubtless in other teleosts as well. If these folds be sensory structures, as they seem to be, they may be the homologues of, or at least represent a certain stage in the development or degeneration of, the tentacles.

### 2. Levatores Arcuum Branchialium.

The levatores arc. branch. (Figs. 52–59, Pls. XXXIV and XXXV) are a group of straight, diverging muscles, arising close together from the well-marked ridge that extends medianward and forward along the side of the skull from the hind end of the posterior process of the intercalar. The surface of origin lies immediately median to and below that of the posterior portion of the adductor hyomandibularis, and immediately lateral to and above that of the anterior end of the trunk muscles, which, in the adult, extend forward beyond the hind margin of the leva-

tores and beyond the vagus foramen to the posterior surface of the slight elevation representing, in Amia, the bulla acustica.

There are, in the adult, seven levatores on each side of the head : two interni, an anterior and a posterior one ; four externi, one to each of the first four arches ; and a fifth muscle, which is inserted on the clavicle instead of on the branchial arches, and hence can be considered as one of this group of muscles simply because of its origin with, or even as a part of, the fourth externus.

The anterior internus (*Labi ᵃ*) is, both at its origin and at its insertion, the anterior muscle of the group. It runs median-ward, downward, and forward across the posterior part of the bulla acustica, external to the glossopharyngeus, the vagus, and the efferent artery of the first arch, and is inserted partly on the bone and partly on the cartilage of the proximal end of the second infrapharyngobranchial. It is innervated by a branch of the glossopharyngeus.

The posterior internus (*Labi ᵖ*) arises external to the posterior portion of the anterior muscle. It runs downward, median-ward, and backward external to the vagus, and is inserted on the upper surface of the third infrapharyngobranchial, near the middle of the piece, the insertion being partly on the hind edge of the bony portion of the piece and partly on the cartilage adjoining it.

The first externus, or levator arc. branch. ext. ad arcum primum (*Labe.I*), the externus anterior of McMurrich, is a short muscle arising immediately external to the anterior internus, and running almost directly downward to its insertion on the posterior edge of the first epibranchial, at the proximal end of the bony portion of the piece. In one specimen the anterior fibres of the muscle had separated as a small separate bundle.

The second, third, and fourth externi (*Labe.II–IV*) arise close together, almost as a single muscle, the surface of origin lying immediately behind that of the first externus and imme-diately external to that of the posterior internus. They are found in 40 mm. specimens as partly separated portions of a single muscle, are considered by McMurrich as such in the adult, and are called collectively by him the externus posterior.

They run downward, backward, and outward, the second muscle lying external to the third, and the third external to the fourth, and are inserted, the second and third muscles on the cartilaginous processes on the posterior edges of the second and third epibranchials, and the fourth muscle at the distal end of the bony portion of the fourth epibranchial, the insertion extending sometimes onto the cartilaginous, distal end of the piece. In one specimen there was an additional muscle, not found in any other dissection. It arose with the fourth levator, apparently as a part of that muscle, but wholly distinct from it, and was inserted on the proximal end of the fourth epibranchial, instead of at its distal end.

The fifth externus (*Labe. V*) arises from the extreme end of the posterior process of the intercalar and runs downward and backward, posterior to and internal to the posterior edge of the fourth externus, to the posterior or outer end of the fourth arch. It then turns downward over the outer end of that arch and that of the fifth, and is inserted by a tendon, often long, to the anterior edge of the clavicle at about one third the length of that bone. It is found in 40 mm. fishes as a part of the fourth levator, and is possibly what McMurrich refers to as a "few fibres" of that muscle "continued toward the degenerate fifth arch." No muscle similar to it is described by Vetter in any of the forms examined by him. It is innervated by a separate branch, or by two delicate branches of, the vagus.

### 3. Interarcuales Dorsales.

The muscles of this group are not all entirely differentiated one from the other; there can, however, be distinguished three obliqui dorsales on each side of the head, two transversi dorsales, an anterior and a posterior one, and a median retractor arcuum branchialium.

#### a. *Obliqui Dorsales.*

The most anterior of the obliqui (*Od*[1], Figs. 52–59, Pls. XXXIV and XXXV) arises from the upper surface of the third infrapharyngobranchial internal to the insertion of the

levator arc. branch. int. post. and external to that of the retractor
arc. branch. It lies in part, at its origin, ventral to the anterior
end of the latter muscle, runs outward and backward across the
dorsal or internal surface of the levator arc. branch. int. post.,
and is inserted on the posterior edge of the median of the three
processes on the proximal end of the fourth epibranchial. The
second obliquus ($Od^2$) arises from the dorsal surface of this
same process, runs almost directly backward to the anterior
end of the pharynx, then turns downward, and is inserted along
the posterior edge of the proximal end of the fifth ceratobran-
chial. The third obliquus ($Od^3$) arises from the upper surface
of the third infrapharyngobranchial median to the posterior
process of the proximal end of the fourth epibranchial, runs
outward and backward across the ligament connecting that
process with the infrapharyngobranchial, then turns downward
and becomes continuous with the constrictor of the pharynx.
The anterior obliquus is innervated by a branch of the vagus
that supplies also the retractor arc. branch. The other two
obliqui are innervated by branches of the vagus that innervate
the constrictor of the pharynx.

### b. *Transversi Dorsales.*

The anterior transversus ($Tda$) arises from the upper surface
of the anterior portion of the third infrapharyngobranchial, the
surface of origin extending to the extreme lateral edge of the
piece, and the muscle lying, in this part, under and external to
the levator arc. branch. int. post. The fibres of the muscle
have a curved course, running at first forward and medianward
under the levator, then medianward, and then medianward and
backward, to the middle line of the head. The fibres in the
middle portion only of the muscle have a fairly straight course
from one side of the head to the other. In its anterior portion,
in the middle line, it is somewhat fibrous, and is here closely
attached to the base of the skull. In 40 mm. specimens the
fibres at this point interlace. The muscle is continuous in its
deeper portion with the retractor, and is innervated by the
nerve that innervates the anterior obliquus and also the retrac-
tor. The transversus posterior ($Tdp$) arises from the posterior

portion of the dorsal surface of the third infrapharyngobranchial, runs directly across the middle line of the head, ventral to the retractor, and is apparently simply an anterior portion of the constrictor of the pharynx, with which it is continuous.

### c. *Retractor Arcuum Branchialium Dorsalis.*

This muscle (*Rabd*, Figs. 52, 53, and 62, Pls. XXXIV and XXXVII) is a large and stout one arising from the sides of the bodies of the third and fourth vertebrae. It runs directly forward along the under surface of the spinal column and of the posterior portion of the skull, and is inserted, on each side of the head, on the median portion of the dorsal surface of the anterior end of the third infrapharyngobranchial, its lateral fibres sometimes turning outward and then backward around the front edge of the levator arc. branch. int. post., ventral to the anterior transversus, to be inserted with the latter muscle on the outer, anterior edge of the infrapharyngobranchial. The two halves of the muscle are, in its anterior part, more or less continuous, and the arrangement of the two differs in different specimens. Some of the fibres of the muscle arising from one side of the spinal column may cross the middle line of the head and be inserted on the infrapharyngobranchial of the opposite side. The muscle lies immediately ventral to the dorsal aorta, but dorsal to the trunk of the united efferent arteries of the third, fourth, and fifth arches on each side, which trunks unite in the middle line to form a common trunk, which runs upward through the muscle at about the middle of its length to join the aorta.

### 4. Adductores Arcuum Branchialium.

There are two of these muscles, one the adductor of the fourth arch, the other that of the fifth (Figs. 48 and 56, Pls. XXXIII and XXXV). The former (*Aab.IV*) arises from the under surface of the distal cartilaginous cap of the fourth epibranchial and from the under surface of the adjoining bony portion of the piece, and is inserted on the entire dorsal surface of the triangular cartilaginous cap of the fourth cerato-

branchial, and somewhat, also, on the bone beyond it. The latter (*Aab. V*) arises from the ventral surface of the thin projecting corner of the triangular cartilaginous cap of the fourth ceratobranchial, and is inserted on the dorsal surface of the cartilaginous cap of the fifth ceratobranchial, a portion of its fibres passing over the end of the cartilage and there contracting into a tendon which is, in part, inserted on the ventral surface of the cap, or of the bone adjoining it, and in part is continuous with the outer, posterior, tendinous end of the transversus ventralis posterior.

The adductores are each innervated by the nerve of its arch, the branch that innervates it passing in each case over the posterior edge of the arch.

### 5. Interarcuales Ventrales.

The muscles belonging to this group in Acipenser are called by Vetter (No. 125, p. 478) the interarcuales ventrales, although but one of them, the first one of the group, is in any way interarcual. In teleosts Vetter retains the same name for the group (No. 125, p. 517), but subdivides it into two portions, the obliqui ventrales and transversi ventrales. McMurrich (No. 76, p. 132) retains for the obliqui muscles in Amia (doubtless because Amia is a ganoid) the name given by Vetter to the corresponding muscles in Acipenser, and so separates the muscles in Amia into two groups, the interarcuales ventrales and transversi ventrales. The names given by Vetter to the muscles in teleosts seeming also the more appropriate for those in Amia, I have used them instead of the ones selected by McMurrich.

### a. *Obliqui Ventrales.*

The obliquus ventralis of the first arch (*Ov.I*, Fig. 47, Pl. XXXIII) arises in the groove on the ventral surface of the first hypobranchial, the surface of origin occupying the full extent of the groove from the bend near the anterior end of the bone to its hind end. Most of the fibres of the muscle converge proximally, that is, outward and backward, toward the middle line of its under surface, and are there inserted on

a longitudinal tendon which extends about one half the length of the muscle, and is itself inserted on the anterior cartilaginous cap of the first ceratobranchial and on the under surface of the bone of that element beyond the cap. A few of the proximal fibres of the muscle have a direct insertion on the cartilaginous cap itself on either side of the insertion of the tendon. In front of this muscle, in the space between the distal end of the hypobranchial and the posterior surface of the hypohyal, McMurrich found a muscle, but I have been unable to find one, or even a trace of one, in any specimen, old or young. A strong interarcual ligament was, however, always found running from the small process on the front edge of the first hypobranchial to the posterior and ventral surface of the hypohyal near its articulation with the ceratohyal, as already described; and internal to it, in the space where McMurrich seems to have found the muscle referred to, I always found a mass of fatty, connective tissue.

The obliquus ventralis of the second arch (*Ov.II*) is very similar to that of the first, but it extends, at its origin, farther forward and it is incompletely separated into a distal and a proximal portion. The proximal portion arises, as does the muscle of the first arch, from the grooved, ventral surface of the shank or straight part of the hypobranchial. The anterior portion arises from the ventral edge of the bent end of the bone, and its fibres form on the ventral surface of the muscle a rounded, nearly separate muscle-belly. In its deeper portion it is continuous with the proximal muscle, and the fibres of both are inserted on a median, longitudinal tendon lying on the ventral surface of the proximal muscle, the tendon and a few fibres of the proximal muscle being inserted, as in the first arch, on the cartilaginous cap and on the anterior end of the bony portion of the ceratobranchial of the arch.

The obliquus ventralis of the third arch (*Ov.III*) resembles that of the second, but the proximal portion of the muscle, owing to the smaller size of the hypobranchial of the arch, is relatively much less important, and the fibres of the distal portion contract more markedly into a tendinous end before their insertion on the median tendon of the muscle. The distal

portion arises from the anterior and ventral surfaces of the small anterior and ventral articular process of the hypobranchial, and between its fibres, on this same process, is inserted one of the two dorsal ends of the ligament from which one of the two arms of the branchiomandibularis arises. The other dorsal end of the ligament is inserted on the second hypobranchial and on the interarcual ligament connecting that hypobranchial with that of the third arch.

The obliquus ventralis of the fourth arch is found as three distinct and separate muscles. One of them ($Ov.IV^1$) corresponds to the outer or proximal portion of the muscles of the other arches. It arises from the anterior half of the ventral surface of the fourth hypobranchial and from the base of the small articular process of that piece, and is inserted on the anterior end of the ceratobranchial of the arch. The second muscle ($Ov.IV^2$) corresponds to the distal portion of the muscles of the other arches. It is a large, spindle-shaped muscle, the largest of all the obliqui, arises from the hind surface of the small articular process of the third hypobranchial, and, running almost directly backward ventral to the proximal portion of the muscle and ventral to the anterior transversus ventralis, is inserted by a long and slender tendon on the ceratobranchial of the fifth arch near its anterior end and along the anterior side of the pharyngo-clavicularis externus. It may arise in part from or be attached to the fourth hypobranchial where it passes across it. The third muscle ($Ov.IV^3$) lies immediately dorsal to the anterior end of the second muscle and is undoubtedly a derivative of that muscle, for in 19 mm. specimens the two are found as a single muscle with no trace whatever of separation. It extends from the posterior surface of the small articular process of the third arch to the anterior surface of the corresponding process of the fourth arch. As all three muscles are innervated by branches of the nerve of the fourth arch, the large ventral one cannot be properly considered as the obliquus of the fifth arch, as McMurrich from its insertion concluded (No. 76, p. 133).

### b. *Transversi Ventrales.*

The transversus ventralis anterior (*Tva*) arises in a line along the middle of the ventral surface of the fourth hypobranchial on one side of the head, internal to and dorsal to the obliquus of the arch but ventral to the artery of the arch, and extends across the middle line, ventral to the second and third basibranchials and dorsal to the sinus arteriosus, to the fourth hypobranchial of the other side. It is always composed of two or more more or less distinct muscles or muscle bundles; the muscle bundles, when they exist, — or when they do not the ventral fibres of the muscle, — always crossing each other at a considerable angle, the muscle fibres or muscle bundle of the right side always lying ventral to that of the left side. The dorsal muscle where the separation is complete, or the dorsal fibres of the muscle where it is incomplete, always run directly across the middle line of the head. The muscle is innervated in all its parts by the nerve of the fourth arch and the muscle in its posterior portion is often continuous with the obliquus of that arch.

The transversus ventralis posterior (*Tvp*) is formed by two muscles, each of which belongs distinctly to its own side of the head and does not, as in the case of the anterior transversus, cross the middle line of the head to be inserted on the opposite side. The muscle, on each side, arises from the ventral edge of the posterior portion of the third basibranchial and from a narrow median aponeurosis extending ventralward from it. It runs outward and backward posterior to the pharyngo-claviculares and is inserted on the ventral surface of the fifth ceratobranchial, the surface of insertion extending nearly the full length of the bone. At its outer, lateral end a portion of its fibres give rise to a tendon which is closely attached to the proximal cartilaginous cap of the fifth ceratobranchial, but continues across that cap and becomes continuous with a similar tendon on the lower end of the adductor of the fifth arch, as already described. In 40 mm. specimens the separation of the muscle into two parts, one on each side of the head, is equally or even more distinctly marked than in the adult, for in young

fish the median aponeurosis from which the muscles in part
arise is not so well developed as in the adult, and there are not
the numerous intercrossing tendinous fibres found in the adult
on the ventral surface of the aponeurosis and of the muscles
themselves adjoining it. In its posterior portion the transversus,
both in the young and in the adult, is continuous with the ante-
rior portion of the constrictor of the pharynx. It is innervated
by the nerve of the fifth arch, closely resembles the fifth inter-
arcual of Acipenser (No. 125, Fig. 6), and is unquestionably the
homologue of the proximal portion of the obliqui muscles of
the anterior arches.

### c. *Pharyngo-Clavicularis Externus and Internus.*

These two muscles (*Pce* and *Pci*) arise close together, usually
as a single muscle, from the anterior surface of the clavicle
immediately external to the dorsal portion of the sternohyoi-
deus. The two muscles separate at once and the externus,
which arises external to and below the internus, runs forward
and medianward across that muscle and is inserted on the pos-
terior or median edge of the ceratobranchial of the fifth arch
near the middle of the bone, lying, at its insertion, median or
posterior to the tendinous end of the second division of the
fourth obliquus, and in front of the transversus posterior.

The internus may be single or double, the two parts, when
it is double, lying closely together but wholly separate except
at their origins. The muscle runs forward and medianward
across the transversus posterior and is inserted between that
muscle and the transversus anterior on the anterior part of the
third basibranchial.

Both muscles are innervated by the nerve of the fifth arch
and they are, in all probability, the homologues of the distal por-
tions of the obliqui muscles of the anterior arches, that is, on
the fourth arch, to muscle *Ov.IV*$^2$. They are called by Schnei-
der (No. 111, p. 116), in ganoids and teleosts, the sterno-
pharyngei, one being a rectus and the other an obliquus; by
Albrecht (No. 2, pp. 29 and 34) they are called, in Acipenser,
the interbranchialis internus VI, or omozonio-branchialis, and,
in Gadus, the branchiretractor inferior and superior.

### 6. Review and Comparison.

In the primitive condition of all fishes there was, according to Vetter (No. 124, pp. 443–446), a single, annular, general constrictor muscle, which later separated into two layers, an outer and an inner one. From the outer layer arose the interbranchial muscles of the visceral arches; from the inner one the adductor and interarcual muscles, and the constrictor of the pharynx. Connected with the primitive general constrictor there were a large number of visceral arches all lying "oberflächlich in der dünnen Leibeswand," their upper ends imbedded in and moved by the "Ringmusculatur des Schlundes." Whether the arches so described lay inside or outside the muscle is not clear either in this statement of Vetter's conclusions, taken alone, or taken in connection with further statements made in two footnotes. The arches would seem to lie inside the muscle were it not that Vetter definitely states his acceptance of Gegenbaur's conclusion that the adductor mandibulae muscle, which is considered as the serial homologue of the adductor muscles of the branchial arches, lay primarily on the inner surface of its arch.

In Acipenser and all teleosts Vetter concludes (No. 125, pp. 485 and 534) that the adductor muscles of the visceral arches become greatly reduced, and that they may, on certain or on all of the arches, disappear entirely. The interarcuales also disappear, or may possibly give origin to the third, fourth, and fifth levatores arch. branch. in Acipenser and to the obliquidorsales in teleosts (No. 125, pp. 485 and 534). The interbranchiales give origin to the levatores arc. branch. ext. and int. of teleosts and to the interarcuales ventrales and levatores arc. branch. (at least the first two) in Acipenser. The transversi dorsales are said to be entirely new formations, or to be derived, possibly, from the retractores; the retractores themselves are possibly the homologues of the subspinalis of Acanthias and Heptanchus.

With these general conclusions of Vetter I do not entirely agree. The arrangement and innervation of the muscles in Amia seem to me to strongly indicate that they all lay primarily

external to the visceral arches. This seems confirmed by
Kaestner's description of the embryological origin of the skele-
togenous elements of a segment, from the inner wall of the
protovertebra of the segment (No. 62, Pl. XII), and by Kupf-
fer's positive statement (No. 71ᵃ, p. 110) that the cartilaginous
arches in selachians arise from the mesoderm of the arch.
That the muscles of the branchial segments lie primarily inter-
nal to the branchial basket in Petromyzon (No. 111, p. 69, and
No. 71ᵃ, Figs.      ) has no bearing on the subject, that basket
being of ectodermal origin and not homologous with the
branchial arches of other fishes (No. 71ᵃ, p. 118).

The general constrictor of the visceral arches lying primarily
external to those arches, the adductor muscle of each arch
must aquire, secondarily, its position on the inner surface of
that arch. This it does, in Amia, in two different ways, either
by passing over the posterior edge of its arch, as on the fourth
arch and probably the fifth also, or over its anterior edge, as
on the mandibular arch. The innervation shows this conclu-
sively, if it be not assumed that the nerve that innervates the
muscle of one or the other of the arches has cut through the
cartilage of its arch.

Before further considering the adductores it will be better
to seek the homologies of the interarcuales, those muscles
being derived by Vetter, in selachians, from the same deep
layer that gives origin to the adductores.

In Acanthias there are, according to Vetter, three interar-
cuales on each of the first three arches. They are called by
him interarcuales I, II, and III. The first of these muscles,
interarcuale I, arises on the upper, posterior process of the
pharyngobranchial of its arch, and is inserted on the corre-
sponding process of the pharyngobranchial of the next follow-
ing arch. The second arises on the epibranchial of its arch,
and is inserted at the base of the posterior process of the pharyn-
gobranchial of its arch. The third arises with the second, on
the epibranchial of its arch, and is inserted on the anterior
process of the pharyngobranchial of the next following arch,
crossing and touching the anterior end of the epibranchial of
that arch. Between the second and third muscles, or, more prop-

erly, immediately median to the third muscle, a branch of the ramus pharyngeus of the next following arch runs downward to the pharynx.

In Heptanchus the interarcuales are found much as they are in Acanthias ; in Scymnus interarcuale I is entirely wanting. In all three fishes the muscles are said, by Vetter (No. 124, p. 443), to be innervated by pharyngeal branches of the vagus nerve that belongs to the corresponding branchial cleft ; hence, necessarily, the nerve of the next posterior cleft, for the glosso-pharyngeus belongs to the cleft in front of the first arch. He, however, only succeeded in definitely tracing the nerves to the muscles in one fish, Scymnus. The corresponding muscles in Mustelus and the rays are first said by Tiesing, in a somewhat uncertain way (No. 123, pp. 111 and 115), to be innervated by the nerve of the arch to which they belong. This statement is then qualified as to the innervation of interarcuale I on the first arch of Mustelus, but not as to the corresponding muscle in the other fishes. Fürbringer refers to this qualification of the first positive statement regarding Mustelus, as confirmation of his own statement (No. 36, p. 133) that the interarcuales I are all innervated by branches of the spino-occipital or spinal nerves, and the interarcuales II and III, on each arch, by the nerve of the arch to which they belong.

There are thus three different innervations given for these muscles. If Vetter were right, each vagus nerve would have to innervate muscles found on the arch in front of its cleft as well as others found on the arch behind that cleft. This is directly opposed to what van Wijhe finds in larvae of Scyllium and Pristiurus, the posttrematic branch alone of each branchial nerve, in those fishes, innervating the muscles of the arch of the segment to which the nerve belongs (No. 130, p. 19). If the branchial clefts were intrasegmental and the arches, there-fore, intersegmental, as Hoffmann says they are in Acanthias (No. 57, p. 647), the innervation given by Vetter would seem possible or even natural. The clefts are, however, said to be intersegmental, and the arches, therefore, intrasegmental in Amphioxus (No. 54, p. 145), in Ammocoetes (No. 54, p. 159), and in Necturus (No. 91, p. 955). The innervation given by

Vetter is, therefore, probably wrong. Between Tiesing and Fürbringer there is no way to choose. The innervation given by Tiesing seems more natural, and if he be right the muscles in selachians can be readily homologized with those in Amia. If Fürbringer is right no comparison seems possible. I therefore assume Tiesing to be correct.

In Amia there are, belonging to each of the first three branchial arches, two dorsal muscles and two dorsal ligaments. One of the two muscles of each arch arises from the side of the skull, and is inserted on the infrapharyngobranchial of the arch next following the arch to which the muscle belongs, the large so-called third infrapharyngobranchial being here considered as the fused third and fourth infrapharyngobranchials. The other muscle, on each arch, arises with the first muscle from the side of the skull, and is inserted on the epibranchial of its own arch. That the muscles belong, in each case, to the arches to which they are assigned is shown conclusively by the innervation. The two dorsal ligaments on each arch are, one, the articular ligament connecting the epibranchial of its arch with the infrapharyngobranchial of the next following arch, and the other, the interarcual ligament connecting the epibranchial of its arch with the infrapharyngobranchial, or epibranchial, of the next following arch. Between these two ligaments, that is, immediately median to the interarcual ligament, the ramus pharyngeus of the next following arch always passes downward to reach its destination.

If, now, muscles I and II in Acanthias should acquire at their upper, anterior ends, an attachment to the side of the skull, and muscle III should become a ligament, an arrangement of muscles and nerves would arise so closely resembling that found on the first three arches of Amia, that the muscles in the two fishes can safely be considered homologous. Muscle I, on each of the first three arches of Acanthias, is thus, in all probability, the homologue of the internal levator of the corresponding arch in Amia, the muscles of the second and third arches, in Amia, being united to form a single muscle, the levator arc. branch. int. posterior, the largest of all the levator muscles. Muscle II, on each arch of Acanthias, is the homo-

logue of the external levator of the corresponding arch in Amia ; and muscle III the homologue of the corresponding dorsal interarcual ligament.   It may here be stated that these latter ligaments in Scomber are still partly muscular.

On the fourth arch, in Acanthias, there is no interarcuale I. As it is found, in Heptanchus, on that arch and the next following ones, Vetter concludes that, in Acanthias, it has been absorbed, before the disappearance of its arch, by the ever advancing constrictor of the pharynx.   It seems to me much more probable that it, either with or without other more posterior muscles of the series, has become the subspinalis of Acanthias and the retractor arcuum branchialium of Amia and teleosts.

In selachians no ventral interarcual muscles are given by Vetter.   He, however, describes them in Acipenser and in teleosts, and derives them, in both, from the interbranchial muscles of elasmobranchs, more particularly from the so-called interbranchiales of Chimaera.   These latter muscles, although considered by Vetter the homologues of the muscles of the same name in selachians and teleosts, differ greatly from those muscles.   They are found in the first three arches only of Chimaera, and arise, on each arch, from a posterior process of the pharyngobranchial of the arch, the surface of origin extending backward beyond the hind end of the process, and, on each of the first two arches, occupying part of the anterior edge of the epibranchial of the next posterior arch.   The muscle runs the full length of its arch, lying on its outer surface, and is inserted on the outer surface of the hypobranchial of the arch.   The muscle has, thus, almost exactly the origin of the interarcuales II and III of selachians, and the insertion of the obliqui ventrales of ganoids and teleosts.   Furthermore, interarcuale II in Scymnus is said to extend outward and downward along the outer surface of its arch, to be inserted on the upper end of the ceratobranchial, thus closely resembling the interbranchiale of Chimaera.   The muscle in Chimaera may accordingly be considered as representing a primitive condition in the differentiation of that deeper layer of the general constrictor to which Vetter assigns the interarcuales and adduc-

tores of all fishes, and not as a specially developed condition of
the interbranchiales of other fishes. From the dorsal end of
this primitive muscle the interarcuales of selachians and the
levatores arcuum branchialium of ganoids and teleosts arise ;
from its ventral end the obliqui ventrales of the latter fishes.

The adductores can now be considered. In Amia and
teleosts they seem to represent, on the branchial arches, the
greatly reduced and disappearing middle portion of the once
continuous interbranchiale of Chimaera. They cannot, how-
ever, always be derived from that muscle, for in Chimaera both
an adductor and an interbranchiale are found on each of the
first three arches. Whether they arise from that muscle, or
with it from the same deeper layer of the general constrictor,
they, in Amia, reach their position on the inner surface of the
arches by passing backward and inward over the hind edge of
the arch. This the innervation shows conclusively.

In the hyoid arch the hyohyoideus superior is unquestion-
ably the true interbranchial muscle of its arch; that is, a
muscle homologous with the interbranchiales of selachians and
not with those of Chimaera. From the dorsal portion of this
muscle the levator and adductor operculi of Amia arise. These
latter muscles are accordingly not the serial homologues either
of the external or the internal levator muscles of the branchial
arches. The homologue of one or both of those muscles is
certainly the so-called adductor of the arch, the adductor hyo-
mandibularis. The origin, insertion, and innervation of the
muscle all indicate this almost conclusively. The other inter-
arcual muscle or muscles of the arch are found, in Amia, in
one or both of the two ligaments on the inner surface of the
hyomandibular. The ligaments, in Amia, have entirely lost
their attachment to the first branchial arch. In certain selachi-
ans they still retain it (No. 38). Other ligaments, in selachians,
connect the hyomandibular with the skull and represent the
levator, or so-called adductor, hyomandibularis, which muscle is
otherwise entirely wanting. The hyohyoideus inferior may be
the obliquus ventralis of the arch.

In the mandibular arch the levator arcus palatini develops
from the superficial portion of the general constrictor, and is,

accordingly, homodynamous with the interbranchiales of sela-
chians, and not with the interbranchiales of Chimaera and the
levatores arcuum branchialium of other fishes. The levator
maxillae superioris, on the contrary, develops from the deeper
portion of the constrictor and is the serial homologue on its
arch, whatever that arch may be, of those muscles. The
adductor mandibulae lies along the outer surface and anterior
edge of the arch, excepting in its ventral portion, where it is
inserted in part on the inner surface of the arch. The nerve
and artery of the arch lie along the anterior surface of the
muscle, as they do along the anterior surface of the inter-
branchiales in Chimaera. The middle portion of that muscle
has accordingly, in the mandibular arch, slipped over the
anterior edge of the arch, instead of over its posterior edge, as
on the branchial arches. The adductor mandibulae is thus not
strictly homodynamous with the adductores arcuum branchia-
lium. The geniohyoideus and intermandibularis are probably
the obliqui ventrales of their arch or arches.

### 7. Nervus Glossopharyngeus.

The nervus glossopharyngeus (*gl*, especially Figs. 25, 52 to
55, and 64, Pls. XXV, XXXIV, XXXV, and XXXVIII) arises,
in the adult, by a single root from the side of the medulla
oblongata, immediately ventral to and a little posterior to the
root of the nervus lineae lateralis vagi. It runs at first out-
ward and backward across the ventral surface of that root, and
then turns outward, backward, and downward immediately in
front of the middle membranous portion of the posterior
bounding wall of the labyrinth recess. It passes above the
ramulus papillae lagenae acustici, under the ramulus ampullae
posterioris acustici, between the sacculus and the sinus utriculi
posterior (Fig. 57, Pl. XXXV), and issues from the cranium
by its foramen (*glfr*, Figs. 9, 10, 11, Pl. XXI), which lies
immediately behind the hind edge of the petrosal, in the angle
between that edge and the ventral edge of the posterior process
of the bone. The foramen lies entirely in the cartilage of the
cranium, but its front and upper edges are formed by the
petrosal.

As the root of the glossopharyngeus issues from under the root of the n. lineae lateralis vagi it receives, from that root, the root of the so-called dorsal branch of the glossopharyngeus. This latter root runs outward with the main root of the nerve, lying immediately behind that root, closely applied to it, and in the adult issues with it, through the same foramen, and enters the same ganglion. As the two roots pass outward between the two terminal branches of the ramus posterior acustici, there is an apparent interchange of fibres, the nature of which could not be satisfactorily determined. The nerve in Amia thus seems to differ in this respect, though not in position, from the nerve in Protopterus (No. 89, p. 316). In larvae, as already stated, the dorsal root usually issues from the cranium by a distinct and separate foramen lying immediately behind the foramen of the main root, and forms a ganglion separate and distinct from the ganglion of that root. From this ganglion arises the ramus dorsalis of the nervus, which runs upward, backward, and outward along the outer surface of the intercalar, internal to the levator arc. branch. int. ant. Immediately below the surface of origin of that muscle, it pierces the intercalar and then the chondrocranium by a special foramen, and issues on the upper surface of the cranium on the bottom of the temporal groove. There it separates into two portions, one of which turns outward, enters the squamosal, and supplies organ 17 infraorbital of the lateral sensory canals. The other portion turns medianward, runs external to and superficial to, or through, the communicating branches from the trigeminus to the vagus, superficial to the anterior extension of the trunk muscles that fills the temporal groove, and supplies the middle dorsal head line of pit-organs (No. 3, p. 516). In Laemargus (No. 31) this dorsal branch of the glossopharyngeus leaves the main root of the nervus proximal to the ganglion of that root, and contains no ganglion cells. It is accompanied by or contains fibres that are distributed to fibrous tissues.

The main ganglion (*ggl*) of the glossopharyngeus, in the adult, is a somewhat elongated structure, lying close against the outer surface of the skull, and extending downward and

forward across the foramen of the nerve and then along the
outer surface of the petrosal. It lies immediately internal to
the levator arc. branch. int. anterior about midway of its length.
From it, in addition to the ramus dorsalis, two main branches
and several smaller ones arise. The main branches are the
ramus posterior, or posttrematicus (*psgl*), and the ramus anterior
(*agl*), which latter soon separates into the ramus pretrematicus
(*prgl*) and the ramus pharyngeus, or palatinus (*pgl*). Of the
smaller branches, one arises from the outer surface of the
ganglion near the base of the ramus posttrematicus and enters
at once the levator arc. branch. int. ant., which it supplies. The
others, two in the specimen used for illustration, arise from the
distal end of the ganglion between the two main branches.
One goes at once to the tissues along the anterior surface of
the distal end of the first arch. The other runs outward and
backward along the upper surface of that arch, parallel to, and
immediately anterior to the ramus posttrematicus, with which
it anastomoses near the middle of the epibranchial.

The ramus anterior runs downward and forward, dorsal to the
efferent artery of the first branchial arch, and to the infra-
pharyngobranchial of that arch, and there separates into its
two portions, or branches, the ramus pharyngeus and ramus
pretrematicus. The former runs forward, downward, and
slightly inward, ventral to the jugular vein and the adductor
hyomandibularis, lateral to and parallel to the common carotid
artery, dorsal to the anterior end of the first infrapharyngo-
branchial, ventral to and external to the hyo-opercularis and
external carotid arteries, and then external to and ventral to
the internal carotid. With this last artery it enters the palatine
canal by the internal carotid foramen at the hind edge of the
lateral wing of the parasphenoid, the foramen lying immediately
in front of the anterior end of the first infrapharyngobranchial.
The common carotid lies ventral to the anterior end of that
bone, the hyo-opercularis dorsal to it, the common carotid
separating into its internal and external branches immediately
beyond the bone. In the palatine canal the ramus pharyngeus
lies on the median side of the ramus palatinus facialis, and at
the point where that nerve separates into its anterior and

posterior branches, the pharyngeus, in one dissection, separated
also into two portions, one of which accompanied each of the
two branches of the palatinus. In other dissections this
separation was not evident, the pharyngeus glossopharyngei
accompanying the anterior branch of the facialis and issuing on
the ventral surface of the vomer to be distributed to dermal
tissues there. The nerve as it passes the external opening of
the pseudobranchial canal lies median to that opening, and
hence in all probability takes no part in the innervation of the
pseudobranch.

The ramus pretrematicus glossopharyngei, after separating
from the ramus pharyngeus, turns outward and forward along the
under surface of the adductor hyomandibularis, and when near
the anterior edge of that muscle, and close to the hind edge of the
pseudobranch, turns outward, downward, and backward along the
under surface of the muscle, and then along the inner surface
of the hyomandibular and symplectic. In this part of its course
it lies ventral to, or even in front of, the truncus hyomandibularis
facialis, and approximately parallel with it and with the ramus
hyoideus facialis. It sends several branches forward and back-
ward to the dermal tissues on the under surface of the hyoman-
dibular, passes beyond the hind edge of the symplectic, and
then turns downward and forward in the dermal fold between
that bone and the upper end of the ceratohyal. At this point,
in larvae, branches of the nerve were traced into the terminal
buds found in the lining membrane of the mouth cavity. The
nerve, in larvae, passes dorsal to the pseudobranchial canal,
dorsal to the hind end of the pseudobranch, lying close to that
organ. No branches of the nerve could be traced directly to
the organ, but a branch was found distributed to the wall of
the spiracular cleft.

The ramus posterior, or posttrematicus, glossopharyngei runs
downward, outward, and backward in front of the levator arc.
branch. int. anterior and the levator arc. branch. ext. primus, and
then along the anterior portion of the upper surface of the first
arch. From the posterior edge of the nerve at its base, or
from the outer surface of the main ganglion itself, one or two
small branches are sent backward to the inner surface of the

levator arc. branch. int. anterior, which they innervate. As the nerve passes in front of the levator arc. branch. ext. primus it sends a nerve to that muscle, and immediately beyond the muscle is joined, on its anterior side, by the small branch that arises directly from the main ganglion, with which it anastomoses, as already stated. Immediately beyond this anastomosis a branch is given off on the anterior side of the united nerve, the branch being larger than the small anastomosing branch, and hence apparently containing fibres from the main nerve. This branch, shortly before reaching the outer end or angle of the arch, turns downward along the anterior surface of the epibranchial of the arch, reaches the dorsal surface of the ceratobranchial, and then runs forward and inward along the dorsal surface of that bone and of the hypobranchial as far as, or farther than, the distal end of the latter element. Many small branches are given off by it in its course.

Immediately beyond the insertion of the levator arc. branch. ext. primus, the ramus posttrematicus glossopharyngei is joined, on its posterior side, by three branches of the ramus pretrematicus vagi primi, and the four nerves continue side by side without anastomosis to the outer end of the arch. They all lie external to and superficial to the efferent artery of the arch, and internal to, that is, deeper than, the afferent artery, the same relations to the arteries being maintained on the ventral portion of the arch as well. At the outer end of the arch the anterior of the three branches of the first vagus crosses externally over the glossopharyngeus, and on the ventral aspect of the arch lies along the anterior side to that nerve. The three remaining nerves pass to the ventral surface of the ceratobranchial, and there anastomose completely, forming at first two nerves and then a single one. From these nerves, before and after the anastomosis, branches are given off on both sides, and, from these branches, branches are sent forward and backward along the entire length of the anterior and posterior edges of the arch. They form numerous anastomoses with each other, as shown in the figures, and anastomose also with that branch of the vagus that crosses externally over the glossopharyngeus to the anterior edge of the arch. The main portion or branch of

the united nerves continues forward along the ventral surface of
the obliquus ventralis primus, sends a branch from its posterior
edge into that muscle, another inward and backward to the
tissues of the floor of the branchial chamber between the ventral
ends of the first and second arches, and then continues forward
and is distributed to the tissues between the ends of the hyoid
and first branchial arches.   Terminal branches of it extend also
to the tissues dorsal to the ventral end of the hyoid.

### 8.   Nervus Vagus.

The nervus vagus (*v*, especially Figs. 25, 52, 53, 59, and 64,
Pls. XXV, XXXIV, XXXV, and XXXVIII), excluding the
nerve of the lateral line which has already been described, arises
by several rootlets which are usually grouped in two main
bundles, the anterior of which arises by one or two rootlets
and the posterior always by three or more.   The most posterior
of these rootlets always arises a little behind the others and
runs at first directly outward, or even outward and a little for-
ward, instead of outward and backward as do the others.   The
long line of origin of the root lies about opposite to, or a little
in front of, the inner edge of the strongly projecting ridge that
forms the posterior wall of the labyrinth chamber.   The root,
owing to its extended line of origin, is at first flattened horizon-
tally, but the posterior edge soon turns downward, thus twisting
the root through about 90°, and so presenting its flattened sur-
face at first upward, backward, and inward, and then nearly back-
ward and inward.   In this twisted position it is joined by the root
of the lateral nerve which lies along its upper, anterior edge, over-
lapping it somewhat.   The united roots then run outward and
backward along the membranous and cartilaginous posterior
wall of the labyrinth chamber, and issue together by the large
vagus foramen.   This foramen (*vfr*, Figs. 9, 10, and 11, Pl.
XXI), as Sagemehl has stated, lies between the intercalar and
the occipitale laterale ; but as the intercalar in Amia is a super-
ficial bone, and probably a membrane bone, the foramen is
properly a perforation of the anterior margin of the occipitale
laterale, nearly inclosed in that bone, but still lying between
it and the cartilage of the skull in front of it.

As the two roots approach the foramen they pass through what is apparently a ganglionic enlargement ($gv^i$), and there is always here, or between the two roots proximal to the enlargement, an interchange, perhaps mechanical, of fibres, for one or more small nerve bundles were always broken in attempting to separate the roots. This apparent interchange of fibres, and a mass of ganglion cells on the root of the vagus, were also found at this point in larvae (No. 3, pp. 517 and 518), the ganglion cells being always confined to the root of the vagus, none of them appearing on the root of the lateral nerve.

Beyond this intracranial ganglion, which lies inside the cranium, the nervus vagus separates immediately into its four main portions, or into three portions, the posterior of which soon separates into the two posterior main branches of the nerve. On each of these branches of the root, after they issue from the cranium, a well-marked ganglion is found, that on the first branch lying close to the side of the skull, the second close to the first, and the third and fourth at some little distance from the other two. From the intracranial ganglion the first branch of the nervus (*stv*) has its apparent origin, and runs outward and backward through the vagus foramen above the four main roots of the nerve. Immediately beyond the ganglion, and ventral to the first branch of the nerve, the nervus lineae lateralis, now well rounded, runs backward across the upper surface of the four main vagus roots, median to their ganglia. On it, in the adult, there is no such ganglionic enlargement as was found in larvae, but a slight difference in the color of the nerve indicates undoubtedly its presence immediately beyond the intracranial ganglion of the main root. From the proximal portion of this discoloration, immediately outside the vagus foramen, or even still inside the cranium, the first branch of the lateral nerve (*nll.sto*) is given off, and joins at once the first branch of the main vagus root, with which it is intimately connected, but apparently without any interchange of fibres whatever. The two nerves issue from the vagus foramen together, turn upward and outward along the side of the skull, pass across the posterior angle of the intercalar median to the posterior process of that bone,

and, lying lateral to, and superficial to, the trunk muscles, and internal to the median process or leg of the suprascapular, which in embryos is simply a ligament, reach the under surface of the extrascapular near its lateral edge. Here the branch of the nervus lineae lateralis separates into two portions, one of which goes to supply organs No. 18, infraorbital in the extrascapular, and No. 19, infraorbital in the suprascapular, and the other to the organs of the supratemporal cross-commissure and those of the posterior dorsal pit line (No. 3, p. 517).

After giving off its first branch the nervus lineae lateralis leaves the vagus, runs backward, outward, and slightly downward, internal to the levatores arc. branch., between them and the trunk muscles, and then slightly upward, internal to the glandular formation which I have already described as probably being the thyroid gland. At the front edge of the supraclavicular the nerve turns downward and backward, then, beyond that bone, directly backward, and, lying slightly imbedded in the fissure between the dorsal and ventral portions of the trunk muscles, continues its slightly undulating course toward the tail. Its branches are distributed to organs 20 and 21, infraorbital, in the supraclavicular, and to the organs of the lateral line and the body pit lines, as already described in my earlier memoir (No. 3, p. 518).

The first branch of the vagus, the one arising from the root of the nerve, gives off a small intracranial branch, and then, after reaching the outer surface of the head with the first branch of the nervus lineae lateralis, separates into two main portions. One of these portions turns forward along the lateral edge of the anterior extension of the trunk muscles and runs directly into and anastomoses completely with one of the branches of the first pair of dorsal branches of the ophthalmicus trigemini, as already described in describing that nerve. This branch of the vagus seems to be the nerve described by Pinkus, in Protopterus, as a communicating branch from the lateralis facialis to the lateralis vagi. Like the nerve in Protopterus, it comes into intimate relations with a branch of the lateral, or so-called dorsal, branch of the glossopharyngeus, as already stated. From it branches are sent medianward and laterally to

the dermal and subdermal tissues in the temporal region of the top of the head. The remaining and larger portion of the nerve turns outward, sends, in all the specimens examined, an important branch forward to unite with the other anterior portion of the nerve, and then, turning downward, breaks up into numerous branches which run downward, forward, and backward in the pigment layer along the inner surface of the operculum, lying immediately external to the terminal ramifications of the ramus opercularis facialis on the outer surface of the levator and adductor operculi. The anterior branches of this part of the nerve run forward into the thick dermal tissue behind the dorsal end of the preoperculum, and even slightly under the free dorsal end of that bone. The entire nerve may, from its distribution, be called the dorsal, or supratemporal branch of the vagus. Its small intracranial branch (*, Figs. 25 and 59) arises more from the main vagus root than from the dorsal branch itself. It, however, arises from the fibres that go to form that branch, and hence can be considered as a branch of it. It runs upward and forward, internal to the root of the lateral nerve, and issues on the top of the chondrocranium by a small foramen (*ltfr*, Fig. 8, Pl. XXI) lying near the middle line of the head, near its hind end. Its further course could not be traced. From its position, at its exit, it would seem to be the ramus lateralis trigemini, or ramus recurrens facialis, of teleosts. Its apparent origin is, however, from the vagus, and not from the trigeminus or facialis. If it be the ramus lateralis trigemini the first pair of dorsal branches of the ophthalmicus superficialis trigemini can not be that nerve, or can only be a part of it. Both nerves, and the main supratemporal nerve as well, are distributed to regions where terminal buds are found, this being especially true of the posterior branch of the main nerve.

The supratemporal branch of the vagus arises almost entirely from the anterior bundle of the root of the nerve. The fibres of that bundle, where they traverse the intracranial ganglion, have, apparently, an important interchange of fibres with the posterior bundle of the root. Viewed superficially and from below, they have, however, the appearance of a separate and

distinct bundle lying on the ventral surface of the intracranial ganglion, near its anterior edge. Immediately beyond the vagus foramen, those fibres of the anterior root that have not entered into the first, or supratemporal branch of the vagus, turn directly outward and enter the first vagus ganglion, which is a well-rounded ganglion lying close to the side of the skull between the levatores arc. branch. int. posterior and anterior.

From this first vagus ganglion ($gv^1$) the truncus branchialis primus n. vagi arises, and close to it, from the posterior surface of the truncus at its base, two small, short nerves which run outward and backward to the inner surface of the levator arc. branch. int. post., which muscle they entirely or in large part supply. The truncus then runs outward and downward between the two levatores arc. branch. int., and in all the specimens examined, excepting one, there separated into a ramus anterior and a ramus posterior. The former separates at once into two portions, the ramus pharyngeus n. vagi primi ($pv^1$), and the ramus pretrematicus n. vagi primi ($prv^1$), the latter being the ramus branchialis posterior arcus primi of Stannius. The ramus pharyngeus turns forward, and then forward and inward, above the efferent artery of the second arch and between the levator arc. branch. int. ant. and the levator arc. branch. ext. primus, passes through the opening between the ligaments that connect the posterior edge of the first epibranchial with the anterior edge of the second infrapharyngobranchial, and, turning inward and forward between the first and second infrapharyngobranchials, is distributed to the tissues on the roof of the branchial cavity.

The ramus pretrematicus ($prv^1$) runs at first forward, outward, and downward, and then separates into four branches, one of which turns inward and forward and joins the ramus pharyngeus, with which it passes through the opening between the articular and interarcual ligaments, connecting the first and second arches. The other three branches turn outward, downward, and backward, and lying close against the posterior surface of the levator arc. branch. ext. primus, reach the upper surface of the first arch beyond that muscle, and have the course and distribution already described.

The ramus posterior, or ramus posttrematicus n. vagi primi ($psv^1$), runs outward and downward immediately in front of the levator arc. branch. ext. secundus, sends a branch to that muscle, and beyond it reaches the upper surface of the second arch, where, like the corresponding nerve on the first arch, it is the anterior and larger of four nerves that run outward and backward on that surface.   From it, as on the first arch, a branch is sent downward across the anterior surface of the epibranchial of the arch to the dorsal surface of the ceratobranchial, and, as also on the first arch, the second nerve of the four crosses over the first nerve at the outer end of the arch, and on the ventral surface of the ceratobranchial lies along the anterior edge of that bone, anastomosing with a branch of the larger nerve.   The other nerves anastomose and branch irregularly, the main portion of the nerve so formed continuing forward and inward onto the ventral surface of the interarcualis ventralis of the arch.   There it sends branches from its hind edge into the deeper and proximal portion of that muscle, and then, turning inward through the superficial and distal bundle of the muscle, or passing around the anterior and then the dorsal surface of that bundle, sends a branch to that bundle, and is then distributed to tissues near the basal line between the second and third arches.   One of the branches of the united nerves is sent along the anterior edge of the arch to tissues between its arch and the next preceding arch.   On each of the four complete arches a similar branch is found.

In one specimen (Fig. 59, Pl. XXXV) what was apparently a second ganglion was found on the truncus primus n. vagi, beyond the first ganglion, at the point where the truncus separated into its several portions.   In this specimen four large nerves arose from this second ganglionic swelling, — a posttrematicus, a pretrematicus, and a pharyngeus, and a fourth nerve which ran directly to the ganglion of the glossopharyngeus, and had on its distal end, where it joined that ganglion, a marked swelling, ganglionic in appearance.   No such nerve or ganglion was found in any other specimen.

The truncus branchialis secundus n. vagi arises from the second vagus ganglion ($gv^2$).   It runs backward, downward, and

outward, internal to, and then outward and downward, behind,
the levator arc. branch. int. post. (to which muscle, in one dis-
section (Fig. 52, Pl. XXXIV), it sent a delicate nerve, appar-
ently innervating it in part), and then separates into an anterior
and a posterior portion. The former, or ramus anterior n.
vagi secundi, turns forward along the outer surface of the
levator, and gives off three branches which together constitute
the ramus pretrematicus of the nerve ($prv^2$). The remainder
of the nerve continues forward, as the ramus pharyngeus n.
vagi secundi ($pv^2$), passes through the large oval opening
between the two ligaments connecting the epibranchial of the
second arch with the infrapharyngobranchial and epibranchial
of the third arch, and then turns inward and forward between
the second and third infrapharyngobranchials, and is distrib-
uted to tissues there. The three branches which together
constitute the ramus pretrematicus of the nerve, the ramus
branchialis posterior arci secundi of Stannius, run outward and
downward close against the posterior surface of the levator arc.
branch. ext. secundus, reach the upper surface of the second
arch beyond the insertion of that muscle, and have the course
and distribution already given.

The posterior portion of the nerve, the ramus posttrematicus
n. vagi secundi ($psv^2$), gives off from its base two nerves, one
of which runs to the levator arc. branch. ext. tertius,
which it innervates, and the other to the levator arc. branch.
ext. quartus, which it innervates in part, but in part only, for
the muscle receives also an important branch from the posttre-
matic branch of the third vagus, which runs downward and
outward to the upper and posterior surface of the muscle.
From this latter nerve a terminal branch is sent into the
adductor muscle that lies between the epibranchial and cerato-
branchial of the fourth arch.

After giving off the two branches described above, the ramus
posttrematicus secundus runs outward and downward in front
of the levator of the third arch, reaches the dorsal surface of
the arch, sends an anterior branch to the dorsal surface of the
ceratobranchial of the arch, and is joined by two or three
nerves, which together constitute the ramus pretrematicus n.

vagi tertii (*prv3*), one of which, as on the other arches, crosses over the posttrematicus to reach its anterior side, and there anastomoses with other anterior branches distributed to the tissues along the anterior edge of the ventral surface of the arch. The main nerve separates, near the distal end of the cerato-branchial of its arch, into two portions, the posterior of which enters and supplies the deeper portion of the obliquus ventralis of the arch, while the anterior one enters and supplies the superficial bundle of that muscle, as on the second arch.

The truncus branchialis tertius n. vagi separates, soon after leaving its ganglion, into two parts, one of which is a ramus pharyngeus, and the other what may be called the united pre- and posttrematic branches of the nerve. The pretrematic branch, however, contains both pharyngeal and pretrematic elements and corresponds, in its relation to the muscles and ligaments of the arches, to the entire ramus anterior of the two preceding vagus nerves. The ramus pharyngeus (*pv3*) runs outward and downward, dorsal to the efferent artery of the third arch, turns backward and inward behind that artery, perforates the cartilage of the third infrapharyngobranchial immediately behind the hind edge of the ossified portion of that element, in front of, or under, the proximal end of the fourth epibranchial, and is distributed to tissues in the region of the pharyngeal bones. In one specimen it sent a branch to the posterior surface of the third arch, and in all the specimens examined it varied somewhat in its arrangement and distribution. The pre- and posttrematic branches of the nerve soon separate. The former sends an important branch, which corresponds to the pharyngeal branches of the two preceding vagus nerves, forward and inward through the opening between the ligaments uniting the third and fourth epibranchials, the rest of the nerve, the true ramus pretrematicus (*prv3*), running outward and backward behind the levator of the third arch to the upper surface of that arch, where it is distributed as already described.

The ramus posttrematicus n. vagi tertii (*psv3*) runs outward, downward, and backward, sends a branch to the posterior surface of the levator of the fourth arch and, on the right side of the specimen used for illustration, another to the second

obliquus dorsalis. From the main nerve, near its base, in one specimen (Fig. 59), a long and delicate branch was sent to the fifth levator. In another specimen, the one used for illustration, this nerve arose from the main truncus of the nerve, or from the united trunks of the third and fourth vagus. The branch that goes to the levator muscle innervates that muscle, and then the adductor of the fourth arch, passing to the latter muscle over the outer and posterior end of the arch. The main nerve, after giving off these branches, passes under and anterior to the levator of the fourth arch, and reaches the upper surface of that arch beyond the muscle. There it is not joined, as the corresponding branch on the other arches is, by pretrematic branches of the next following vagus nerve. It, however itself, separates, on the ventral aspect of the arch, into two, three, or four branches, the smaller ones of which lie along the anterior and posterior edges of the arch, the main central portion running forward and inward onto the ventral surface of the obliquus ventralis of the arch. There it breaks up into several branches which enter and supply the obliquus of the arch, the two divisions of the so-called obliquus of the fifth arch, and the several divisions of the transversus ventralis anterior. Other branches are doubtless distributed to the cutaneous tissues between the fourth and fifth arches, though this was not definitely traced.

The fourth vagus ganglion ($gv4$) is a large one, either well rounded or somewhat elongated, and from it arise two nerves. The outer or anterior of these nerves is apparently the truncus pharyngeus inferior of Stannius, the other the united truncus pharyngeus superior and truncus intestinalis. The truncus pharyngeus inferior separates at once into two branches, the outer or anterior of which is, from its distribution to the muscles of the fifth arch, the ramus branchialis anterior of that arch, or the ramus posttrematicus n. vagi quarti ($prv4$). The other branch is the ramus pharyngeus inferior of Stannius, but as it is in part, at least, a motor nerve, it cannot be compared with the pharyngeal branches of the other vagus nerves. The two nerves run backward and downward, close together, to the hind end of the branchial arches, lying

internal to the fourth and fifth levators, and dorsal to, and
then posterior to, the posterior of the two upper ends of the
efferent artery of the fourth arch.  Behind that artery and
behind the hind end of the fifth ceratobranchial, the ramus
posttrematicus turns downward, sends a branch to the adductor
muscle of the fifth arch, and then runs forward and median-
ward along the ventral surface of the fifth ceratobranchial.  It
crosses the lateral end of the transversus ventralis posterior,
sends branches to that muscle, and then to the pharyngo-
claviculares externus and internus, and ends in several delicate
branches, which enter the tissues near the basal line.  McMur-
rich says (No. 76, p. 138) that the claviculares in Amia are
innervated by branches from the first spinal nerve.  This is
an error due, doubtless, to the fact that the fourth occipital
nerve, mistaken by him for the first spinal, sometimes enters,
but simply transverses, or gives off a branch which transverses,
the inner of the two muscles.  The nerve does not innervate
the muscle ; it simply perforates it.

The ramus pharyngeus inferior ($piv^4$) turns downward at
the hind end of the branchial apparatus, then medianward, and
separating usually into two main portions, sends branches
forward and backward along the outer surface of, and into,
the constrictor oesophagei.

The truncus pharyngeus superior is the median and posterior
of the two divisions of the median and posterior of the two
main nerves that arise from the fourth vagus ganglion.  It runs
medianward, backward, and downward toward the front end of
the oesophagus, where it turns directly medianward ventral to
the efferent artery of the third and fourth arches, and passes
between the transversus dorsalis posterior and the retractor arc.
branch. dorsalis.  It then turns forward and medianward toward,
and then along, the median edge of the third infrapharyngo-
branchial, sending in its course some branches through that
element, the branches perforating first the transversus posterior,
or the anterior end of the constrictor oesophagei.  It is distrib-
uted mainly, if not entirely, to dermal tissues near the hind and
median edge of the pharyngeal bone.  On the left-hand side
of the specimen used for Fig. 52, a branch of this nerve

formed a loop with a branch of the truncus intestinalis, and
another branch entered, and apparently innervated, the second
obliquus dorsalis. The second obliquus on the right side was
apparently innervated by a branch of the ramus posttrematicus
of the third vagus, as already stated.

From the base of the truncus pharyngeus superior, or even
from the main fourth ganglion itself, one or two branches are
sent medianward and backward to the dorsal surface of the
retractor arc. branch. dorsalis, and then forward and backward
upon that muscle and the transversus dorsalis anterior, inner-
vating those two muscles and also the anterior obliquus dorsalis.

The truncus intestinalis runs downward, medianward, and
backward to the side of the oesophagus, sends several branches
to the dorsal surface of the constrictor oesophagei, and then
continues backward, its further course and distribution not
being traced.

## IV. MUSCLES INNERVATED BY THE POSTVAGAL OR OCCIP-
## ITAL NERVES, AND THE NERVI OCCIPITALES.

### 1. Branchiomandibularis.

The branchiomandibularis (*Bm*, Figs. 44–47, and 58, Pls.
XXXI–XXXV) is a long muscle, double at its origin and at its
insertion, but single in its middle portion, where it lies in the
median plane of the body, immediately ventral to the tough, semi-
cartilaginous mass that lies between, and in front of, the hypohy-
als, and forms the tongue of the fish. Posterior to this mass, and
posterior to and between the hypohyals, the muscle, when at
rest, turns directly downward in front of the anterior tendinous
edges of the hyohyoidei, and passes through a large vascular
or lymphatic space, which, in the adult, forms an annular vein-
like vessel, tightly holding the muscle, about midway of its
length, and compressing its fibres into a small, round neck.
This neck lies nearly perpendicular to the anterior ventral por-
tion of the muscle, and somewhat in front of the posterior
extremity of that portion, the ventral fibres of the muscle
turning upward and then forward to enter it, as if, in the com-
pression of the fibres here, the dorsal ones had been more

closely and more tightly gathered in than the ventral ones, these latter being thus left to project loop-like beyond the neck.

The anterior, ventral portion of the muscle lies nearly horizontal, running forward from the neck of the muscle, immediately dorsal to those portions of the geniohyoidei that have their insertion in median aponeurosis of that muscle, and immediately dorsal to the intermandibularis, but ventral to those portions of the superior geniohyoidei that have their insertions as a tendinous sheet on the inner surface of the mandible. It is double throughout the greater part of its length, the two parts lying close together at the middle line of the head, except near their front ends, where they diverge slightly, and are inserted, in part, on the dorsal surface of the hind margin of the intermandibularis, and in part beyond that margin, on the inner surface of the mandible, near the symphysis. That portion of the muscle that has its insertion on the mandible is mainly or entirely tendinous, and forms a thin, tendinous sheet, which, in the adult, is closely applied to, and not easily separated from, the ventral surface of the anterior, tendinous ends of the geniohyoidei.

The posterior portion of the muscle runs upward and backward from the neck of the muscle, in front of the anterior tendinous margins of the hyohyoidei, and between the two tendons of the sternohoideus, being compressed somewhat by these latter tendons as it passes between them. It then separates at once into two portions, which diverge to the right and left, and are inserted on either side to the arms of a Y-shaped tendon (*Caa4*, Figs. 46 and 47, Pls. XXXII and XXXIII), which arises, by its median portion, from the symphysis of the clavicles, or from the median aponeurosis of the sternhyoideus, and is inserted, by its two arms, to the second and third hypobranchials on each side of the head. This ligament, therefore, corresponds to one or both of those portions of the coraco-arcuales anteriores of Acipenser (No. 125, p. 480) that have their insertions on the second and third arches. As the insertion on the third arch, in Amia, is apparently the most important, and as the branchiomandibularis, in Acipenser, is in intimate relation with the tendon to the third arch, I have called the tendon or

ligament in Amia, as Vetter does the muscle in Acipenser, the coraco-arcualis anterior[4].

The branchiomandibularis, in Amia, varies greatly in different individuals. The posterior portion of the muscle, both in old and young specimens, is as often single as double, the insertion, when single, being to one arm only of the Y-shaped tendon, no trace of the other arm of the muscle being found. In some instances the second arm of the muscle is found reduced in length, and ending without insertion of any kind, and in one specimen both arms were wholly wanting, the muscle ending, without insertion of any kind, a little beyond its neck. The anterior portion of the muscle, which usually, in the adult, has, in part, a tendinous insertion on the inner surface of the mandible, sometimes extends only as far as the intermandibularis, and in larvae it can never be traced definitely beyond that muscle. The anterior, tendinous portion of the superior division of the geniohyoideus, that portion that has its insertion on the mandible, can also not be traced in larvae. In 10 mm. specimens, the branchiomandibularis extends but little beyond the large vascular space through which the muscle passes, and is there lost in the general tissues. In one young specimen it passed above the fold in the lining membrane of the mouth cavity at the base of the tongue, instead of below it, as it is usually found, and was lost in the connective tissues toward the tip of the hyoid and at the base of the tongue. It seems therefore probable, that the tendinous insertions of the geniohyoideus and branchiomandibularis on the inner surface of the mandible are acquired in postlarval, or even in adult life, Acipenser (No. 125, p. 480), where the branchiomandibularis is firmly attached to the integument of the floor of the mouth, representing an intermediate stage. The general relations of the branchiomandibularis to the tongue, in larvae, and the actual termination of the muscle in one young Amia in that organ, seem also to corroborate McMurrich's statement (No. 76, p. 152) that that muscle, in lower forms, corresponds to the muscle from which the tongue muscles of Amphibia are developed. The tongue of the adult Amia may, therefore, represent a condition of that organ in which its partial muscularization has been lost,

rather than not yet acquired, as Gegenbaur (No. 45) states to
be the case for fishes in general.   That such a condition should
be found in Amia is only what might reasonably be expected
if amphibians and ganoids both descend from some proto-
urodelian type, as the muscles and nerves of the eyeball indi-
cate (Fig. 12, Pl. XXII).   It is perhaps worthy of note, in this
connection, that the branchiomandibularis is found in Acipenser
(Vetter) and Polypterus (Pollard), that it is not found in teleosts,
and that it is found, undifferentiated, as Muscle $Cm_1$, or $Cm_2$,
in Chimaera (Vetter).

The branchiomandibularis is innervated, in Amia, by a deli-
cate terminal branch (*r.bm*) of the fused ventral branches of
the first, second, and third occipital nerves.

### 2.  Sternohyoideus.

The sternohyoideus (*Sh*, Figs. 33–35, 46, and 57, Pls.
XXVIII–XXXV) arises from the dorsal and anterior surface of
the lower half of the clavicle, the surface of origin extending
from the symphysis of the bones up to the place of origin of
the pharyngo-claviculares.   It is a strong, conical muscle lying
immediately below the bulbus arteriosus, and tapering grad-
ually into a stout, rounded tendon which is inserted on the
ventral surface of the ossified portion of the hypohyal.   It is
closely united with its fellow of the opposite side of the head
from its origin forward, through about one half its length, the
two muscles being separated by a median aponeurosis, from
which the fibres of both in part arise.   This aponeurosis is
strongest toward the dorsal surface of the muscle, and from its
anterior end arise the two arms of the tendon $Caa^4$, to which
the branchiomandibularis is inserted.

Through each sternohyoideus there are two transverse inter-
muscular septa, resembling the intermuscular septa of the
trunk muscles, and dividing each muscle into three parts.
The middle point of each septum (Fig. 35) is pulled forward
into a pocket, and on the anterior face of the septum, around
the pocket, there is a conical tuft of connective tissue.   A
similar tuft is found on the corresponding point of the ante-

rior face of the clavicle. In the skin on the outer surface of the muscle, opposite the posterior septum, the anterior end of the posterior flagellum (*flp*) has its attachment. No special attachment to the clavicle, such as Sagemehl describes (No. 106, p. 63), could be found, nor were there what could be called special muscles connected with it. Certain fibres of the sterno-hyoideus were, however, inserted on the inner surface of its anterior end, or base, as McMurrich has described. The anterior flagellum (*fla*) is attached to the skin alone, a little in front of the anterior septum.

The trunk muscles, although projecting, internal to the clavicle, slightly beyond the inner, curved edge of that bone immediately above the sternohyoideus, are in no place continuous with that muscle. No indication was found of a separation of the sternohyoideus into a hyodorsalis and hyoventralis, such as Schneider describes in Esox (No. 111, p. 116).

The sternohyoideus is innervated by the united ventral branches of the first three occipital nerves, and by the ventral branch of the fourth occipital nerve, or by a branch of that branch. The branch of the fourth occipital nerve innervates the posterior segment of the muscle, the first three nerves innervate the two anterior segments, a terminal branch of these united nerves being sent to the branchiomandibularis as already stated.

The muscles of the pectoral fin are innervated by branches of the ventral branches of the first six spinal nerves, and, in the two dissections made, by a corresponding branch also of the ventral branch of the last, or fourth, occipital nerve.

### 3. Occipital Region of the Skull.

The occipital region of the skull, in the adult of the lower vertebrates, and of the head, in their embryos, is not defined and limited in the same way by different authors. Gegenbaur (quoted No. 104, p. 189) defines it, definitely, in adult selachians, as that part of the skull that lies posterior to the vagus foramen ; and Froriep (No. 34, p. 226), in chick embryos, as that part of the vertebral column that is included between

the first cervical nerve and the vagus. By these definitions that part of the skull that lies between the glossopharyngeal and vagus foramen, in the adult, and the glossopharyngeal somite or segment of the head, in embryos, are definitely excluded from the region. In selachian embryos the glosso-pharyngeal somite is similarly excluded from the occipital region of the head by van Wijhe (No. 130), Kaestner (No. 62), Killian (No. 63), and others, Hoffmann (No. 57) even excluding with it the first vagus somite. Sagemehl (No. 104, p. 189), in opposition to these views, defines the occipital region of the skull, in Amia and in all bony ganoids and teleosts, as that part of the skull that lies posterior to the glossopharyngeal foramen and to the hind edge of the petrosal, the reason given for this definition being that, in some rare instances in teleosts, the glossopharyngeus and vagus issue through the same foramen. Mollier (No. 82), in embryos of reptiles, gives practically the same definition as Sagemehl, for he takes the hind edge of the auditory vesicle, which lies immediately in front of the glossopharyngeus, as the anterior limit of the occipital region of the skull. Froriep, in a recent work on embryos of mammals, birds, and reptiles (No. 35, p. 575) apparently assigns the same anterior limit to the region, though he does not definitely so state. This latter definition differs, therefore, from his earlier one. According to it, and to the definitions of Mollier and Sagemehl, the glossopharyngeal somite must either be absorbed by the auditory vesicle or be included in the occipital somites of the head. In either case, occipital and postauditory would be, in the adult, synonymous terms, and as such I consider them in Amia in all stages included in this investigation.

In the interior of the skull of the adult Amia, there is a large postauditory portion, or chamber, occupying almost one third the length of the cavum cranii. This chamber is sepa-rated from the labyrinth recess by a ridge of cartilage which projects, in a curved line, forward and inward from the inner surface of the lateral wall of the skull. This ridge of cartilage is, in young larvae, the bulging hind wall of the auditory cap-sule and of the skull, for, as will be later shown, that part of

the skull that lies posterior to the capsule develops, in its dorsal portion, later than, and independently of, that capsule, and then fuses with it to form the occipital or postauditory region. Whether the skull of these young larvae, that is, that part of the skull of the adult that lies anterior to the hind wall of the auditory capsule, is the protometameric skull of Sagemehl (No. 105, p. 527), the equivalent of the skull of adult amphibians and selachians, or is equivalent to that skull less the occipital vertebra of Stöhr, I am unable to judge, not having been able to see the particular publication referred to by Sagemehl. My work, however, strongly inclines me to accept the assertion attributed to Stöhr, that a vertebra, lying originally between the vagus and first spinal nerves, has fused with the hind end of the amphibian skull, and also to consider the skull, as first formed in young Amia, as the true protometameric skull of Sagemehl, but as something less than that skull as defined by him.

The anterior limit of the ventral portion of the postauditory, or occipital chamber of the adult Amia is marked by the transverse ridge of cartilage that forms the hind wall of the eyemuscle canal, and gives support to, and doubtless also origin to, the median processes of the petrosals. The antero-ventral end of the lateral bounding ridge of the chamber, the hind wall of the labyrinth recess, lies opposite the ventral ridge, and opposite, or immediately in front of this point, the glossopharyngeus and vagus have their origins from the brain. The glossopharyngeus perforates at once the median, membranous wall of the auditory cavity, and transverses that cavity to reach its foramen. The vagus, on the contrary, lies always median to that membranous wall, running outward and backward along it, and then along the posterior surface of the hind wall of the labyrinth recess, to its foramen, which lies immediately behind the point where the wall or ridge arises from the inner surface of the skull. The nerve of the lateral line has a similar course. Both nerves, therefore, lie morphologically behind the hind wall of the auditory chamber, or, in larvae, behind the hind wall of the skull. In the occipital chamber the occipital nerves of Sagemehl arise, all of them running outward and backward, in the chamber, to their points of exit.

The course and position of all these nerves indicate that the skull in its posterior portion, as in its anterior portion, develops more rapidly than the inclosed brain, and that posterior to some central point, approximately the nervus acusticus, the issuing nerves are, by that development, pulled or pushed backward, just as in front of that point they are pulled forward. The glossopharyngeus is an exception to this rule, for it runs almost directly outward through the auditory cavity instead of behind it. The auditory vesicle, which "develops between the facial and glossopharyngeal nerve roots" (No. 4, p. 232), seems, in fact, as it developed, to have invaded and occupied the glossopharyngeal region of the head, and, in so doing, to have pushed the vagus and lateral nerves before it, but to have left the glossopharyngeus in its natural position, the sinus utriculi posterior pushing backward above that nerve, and the sacculus below it. In the tadpole, the glossopharyngeus is apparently not treated with similar consideration, for Strong shows it lying, with the vagus, posterior to the auditory capsule.

The brain, in the adult Amia, does not lie on the floor of the occipital chamber; on the contrary, it traverses the chamber at such a height that about one fourth the chamber lies below its ventral surface. The extreme posterior end of this ventral portion of the chamber ends (Fig. 11, Pl. XXI) in a blunt point in the line of the center of the vertebral column, and hence, of the center of the notochord. This part of the chamber lies on a level with the main portion of the eye-muscle canal, and, like that canal, is developed in late larval or postlarval stages. It is filled, in the adult, with fatty tissue.

In the walls of the occipital chamber there are three large ossifications, which extend from the outer to the inner surface of the skull. They are the two occipitale laterale, the exoccipitals of Bridge, one on each side of the head, and the median basioccipital.

The basioccipital (*BO*, Figs. 8–11, Pl. XXI) is described by Sagemehl as having the shape of a muscle-shell. It is a stout bone, has approximately the length of the first six vertebrae, and its posterior third is solid and shaped like a vertebra (Sagemehl). Both ends of this solid portion are concave, and

its posterior portion is marked off from the rest of the bone by
a groove, sometimes faint and sometimes well defined, which
runs across the ventral surface of the bone and then upward
and forward along its sides, but not across its dorsal surface.
In front of this groove, on the ventro-lateral surfaces of the
bone, there are two large, oblong, flattened surfaces, one on
either side of the middle line. They are inclined at an angle
to each other, raised somewhat above the level of the rest of
the bone, and extend to its anterior end. On them the
hind ends of the parasphenoid rest, and the development of
this part of the skull shows that these flattened elevations, in
their posterior portion at least, must be, like portions of the
vertebrae, of membranous, and not of cartilaginous, origin.
Above the dorso-lateral edges of the two surfaces, and in front
of the posterior, solid end of the bone, the basioccipital becomes
thin, and its thin upper edges diverge from behind forward.
Between the two surfaces, on the ventral surface of the bone,
there is a median, depressed portion, or groove, and at the
anterior end of this groove, in the middle line of the head and
extending directly forward, there is a long, flat, thin process,
which, viewed from beneath, has the appearance of a thin
wedge or blade of bone driven into the basioccipital from
its ventral surface, and slightly separating the bone into two
halves. The anterior end of this process almost reaches the
posterior end of the hypophysial fenestra.

Between the hind ends of the two flattened and slightly
elevated ventro-lateral surfaces of the basioccipital, and hence
between the hind ends of the parasphenoid, are the two little
openings (*cai*) described by Sagemehl (No. 104, p. 195), one
on each side of the middle line of the head. From each of
them arise one or two canals which run outward, upward, and
forward, and have their dorsal opening, or openings, at about
the middle of the lateral surface of the basioccipital, dorsal to
the flattened, ventro-lateral surface of that bone, and hence
dorsal to the lateral edge of the parasphenoid. The anterior
of the two canals, where there are two, is the smaller, and
through it a small sympathetic nerve passes ; through the
posterior and larger canal a spinal artery passes. This artery

arises from the dorsal aorta, traverses its canal, runs upward, outward, and forward along the lateral surface of the basioccipital and then of the occipitale laterale, and then inward along the dorsal surface of the latter bone. It apparently receives branches from all the prespinal muscle segments, but this was not definitely established. In 20 mm. larvae it lies wholly outside the cartilage of the base of the skull, passing through a region where membrane bone is forming, internal to the parasphenoid.

Immediately posterior to the ventral openings of the two canals, above described, there are, on the ventral surface of the basioccipital, on each side of the middle line, one or two slightly projecting processes of cartilage ($vp°$). The posterior of these two processes is always found, and has been described by Sagemehl (No. 104, p. 195). The anterior one, which is sometimes wanting, and is sometimes fused with the posterior one, to form a single elongated piece or process is not described by him. The posterior process lies on the posterior, vertebra-like, terminal piece or portion of the basioccipital; the anterior one lies immediately in front of the groove or line separating that posterior portion from the anterior portion of the solid end of the bone. Sagemehl states that these little cartilaginous processes correspond to similar processes found on the ventral surfaces of all the vertebrae. This statement I can confirm for the six anterior trunk vertebrae. On the vertebrae of the tail, which unfortunately I have been unable to examine, I am inclined to think the processes become the ventral arches, which is evidently not what Sagemehl intended to state. It seems to me also extremely probable that these processes, contrary to Sagemehl's statement (No. 102, p. 516), are the homologues of the pharyngeal process of Cyprinoids, and that these latter processes are thus also the homologues of the ventral arches of the tail. One reason given by Sagemehl for not so considering them is, that the pharyngeal process in Chondrostoma nasus is not performed in cartilage. This may be, in part, an error, as are certainly his statements that the intercalar in Amia is so performed, and that the prefrontal in Amia is in part a dermal bone. The other important reason given by him for

not considering the pharyngeal process as formed by the fusion of ventral arches, is, that the ribs in Amia are ventral arches, and that they inclose the body cavity instead of simply the aorta, as the process does. As will be seen later, the statement that the ribs in Amia are ventral arches is not wholly above question. If the pharyngeal process of Cyprinoids is not homologous with the cartilaginous processes of Amia, it must have its homologue in Amia in the bony pads that give support to the hind ends of the parasphenoid.

On the dorsal surface of the solid end of the basioccipital, extending longitudinally the full length of that end, there is, on each side of the middle line, a projecting strip of cartilage ($dp^o$, Fig. 18, Pl. XXII), which has its origin slightly below the outer surface of the bone. This strip is thickest along its median edge, and has, extending transversely across it, two wedge-shaped ridges. The anterior ridge is much the larger of the two, its summit corresponding with the hind edge of the occipitale laterale. The posterior ridge lies on the dorsal surface of the posterior, vertebra-like, terminal portion of the basioccipital. Between the two processes lies the anterior occipital dorsal arch ($DA^o$) of Sagemehl; between the posterior process and the first vertebra, the second or posterior occipital arch. This second occipital arch of Sagemehl, although fused with the hind end of the skull, is possibly, though not probably, the first spinal arch, as will be later shown.

From the lateral surface of the basioccipital, immediately dorsal to the parasphenoid, two ligaments arise ($loc^a$ and $loc^b$), one from the posterior, vertebra-like, terminal portion of the bone, and the other just in front of that terminal portion. The posterior ligament is much the stronger of the two. It runs outward, downward, and backward in the first prespinal or last occipital intermuscular septum (Figs. 33–35, Pls. XXVIII and XXIX), and is inserted in dense tissue lying between the lower, posterior end of the supraclavicular, and the upper end of the clavicle, the attachment being rather to the former, than to the latter, bone. The anterior ligament runs outward, downward, and backward in the second prespinal intermuscular

septum, and is inserted on the inner surface of the supraclavicular. In young larvae the large ligament is well developed and easily traced in sections ; the small ligament, on the contrary, could not be distinguished from the septal tissues in which it lies. In one larva the large ligament lay in the second prespinal septum instead of in the first.

The occipitale laterale (*LO*) has two exposed surfaces, a lateral and a dorso-posterior. The two surfaces lie almost at right angles to each other, and form, where they unite, the dorso-lateral edge of the bone and of the hind end of the skull. The posterior and lower end of this dorso-lateral edge is thickened, and rests upon, or abuts against, the anterior face of the anterior wedge-shaped process of the cartilaginous strip on the dorsal surface of the basioccipital. The occipitale laterale is thus separated entirely by cartilage from the solid vertebral portion of the basioccipital, excepting, possibly, along its extreme outer edge. The antero-dorsal end of the dorso-lateral edge of the bone is also thickened, and that portion of the lateral surface of the bone that lies between this thickened upper corner and the upper edge of the vagus foramen is raised slightly above the level of the rest of the bone, and has its outer surface flat and roughened. On this roughened surface, which resembles, in general appearance, the surfaces found elsewhere, in Amia, between the dermal and cartilaginous bones, the lower, posterior corner of the intercalar rests. The intercalar thus, in this particular, presents the characteristics of a membrane rather than of a cartilage bone. It is also easily detached from the skull, as the dermal and membrane bones are, and it leaves beneath it, when removed, a clean, unbroken surface. In larvae its first appearance is decidedly that of a membrane bone. Sagemehl's positive statement (No. 107, p. 556) that it is, in Amia, a primary ossification is, therefore, greatly to be doubted. It is much more probable that it is of exactly the same character as the corresponding bone in the Cyprinidae, that is, simply a "Deckknochen."

In front of the thickened postero-ventral end, or base of the occipitale laterale, the ventral edge of the bone, is thin, and

rests directly upon the thin, dorsal edge of the anterior portion of the basioccipital. Through this thin portion, and hence in front of the thickened base of the bone, there is, as Sagemehl states, a foramen (*ofr*[2]) giving passage to the ventral root of a spinal-like occipital nerve; the foramen is, however, strictly on the lateral face of the bone instead of on its dorsal surface, as his figure shows. In front of this foramen, slightly above it, and about halfway between it and the vagus foramen, there was, in all the specimens I examined, another very small foramen (*ofr*[1]) giving passage to another similar but very delicate ventral root. Between these two foramen there is no thickening of the bone, but opposite the space between them, on the median edge of the bone, directly above the foramen magnum, there is a considerable thickening. Along the lateral surface of the basioccipital, starting from the exit foramen of the spinal artery already described, there is a slight depression or groove which continues upward and forward onto the occipitale laterale, across the posterior occipital foramen, and then onto the dorsal surface of the bone. It apparently marks the course of the spinal artery, and is apparently the line shown by Sagemehl in his Fig. 3, and considered by him as of segmental value.

The first vertebra (*V*[1]) is, as Sagemehl states, slightly convex in front and strongly concave behind. It is thinner longitudinally than the second vertebra, the second is thinner than the third, and the third thinner than the fourth. The progression is regular, and there is no reason whatever, from their dimensions alone, to consider the two first vertebrae as half vertebrae (Schmidt, No. 110). The first six vertebrae, the only ones that were examined, had each, on each side, a dorsal and a ventral cartilaginous process (*dp* and *vp*), and all excepting the first had also a lateral, bony process (*lp*) directed downward, outward, and backward, tipped with cartilage, and giving articulation to a rib. Schmidt (No. 110, Fig. 2) shows the first lateral process and the first rib on the third vertebrae. I have always found them on the second. Arising from the lateral surface of the first vertebra, exactly in line with the lateral processes and ribs of the other vertebrae, there is always a small ligament (*lvl*), similar to and in line with those

arising from the posterior end of the basioccipital, and, like them and like the ribs, lying in an intermuscular septum.

The ventral cartilaginous process on each vertebra is small, is round or oval, arises slightly beneath the surface of the vertebra, and projects but slightly beyond that surface. The dorsal process is much larger and much more important than the ventral one. It arises, like the ventral one, slightly beneath the surface of the vertebra, but it projects, at its median edge, considerably above that surface. It extends longitudinally across the dorsal surface of the vertebra, and is wedge-shaped, the edge of the wedge lying transversely, as do the edges of the wedge-shaped projections on the dorsal surface of the basi-occipital. The posterior face of each process is intimately united with a dorsal spinal arch (*DA*), so intimately, in fact, that the two form a single piece and cannot be separated without fracture. A surface line, however, indicates a plane of separation between them.

The first six spinal arches, the only ones examined, are wedge-shaped at their lower ends, and fit in between the wedge-shaped dorsal processes of two adjoining vertebrae. They are therefore, in position, strictly intervertebral, as Franque described them (quoted and contradicted by Schmidt, No. 110, p. 751). They are capped above and below with cartilage, and on the median side of the ventral end of each there is an indentation which extends entirely through the cartilaginous cap of the piece, and, on the sixth arch, slightly into the bone itself. On the sixth arch the indentation or groove extends from the median face of the arch almost to its lateral surface. The cartilaginous cap on this arch thus had the appearance of two oval pieces placed transversely, and connected, at their lateral edges, by a narrow strip of cartilage. Whether this is an indication that the arches are double in origin, as Goette (No. 49) finds them in reptiles, or not, I am unable to state. Nothing else in their development, in Amia, from 12 mm. larvae upwards, indicates such a double origin. The indentation on all the arches is filled with ligamentous tissue connected with, and doubtless derived from, the ligamentum longitudinale dorsale inferior. The anterior face of the cartilaginous

cap of each arch sits directly upon the posterior face of the dorsal process of the next preceding vertebra, and is so intimately connected with that face that, as already stated, it cannot be separated from it, without fracture, even in specimens that have been treated with acids. The posterior face of the cap rests upon the anterior face of the dorsal process of the next following vertebra, but is always separated from that face by a thin, flat pad, which, in the one specimen examined, was of tough, semi-cartilaginous tissue on the first two vertebrae, but, to all appearance, of true cartilage on the posterior ones. The pad or plate is, at its lower anterior end, continuous with, or connected with, the ligamentous tissue filling the median indentation in the base of the arch. It is also connected, at its lateral edge, by tendinous tissue, extending downward and backward along the lateral surface of the vertebra next posterior to the arch, with the intermuscular septum of that vertebra.

The intermuscular septum on each vertebra runs from the rib of that vertebra directly upward toward the top of the vertebral body, where it turns sharply backward onto and across the lower, or lower and posterior, end of the dorsal arch that lies next behind that vertebra. It then runs in a nearly horizontal direction across the lower ends of the two dorsal arches next posterior to that arch, onto the third posterior arch. There it turns upward and backward along that arch, and onto the processus spinosus of the arch. At the distal end of the spine it turns sharply forward and upward, and reaches the dorsal surface of the body about opposite the vertebra to which it belongs. The angle formed where the septum first turns backward from its own vertebra, onto and across the dorsal arch next posterior to that vertebra, is much sharper on the anterior of the six vertebrae than on the posterior ones. It is at this angle that each septum receives the tendinous formation from the semi-cartilaginous pad that lies between its vertebra and the dorsal arch that lies next anterior to that vertebra.

In larvae from 20 mm. to 50 mm. in length, the six vertebral processes of the adult, above described, are found as relatively large blocks of cartilage, three on each side of each vertebra,

and they form, with the notochord, the principal part of each vertebral body.  Membrane bone has, however, even in 20 mm. larvae, begun to form between the processes, and between those of opposite sides.  There is not the slightest indication of a separation of the vertebra into two lateral halves, or into a dorsal and a ventral half.

The dorsal process, on each side, is much larger than the other processes, and is wholly independent of them.  Of the other two the lateral one is the larger, but it is not independent of the ventral one, the two being connected by a thin strip of cartilage, which extends downward from the anterior end of the lateral process to the posterior end of the ventral one.  The ventral processes lie on either side of the dorsal aorta, and, in old larvae, are sometimes continuous at their hind ends, dorsal to the aorta.

The dorsal arches lie, as in the adult, with their bases wedged in between two succeeding dorsal processes, but they are not continuous with either process.  The fusion of the arches, at their bases, with the dorsal process next in front of them, is thus a secondary arrangement, arising in post-larval stages.  That this late and secondary fusion of the arch proper with the vertebral body is not peculiar to Amia, and that it is probably the regular method of development in all bony ganoids and teleosts, if not in all animals, is shown by Sagemehl's statement (No. 107, p. 587) that even in the adult of the Cyprinidae and Characinidae, the dorsal arches are not "fest verbunden" with the vertebral bodies.  The cartilaginous pad found in the adult Amia, between each arch and the next posterior dorsal process, is not found even in 50 mm. specimens.  It, also, must therefore develop in later stages.

The vertebral processes all rest, at their bases, upon a thin layer of strongly refractive material, apparently bone, formed around the notochord.  This layer is considered by Schmidt, in the adult Amia, as the "selbständig verknöcherte äussere zellige Chordascheide" (No. 110, p. 754), and by Hasse (No. 52, Fig. 14), as the "Faserscheide der Chorda," a part of the cuticula chordae.  What is apparently the same layer in teleosts and selachians is called by Göppert (No. 48) the "Chorda-

scheide," while in Amphiuma it is considered by Field (No. 33, p. 346) as the cuticula sceleti. This layer in Amia, whatever its origin and character, is, with the notochord, in 30 mm. specimens, strongly constricted. There are, however, no breaks in it, as there are in Amphiuma, and no indication whatever of intervertebral or intercuticular cartilage, as in that animal. The vertebral constriction and subsequent segmentation of the notochord in Amia are, therefore, due to the compressive action of the cartilaginous processes, which always lie directly opposite and in the constrictions, and not to an intervertebral expansion of the notochord, as in Amphiuma (Field). The vertebral processes in Amia are, accordingly, in origin as well as in position, strictly vertebral; the dorsal arches, which lie between the dorsal processes and directly opposite the crests of the notochordal constrictions are, on the contrary, so far at least as their position is concerned, as strictly intervertebral.

These relations do not change in 14 mm. or 12 mm. larvae. The dorsal processes on each side in 12 mm. larvae are, however, found forming a continuous line, the dorsal "Längleiste" of Göppert (No. 48, p. 168). In this line the dorsal arches are imbedded at their bases. The outlines of their bases are, however, distinctly indicated, and they lie opposite the places where later the crests of the notochordal constrictions appear.

Whether there is also a continuous ventral line, such as Göppert describes, formed by the fusion of the lateral processes, or two such lines, one representing the line of the lateral processes, and the other that of the ventral processes, was not investigated.

From the literature at my disposal I am unable to satisfactorily homologize the cartilaginous portions of the vertebrae in Amia with those described by others in other animals. The lateral processes of all, or at least most, fishes — Amia being especially mentioned — are called by Baur (No. 7, p. 119) parapophyses, and are defined by him as the proximal, independent ends of the ribs or haemapophyses. What, then, are the ventral processes in Amia which Baur does not mention? They have

exactly the position of ventral arches, and structures resembling them in every respect in larvae of Salamandra maculosa are considered by Göppert (No. 47, p. 444) as probable remnants of such arches. They are also, almost unquestionably, the homologues of the two little bony processes described by Göppert on the ventral surface of the body vertebrae of Cala-moichthys (No. 48, p. 150). These processes in Calamoichthys become, in the tail, the basal processes (Basalstümpfe) of the haemal arches, the remaining, distal portion of those arches being formed by the " Pleuralbögen," or lower ribs, the haema-pophyses, therefore, of Baur, which shift from the under surface of the lateral processes onto the distal ends of the ventral ones. In the trout, in marked distinction to Calamoichthys, no such shifting process takes place (No. 48, p. 187). The lower ribs, or Pleuralbögen, in that fish, simply disappear, and the haemal arches are formed by direct and independent growth from the ventral surface of the base of the Basalstümpfe, the parapophyses of Baur. Judging simply from Schmidt's figures, Amia must agree with the trout in this respect. The haemal arches in the tail of Amia would then be homodynamous with the ventral processes found on the trunk vertebrae, and with those processes only. The ribs would then take no part in the formation of the haemal arches in Amia, contrary to what Göppert states to be the case in all ganoids excepting Calamo-ichthys (No. 48, p. 153), and would agree with those of teleosts (No. 48, p. 161). The ribs in Amia may, therefore, be the homologues of the upper ribs of Calamoichthys, and not of the lower ones. If this be so, the ventral processes in Amia are the parapophyses of Baur. The lateral processes would then of necessity be, as in Polypterus, diapophyses (Baur), and the ribs pleurapophyses or true ribs.

Göppert states (No. 48, p. 153), based on the observations of Balfour and Parker, that the ribs in Lepidosteus present some of the characteristics of upper ribs ; and Scheel states (No. 109, *Abstr.*) that the ribs in teleosts are such ribs, and not lower ribs. The ribs in teleosts lie in the lining wall of the peritoneal cavity. If they be upper ribs they must therefore have lost the position assigned by origin to them, that is, in the horizontal

muscular septum where that septum is crossed by the transverse intermuscular septa (Rabl, Göppert). In Lepidosteus, on the contrary, they still lie in the horizontal septum. A condition intermediate between these two is indicated in the anterior ribs of larvae of Amia, which lie buried, at their proximal ends, in the trunk muscles, approximately at the level of the horizontal septum. A still less advanced condition is shown in the rib-like ligaments which lie in front of the anterior ribs in Amia, for although lying below the horizontal septum they lie, even in the adult, entirely in the trunk muscles.

The horizontal muscle septum is said to be a relatively late acquisition (Rabl, quoted No. 48, p. 166). Being such, one would naturally suppose that the " Anlagen " of the upper ribs would lie in the transverse septa, immediately ventral to the horizontal septum, rather than in the latter septum itself. Why, then, should a relatively slight difference of position, in those septa, be considered of such great morphological importance that ribs found in the horizontal septum should be called upper ribs, and ribs found out of it, in totally different classes or orders of animals, lower ribs ? Göppert says (No. 48, p. 196) that " Gleichzeitig mit dem Auftreten der Pleuralbögen hebt sich das Niveau des horizontalen Septums." Why not say that as the horizontal septum began to rise in level, the ribs, developed primarily in connection with it, began to descend, relatively to it, and finally acquired a position in the wall of the peritoneal cavity ? That in such a process, in certain fishes, small remnants of the original cartilaginous " Anlage " should remain attached to the horizontal septum, and there appear as cartilaginous parts of the " Seitengräten," or as the cartilagines intermusculares, would not seem improbable ; nor that, in other fishes, ribs should be occasionally found on the first tail vertebrae, as in Salmo (No. 48, p. 160). As the true upper ribs thus acquired a new position, the lower ribs, which Göppert considers as the more primitive structures, would naturally disappear. They are still retained in Polypterus and Calamoichthys, which thus, in this as in many other things, show a more primitive organization than that of Amia or the teleosts. In selachians also, remnants of them seem sometimes to be still retained, as in the

little pieces of cartilage described by Gegenbaur in Cestracion (quoted No. 48, p. 154).

For the dorsal processes of Amia Baur gives no name, and I find no description, that I can satisfactorily identify, of such processes in any fish or other animal. They may be centra or pleurocentra, or the interarcualia of Klaatsch in Chimaera (No. 64, *Abstr.*), or possibly the basi-dorsals of Gadow and Abbott (No. 39). They are certainly not rudimentary dorsal arches (Schmidt), nor can they be considered as the proximal ends of dorsal arches, for they are segmented, after the formation of those arches, from a continuous cartilaginous layer, or "Anlage," and they lie, in larvae, between the dorsal arches. The arches themselves are certainly neuropophyses, but they are not, in their origin, processes of the vertebrae. The whole subject is to me much too obscure to venture upon names.

The dorsal portions of the anterior intermuscular septa, in larvae of Amia, differ considerably, in their relations to the vertebral processes, dorsal arches, and dorsal spines, from what has been described in the adult. In 12 mm. larvae they run downward and forward along the antero-lateral edges of the dorsal arches, then turn sharply forward, practically at a right angle, as is well shown in vertical sections, onto the dorsal process in front of the arch to which they belong, and then downward, at a right angle again, onto the corresponding lateral process and rib. Ventral to the rib they turn backward, and seem to have their attachment, or origin, in the region where, later, the crest between two constrictions of the notochord will appear. They are thus, at this age, restricted to a single dorsal arch, and are, in their central portions, vertebral in position. In their dorsal portions they are intervertebral in position, as the dorsal arches themselves are.

Between the age represented by 12 mm. larvae, and the adult, there must, therefore, have been a gradual backward displacement of the middle portion of that part of each of the anterior intermuscular septa that lies above the level of the vertebral column, the displacement becoming continually more pronounced up to its complete development. The amount of this displacement certainly varies, not only at different ages, but also in

different parts of the vertebral column and of the body. Where it first begins, at what age, and the amount of it at different ages, I have not attempted to determine, as it belongs more properly to a later study. It certainly begins at very early ages, for even in 12 mm. larvae it is already plainly evident. The course alone of the septa, in part vertebral and in part intervertebral, sufficiently indicates this, for it it generally accepted that the septa are, primarily, through their entire length, strictly vertebral in position. As the dorsal arches arise in the septa, even these arches must, in 12 mm. larvae, have shifted somewhat from their original position. In this shifting, the arch and septum would naturally carry with them the nerve that innervates the muscle segment next posterior to them. Such at least is the case in Amia ; for in larvae, as in the adult, the nerve that issues behind an arch innervates the muscle segment that lies behind the septum of the vertebra next anterior to that arch. My work thus tends to confirm Kollmann's conclusion (quoted No. 24, p. 20) that the dorsal arches in human embryos shift backward relatively to their vertebrae.

Schmidt, from an anatomical and histological investigation of the vertebral column of the adult Amia, concludes (No. 110, pp. 751 and 755) that there were, originally, in each body segment, two vertebral " Anlagen." These " Anlagen " in the trunk fuse to form a single complete vertebra, the dorsal arch of the posterior half-vertebra, or intercentrum, remaining as the dorsal arch of the complete one, and that of the anterior half-vertebra, the centrum, persisting as a cartilaginous process, on the dorsal surface of the vertebra, immediately in front of that arch. This process, or rudiment of a dorsal arch, as he considers it, can be nothing more nor less than the anterior half of what I have described as the dorsal cartilaginous process of the vertebra, a part of that vertebra and not of an arch. In larvae, as in the adult, there is not the slightest indication of an arch that has disappeared, or of two vertebral bodies that have fused. Schmidt's conclusion, which simply confirms Ihering's earlier statement (No. 61), thus finds no confirmation in my work, so far as it has gone. Regarding Gadow and Abbott's conclusions (No. 39, p. 298), based on

the study of a 57 mm. Amia, I can form no opinion as yet. It is to be noted, however, that they consider the precentrum, or arch-bearing element of a complete vertebra, the intercentrum, therefore, of Schmidt, as the anterior of the two elements of a complete vertebra, instead of the posterior. This anterior element of the complete tail vertebra is described by them as bi-protovertebral or bi-myomeric in origin, being formed by the fusion of "the basidorsal of one sklerotome and the basiventral of the next previous sklerotome." The posterior, archless disk of a complete tail vertebra, the postcentrum, is formed by the interdorsalia and interventralia of the same sklerotome. "The intermuscular septum runs obliquely across the precentrum." The complete trunk vertebrae are thus, according to them, partly bi-myomeric, instead of being myomeric, as Schmidt finds them ; and the dorsal arch that belongs to any particular vertebra must be, in 57 mm. specimens, and hence also in the adult, the one that lies in front of that vertebra instead of the one that lies behind it. Gadow and Abbott, therefore, assign the arches to the vertebrae next posterior to the ones to which Schmidt assigns them. Schmidt's conclusions in this respect agree with the earlier statements of Sagemehl (No. 104) and Ihering (No. 61).

Although my own work as yet warrants no opinion on this subject, I cannot avoid the impression that each "Anlage" of a dorsal arch, arising, as it is said to, from parts of two adjoining somites, must, because of this double origin, have inherently in itself the possibility of forming two separate pieces ; and that these two pieces may remain separate, giving origin to two arches, or that they may fuse, or that one of them may disappear, thus giving origin in either case to a single arch. The single arches so formed would doubtless always remain septal at their bases ; they would, however, tend, because of their different origin in different species, to occupy different positions on, or relative to, their vertebrae. Those vertebrae, also, even in nearly related forms, may not be homologous structures, for Gadow and Abbott (No. 39, p. 298) state that the vertebrae of Lepidosteus are truly bi-myomeric, while those of Amia are not so.

In addition to these possibilities of some difference in the developmental composition of the parts concerned, there is the strong probability, already explained, that the dorsal arches may, in early stages of development, shift somewhat, with their septa, relatively to their vertebrae. Can a dorsal arch, then, be definitely assigned in every case, without proper investigation, to the vertebra to which it has, in the adult, its apparent attachment? It seems to me not, and yet it is evidently of the first importance to know, definitely, to which of two adjacent vertebrae a dorsal arch should be assigned, when the vertebral composition of the occipital part of the skull is under consideration. It is also of first importance in the naming or numbering of the spinal and occipital nerves.

The spinal nerves are usually assigned to the vertebrae that bear the dorsal arches next in front of them, and named in consequence. This does not, however, apply to the complete serial numbering of the nerves, for the nerve in front of the first vertebra, between that vertebra and the hind end of the skull, is usually counted as the first spinal nerve. Contrary to this generally accepted method Willis considered it in man as the last cranial nerve, and Froriep (No. 34, p. 230) considers the corresponding nerve in the chick as the last occipital nerve, or nervus postoccipitalis. That the nerve, in Amia, is in reality the first spinal nerve, and not the last occipital one, seems certain if Ihering is correct in his statement (No. 61) that in the tail of Amia, the nerve of each segment issues in front of the arch of that segment. It is evident that the general distribution of the nerve itself can give no definite solution of the problem, for the nerve belongs, in that distribution, to the muscle segment between two vertebral septa, and not to the vertebrae to which those septa were primarily attached.

Unable from my own work to arrive at any satisfactory conclusion on this subject, I follow, for the present, Schmidt in the serial numbering of the arches, and Ihering — that is, the generally accepted method — in the numbering of the nerves. The first spinal arch in all my figures and descriptions is, therefore, the arch that lies between the first and second vertebrae,

the first spinal nerve the nerve that lies between that arch and the hind end of the skull.

In front of the first spinal dorsal arch, defined as above, between it and the occipitale laterale, there are still, in Amia, two dorsal arches, the occipital arches ($DA^o$) of Sagemehl. The anterior of these two arches rests in the hollow between the two transverse wedge-shaped ridges on the cartilaginous strip on the dorsal surface of the basioccipital, the posterior one in the hollow between the posterior ridge and the dorsal cartilaginous process on the first vertebra. These transverse occipital ridges are, therefore, dorsal vertebral processes, morphologically similar to the dorsal processes of the vertebrae. The posterior occipital arch is fused with the posterior face of the posterior process, while the anterior arch is fused, not with the posterior face of the anterior process, but with the hollow between the two processes. This hollow lies, as already stated, in the plane of the slight groove or line that separates the posterior, vertebra-like end of the basioccipital from the rest of that bone.

Between the dorsal end of the two pieces forming each occipital arch, there was, in all young specimens, a processus spinosus ($PSP$), performed, in part at least, in cartilage, as are the corresponding processes on the spinal arches. In some of the adult specimens there were, also, two such occipital processes, as Sagemehl has already stated (No. 104, p. 190). In others there was but one, the process, or plate (Sagemehl), in such cases, having two upper ends or spines, one directed backward and the other forward. No separate piece was found, in any specimen, between the first spine and the occipitale laterale, as shown in Sagemehl's Fig. 3 (No. 104), and described by him in a footnote to page 190. Sagemehl suggests (No. 107, p. 524), that one of these two occipital spines may fuse with, or become incorporated in, the skull of teleosts and Amniota, and there give origin to the supraoccipital. He also makes the general statement (No. 107, p. 526), that the latter bone is found in all vertebrates in which one or more nerves issue from the cranium posterior to the vagus. This is certainly not entirely true, for two such nerves issue from the cranium of Amia, and Amia does

not possess the bone. Sagemehl himself was well aware that
one such nerve is found in Amia, for he himself first described
it. Doubting the origin of the bone assigned to it by Sagemehl,
I am inclined to consider it as primarily of membranous origin,
developed in connection with the tendinous attachment of
muscles, as the intercalar evidently is. That it should not be
developed in ganoids, dipnoids, selachians, or amphibians is not
singular, if the schema given in Fig. 12, Pl. XXII, is correct.
The bone is, in that case, simply a late acquisition, and hence
found naturally, and developed independently, in teleosts and
Amniota.

The intermuscular septum that has its attachment on the
posterior occipital arch runs downward onto the vertebral-like
end of the basioccipital, and then onto the large ligament that
arises, rib-like, from that end. The intermuscular septum that
has its attachment on the anterior occipital arch runs down-
ward in front of the vertebra-like end of the basioccipital, onto
the small anterior occipital ligament.

Starting from the hind end of the skull, partly from the
cartilage of the skull, and partly from the adjoining median
edges of the two occipitalia lateralia, and running directly
backward between the dorsal arches, there is a strong liga-
mentum longitudinale vertebrale dorsale superior (*lvd$^s$*, Fig. 9,
Pl. XXI). In the 50 mm. larva examined in transverse sections,
there were, immediately below this ligament, between the two
halves of the last dorsal spinal arches included in the sections,
two independent pieces of cartilage, one on each side, forming
a bridge between the arches, and between the ligament and the
spinal cord. These little pieces are described by Scheel (No.
109) in Trutta and other teleosts, and are considered by him
as the " ursprüngliche Fortsetzung der Neuralbogen."

The ligamentum longitudinale dorsale inferior (*lvd$^i$*, Fig. 18,
Pl. XXII) starts from the front edge of the dorsal surface of
the solid end of the basioccipital, and runs backward between
the dorsal occipital processes, and then between the dorsal
spinal processes. From it arise, as already described, the little
lateral projections that fill the indentations in the bases of the
dorsal arches.

We thus have in the solid end of the basioccipital of the adult Amia all the parts of two vertebrae, or vertebral segments; the longitudinal ligaments, the intermuscular septa, the lateral processes and ribs represented by the two occipital ligaments, the dorsal arches, the dorsal processes, and the ventral processes. That still other vertebrae also have fused with the skull anterior to these two vertebrae is indicated by the nerves and muscle segments, and by the development of the occipitale laterale.

### 4. Occipital Nerves and Corresponding Muscle Segments.

From each of the spinal ganglia (*gsp*) in the adult Amia four well-developed nerves or branches arise (Fig. 35, Pl. XXIX): a dorsal branch (*spd*), which runs upward and backward along the posterior face of the septum in front of the segment to which the nerve belongs ; a horizontal branch (*sph*), which runs outward to, and then outward and backward along, the same face of the same septum, lying always above the horizontal septum between the dorsal and ventral muscles ; a small branch (*spa*), which runs upward and backward, but morphologically forward, through the septum in front of the segment to join the dorsal branch of the second anterior spinal nerve ; and a large ventral branch (*spv*), which runs downward and backward in front of the septum posterior to its segment, along the anterior edge of the rib that lies in that septum.

In larvae the spinal ganglia lie wholly on the dorsal root of their nerve. The ventral root issues close to the ventral end of the ganglion, but does not touch it. Here it gives off the dorsal and horizontal branches above described, which are, respectively, the ramus dorsalis and ramus ventralis lateralis of von Lenhossék (No. 73, p. 173). It then turns downward to form the principal part of the large ventral branch of the nerve, the ramus ventralis medialis of von Lenhossék. The remaining portion of this ramus medialis is formed by a large nerve which arises from the ventral end of the ganglion of the dorsal root, and runs downward and slightly outward in front of the dorsal and horizontal branches of the ventral root. No fibres of this

nerve were seen going to either of the other two branches of the ventral root, but they may nevertheless exist, as in the chick (von Lenhossék), for my sections not being especially prepared for such work, I made no special search for them. I also did not attempt to find whether the small communicating, or anterior, branch of the nerve arose from the dorsal or from the ventral root. This communicating branch in young larvae runs directly forward instead of upward and backward as in the adult. The sympathetic ganglia did not lie, as in the chick, upon the ventral branches of the ventral roots. From the dorsal end of the spinal ganglion, in some sections, there was a dorsal prolongation and possibly also a delicate dorsal branch. I could not, however, trace the latter either in larvae or in the adult.

The spinal nerves in Amia thus seem to differ considerably from the schema given by Hatschek of the nerves in Amphioxus (No. 54, p. 140).

The nervus postoccipitalis, or first spinal nerve, agrees in every respect with the other spinal nerves, and in front of it there are still two nerves ($oc4$ and $oc3$, Figs. 33–35, 63, and 64, Pls. XXVIII, XXIX, XXXVII, and XXXVIII) also agreeing in every respect with those nerves. They issue and lie, one in front of each of the two occipital arches, and are described by Sagemehl (No. 104, p. 193) as occipital nerves. Sagemehl, however, did not find, or at least does not mention, the ganglion found on the dorsal root of each of them. In front of these two, complete, spinal-like, occipital nerves there are two ventral roots ($oc2$ and $oc1$), one of which, the posterior one, is described by Sagemehl as the first occipital nerve. The other he did not find, nor did he find the foramen by which it issues from the skull. No other still more anterior nerve being found, I have called these nerves the first and second occipital nerves. As they belong, as will be shown later, to the second and third occipital muscle segments, they might, perhaps with better reason, be called the second and third occipital nerves. From each of them arise the dorsal, horizontal, and ventral branches found on the ventral roots of all the spinal nerves. The small, anterior, communicating branch found on all the spinal nerves was not, however, found on them.

In young larvae the two anterior occipital nerves arise directly ventral to the two posterior roots of the vagus, often lying in the same transverse section with those roots. These two roots of the vagus arise, even in large larvae, considerably behind the other roots of the nerve, and run forward a relatively long distance internal to the occipitale laterale to join those roots. In one 12 mm. larva, cut in vertical sections, all the roots of the vagus were cut in one section. In that specimen there were six distinct roots, all of fairly equal size, and all converging together to form the common root of the nerve. The posterior of these six roots was formed by the fusion of the two roots above described, and on these roots was found a considerable part of the intracranial ganglion of the nerve. If these two roots are, as they seem to be, the dorsal roots corresponding to the two independent ventral ones, we have, in Amia, four complete spinal-like occipital nerves, and a postoccipital, or first spinal nerve which must also be assigned, as its dorsal arch is, to the occipital region of the skull. The nerves thus indicate five vertebral segments fused with the hind end of the skull. If the last occipital arch be assigned to the first vertebra, there would be but four.

In 12 mm. larvae the occipitale laterale is a piece of cartilage running upward, outward, and forward, touching, or almost touching, at its upper end, the hind wall of the auditory capsule, but not fused with that wall. It is, however, connected with the wall by a line of tissue, in which cartilage later develops. At its lower end, which is much enlarged, it is imbedded, like the occipital and spinal dorsal arches, in the as yet unsegmented cartilaginous line of the dorsal processes. On the dorsal surface of its lower end there is a slight elevation, so that, in vertical sections passing through this portion, it has exactly the shape and appearance of the following occipital and spinal arches. It is, however, inclined forward, instead of backward as these arches are. The cartilaginous line of the dorsal processes here drops gradually from near the dorsal surface of the notochord to its lateral aspect, and in front of the occipitale laterale it is continuous with, or becomes, the base of the skull. The notochord everywhere lies

between and separates the two sides of the hind end of the skull, the cartilage in no place passing either above it or below it. Farther forward, where the notochord becomes smaller, the sides of the skull recede from it, and a wide cleft is left which continues beyond the notochord, and there becomes the hypophysial fenestra of the adult. In later stages the median processes of the petrosals develop, approximately at the anterior end of the notochord, and the hypophysial fenestra, or anterior portion of the cleft, is thus separated from its posterior, occipital portion. The ventral portion of the original cleft persists, however, in this intermediate portion, as a groove, and is found as such even in the adult. The occipital portion of the cleft is filled, in the adult, by the median, anterior process of the basioccipital. That structure may, therefore, be the ossified notochord, if that structure ossifies, or membrane bone, if it simply disappears.

The hypophysial fenestra of the adult Amia seems to be the opening, the posterior end of which, in the chick, is said, by Froriep (No. 34), to mark the anterior limit of the occipital region of the skull. This limit, in the Characinidae, is marked, according to Sagemehl, by the anterior end of the basioccipital, which extends forward to the hind edge of the petrosal (No. 106, pp. 42 and 61). In Amia it is marked by the bases of the transverse processes of the petrosals. The occipital cleft of Amia is, therefore, not found in adult teleosts or in embryos of the chick. In the latter the notochord and parachordalia seem, from Froriep's description, to extend as far forward as the sphenoid bone, the basisphenoid apparently of Amia. The parachordalia in Amia, whatever their anterior limit may be, are, posteriorly, a direct continuation of the cartilaginous line of the dorsal processes of the vertebrae.

Antero-ventral to the occipitale laterale in 12 mm. larvae, in the triangular space inclosed between it, the base of the skull, and the bulging hind end of the auditory capsule, the nervus vagus and the two anterior occipital ventral roots have their exit. The vagus issues at the upper, anterior angle of the triangle, the second occipital nerve near its lower, posterior angle, and the first occipital nerve about midway between them. The

two ventral occipital roots and the nervus vagus thus issue, at this age, through the same foramen.   The occipitale laterale, at this age, may therefore be considered as a dorsal occipital arch that has inclined forward against the hind end of the auditory vesicle, or as parts of three such arches that have fused with each other and are about to fuse with the auditory vesicle, as later stages show.   In the former case, the arch, inclining forward, has pushed before it, against the vagus, the dorsal roots of two occipital nerves ; in the latter, the two dorsal occipital roots, if they do not abort, have, in joining and fusing with the vagus, cut through the ventral ends of the "Anlage" of two dorsal arches, so retarding or deranging their development that no trace of their ventral ends is found in 12 mm. larvae.   The vagus, under either supposition, would lie immediately behind the hind wall of the auditory vesicle, and would, if the dorsal occipital roots have not entirely disappeared, be formed by the fusion of the dorsal roots of three or more postauditory nerves.

That the occipitale laterale represents three, rather than one, dorsal arch is shown by its relations to the muscle segments and intermuscular septa.   The first septum, the one that lies between the first and second segments, has its attachment, in 12 mm. larvae, near the upper, outer end of the occipitale laterale ; the second has its attachment near the middle of that piece, and the third, to the elevated portion of its lower end.   The fourth septum is attached to the first occipital arch, the fifth to the second occipital arch, the sixth to the first spinal arch, etc.

The dorsal portion of the first muscle segment, the segment lying in front of the first septum as above defined, arises, at its anterior end, from the hind wall of the auditory capsule, and extends across the upper, unformed end of the occipitale laterale.   The ventral portion of this same segment arises from that thickened portion of the base of the skull that forms the hind boundary of the eye-muscle canal, and lies along the ventral portion of the lateral surface of the hind end of the skull, appearing, in vertical sections, below the notochord.   It extends nearly, if not quite, to the anterior end

of the notochord, and it lies, in sections, approximately opposite the united vagus roots. Opposite the second segment, and distributed to that segment, lies the anterior occipital nerve ; opposite the third, the second nerve ; and opposite the fourth and fifth, the first and second occipital ganglia. No ventral root was found opposite to, or belonging to, the first muscle segment, nor could the innervation of that segment by branches of other roots be established. It seems, however, probable that it is innervated by branches of the first occipital nerve.

In 20 mm. larvae the occipitale laterale has fused, at its upper end, with the hind end of the auditory capsule, and at its lower end, with the cartilaginous base of the skull. In certain vertical sections through it, it is seen to have two thin portions, with a third thin portion between it and the skull. It therefore presents three transverse enlargements. From the hind end of the first or anterior of these enlargements the first intermuscular septum arises; from the hind end of the second, the second septum; and from the third, the third septum. The third enlargement is fused with, and forms part of, the enlarged hind end of the base of the skull. Posterior to that base, between it and the first separate cartilaginous, dorsal vertebral process, lies the first dorsal occipital arch, and other arches and processes follow regularly. The dorsal process between the two occipital arches is thus, at this age, not fused with the hind end of the cartilaginous base of the skull, and we have a vertebra or part of a vertebra in process of absorption.

The vagus foramen in 20 mm. larvae is beginning to be enclosed by a cartilaginous process which forms between it and the first occipital nerve. In 25 mm. larvae it is entirely inclosed, and at 30 mm. the two first occipital nerves are also separated by a line of cartilage. The other arrangements and relations of the parts here under consideration are the same as at 20 mm., except that the trunk muscles already push forward somewhat into the temporal groove, and that a thick, tough line of tissue begins to be evident extending from the upper, hind edge of the skull backward into the first muscle segment.

This ligament is found strongly developed in the adult, arising from the hind edge of the parietal under the extrascapular.

The suprascapular lies opposite the second muscle segment, and the nerve innervating the lateral sense organ contained in it, organ No. 19 infraorbital, lies opposite the outer edge of the first intermuscular septum. The pedicle of the suprascapular is, at this age, still a ligament, lies opposite the first septum, and has its attachment to the skull immediately in front of that septum. It may, therefore, be homologous with the ligaments that arise from the hind end of the basioccipital and lie in the fourth and fifth septa. The upper end of the supraclavicular lies opposite the third and fourth segments, and the nerves to organs 20 and 21 infraorbital lie opposite the second and third septa.

In the adult the muscle segments (*Ms*) have not the simple arrangement found in young larvae. The attachment of the intermuscular septa (*ims*) to the vertebral column, and the line followed by their median, inner edges has already been given. Their outer edges do not have such a zigzag course. Starting from the mid-dorsal line they run first strongly backward, and then curve downward and forward to a point approximately in the line of the lateral edge of the skull. There they turn backward and downward in a gentle curve, cross the line of the lateral-line canal, turn downward and slightly forward to the line of the outer ends of the ribs, and then curve gently forward and downward toward the mid-ventral line. As they cross the lateral line and the line of the outer ends of the ribs there is a slight notch or interruption in the line of the curve.

The septa and segments in front of the first rib are always short, and while they cannot strictly be said to be irregular, they vary somewhat in different specimens and present a much more complicated arrangement than the posterior segments.

Ventral to a radial, longitudinal plane passing, approximately, through the line of the lateral edge of the skull, the septa and muscle segments incline regularly outward and backward, their outer ends lying posterior to the inner ones. Above that plane they are not so regularly arranged. The septa in the middle point of the muscle mass so defined have been pulled

backward, so as to form a pocket, the hind end of which lies considerably posterior both to the outer and to the inner edges of the septa. The septa and segments in this part run inward and backward from the outer surface of the body to the hind end of the pocket, and then inward and forward to the mid-vertical plane of the body. The cause, whatever it may be, that has led to the formation of these pockets is certainly the one that has also caused the shifting of the inner edges of the septa. A good and sufficient reason would seem to be found in the fact that the tail is capable of, and subject to, violent movements in early larval stages when the egg-yolk has not yet been absorbed. The trunk, or at least its anterior portion, is held by the yolk practically immovable, and as something must yield when the muscles contract, it is apparently the trunk septa, which are thus pulled backward by the functionally more active muscles of the tail. If the tail movements began before the dorsal arches had become fixed in position, those arches would doubtless yield and shift with their septa, the amount of the shifting depending upon the state of development of the vertebral column when the tail movements first began. The varying and perplexing relations of the dorsal arches to the vertebral bodies might thus be simply and naturally accounted for.

At its anterior end the limited muscle mass here considered extends into the temporal groove and above and below the vagus foramen, the vagus and nervus lineae lateralis, as they issue, lying in a notch in the front edge of the mass. By this notch the first muscle segment is always cut into two portions, and the second segment almost but not entirely so. The first intermuscular septum, the septum between the first and second segments, is not, however, usually cut through; it is simply interrupted and pressed back by the issuing nerves toward or against the second septum, so that the second segment is pinched off at that point. Amia thus shows, in this, practically the same arrangement as that described by Hoffmann (No. 57) in Acanthias. It seems to me, however, that the vagus cuts into the muscle segments, rather than that the latter push forward above and below the nerve.

Above the vagus, the first intermuscular septum, follows the postero-lateral edge of the skull to its upper surface, and then runs medianward along the hind edge of the squamosal and parietal under the extrascapular. There it is somewhat broken, so that two or three parts of it appear as short ligaments, varying slightly in every specimen. Below the line of its attachment to the dorsal edge of the skull it is pushed forward into the temporal groove, crowding out the first muscle segment, so that it is the anterior surface of this part of the first septum that forms the upper surface of the muscle mass that fills that groove. In the first septum there are thus two pockets, a median one directed backward, as in the other septa, and a lateral one directed forward into the temporal groove. Similar but much less marked lateral pockets are found in the second, third, and following septa. The second septum, and even the third, may acquire, with the corresponding muscle segments, an insertion in the temporal groove.

Into the posterior pocket of the first septum, from the hind edge of the parietal, a strong, flat, ligamentous structure extends, and ends free without insertion of any kind. From its dorsal and ventral surfaces muscle fibres arise, and have their insertions on the adjoining surfaces of the pocket. They represent all that can be found of that part of the first muscle segment that lies above the vagus. Below the vagus the first muscle segment is represented by a small pocket of muscle fibres found at the anterior end of the trunk muscles. Ventral and median to this point the edge of the trunk muscle runs backward and inward, and opposite the hind end of the basioccipital almost reaches the ventral cartilaginous processes of that bone (Fig. 63, Pl. XXXVII). At this point the trunk muscles cover ventrally the lateral occipital ligaments. The edge of the muscle mass then turns outward and backward, but the mass still covers ventrally the lateral ligament on the first vertebra and the lateral process and proximal end of the rib on the second. The lateral process and rib on the third vertebra are left fully exposed. One rib and three rib-like ligaments thus lie imbedded at their proximal ends in the upper end of the ventral portion of the trunk muscles.

The course of the outer edge of the first intermuscular septum has been given. The second runs from the mid-dorsal line, outward and backward, approximately along the posterior edge of the extrascapular. It then turns abruptly downward and forward, and runs between the posterior end of the supra-scapular and the anterior and upper end of the supraclavicular. The third septum reaches the under surface of the supracla-vicular near its upper end. It then turns downward and back-ward, and is attached to the corium, approximately opposite the anterior end of the first scale of the lateral line. The same part of the fourth septum corresponds, approximately, to the anterior end of the second scale of the lateral line, etc. The seventh septum lies close against the inner, posterior sur-face of the muscles of the pectoral fin, and is the first septum that extends unbroken from the mid-dorsal to the mid-ventral line. Between the ventral end of this septum and the ventral end of the clavicle, below the fin, there were, in the several specimens dissected, but one muscle segment and no muscle septum. The sixth septum always ended as a ligament attached to the clavicle at the lower edge of the fin, and the fifth as a similar ligament attached to its upper edge. The fin always lies in a deep impression in the trunk muscles, the muscle mass being pushed inward so that its anterior edge, at this place, lies internal to the inner edge of the clavicle. By this arrange-ment the sixth segment is apparently cut off. As there are, however, muscle fibres between the sixth septum and the clavi-cle, above the fin, the clavicle, at its ventral end, probably corre-sponds to the fifth intermuscular septum, that is, to the septum that has its attachment to the second occipital arch. The sternohyoideus, which lies in front of the clavicle, thus belongs, in its relation to the muscle segments, as it will be shown that it does in its innervation, entirely to the region of the head, and not in part to the trunk.

In one specimen shown in Fig. 34, Pl. XXVIII, the septa were still further complicated, for the seventh septum had been pushed back by the fin against the eighth septum, and was found partly fused with that septum. The eighth septum had a surface septal connection with the ninth septum. In front

of the ventral end of the seventh septum there was a small piece of septum and a small muscle pocket which doubtless represented the missing sixth segment.

The ventral branches (*ocv* and *spv*, Figs. 33, 34, and 57, Pls. XXVIII and XXXV) of the four occipital and first spinal nerves come to the outer surface of the trunk muscles posterior to the segments to which they belong, and vary greatly in their points of exit. The ventral branch of the first occipital nerve is a very delicate nerve which usually issues at, or immediately behind, the third septum. It always joins and fuses with the ventral branch of the second occipital nerve, which issues from the fourth segment or at the fourth septum. The two nerves united join the ventral branch of the third occipital nerve, which issues between the fourth and fifth septa; and the nerve or trunk so formed sends a communicating branch to the ventral branch of the fourth occipital nerve. It then turns downward and forward, behind the hind end of the visceral arches, along the anterior surface of the ventral portion of the trunk muscles, and near the inner, anterior edge of the clavicle. It passes internal to both the pharyngo-claviculares, and reaches the dorsal surface of the sternohyoideus near the median line of the body. It there gives off several cutaneous or general branches, and, continuing forward, sends branches first to the middle, and then to the anterior, divisions of the sternohyoideus, and ends in a delicate branch which innervates the branchiomandibularis.

In one specimen, and on one side only of that specimen, a large branch was found which arose from the third occipital nerve, and passed dorsal to, that is, internal to, the second occipital nerve. There it received an anastomosing branch from that nerve, and then ran outward and upward internal to the nervus lineae lateralis. As the specimen had been cut, in dissection, above that point, the ultimate destination of the nerve could not be determined. Nothing anything like it was found in any other fish, and I can only think that it was some marked irregularity due to accident of some kind.

The ventral branch of the fourth occipital nerve issues, as it should, in its own segment, the fifth, along the anterior

surface of the septum posterior to that segment or imme-
diately behind that septum.    It always has a double dis-
tribution, going in part to the posterior segment of the
sternohyoideus and in part to the pectoral fin.    In some
specimens it separates into two parts, one going directly to the
posterior segment of the sternohyoideus, and the other joining
and fusing with the ventral branch of the nervus postoccipi-
talis, or first spinal nerve.    In others it touches and anasto-
moses with, or receives an anastomosing branch from, the ven-
tral branch of the third occipital nerve, and then joins the first
spinal nerve, or the contrary, anastomosing with the first
spinal nerve, and then sending a branch to the sternohyoideus.
The branch that goes to the sternohyoideus accompanies closely
the united ventral branches of the first, second, and third
occipital nerves, running sometimes, with those nerves, internal
to the pharyngo-claviculares, but sometimes perforating the
internal one of those two muscles.    It always gives off one or
more general cutaneous branches, one of which either perfo-
rates the pharyngo-clavicularis internus, or passes around the
anterior edge of that muscle to its outer surface.    The nerve
then enters the posterior division of the sternohyoideus along
its median face.

The ventral branches of the first spinal and last two occipi-
tal nerves often separate, in dissection, into two strands, and
the two strands of one or more of the nerves may be found
issuing as separate nerves from the trunk muscles.    This is
often confusing, and to be certain of the number of nerves
under consideration, the dissection must always be carried to
the ganglia themselves.

There are thus in Amia, in the adult, five muscle segments
and five intermuscular septa in the postauditory region of the
skull.    In larvae there are but four.    The sixth intermuscular
septum, in the adult, is attached to the first vertebra, and the
sixth muscle segment extends from it to the fifth septum,
which is attached to the hind end of the skull.    In larvae it is
the fifth septum and segment that have that position.    If the
intermuscular septa are vertebral, as is generally conceded, the
five postauditory septa of the adult must indicate five vertebrae

fused with the hind end of the skull to form the postauditory or occipital region. In larvae but four such vertebrae are indicated. ·Whether the dorsal arches may have shifted from the anterior end to the posterior end of their respective vertebrae, or not, makes no difference in the count. If, however, the last occipital arch of the adult is to be assigned to the first vertebra, the count would be diminished by one. The septa seem to preclude such a supposition.

### 5. Review and Comparison.

In comparing the occipital region in Amia with that in other animals, some fixed point must first be found. The anterior end of the first muscle segment seems to be such a point ; but there may have been still other, more anterior, muscle segments that have disappeared, as there certainly is one nerve that has disappeared, that of the first existing muscle segment. Another point that would seem to be fixed, and wholly independent of the possible disappearance of such segments, is the fifth intermuscular septum. This septum seems to mark, in both fishes and reptiles, the posterior limit of the muscle segments, or myotomes, from which the muscles of the tongue primarily develop; and the anterior limit, in fishes, of the segments that enter into the formation of the muscles of the pectoral fin.

In Scyllium and Pristiurus the coraco-hyoideus, or sterno-hyoideus, develops, according to van Wijhe (No. 130, p. 16), from ventral prolongations of the last three head myotomes and the first trunk myotome. Corning (No. 21), in Scyllium, assigns the same posterior limit to the myotomes so concerned, but adds the first head myotome to the number. In teleosts, also, Corning finds the "Hypoglossusmusculatur" arising from the first five myotomes, confirming in this Harrison's earlier observation (quoted by Corning). In reptiles both he and Mollier (No. 82) find the muscles of the tongue arising from the first five myotomes, but Corning states that in late stages these muscles receive additions from the sixth and seventh myotomes. In Acanthias the coraco-hyoideus develops, according to Hoffmann (No. 57, p. 639), from the fourth occipital myotome, that

is, from the tenth somite, or first trunk myotome of van Wijhe. The tenth somite of van Wijhe thus corresponds, in all these forms, in so far as its relations to the sternohyoideus are concerned, with the fifth muscle segment in Amia. In all these forms, also, the muscles of the pectoral fin in fishes, and of the neck and anterior extremity in reptiles, develop from myotomes posterior to van Wijhe's tenth somite; that is, in Scyllium (Corning), from the sixth to the fourteenth myotomes; in teleosts (Corning) and reptiles (Corning and Mollier), from the sixth to the thirteenth. In Scyllium the "Vornierenbläschen," and in reptiles the "Urniere," begin at the sixth segment. The fifth intersegmental space in all these animals seems, therefore, to mark a definite limit, and this limit corresponds to the fifth intermuscular septum in Amia. The first muscle segment in Amia is therefore, in all probability, the sixth somite of van Wijhe, and there are, in Amia, the same number of head segments as in Acanthias (Hoffmann), that is, one more than in the head of Scyllium, of Pristiurus, and of reptiles. The one additional segment in Amia fuses with the hind end of the skull in post-larval stages.

Opposite the sixth somite in Amia, as in Scyllium and Pristiurus (van Wijhe) and in teleosts (Harrison, quoted by Corning), lie the united vagus roots with no corresponding ventral root; opposite each of the next two somites lies a ventral root, as in Scyllium and Pristiurus (van Wijhe) and Acanthias (Hoffmann). Directly dorsal to these two ventral roots in Amia, there arise two distinct and separate roots of the vagus, in all probability the dorsal roots of the somites, and on these roots there is part of an intracranial ganglion. Opposite these two somites in Scyllium and Pristiurus, van Wijhe, in his earlier work (No. 130), also found separate roots of the vagus. In later work he found a root opposite the seventh somite only (quoted by Rabl, No. 101, p. 118). In Acanthias, opposite the seventh somite, Hoffmann finds the posterior root of the vagus with a part of the vagus ganglion; opposite the eighth somite he finds a rudimentary ganglion which soon disappears. Opposite the ninth somite, or fourth muscle segment, in Amia, there is a spinal nerve with dorsal and ventral roots,

and a dorsal ganglion. Similar roots and ganglia are found in Scyllium (Corning), Pristiurus (Kaestner, No. 62, p. 188), and reptiles (Corning), while in Acanthias (Hoffmann) there is simply a rudimentary ganglion which soon disappears. Van Wijhe did not find in Scyllium the dorsal root or ganglion described by Corning in this somite, and Mollier also (No. 82, p. 483), in reptiles, seems to have found the first ganglion opposite the fifth segment and not opposite the fourth.

Amia, therefore, in its occipital region presents nothing new or different from what has already been described in selachians, teleosts, and reptiles. With Protopterus, as described by Pinkus, it is impossible to make any comparison, for in Protopterus (No. 89, p. 325) there are, in the occipital region of the head, three ventral roots, and three complete spinal nerves, two of which are called by Pinkus the first and second hypoglossi. The two posterior ventral roots are distributed to the "umliegende Visceralmuskulatur." The anterior one is simply said to run backward along the under side of the basis cranii, but as it joins, in the figures, the two posterior roots, it probably has the same distribution that they have. The two hypoglossi are distributed in part to the first and second trunk muscle segments, in part to the tongue, and in part they join branches of the first three spinal nerves to form the brachial plexus and take part in the innervation of the fin.

### GENERAL SUMMARY.

1. The eye-muscle canal of Amia and its tall, orbital opening present, in certain respects, striking resemblances to the pituitary fossa, or sella turcica, and the sphenoidal fissure of the human skull. The pituitary fossa, in man, is bounded behind by the dorsum sellae of the sphenoid bone, and in front by the olivary eminence of the same bone. In Amia the eye-muscle canal is bounded behind by the median, horizontal wings of the petrosals, and in front by a transverse cartilaginous bar, on the sloping, dorsal surface of which the optic chiasma rests. On this sloping surface, in Amia, as on the olivary eminence, in man, the optic nerves pass outward to their foramina, accompanied by the ophthalmic arteries. In the

fossa, in man, on each side of the pituitary body, are the
cavernous sinuses. These sinuses receive, anteriorly, from the
orbits, through the sphenoidal fissures, the ophthalmic veins ;
and posteriorly, behind the pituitary body, they communicate
with each other by the intercavernous sinuses. In Amia, in
the same position, the eye-muscle canal lodges, on each side, the
ophthalmic veins, which communicate with each other, in the
adult, under the hypophysis, but in embryos behind it. In
the cavity of each sinus, in man, lie the internal carotid artery
and the sixth nerve ; in the canal, in Amia, lies the latter one
only of these two structures, — the internal carotid, however,
lying near the edge of the hypophysial fenestra, in such a posi-
tion that it would of necessity enter the canal if the fenestra
were slightly enlarged. In the outer wall of each cavernous
sinus, in man, lie the third and fourth nerves and the ophthalmic
and superior maxillary divisions of the fifth nerve ; in the upper,
lateral chamber of the canal, in Amia, are found the same
nerves. In man, after traversing the fossa, the internal carotid
artery turns upward, between the optic and third nerves, and
internal to the anterior clinoid process ; in Amia it has the
same relation to the same nerves and to the hind end of
the basisphenoid. In man, the sphenoidal fissure transmits to
the orbit the third, fourth, and sixth nerves, the ophthalmic
division of the fifth nerve, and the ophthalmic veins ; in Amia,
the orbital opening of the canal similarly transmits the third,
fourth, and sixth nerves, the ophthalmic veins, and the radix
profundi, which latter nerve is, in all probability, the homologue
of the ophthalmic division of the fifth nerve in man. The
superior maxillary nerve, both in man and in Amia, does not
issue from the fossa with the other nerves, through the sphe-
noidal fissure, but through a separate and more posterior fora-
men. This foramen, in man, is said by Thane (No. 100) to be
cut off from the sphenoidal fissure ; in Amia it is certainly not
so cut off. The recti muscles arise, in man, from the sphenoid
bone, near the lower end of the sphenoidal fissure ; in Amia
they arise from the basisphenoid, at the lower end of the
orbital opening of the eye-muscle canal, or from the floor of
that canal, inside the opening.

2.  The canalis transversus of selachians is, in Amia, a groove extending transversely from orbit to orbit, immediately in front of the transverse, cartilaginous bar that marks the anterior limit of the eye-muscle canal, and immediately in front of the two ossifications of that bar, which are said to represent, in Amia, the basisphenoid of teleosts.

The eye-muscle canal of Amia and teleosts is not, therefore, the canalis transversus of selachians, as Sagemehl concluded it to be.   In Amia it is a cavity or space formed in late larval stages around the hypophysis cerebri and saccus vasculosus ; and entrance to it, for the muscles of the eye, has been acquired through the foramen of the nervus abducens, or along a canal which serves for the passage of an important ophthalmic vein.   This vein arises from the under surface of the hypophysis, in connection with the corresponding vein of the opposite side of the head, and is almost directly continuous, anteriorly, with another vein, which arises from the choroid gland.   Both veins are branches of that part of the orbital venous sinus that lies in the upper, lateral chamber of the eye-muscle canal.

3.  The internal carotid artery enters the cranial cavity through a special canal which traverses the basis cranii along the median edge of the basisphenoid of its side of the head. The basisphenoid of Amia thus lies lateral to the artery, while in Scomber and Perca it lies median to it.   While traversing its special canal, the artery, in Amia, sends a communicating branch to the efferent pseudobranchial artery, and in the cranial cavity, after giving off optic and olfactory branches, it forms a circulus cephalicus opposite the auditory region and behind the lobi inferiores.

4.  The external carotid artery enters the upper, lateral chamber of the eye-muscle canal by a special foramen through the petrosal, but does not, so far as could be determined by sections, enter or send any branches into the cranial cavity proper.   Branches of it issue from the eye-muscle canal with branches of the trigeminus and facialis, which they accompany.

5.  The efferent pseudobranchial artery enters the orbital opening of the eye-muscle canal by a special foramen, the

foramen lying immediately in front of the lateral wing of the parasphenoid. It thus, like the external carotid, pierces a part of the chondrocranium, but does not enter the cranial cavity proper. In the orbital opening of the eye-muscle canal it receives a small branch from the internal carotid, and issues into the orbit with the recti muscles.

6. The hypophysis cerebri and saccus vasculosus, in the adult, are both glandular structures. Both receive a considerable nervous supply directly from the base of the infundibulum, and the glandular cavities of both communicate directly with the infundibular cavity.

7. The olfactory nerve, contrary to Sagemehl's statement, lies exposed to the orbit through a limited part of its course. The opening through which it is so exposed lies at the extreme front and upper end of the orbit, and gives passage to a vein coming from the nasal pit. The olfactory canal in front of the opening is, therefore, formed by the fusion of two canals, the anterior part of the olfactory canal proper, and what is probably the homologue of the orbito-nasal canal of selachians. The opening can, therefore, be called the orbito-nasal opening or fenestra.

8. The "hitherto undescribed cranial nerve" of Pinkus, in Protopterus, is found in Amia, part of its fibres arising with the olfactorius and part of them having the intercranial course described by Pinkus, though their definite origin from the brain, as in Protopterus, was not satisfactorily determined. In the nerve the large cells described by Pinkus are found, scattered along the nerve in old larvae, but in 12 mm. larvae gathered into a knob-like protuberance on the under surface of the nervus olfactorius at about the middle of its length. In the latter larvae the collection of cells on the olfactorius resembles, in general histological appearance, the ciliary ganglion found on the nervus oculomotorius in much the same relation to that nerve. As the ciliary ganglion is unquestionably, in part at least, a sympathetic ganglion, the ganglion on the olfactorius is, perhaps, of a similar character, and is possibly the sphenopalatinum of higher vertebrates.

9. The muscles rotating the eyeball in the several orders of

the Ichthyopsida are not homologous structures, if existing descriptions of their innervation can be relied upon. On the contrary, the group Ichthyopsida (the Pharyngobranchii excluded) can be divided by the innervation of the muscles of the eye into two great groups and other sub-groups, which, if reversions have not taken place, indicate distinct and definite lines of descent.

In one of the two great groups, represented by the Cyclostomata alone, the nervus abducens innervates two recti muscles, the inferior and the externus. In the other group that nerve innervates but one rectus muscle, the externus, but it innervates also a retractor bulbi when that muscle is found.

The second group is subdivided into two sub-groups, in one of which the superior branch of the oculomotorius, lying dorsal to the ophthalmicus profundus, innervates two recti muscles, the superior and the internus, while in the other it innervates but one, the superior. In the first sub-group are found Elasmobranchii, Dipnoi, and Urodela; in the second, Ganoidei, Teleostei, and Anura. The Amphibia are thus separated into two sub-groups, corresponding to the two sub-groups of Pisces. In the prototype of this second group there must have been an arrangement of muscles and nerves most nearly represented by that found in the Holocephala, where the rectus internus arises near the front end of the orbit, as in Petromyzon, and the obliquus superior from the edge of the orbit, as in Petromyzon. From this prototype two lines lead to the two sub-groups of Pisces and two to the two sub-groups of Amphibia.

Between the two lines leading to Amphibia lies Ichthyophis, in which the muscles rotating the eyeball are innervated as in Anura, but in which a retractor tentaculi has been formed from one of the muscles of the eye (Sarasins), probably from the rectus internus of Urodela, and not from the retractor bulbi, as the Sarasins suggest. Ichthyophis seems therefore, in the arrangement of the muscles of the eyeball, to represent the beginning of the line leading to higher vertebrates, as Burckhardt states that it does in the arrangement of the parts of the brain.

10. The profundus ganglion is found both in larvae and in the adult as a separate and distinct ganglion, connected with the ciliary ganglion by a radix longa, and with the brain by a profundus root, which, in larvae, is wholly separate and distinct from the root of the trigeminus, but, in the adult, is somewhat fused with that root.

From the ganglion arise, in addition to the radix longa, two ciliares longi, a large and important portio ophthalmici profundi, and often, but not always, a small, delicate, and apparently degenerating nerve.

11. The portio ophthalmici profundi may be single, double, or even triple. It runs forward and upward, dorsal to all the muscles of the eyeball and the nerves innervating them, and joins and completely fuses with the ramus ophthalmicus superficialis trigemini, while that nerve is still in the orbit. Its position thus shows that it cannot be the homologue of the ramus ophthalmicus profundus trigemini of selachians and other animals, that nerve always lying below the superior branch of the oculomotorius, and below the nervus trochlearis. It must, therefore, correspond to certain of those frontal branches of the ophthalmicus profundus of selachians that arise from that nerve before it passes under the rectus superior.

There is, therefore, no true ramus ophthalmicus profundus in Amia, that nerve being apparently represented by the small and delicate nerve often found arising from the profundus ganglion.

12. The ciliary ganglion is a separate and well-developed ganglion, connected with the profundus ganglion by a long radix longa, and with the nervus oculomotorius by short fibres, which represent the radix brevis. From the ganglion a single nerve arises, the ciliaris brevis.

13. The trigemino-facial ganglionic complex lies in the upper, lateral chamber of the eye-muscle canal, and consists of three parts between which no communicating fibres could be traced. The three parts are the profundus ganglion, which is entirely separate and distinct, the main trigemino-facial ganglion, and the ganglion of the buccalis and ophthalmicus facialis. The latter ganglion lies on, and partly imbedded in, the upper surface of the anterior part of the main ganglion.

The main ganglion is in young larvae imperfectly separated into three parts or regions, an anterior and upper, a median, and a posterior one.

The anterior and upper of these three portions of the ganglion is connected with the brain by the anterior, or trigeminal, root of the ganglion. This root arises from the anterior and lateral edge of the medulla, and is there distinctly double, its dorsal and posterior portion arising superficially in the brain, its ventral and anterior portion deeper in the brain, in part from the posterior longitudinal fasciculus. These latter fibres, which are the motor fibres of the root, either alone or with other of the deeper fibres, traverse the anterior part of the main ganglion as its anterior commissure, and enter the truncus maxillaris trigemini.

The median and posterior portions of the main ganglion are connected with the brain by the posterior, or facial, root of the ganglion, which arises from the lateral surface of the medulla in front of, and close to, the root of the nervus acusticus. It arises as two bundles, which immediately receive a third bundle coming from the root of the ganglion of the buccalis and ophthalmicus facialis. The anterior of the two bundles of the root proper arises at a high level in the brain, apparently from the fasciculus communis tract, and enters mainly or entirely into the median portion of the main ganglion. The posterior of the two bundles arises deep in the brain, and contains the motor fibres of the root. It enters the posterior portion of the main ganglion, and, with the third bundle, traverses it as its posterior commissure. The third bundle contains the lateral, or sense-organ, elements of the root.

The root of the ganglion of the buccalis and ophthalmicus facialis is the lateral or sense-organ root of the ganglionic complex. It arises from a slight swelling on the lateral surface of the medulla, close to, and almost as a part of, the root of the nervus acusticus. The branch of this root that is sent to the posterior root of the main ganglion, may arise as a separate root or bundle.

14. The rami ophthalmicus superficialis and buccalis facialis arise from their own special ganglion; the ramus oticus facialis

from that same ganglion, but as a part of the ramus buccalis. The ophthalmicus superficialis innervates the sense organs of the supraorbital sensory canal, and the anterior head line of pit organs ; the buccalis innervates the sense organs of the suborbital and antorbital parts of the main infraorbital canal ; and the oticus, the otic part of that canal, and the sense organ of the spiracular canal.

15. The ramus ophthalmicus superficialis trigemini arises, in young larvae, from the upper, anterior end of the main trigemino-facial ganglion, but many of its fibres can be traced backward to the superficial portions of the median portion of the ganglion. They accordingly belong to the fasciculus communis portion of the posterior root of the ganglion, and not to the anterior root. The nerve is concerned largely in the innervation of the terminal buds found on the top of the head and snout, and its branches are sent to the outer surface of the head on both sides of the ophthalmicus facialis, which nerve it closely accompanies, lying immediately beneath it.

16. The truncus maxillaris trigemini arises directly from the anterior commissure of the main trigemino-facial ganglion, but receives important additions to its fibres from the median, fasciculus communis portion of that ganglion. From it arise the rami maxillaris superior and inferior, and from it, also, or from the main ganglion itself, several accessory trigeminal nerves which seem destined to supply the terminal buds on the side of the cheek.

17. The ramus maxillaris superior trigemini closely accompanies the buccalis facialis in its course forward, behind, and below the eye. It lies immediately internal to and below the buccalis, its branches are given off on both sides of that nerve, and from its distribution it seems destined in part to the innervation of terminal buds. Its branches form anastomoses both with the posterior and the anterior portions of the ramus palatinus facialis.

18. The ramus maxillaris inferior trigemini innervates all the levator and adductor muscles of the mandibular arch, the dilatator operculi, and probably also the intermandibularis, and all, or a part of, the inferior division of the geniohyoideus.

The branch that innervates the four divisions of the levator maxillae superioris arises from the base of the nerve, or even from the main truncus maxillaris, and may be double throughout its entire length.

Certain branches of the ramus have a peculiar and circuitous course, and are distributed to regions where terminal buds abound. One of these branches becomes a mandibularis internus trigemini, distributed to the inner surface of the mandible; another goes to the tissues of the mouth cavity near the upper end of the ceratohyal; and two others pass under the ramus mandibularis externus facialis, and are distributed to the outer, ventral surface of the head, where they form anastomoses with branches of the ramus hyoideus facialis. These branches of the trigeminus are all prespiracular nerves, and are all distributed to regions where terminal buds are found. The mandibularis internus trigemini, with or without other of these special branches, seems to correspond to the inferior branch of the palatinus facialis in Protopterus and Polyodon, and to be the homologue of the chorda tympani of higher animals.

19. The truncus hyoideo-mandibularis facialis arises largely from the posterior commissure of the main trigemino-facial ganglion. Its rami opercularis and hyoideus innervate the adductor and levator muscles of the hyoid arch, the hyohyoideus, and probably also the superior division of the geniohyoideus, and a part, at least, of the inferior division of that muscle. Its ramus mandibularis externus contains all the lateral fibres of the nerve, and innervates the sense organs of the operculo-mandibular portion of the lateral canal system. Its ramus mandibularis internus is distributed to the inner surface of the hyoid and mandibular arches. As this last nerve is a postspiracular nerve, it seems impossible that it should be the homologue of the chorda tympani, which is apparently a prespiracular nerve.

No branches of any of these nerves could be traced definitely to the pseudobranch.

20. The ramus palatinus facialis arises, in larvae, from the median, or fasciculus communis, portion of the main trigeminofacial ganglion. It separates into an anterior and a posterior

portion, both of which are distributed to the upper and lateral surfaces of the mouth cavity. Both portions form anastomoses with branches of the ramus maxillaris superior trigemini, and the anterior portion, at its anterior end, pierces the basis cranii to reach the upper surface of the anterior end of the chondrocranium. One of the branches of this nerve was traced into one of the large premaxillary teeth.

21. The nervus acusticus arises, in larvae, from the summit of the tuberculum acusticum by three distinct roots, on each of which a somewhat distinct part of the large acoustic ganglion is formed. The more dorsal of the three roots forms the posterior root of the nervus, and gives origin to the ramus cochlearis. The other two roots together form the anterior root of the nervus, and give origin to the ramus vestibularis. The two nerves innervate the cristae ampullae as in other fishes.

No ductus or saccus endolymphaticus could be recognized in any of the specimens examined, old or young.

22. The nervus lineae lateralis vagi arises, in larvae, immediately posterior to the tuberculum acusticum. It either traverses in part, or is traversed by, the root of the nervus glossopharyngeus, an important interchange of fibres there taking place, the fibres apparently running from the nervus lateralis into the glossopharyngeus, and there giving origin to the so-called dorsal root of that nerve.

The nervus issues from the cranial cavity with the nervus vagus through the vagus foramen, enters its own ganglion, and is then distributed to the lateral sense organs of the supratemporal cross-commissure, and to those of that part of the lateral sensory system that lies posterior to that commissure.

23. The nervus glossopharyngeus, immediately after its exit from the brain, pierces the median, membranous wall of the auditory cavity and traverses that cavity, lying, in its passage, behind the membranous ear, between the sacculus and the sinus utriculi posterior, and between the two terminal branches of the ramus cochlearis acustici. It apparently receives fibres both from the root of the nervus lineae lateralis and from the ramulus ampullae posterioris, the fibres so received going to

form the so-called dorsal root of the nervus. On this dorsal root, in larvae, a separate ganglion is formed, and from it arises the dorsal or suprabranchial branch of the nervus, which branch is destined entirely to supply certain of the sense organs of the lateral system. From the ganglion of the main root arise pharyngeal, pretrematic, and posttrematic branches, which have the distribution indicated by their names. The ramus pharyngeus and ramus pretrematicus traverse regions where terminal buds are found. No branch of the nervus could be traced definitely to the pseudobranch.

24. The vagus arises by several roots, all of which traverse an intracranial ganglion, and then separate into four portions, on each of which an extracranial ganglion is formed. From each of the first three of the latter ganglia arise pharyngeal, pretrematic, and posttrematic branches ; from the fourth arise the superior and inferior pharyngeal nerves, and the nervus intestinalis. The pharyngeal branches of the first three nerves pass downward, to their destination, between the articular and interarcual ligaments that connect the infrapharyngobranchial, or epibranchial, of the arch of the nerve, with the epibranchial of the next preceding arch.

From the anterior fibres of the root of the nervus, or in part from its intracranial ganglion, a supratemporal branch arises, which accompanies closely the supratemporal branch of the nervus lineae lateralis, and is distributed to regions where terminal buds abound. A part of this nerve fuses completely with a branch arising from one or both of the first pair of dorsal branches of the ramus ophthalmicus superficialis trigemini. Another branch, arising from the base of the nerve, or perhaps from the main vagus root itself, has an intracranial course, and issues on the top of the cranium, about where the ramus lateralis trigemini, or ramus recurrens facialis, of other authors issues. It may represent that nerve or a part of it, the rest of the nerve being represented in the main supratemporal branch of the vagus, or in that nerve and the two branches of the ophthalmicus trigemini together.

25. Terminal buds are said to represent a condition through which the canal organs of the lateral lines have passed in

their development. They and the nerves innervating them should, therefore, arise from, or in connection with, sensory, ectodermal thickenings, as do the canal organs and their nerves. Certain pharyngeal or pretrematic nerves are known to innervate certain terminal buds, and the pharyngeal and pretrematic branches of all the cranial nerves are said to arise from, or in connection with, epibranchial or pretrematic ectodermal thickenings. Terminal buds should, therefore, arise from, or in connection with, those same thickenings.

Those trigeminal and facial nerves, in Amia, that are known to innervate terminal buds, or that innervate regions where those buds abound, all arise either from the median, fasciculus communis portion of the main trigemino-facial ganglion, or receive important additions to their fibres from that portion of that ganglion. It seems, therefore, probable, as Strong has suggested, that the fasciculus communis tract of the brain is largely or entirely concerned in the innervation of terminal buds. The arrangements presented by different fishes indicate that the fibres of this tract may issue from the brain as a somewhat separate root, and have a somewhat separate ganglion, or that they may issue as parts of the roots of certain of the cranial nerves, their ganglia, in that case, being completely fused with the ganglia of the nerves with which they issue. The nerves, their ganglia, and the organs they innervate thus form an epibranchial or pretrematic series comparable to the nerves, ganglia, and organs of the lateral or dorso-lateral series. The organs and nerves of the epibranchial series are formed before those of the lateral series, for the nerves of the former lie always below those of the latter, and may hold them between branches issuing on both sides of them.

26. The eye is said to belong to the line of epibranchial thickenings. The lens of the eye may, therefore, be a modified terminal bud or buds, and one or more of the ciliary nerves the nerve innervating it.

27. The chorda tympani is distributed to the tongue, and contains taste fibres. As taste bulbs are supposed to be developed from terminal buds, the chorda probably belongs, in part, at least, to the fasciculus communis nerves. As it is

apparently a prespiracular nerve, it cannot be the ramus mandibularis internus facialis of Amia and other fishes, as that nerve is a postspiracular nerve. It, therefore, seems probable that it is represented, in Amia, by the mandibularis internus trigemini, and in Protopterus and Polyodon by the inferior branch of the palatinus facialis.

28. The ramus ophthalmicus superficialis trigemini, in Amia, arises largely from the fasciculus communis portion of the trigemino-facial ganglion, and is largely or entirely concerned in the innervation of the terminal buds found on the top of the head and snout. Where those buds are few or entirely wanting in that part of the head, as in selachians and amphibia, the nerve is, naturally, small or wanting.

29. The suprapharyngeal elements of the branchial arches, in Amia, are usually found as special processes on the posterior edges of the epibranchials of their arches, and not as separate elements. They lie at the distal edge of the surface of insertion of the external levator muscle of the arch to which they belong; behind, rather than above, the efferent artery of the arch; behind the posttrematic branch of the nerve of the arch; and in front of pretrematic branches of the nerve of the next posterior arch.

The infrapharyngeal elements lie proximal to, or in front of, the external levator muscles of their respective arches, and give insertion to the internal levator muscle of the next preceding arch, when that muscle exists. They lie, on each arch, below the efferent artery, and below the posttrematic and pharyngeal branches of the nerve of the arch.

The epibranchial of each arch is connected, normally, with the infrapharyngobranchial of the next posterior arch by articular and interarcual ligaments. Where the infrapharyngobranchial of the next posterior arch is not normally developed, the connection is wholly or in part with the epibranchial of that arch.

30. In the hyoid arch the hyomandibular and symplectic have practically the same relations to the muscles, nerves, artery, and ligaments of their arch, that the suprapharyngobranchials and infrapharyngobranchials, respectively, have to

those structures in their arches. They seem, therefore, to be those elements of the hyoid arch, and the interhyal, or stylo-hyal, to be the epal element.

The opercular bones and the branchiostegal rays, from 12 mm. larvae upward, contain no trace of cartilage, and give no indication whatever of having been preformed in cartilage. They all lie external to the nerve and artery of the arch. They are, accordingly, probably entirely of dermal origin, and not homologous with the gill-rakers of selachians.

31. In the mandibular arch the metapterygoid process of the metapterygoid and the anterior process of the same bone fulfil even better the same conditions. They seem, therefore, to be the supra- and infrapharyngeal elements, respectively, of the mandibular arch, and the quadrate to be the epal element. The palatine is then, probably, part of a premandibular arch, the coronoid process of Meckel's cartilage being possibly an-other part of the same arch.

32. The muscles of the visceral arches probably all lay primarily external to those arches, as a simple constrictor muscle. From that muscle the arcual and interbranchial muscles arose, by a separation of the simple constrictor into an inner and an outer layer. From the arcuals arose the adduc-tors and the interarcuals. The adductors, however, on the different arches, probably arose in different manners, and thus are not all strictly homodynamous structures. The same is true of the levators, which probably arose in part from the interarcual muscles, and in part from the interbranchials.

33. The adductor mandibulae probably arose from a muscle resembling the so-called interbranchiale of Chimaera. That muscle in Chimaera is the simplest form of arcual muscle known, and, in giving origin to the adductor mandibulae, it had simply to slip over the anterior edge of the mandibular arch into its actual position on that arch.

In Amia the adductor mandibulae is in large part a con-tinuous muscle extending from the upper ends of the hyoid and mandibular arches downward and forward, internal to the coronoid process of Meckel's cartilage, into the hollow of the mandible. The muscle has become entirely tendinous in its

middle portion, where it passes into the mandible, and is almost completely separated into two portions, a deeper and a superficial one. The latter, in its superior portion, is again incompletely separated into two or three portions. The most superficial of these portions is probably the homologue of the entirely independent superficial muscle of some teleosts.

34. The adductor hyomandibularis, adductor operculi, and levator operculi are derived from the dorsal half of the general constrictor of the hyoid arch, the adductor hyomandibularis from the deeper layers of that muscle, the other two, or at least the levator operculi, from its superficial portions. The adductor hyomandibularis is probably developed from a muscle comparable to one or more of the interarcual muscles of the branchial arches of selachians, and is thus homodynamous with the levators of the branchial arches of teleostomes, and not with the adductor mandibulae. The adductor operculi and levator operculi, at least the latter, are derived from the interbranchial muscles of their arch, and are thus homodynamous with the levator arcus palatini, and not with the levator muscles of the branchial arches.

35. The adductors of the branchial arches arise from, or in connection with, the arcuals of their respective arches, muscles comparable to the so-called interbranchiale of Chimaera. They represent the middle portion only of that muscle, and have acquired their actual position on their respective arches by passing over the posterior edges of those arches. They are thus not homodynamous either with the adductor of the mandibular arch or with that of the hyoid arch.

36. The levator arcus palatini and the dilatator operculi are, in Amia, as in teleosts, separate and distinct muscles. They are developed from a single muscle derived from the dorsal half of the general constrictor of the mandibular arch. Their innervation and position indicate that they are, together, the homologue of the muscle called by Vetter, in selachians, *Add*γ, and that they, and that muscle, arise from the dorsal portion of the interbranchial muscle of their arch. They are thus homodynamous with the levator operculi, and not with the levators of the branchial arches.

37. The levators of the branchial arches probably arise from the first and second interarcuals of selachians. There are, normally, two of them to each arch, — an external muscle, inserted on the epibranchial of its own arch, in close connection with the suprapharyngobranchial of that arch, and an internal muscle, inserted on the infrapharyngobranchial of the next following arch. As the posterior arches, or the pharyngobranchials of those arches, disappeared, the internal levators, or interarcuales I, of those arches, seem to have acquired attachment to the under surface of the spinal column, and thus to have given origin to the retractor arcuum branchialium.

Interarcuales III of selachians are found, in Amia, as a series of dorsal interarcual ligaments.

38. The four muscles called by McMurrich the second, third, fourth, and fifth divisions of the levator arcus palatini are probably not parts of that muscle. The second and third of these muscles are probably derived from the levator maxillae superioris of selachians, or from that muscle and one of the spiracle muscles. The fourth and fifth muscles are undoubtedly derived from the muscle called by Vetter, in selachians, *Addβ*. As all four muscles are, in Amia, innervated by branches of a single nerve, I have called them the second, first, third, and fourth divisions, respectively, of the levator maxillae superioris. This name is, however, probably not a proper one for the third and fourth muscles.

The nerve that innervates the muscles was found double in one Amia, and it is frequently, if not always, double in selachians. It arises, both in Amia and in selachians, from the truncus maxillaris trigemini, and is sometimes assigned to the inferior maxillary nerve and sometimes to the superior maxillary. The muscles may, therefore, belong in part to one or more preoral arches. They apparently belong to the interarcual, and not to the interbranchial muscles.

In teleosts these muscles either disappear entirely, are entirely absorbed by the adductor mandibulae, or persist as what are called special insertions or special divisions of the latter muscle.

39. No interarcuales ventrales are described by Vetter in

selachians.   In Acipenser and in teleosts he describes them, calling them, in the latter, the obliqui ventrales.   In Amia these muscles resemble, in a general way, the dorsal inter-arcual muscles ; that is, there is on each arch an interarcual ligament, a muscle that has its insertion on its own arch, and another that tends to have its insertion on the next posterior arch.   They therefore probably arise from the ventral portion of the interbranchiale of Chimaera, as Vetter concludes, but they belong, with that muscle, to the deeper layer of the primitive constrictor, and not to the superficial layer.

The pharyngo-claviculares externus and internus are probably the obliqui muscles of the fifth arch.

40.  The geniohyoideus and intermandibularis arise together. The geniohyoideus has an inferior and a superior portion. From, or in connection with, the anterior end of the inferior portion, the intermandibularis arises.   The latter muscle is double, both parts extending from mandible to mandible.   The superior division of the geniohyoideus extends from mandible to hyoid, and seems to be one of the obliqui ventrales of the mandibular arch.   The inferior division occupies a position intermediate between that of the superior division and that of the intermandibularis, and may also be an obliquus ventralis, or more probably the interbranchial muscle of the arch, in which case the intermandibularis probably belongs to the same muscles.

41.  The hyohyoideus, in its superior portion, is the inter-branchial muscle of its arch.   In its inferior portion it may be partly the obliquus ventralis.

42.  The branchiomandibularis is found in Acipenser and Polypterus as well as in Amia.   It is represented in Elasmo-branchii by the coraco-mandibularis, or by part of that muscle. In teleosts it is not found.   In Amia it varies greatly in dif-ferent individuals, and undoubtedly represents a muscle in process of deterioration and disappearance.   It is probably, as McMurrich has suggested, a muscle from which the muscles of the tongue, in higher vertebrates, have developed.   It is inner-vated by a terminal branch of the united first, second, and third occipital nerves.

43. The sternohyoideus is separated by two transverse intermuscular septa into three portions. The posterior of these portions is innervated by a branch of the fourth occipital nerve, and thus belongs to the fifth and last occipital muscle segment, which is innervated by the same nerve. The two anterior portions of the muscle are innervated by branches of the united first, second, and third occipital nerves, and thus belongs to the fourth, and one or more anterior, occipital muscle segments.

44. The development of the occipitale laterale indicates that that bone is formed of parts of three occipital dorsal arches. It is pierced by two ventral occipital nerves, and the nervus vagus issues along its anterior margin between it and the hind wall of the auditory capsule. It gives attachment to three intermuscular septa. Posterior to it, on the dorsal surface of the basioccipital, there are two complete dorsal arches, each giving attachment to an intermuscular septum. In front of each of these two arches there is a complete spinal nerve with dorsal and ventral roots and dorsal ganglion. There are thus five vertebral segments indicated in the occipital region of the skull of Amia, the first, or most anterior, segment not being represented by a nerve, unless the vagus be, in part, that nerve. The posterior of these five segments fuses with the hind end of the skull in post-larval stages.

45. A complete trunk vertebra, in larvae of Amia, contains six blocks of cartilage, three on each side. The dorsal block, on each side, is wholly independent of the other two, and is fused, in the adult, with the ventral end of the dorsal arch that lies next posterior to it in larval stages. The lateral block is, in larvae, connected at its anterior end with the posterior end of the ventral block. It bears at its outer end the rib. The ventral blocks lie on either side of the aorta, and resemble vertebral processes found in Calamoichthys and Salamandra, which, in those animals, are said to become the "Basalstümpfe" of the haemal arches. If they be the homologues of those structures, as seems most probable, they are haemapophyses, and the ribs of Amia may be true ribs, or pleuropophyses.

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## EXPLANATION OF PLATES.

### Index Letters.

$a$. ossicle a of Bridge.

$aa$. ampulla anterior.

$A_2$. superficial portion of adductor mandibulae; $A_2'$, $A_2''$, $A_2'''$, its three divisions.

$A_3$. deeper portion of adductor mandibulae.

$A\omega$. mandibular portion of adductor mandibulae; $A\omega'$, $A\omega''$, its two divisions.

$A_2A\omega'$. tendon connecting $A_2$ and $A\omega'$.

$A_3A\omega''$. tendon connecting $A_3$ and $A\omega''$.

$Aab. IV, Aab. V$. adductores arcuum branchialium of fourth and fifth arches.

$ab$. nervus abducens.

$abfr$. foramen for nervus abducens.

$ac$. nervus acusticus.

$acvfr$. foramen for anterior cerebral vein.

$ae$. ampulla externa.

$agl$. ramus anterior glossopharyngei.

$Ah$. adductor hyomandibularis.

$allg$. accessory lateral-line groups of pit organs.

$ana$. anterior nasal aperture.

$ANT$. antorbital (Sag.), preorbital (Bridge).

$Ao$. adductor operculi.

$ap$. ampulla posterior.

$aps$. afferent pseudobranchial artery.

$ART$. articular.

$AS$. alisphenoid.

$AUP$. autopalatine.

$b$. ossicle b of Bridge.

$BB.^{1-3}$ first, second, and third basibranchials.

$bf$. ramus buccalis facialis.

$bf.io.^{1-14}$ branches of buccalis facialis to infraorbital sense organs, Nos. 1 to 14.

*Bm.*    branchiomandibularis.

*BO.*    basioccipital (Bridge), occipitale basale (Sag.).

*BRG.*    branchiostegal rays.

*BS.*    basisphenoid.

*c.*    ossicle c of Bridge.

*ca.*    carotid artery.

*caa.*[4]    coraco-arcualis anterior[4].

*cai.*    canal for intervertebral artery and sympathetic nerve.

*cb.*    ciliaris brevis.

*CB. I–V.*    first to fifth ceratobranchials.

*cc.*    common carotid artery.

*CH.*    ceratohyal.

*CL.*    clavicle.

*cl.*    ciliares longi.

*CP.*    coronoid process.

*Cp.*    constrictor pharyngis.

*csa.*    anterior semicircular canal.

*cse.*    external semicircular canal.

*csp.*    posterior semicircular canal.

*ct.*    canalis transversus.

*cus.*    canalis utriculo-saccularis.

*D.*    dentary.

*d.*    ossicle d of Bridge.

*DA.*[1–6]    dorsal arches of first six vertebrae.

*DA.*[o]    dorsal occipital arches.

*da.*    dorsal aorta.

*Do.*    dilatator operculi.

*DP.*    dermopalatine.

*dp.*[o–6]    dorsal cartilaginous occipital and vertebral processes.

*ea. I–IV.*    efferent arteries of first to fourth branchial arches.

*EB. I–V.*    first to fifth epibranchials.

*ec.*    external carotid artery.

*ecfr.*    foramen for external carotid artery.

*ecm.*    mandibular branch of external carotid artery.

*ecop.*    branch of external carotid accompanying ophthalmic branches
of trigeminus and facialis.

*ECP.*    ectopterygoid.

*EH.*    epihyal.

*emc.*    eye-muscle canal.

*emc′.*    upper lateral portion of eye-muscle canal.

*ENP.*    entopterygoid.

*EO.*    exoccipital, occipitale externum (Sag.), epiotic (Bridge).

*ep.*    epiphysis.

*eps.*    efferent pseudobranchial artery (arteria ophthalmica, Sag.).

*epsfr.*    foramen for efferent pseudobranchial artery.

*ESC.*    extrascapular (Sag.), supratemporal (Bridge).

*ETH.*    ethmoid.

*fb.*    fore-brain.

*fc.*    facial canal through the hyomandibular.

*ffr.*   facial foramen.
*fla.*   anterior flagellum.
*flp.*   posterior flagellum.
*fm.*   foramen occipitale magnum.
*FR.*   frontal.
*G.*   gular.
*gc.*   ciliary ganglion.
*gg.*   Gasserian ganglion.
*ggl.*   glossopharyngeal ganglion.
*Ghi.*   inferior portion of geniohyoideus.
*Ghs.*   superior portion of geniohyoideus.
*gl.*   nervus glossopharyngeus.
*glfr.*   glossopharyngeal foramen.
*gobf.*   ganglion of rami ophthalmicus superficialis and buccalis facialis.
*goc.*[3-4]   ganglia on third and fourth occipital nerves.
*gp.*   profundus ganglion.
*gsp.*[1-6]   spinal ganglia.
*gtf.*   trigemino-facial ganglion.
*gvi.*   intracranial vagus ganglion.
*gv.*[1-4]   ganglia of first to fourth vagus.
*hb.*   hind-brain.
*HB. I-IV.*   first to fourth hypobranchials.
*hf.*   ramus hyoideus facialis.
*hfn.*   hypophysial fenestra.
*hf.sc.*   subcutaneous branches of ramus hyoideus facialis.
*HH.*   hypohyal.
*Hhi.*   inferior portion of hyohyoideus.
*Hhs.*   superior portion of hyohyoideus.
*hl.*   horizontal pit line of cheek.
*HMD.*   hyomandibular.
*hmf.*   truncus hyoideo-mandibularis facialis.
*hop.*   hyo-opercularis artery.
*hy.*   hypophysis.
*IC.*   intercalar (Sag.), opisthotic (Bridge).
*ic.*   internal carotid artery.
*icc.*   internal carotid canal.
*icfr.*   internal carotid foramen.
*ie.*   ethmoid incisur.
*ig.*[1-21]   infraorbital groups of pores Nos. 1 to 21, or trunk canals leading to those pores.
*Im.*   intermandibularis.
*ims.*[1-9]   intermuscular septa.
*ioc.*   infraorbital lateral canal.
*IOP.*   interoperculum.
*ip.*   preorbital incisur.
*IPB. I-III.*   first to third infrapharyngobranchials.
*i*[15]*s*[7]*g.*   double trunk at union of infraorbital and supraorbital canals.
*JG.*   jugal.
*LA.*   lachrymal.

| | |
|---|---|
| *Labe. I–V.* | levator arcus branchialis externus of first to fifth branchial arches. |
| *Labiª.* | levator arcus branchialis internus, anterior muscle. |
| *Labiᵖ.* | levator arcus branchialis internus, posterior muscle. |
| *Lap.* | levator arcus palatini. |
| *li.* | lobus inferior. |
| *lid.h. to lid. III.* | ligamenta interarcualia dorsalia of hyoid and first three branchial arches. |
| *liv.h. to liv. IV.* | ligamenta interarcualia ventralia of hyoid and first four branchial arches. |
| *llg.* | lateral-line groups of pores. |
| *lmb.* | ligament from mandible to first branchiostegal ray. |
| *lmh.* | ligamentum mandibulo-hyoideum. |
| *lmi.* | ligamentum mandibulo-interoperculum. |
| *Lms.*[1-4] | first to fourth divisions of the levator maxillae superioris. |
| *Lo.* | levator operculi. |
| *locª.* | anterior lateral occipital ligament. |
| *locᵖ.* | posterior lateral occipital ligament. |
| *lol.* | lobus olfactorius. |
| *lp.*[2-6] | lateral cartilaginous vertebral processes. |
| *ltfr.* | foramen of ramus lateralis trigemini, found in Amia as a branch of the vagus. |
| *lvdi.* | ligamentum longitudinale vertebrale dorsale inferior. |
| *lvdˢ.* | ligamentum longitudinale vertebrale dorsale superior. |
| *lvl.* | lateral vertebral ligament. |
| *M.* | Meckel's cartilage. |
| *MM.* | mentomeckelian ossification. |
| *mb.* | mid-brain. |
| *mdc.* | mandibular lateral canal. |
| *mef.* | ramus mandibularis externus facialis. |
| *mefc.* | canal in articular for ramus mandibularis externus facialis. |
| *mef.hl.* | branch of r. mandibularis externus facialis to horizontal cheek line of pit organs. |
| *mef.mdl.* | branch of r. mandibularis externus facialis to mandibular line of pit organs. |
| *mef.omo.*[1-16] | branches of r. mandibularis externus facialis to operculo-mandibular sense organs, Nos. 1–16. |
| *mef.vl.* | branch of r. mandibularis externus facialis to vertical cheek line of pit organs. |
| *mf.* | truncus mandibularis facialis. |
| *mif.* | ramus mandibularis internus facialis. |
| *mit.* | ramus maxillaris inferior trigemini. |
| *mitfr.* | foramen for ramus maxillaris inferior trigemini. |
| *MP.* | metapterygoid. |
| *Ms.*[1-11] | first eleven muscle segments of trunk. |
| *mst.* | ramus maxillaris superior trigemini. |
| *mst.mx.* | maxillary branch of ramus maxillaris superior trigemini. |
| *MX.* | maxillary. |
| *mx.* | maxilla. |

| | |
|---|---|
| *n.* | nerve " n " of Pinkus. |
| *NA.* | nasal. |
| *nct.* | ramus nasalis or nasociliaris trigemini. |
| *ne.* | nasal epithelium. |
| *nll.* | nervus lineae lateralis. |
| *nll.all.* | branches of n. lineae lateralis to pit organs of accessory lateral line. |
| *nll.dl.* | branches of n. lineae lateralis to pit organs of dorsal body line. |
| *nll.io.*[18-21] | branches of n. lineae lateralis to infraorbital sense organs, Nos. 18–21. |
| *nll.llo.* | branches of n. lineae lateralis to sense organs of lateral line. |
| *nll.pl.* | branches of n. lineae lateralis to pit organs of posterior line of head. |
| *nll.sto.*[1-3] | branches of n. lineae lateralis to sense organs of supratemporal commissure. |
| *nt.* | nasal tube. |
| *o.* | nervus opticus. |
| *OB.* | occipitale basale (Sag.), or basioccipital (Bridge). |
| *oc.*[1-4] | occipital nerves. |
| *oca.*[2-4] | anterior or communicating branches of occipital nerves. |
| *ocd.*[1-4] | dorsal branches of occipital nerves. |
| *och.*[1-4] | horizontal branches of occipital nerves. |
| *ocv.*[1-4] | ventral branches of occipital nerves. |
| *ocm.* | nervus oculomotorius. |
| *ocmi.* | inferior branch of nervus oculomotorius. |
| *ocms.* | superior branch of nervus oculomotorius. |
| *Od.*[1-3] | obliqui dorsales. |
| *of.* | ramus oticus facialis. |
| *ofc.* | canal for ramus oticus facialis. |
| *of.io.*[15-16] | branches of ramus oticus facialis to sense organs, Nos. 15 and 16, of infraorbital canal. |
| *ofn.* | optic fenestra. |
| *ofr.*[1-2] | foramen of first and second occipital nerves. |
| *of.spo.* | branch of oticus facialis to sense organ of spiracular canal. |
| *oi.* | obliquus inferior. |
| *OL.* | occipitale laterale (Sag.), or exoccipital (Bridge). |
| *ol.* | nervus olfactorius. |
| *olc.* | olfactory canal. |
| *olfr.* | olfactory foramen. |
| *olp.* | olfactory pit. |
| *omc.* | operculo-mandibular lateral canal. |
| *omg.*[1-16] | operculo-mandibular groups of pores, Nos. 1–16, or trunk canals leading to those pores. |
| *onc.* | orbito-nasal canal. |
| *onfn.* | orbito-nasal fenestra. |
| *OP.* | operculum. |
| *op.* | optic perforation of sclerotic. |
| *opf.* | ramus ophthalmicus superficialis facialis. |
| *opf.al.* | branch of ramus ophthalmicus superficialis facialis to pit organs of anterior line of head. |

*opfr.* ophthalmic foramen.

*opf.so.*[1-7] branches of ramus ophthalmicus superficialis facialis to sense organs of supraorbital canal.

*opp.* ramus ophthalmicus profundus trigemini.

*oprf.* ramus opercularis facialis.

*opt.* ramus ophthalmicus superficialis trigemini.

*opt.sc.* subcutaneous branches of ramus ophthalmicus trigemini.

*optstv.sc.* fused subcutaneous branches of ramus ophthalmicus trigemini and supratemporal branch of vagus root.

*OS.* orbitosphenoid.

*os.* obliquus superior.

*Ov. I–III.* obliqui ventrales of first three branchial arches.

*Ov. IV.*[1-3] three divisions of obliquus ventralis of fourth arch.

*ov and vo.* vein coming from eyeball.

*ov′ and vo′.* vein coming from eyeball.

*p.* pectoral fin.

*PA.* parietal.

*paf.* ramus palatinus anterior facialis.

*paffr.* foramen for ramus palatinus anterior facialis.

*pc.* palatine canal.

*pce.* pharyngo-clavicularis externus.

*pci.* pharyngo-clavicularis internus.

*PE.* petrosal.

*pf.* ramus palatinus facialis.

*pfr.* palatine foramen.

*pgl.* ramus pharyngeus glossopharyngei.

*ph.* pit for hypophysis cerebri and saccus vasculosus.

*piv.*[4] ramus pharyngeus inferior n. vagi quarti.

*PMX.* premaxillary.

*pn.* prenasal process (Bridge).

*pna.* posterior nasal aperture.

*POP.* preoperculum.

*popp.* portio ophthalmici profundi.

*POR.*[1] first postorbital.

*POR.*[2] second postorbital.

*ppc.* canal for ramus palatinus posterior facialis between the auto- and dermo-palatine bones.

*ppf.* ramus palatinus posterior facialis.

*ppffr.* foramen for ramus palatinus posterior facialis.

*pq.* cartilaginous portion of palato-quadrate.

*pr.* preorbital process.

*PRE.* prefrontal, or antorbital ossification.

*prgl.* ramus pretrematicus glossopharyngei.

*prv.*[1-4] pretrematic branches of first to fourth vagus nerves.

*PS.* parasphenoid.

*psb.* pseudobranch.

*PSF.* postfrontal (Sag., Allis), dermo-postfrontal (Bridge).

*psgl.* ramus posttrematicus glossopharyngei.

*PSP.* processus spinosus.

*PST.*   postorbital ossification (Allis), postfrontal (Sag.), sphenotic (Bridge).

$psv.^{1-3}$   posttrematic branches of first to third vagus.

$psv.^4$   ramus pharyngeus superior n. vagi quarti.

$pv.^{1-3}$   pharyngeal branches of first to third vagus.

*Q.*   quadrate.

*r.ca.*   ramus cochlearis acustici.

*r.va.*   ramus vestibularis acustici.

*r.aa.*   ramulus ampullae anterioris.

*r.ae.*   ramulus ampullae externae.

*r.ap.*   ramulus ampullae posterioris.

*r.mfu.*   ramulus maculae acusticae fundi utriculi.

*r.ms.*   ramulus maculae acusticae sacculi.

*r.mu.*   ramulus maculae acusticae recessus utriculi.

*r.pl.*   ramulus papillae lagenae.

*r.a., r.ac., r.b., r.c., r.cde., r.ce., r.d., r.de., r.e.*   } accessory branches of nervus trigeminus.

*r.am.*   branch of r. max. inf. trig. to $A_2$ and $A_3$.

*r.bm.*   branch of occipital nerves to Bm.

*r.ghi.*   branch of r. max. inf. trig. to Ghi.

*r.ghs.*   branch of r. max. inf. trig. to Ghs.

*r.im.*   branch of r. max. inf. trig. to Im.

*r.lap.do.*   branch of r. max. inf. trig. to Lap. and Do.

*r.lms.*   branch of r. max. inf. trig. to Lms.

*r.mdit.*   ramus mandibularis internus trigemini.

*r.mit.sc.*   subcutaneous branches of r. max. inf. trig.

*r.mst.sc.*   subcutaneous branches of r. max. sup. trig.

*Rabd.*   retractor arcuum branchialum dorsalis.

*RB.*   ribs.

*rb.*   radix brevis.

*rbb.*   retractor bulbi.

$rdoc.^{3-4}$   dorsal roots of third and fourth occipital nerves.

*re.*   rectus externus.

*rgl.*   root of nervus glossopharyngeus.

*rif.*   rectus inferior.

*rit.*   rectus internus.

*rl.*   radix longa.

*robf.*   root of rami ophthalmicus superficialis and buccalis facialis.

*rp.*   radix ophthalmici profundi.

*r.phgl.*   ramus pharyngeus glossopharyngei.

*rs.*   rectus superior.

*rtfa.*   anterior root of trigemino-facial ganglion.

*rtfp.*   posterior root of trigemino-facial ganglion.

$rvoc.^{3-4}$   ventral roots of third and fourth occipital nerves.

*S.ANG.*   supra-angular.

*SC.*   suprascapular.

*sc.*   sacculus.

*SCL.*   supraclavicular.

*sg.*<sup>1-8</sup> supraorbital groups of pores, Nos. 1–8, or trunk canals leading to those pores.

*Sh.* sternohyoideus.

*SMX.* septomaxillary.

*SOP.* suboperculum.

*SOR.*<sup>1</sup> first suborbital.

*SOR.*<sup>2</sup> second suborbital.

*SP.* splenial.

*sp.*<sup>1-6</sup> first six spinal nerves.

*spa.*<sup>1-6</sup> anterior or communicating branches of first six spinal nerves.

*spd.*<sup>1-6</sup> dorsal branches of first six spinal nerves.

*sph.*<sup>1-6</sup> horizontal branches of first six spinal nerves.

*spv.*<sup>1-6</sup> ventral branches of first six spinal nerves.

*SPB. II–III.* second and third suprapharyngobranchials.

*spc.* spiracular canal.

*SQ.* squamosal.

*stgl.* supratemporal branch of nervus glossopharyngeus.

*stglc.* canal for supratemporal branch of nervus glossopharyngeus.

*stgl.io.*<sup>17</sup> branch of supratemporal branch of nervus glossopharyngeus to sense organ, No. 17, of infraorbital canal.

*stgl.ml.* branches of supratemporal branch of nervus glossopharyngeus to pit organs of middle head line.

*stv.* supratemporal branch of vagus root.

*stv.sc.* subcutaneous branches of supratemporal branch of vagus root.

*sup.* sinus utriculi posterior.

*sus.* sinus utriculi superior.

*sv.* saccus vasculosus.

*SY.* symplectic.

*sy.* sympathetic nerves.

*Tda.* transversus dorsalis anterior.

*Tdp.* transversus dorsalis posterior.

*tfr.* trigeminal foramen.

*tgr.* temporal groove.

*tmt.* truncus maxillaris trigemini.

*tr.* nervus trochlearis.

*tt.sc.* subcutaneous branches of truncus trigeminus, or of accessory trigeminal nerves.

*tv.* truncus intestinalis n. vagi.

*Tva.* transversus ventralis anterior.

*Tvp.* transversus ventralis posterior.

*V.*<sup>1</sup> first spinal vertebra.

*v.* nervus vagus.

*vfr.* vagus foramen.

*VO.* vomer.

*vo.* vein coming from eyeball.

*vo'.* vein coming from eyeball.

*vp.*<sup>0-6</sup> ventral cartilaginous occipital and vertebral processes.

*w.* transverse "Wulst" or bar of cartilage.

* branch of vagus apparently corresponding to the ramus lateralis trigemini.

EXPLANATION OF PLATE XX.

FIG. 1.   Side view of complete skull of adult Amia.   × 1½.
FIG. 2.   Side view of pterygo-palatine arch, hyoid arch, and Meckel's cartilage.
× 1½.
FIG. 3.   Side view of quadrate.   × 1½.
FIG. 4.   Side view of symplectic.   × 1½.
FIG. 5.   Inside view of upper end of hyoid arch with opercular bones attached.
× 1½.
FIG. 6.   Inside view of right mandible.   × 1½.
FIG. 7.   The same with splenial removed.   × 1½.

Pl. XX.

EXPLANATION OF PLATE XXI.

FIG. 8. **Top** view of primordial cranium and first three vertebrae of adult Amia. The spinal and occipital dorsal arches have been removed and also the intercalar bone on the left side of the skull. × 1½.

FIG. 9. Side view of same, the spinal and occipital dorsal arches in place. × 1½.

FIG. 10. Bottom view of same. × 1½.

FIG. 11. Inside view of primordial cranium, vertically bisected. × 1½.

Pl. XXI
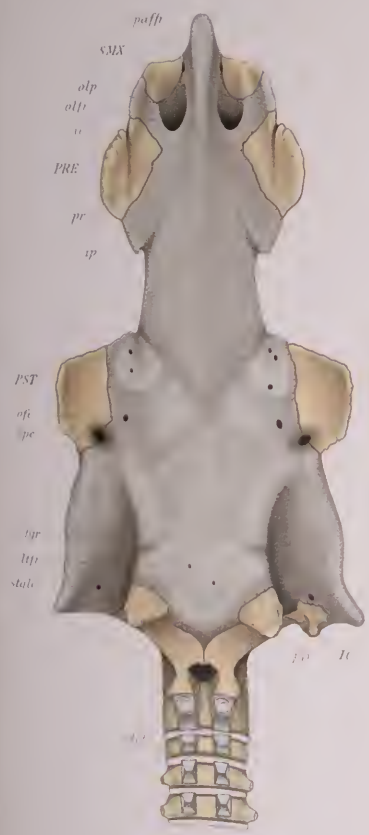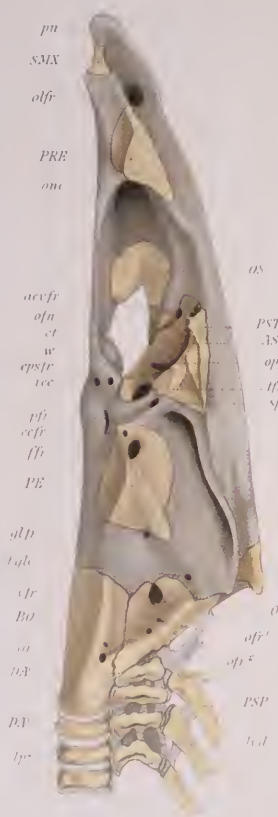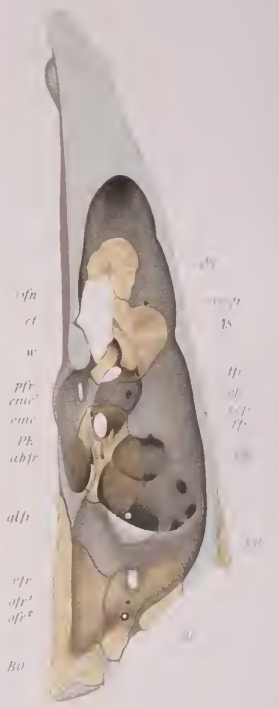
EXPLANATION OF PLATE XXII.

FIG. 12.   Genealogical tree based on the innervation of the muscles of the eye-ball.

FIG. 13.   Side view of basisphenoid of adult Amia.   × 1½.

FIG. 14.   Top view of same.   × 1½.

FIG. 15.   Bottom view of same.   × 1½.

FIG. 16.   Rear view of primordial cranium, looking slightly from above.   Same specimen as Figs. 8–11.   The intercalar bone removed on left side.   × 1½.

FIG. 17.   Top view of parasphenoid of a different and larger specimen.   × 1½.

FIG. 18.   Top view of hind end of cranium and first six vertebrae of a third specimen.   On the right side the occipitale laterale and the dorsal, spinal, and occipital arches have been entirely removed.   On the left side the ventral half of the occipitale laterale and the ventral, cartilaginous ends of the dorsal arches are left in place.   × 2.

Pl XXII.
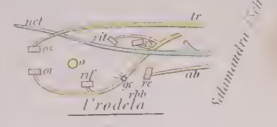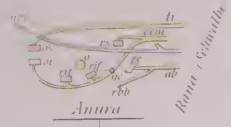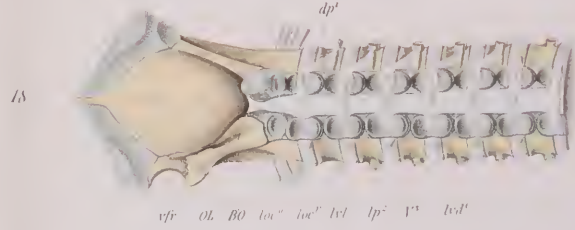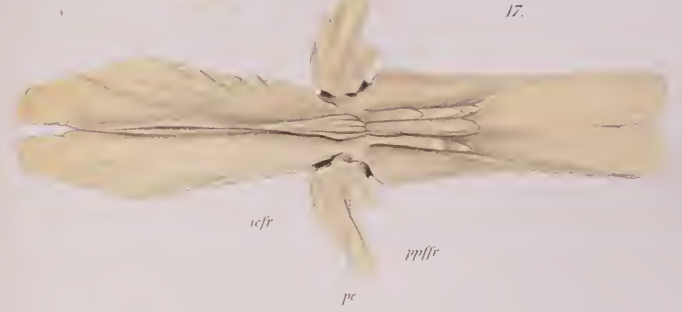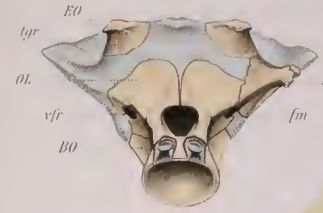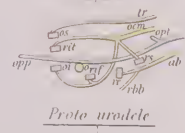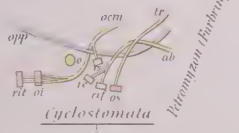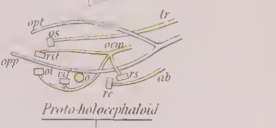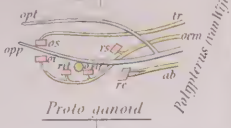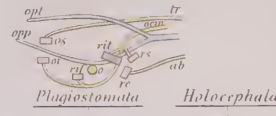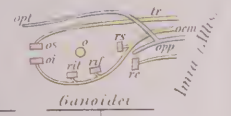
EXPLANATION OF PLATE XXIII.

FIG. 19. Top view of head of adult Amia. On the left side the dermal bones have been entirely removed ; on the right side they have been left in place, but scraped so as to expose the lateral canals and the ends of the nerves that innervate the canal organs. On the left side the anterior extension of the trunk muscles that fills the temporal groove has been removed. × 1½.

FIG. 20. The same, a deeper dissection. The eyeball has been removed on the left side, the surrounding tissue being left in place and represented as transparent in its ventral portion so that the nerves below it can be seen. × 1½.

Pl. XXIII

## EXPLANATION OF PLATE XXIV.

Fig. 21. Same as Figs. 19 and 20, but a still deeper dissection. On the left side only a thin outer shell of the chondrocranium has been left, and this shell has been cut through in one place to show the full course of the supratemporal branch of the glossopharyngeus. The left eyeball, which was entirely removed in Fig. 20, is shown cut in this figure. × 1½.

Fig. 22. Top view of right eyeball of another, unusually large specimen, with the associated nerves and muscles ; the rectus superior cut and turned back. × 2.

Fig. 23. Bottom view of same, the rectus inferior cut and turned back. × 2.

Fig. 24. Top view of cartilaginous capsule (sclerotic) of same, the dorsal part of capsule removed. × 2.

Pl. XXIV

## EXPLANATION OF PLATE XXV.

FIG. 25.  Same as Figs. 19, 20, and 21, but a still deeper dissection.  Both eyeballs have been entirely removed.  On the left side the adductor mandibulae, the levator arcus palatini, the dilatator operculi, and the upper half of the hyomandibular have also been removed.  × 1½.

FIG. 26.  Top view of inside of cranium of specimen used for Figs. 22–24.  The brain has been entirely removed, but the spinal cord and the roots of all the nerves have been left in place.  The trochlearis is cut near its place of exit from the cranium.  × 1½.

FIG. 27.  Same as Fig. 22, but showing the nerves only.  × 2.

FIG. 28.  Reconstruction from a series of transverse sections of a 35 mm. Amia to show the dorsal aorta and its branches.

Pl. XXV.

## EXPLANATION OF PLATE XXVI.

Fig. 29.   Side view of specimen used for Figs. 19, 20, 21 and 25.   The skin and dermal bones have been removed, the dissection corresponding nearly with that shown in Fig. 19.   × 1½.

Fig. 30.   Same as above, a deeper dissection, corresponding nearly with that shown in Fig. 21.   × 1½.

Pl. XXVI.
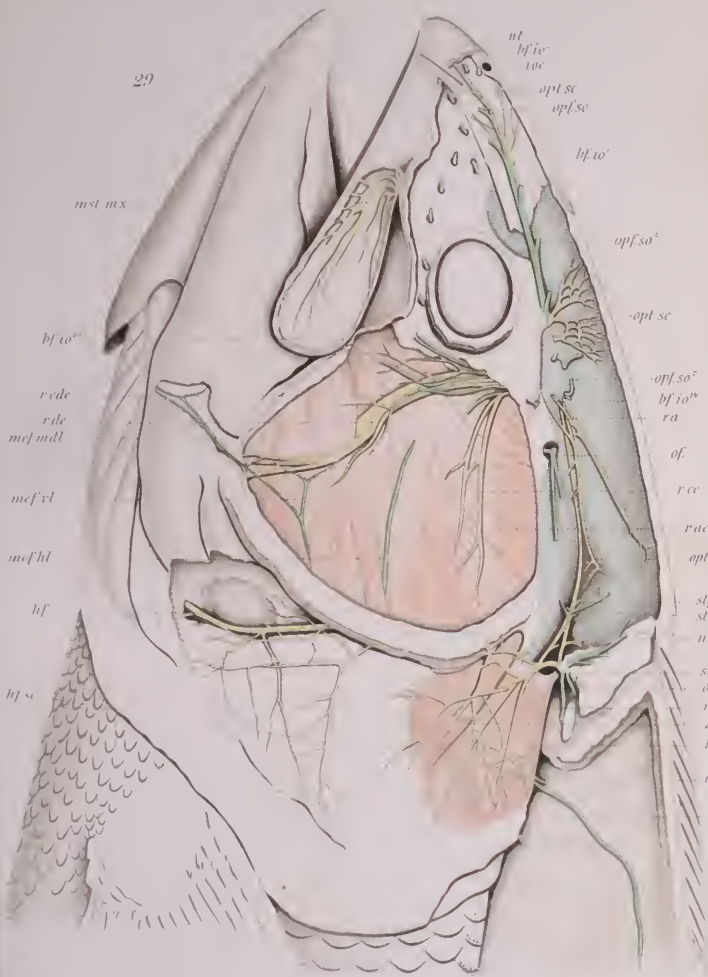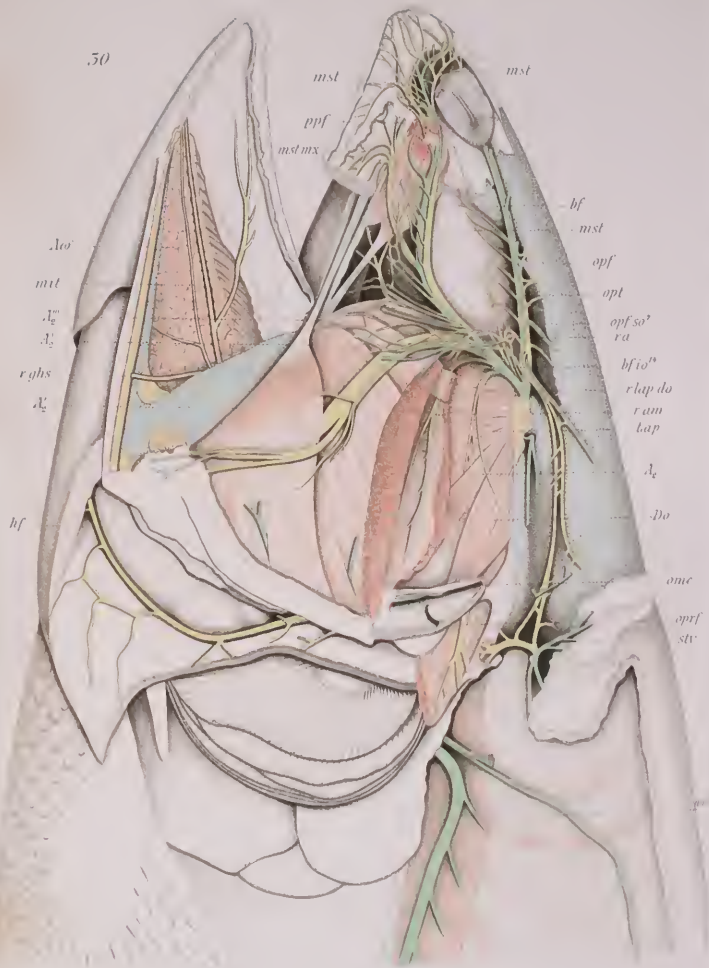
EXPLANATION OF PLATE XXVII.

FIG. 31. Same as Figs. 29 and 30, but a still deeper dissection. The coronoid process of Meckel's cartilage has been removed, the lateral canals in the preoperculum exposed, and the gill filaments removed from the branchial arches. $\times$ 1½.

FIG. 32. Same as above, but still deeper. The hyoid and pterygo-palatine arches have been almost entirely removed so as to expose the branchial arches and the levatores arcuum branchialium. $\times$ 1½.

Pl. XXVII.



31.

32.
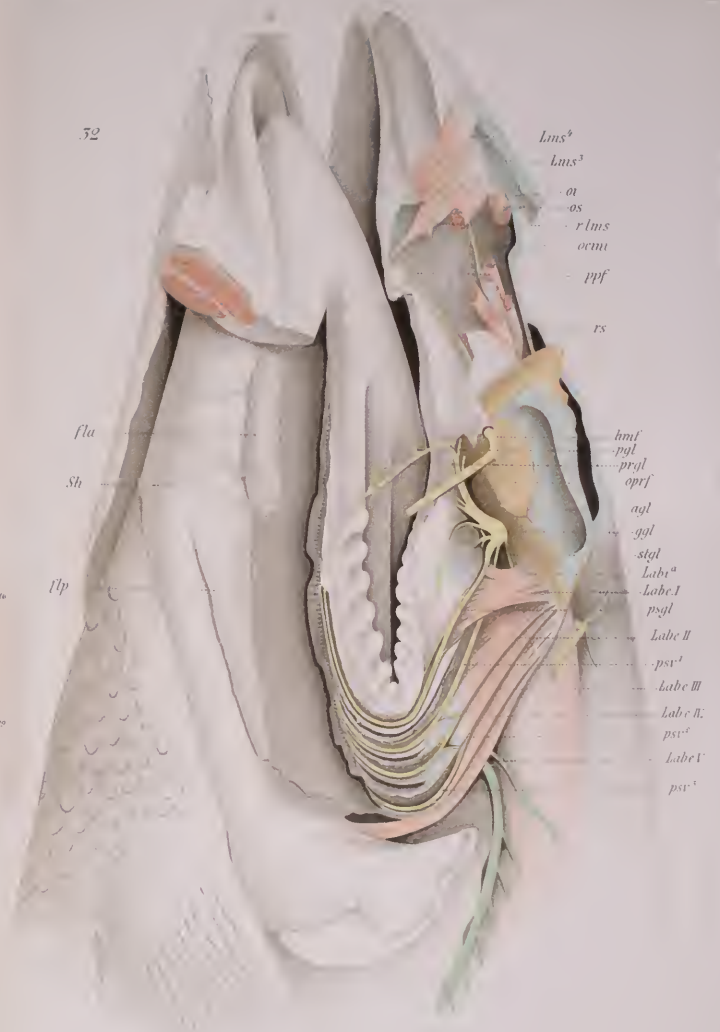
EXPLANATION OF PLATE XXVIII.

FIG. 33. Side view of occipital and anterior trunk regions of adult Amia. The branchial arches, excepting only their ventral ends, the corresponding portion of the clavicle with the pectoral fin, and the dermal bones between the clavicle and the skull and those on the top of the latter have been entirely removed. The vagus and glossopharyngeal nerves are cut near their exits from the skull, the spinal nerves near the points where they enter the pectoral fin. The occipital nerves are left entire. The pharyngo-claviculares are shown, the externus entirely cut, the internus partially so. × 1½.

FIG. 34. Same as Fig. 33, but another specimen. The occipital and spinal nerves have been pulled down out of their natural positions so as to show their anastomoses. The position of the pectoral fin is indicated by dotted lines, as is also the position of the deeper portion of the eighth intermuscular septum. The muscle fibres between the dorsal portions of the intermuscular septa have been removed so as to show the pockets in the latter. × 1½.

33.

34.

EXPLANATION OF PLATE XXIX.

Fig. 35. Side view of occipital and anterior trunk regions of a third specimen. The muscle fibres of the muscle segments have been entirely removed, leaving the nerves and intermuscular septa in position, but considerably cut. The intermuscular septa of the sternohyoideus are shown in a similar manner. The horizontal intermuscular septum, in the twelfth muscle segment, has been cut so as to show the ventral branch of the spinal nerve of the segment in position. × 1½.

Fig. 36. Side view of the deeper muscles on the side of the head, the two divisions of the adductor mandibulae turned downward and forward. × 1½.

Fig. 37. The same, the upper end of the hyomandibular, with the levator arcus palatini and dilatator operculi, removed. × 1½.
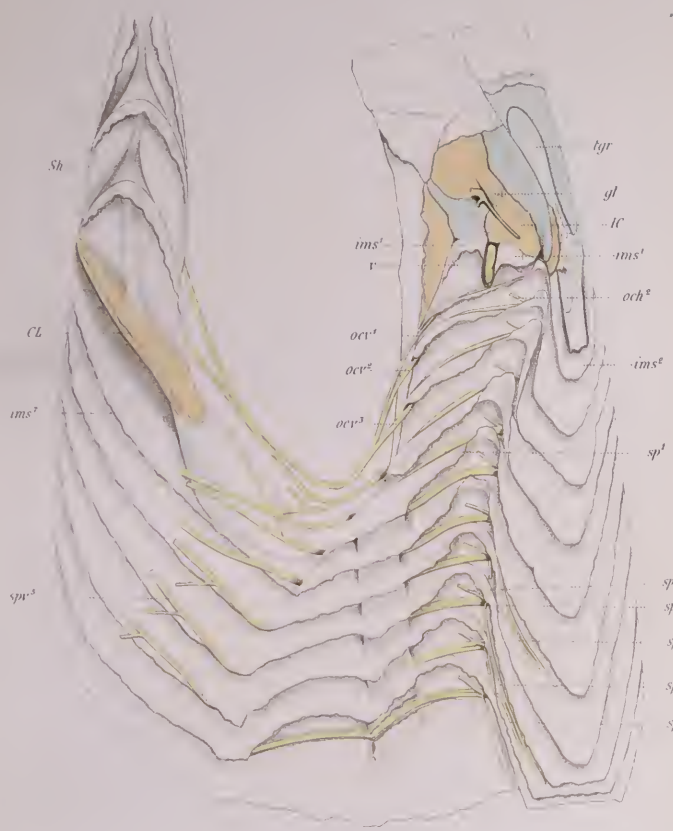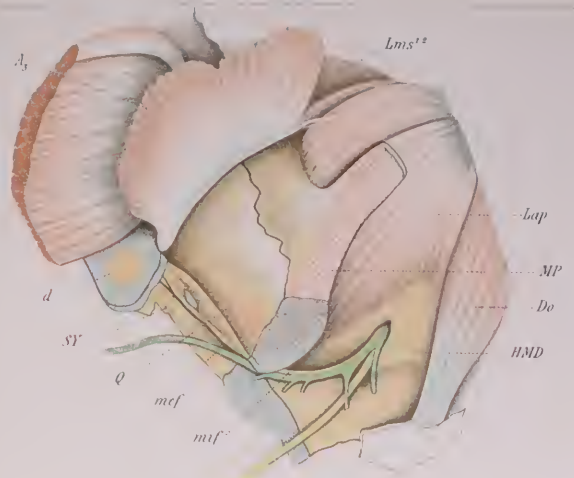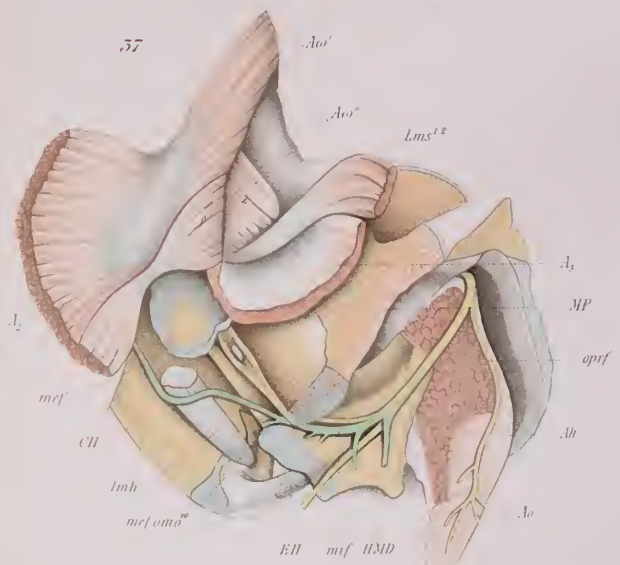
EXPLANATION OF PLATE XXX.

FIG. 38. Enlarged side view of trigemino-facial ganglionic complex, with nerves arising from it. The hyomandibular and palatine branches of the facialis are not shown. Same dissection as shown in Figs. 20 and 30.

FIG. 39. The same, still more enlarged. The ophthalmic and buccal branches of the facialis have been turned back to expose the Gasserian ganglion, and the accessory trigeminal nerves have been turned forward and pulled apart to show their origins and interconnections.

FIG. 40. Side view of Meckel's cartilage, and the adductor mandibulae and levator maxillae superioris muscles. $\times$ 1½.

FIG. 41. Inside view of same. $\times$ 1½.

FIG. 42. The same with muscle $A_3$, and the second and third divisions of the levator turned down. $\times$ 1½.

Pl. XXX

EXPLANATION OF PLATE XXXI.

FIG. 43. Bottom view of head of adult Amia, the skin, gular plate, and part of right mandible removed. × 1½.

FIG. 44. The same, a deeper dissection. The hind corner of the right mandible and ramus *r.ghi* turned outward so as to show the internal mandibular branches of the trigeminus and facialis. × 1½.

Pl. XXXI
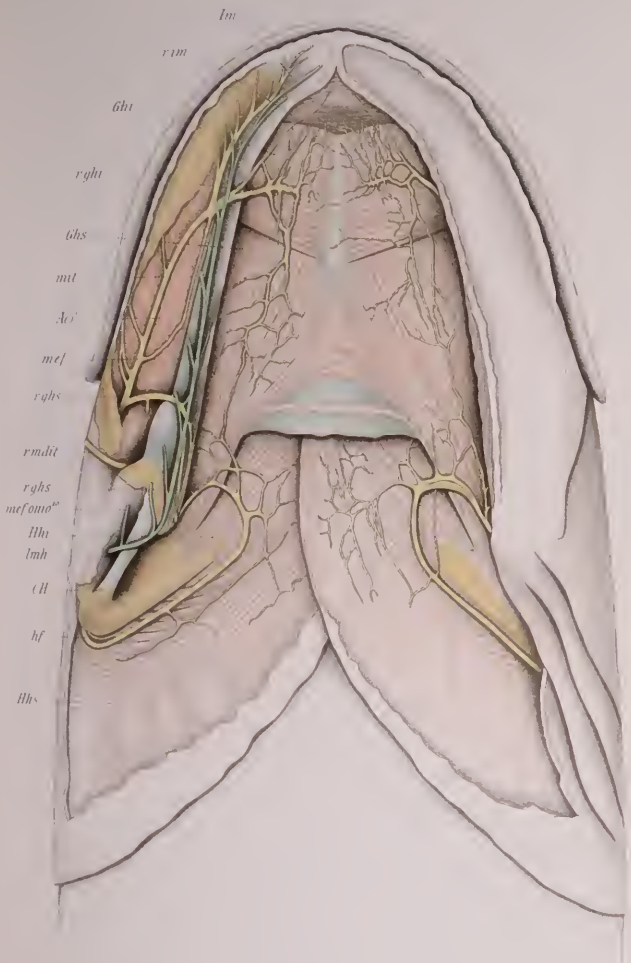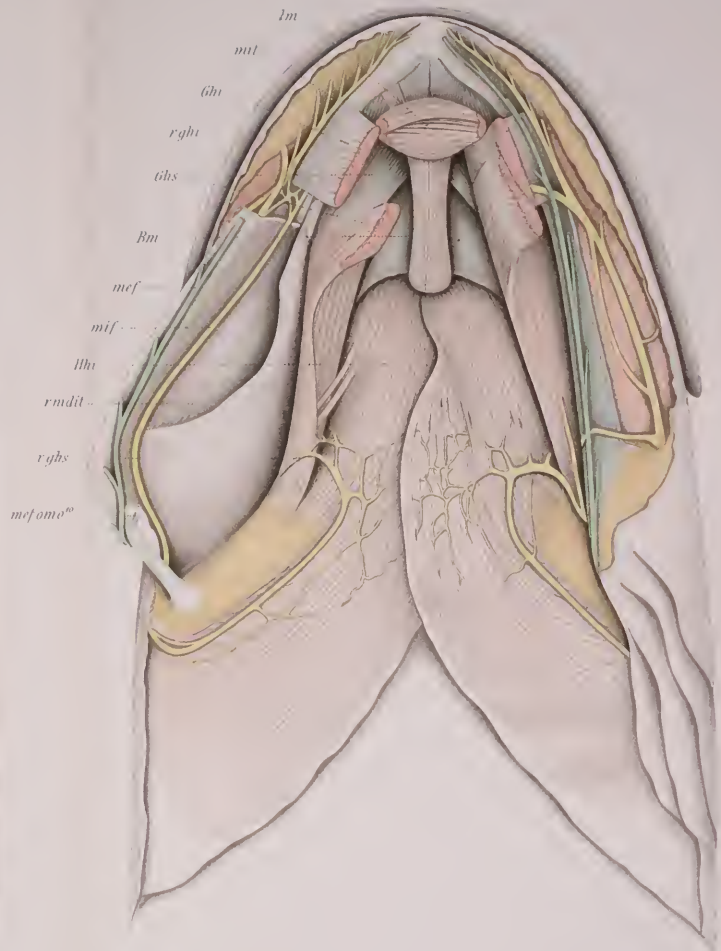
## EXPLANATION OF PLATE XXXII.

FIG. 45. The same as Figs. 43 and 44, with the geniohyoidei, the anterior half of the branchio-mandibularis, and part of the tendinous ends of the hyohyoidei removed. × 1½.

FIG. 46. The same, the hyohyoidei and one half of the sternohyoideus removed. × 1½.

Pl. XXXII.

45.

46.



Sh

Bm

Hh

Ghs

Hh

Ch

Bm

Sh

r bm

Caa⁴
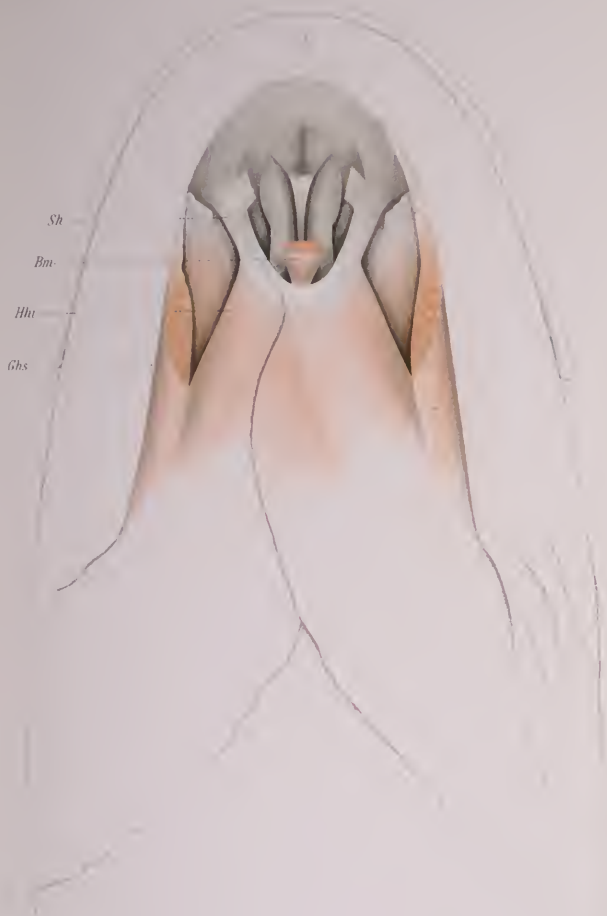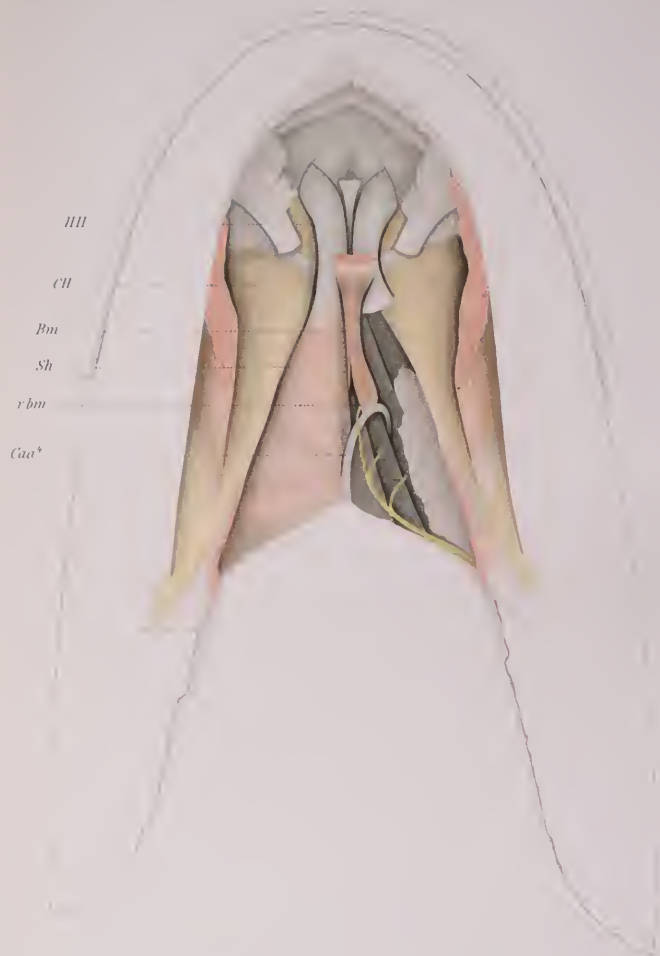
EXPLANATION OF PLATE XXXIII.

FIG. 47. The same as Fig. 46, but still deeper. The hyoid arches and sterno-hyoidei have been cut so as to leave only their anterior ends, and the clavicles and the heart and blood-vessels have been entirely removed, so as to expose the under surfaces of the branchial arches and basal line, with the nerves and muscles that lie upon them. × 1½.

FIG. 48. The same as Fig. 47, showing the ventral surfaces of the fourth and fifth ceratobranchials of the right side of the head, and the muscles associated with them. × 2.

FIG. 49. Dorsal view of the branchial and hyoid arches of the left side of the head. × 1½.

FIG. 50. Ventral view of the branchial and hyoid arches of the right side of the head. × 1½.

FIG. 51. Side view of the basal line. × 1½.
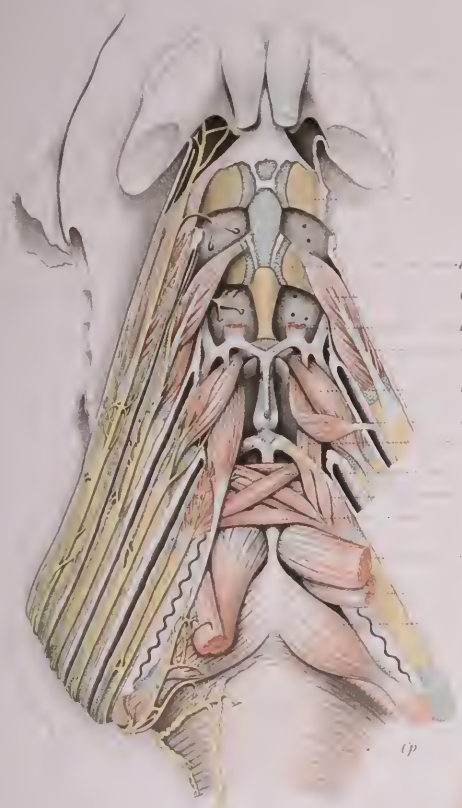
Pl. XXXIII



47

Sh

CH

HB.I

HB.II

Ov.II

Bm

Caa⁴

Ov.III

Ov.IV²

Ov.IV³

Tva

Ov.IV¹

Tva

Pci

Pce

Tvp

48

Op

Tvp

CB.V

CB.IV

Od¹

Aab.V

Aab.IV

49

EB.I

E.B.II

E.B.III

E.B.IV

E.B.V

IPB.II

IPB.III

CB.I

IPB.I

CH

HB.I

HII

liv.IV    liv.III    liv.II    liv.I    liv.h

50

CH

HB.II

HII

Sh

E.B.V

CB.V

51

BB.V    BB.²    BB.¹
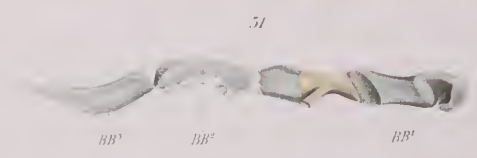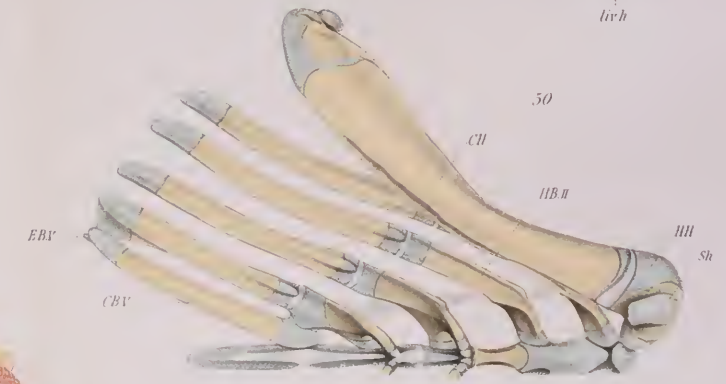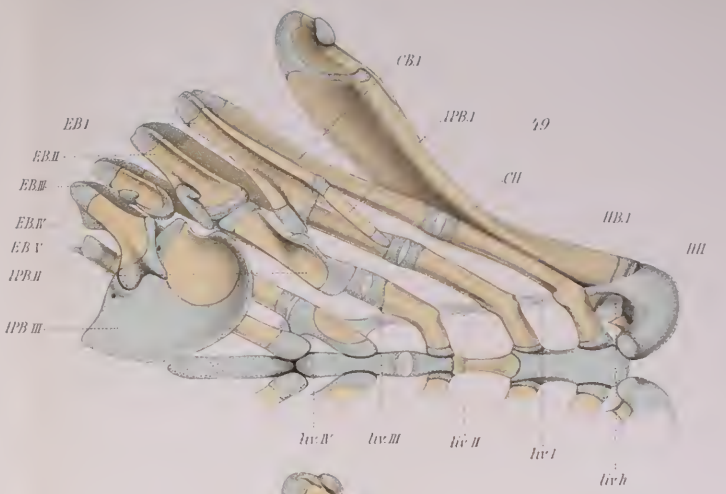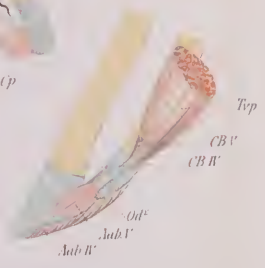
EXPLANATION OF PLATE XXXIV.

FIG. 52. Dorsal view of the branchial arches and the floor of the mouth cavity, with the muscles and nerves in place. The cranium has been entirely removed excepting a small piece where the levator muscles have their origin. On the left side the transversus dorsalis and the retractor arc. branch. dors. have been removed. × 1½.

FIG. 53. Same as above, with the muscles entirely removed on the left side so as to show their insertions on the branchial arches. On the right side the levator muscles are cut about the middle of their length, and the vagus nerve is turned medianward and downward so as to expose its branches. The ventro-lateral surface of the nerve is thus shown in the drawing. × 1½.

Pl. XXXIV



52.

53.

ec

hor

ca

ggl
stgl
Labi^a
gv^1
gv^1

Labi^p
Td^1
Od^1
Labc IV

Od^2
Td^2
Od^3

Labc V

Cp

IPB 1

lid I

EB.1

lid II

lid III

Rabd

Labc I
gv^1

gv^2
gv^3
gv^4

Labc IV

Labc V
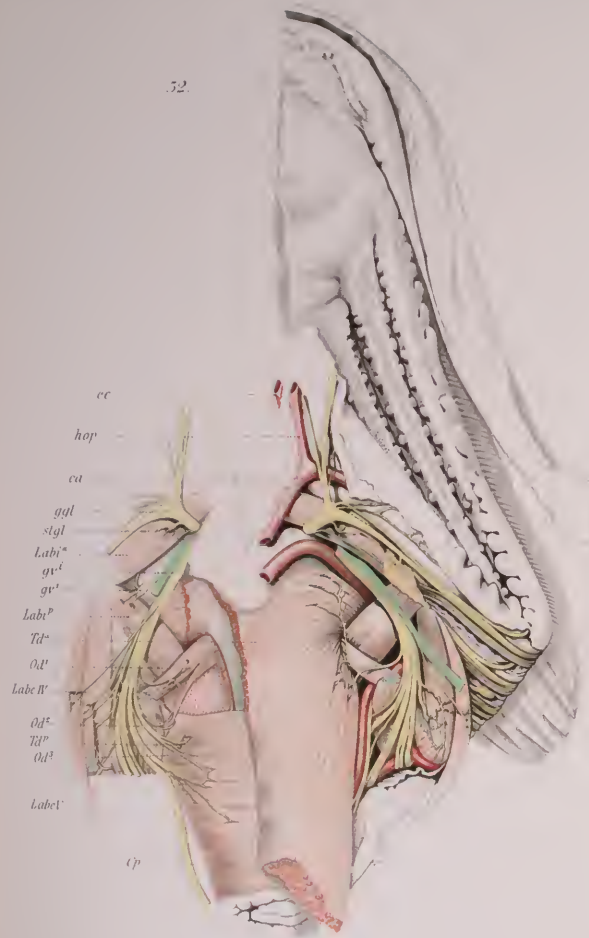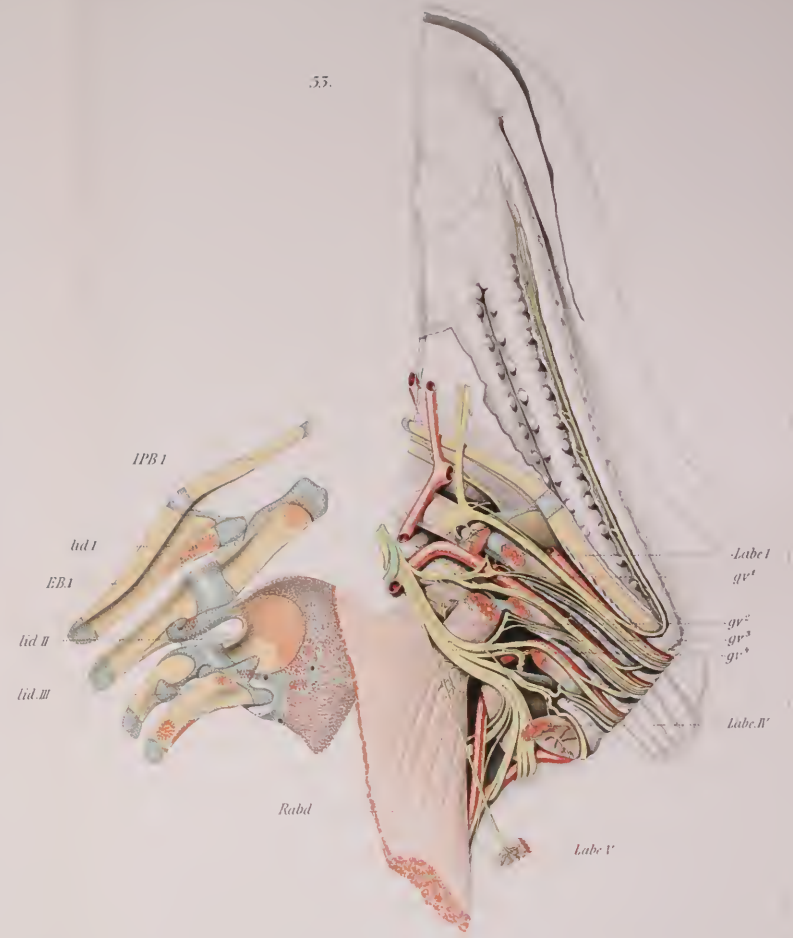
EXPLANATION OF PLATE XXXV.

Fig. 54. Same dissection as Figs. 52 and 53. Shows the dorsal surfaces of the branchial arches on the right side of the head, the muscles and arteries entirely removed, and the nervus vagus turned medianward and downward as in Fig. 53. × 1½.

Fig. 55. Same as above, but showing the arches and efferent arteries only, with the sympathetic nerves that surround and follow the arteries. × 1½.

Fig. 56. Top view of the outer ends of the fourth and fifth arches, with the adductor muscles and the second obliquus dorsalis. × 2.

Fig. 57. Top view of the left ear with the associated nerves. × 2.

Fig. 58. Top view of the sternohyoideus, showing the distribution of the occipital nerves. × 1½.

Fig. 59. Top view of the vagus and glossopharyngeal nerves in a specimen in which the first vagus nerve and the glossopharyngeus were united by a communicating branch. The glossopharyngeus has been cut away between its ganglion and its point of exit from the brain, and pulled slightly forward. × 3.
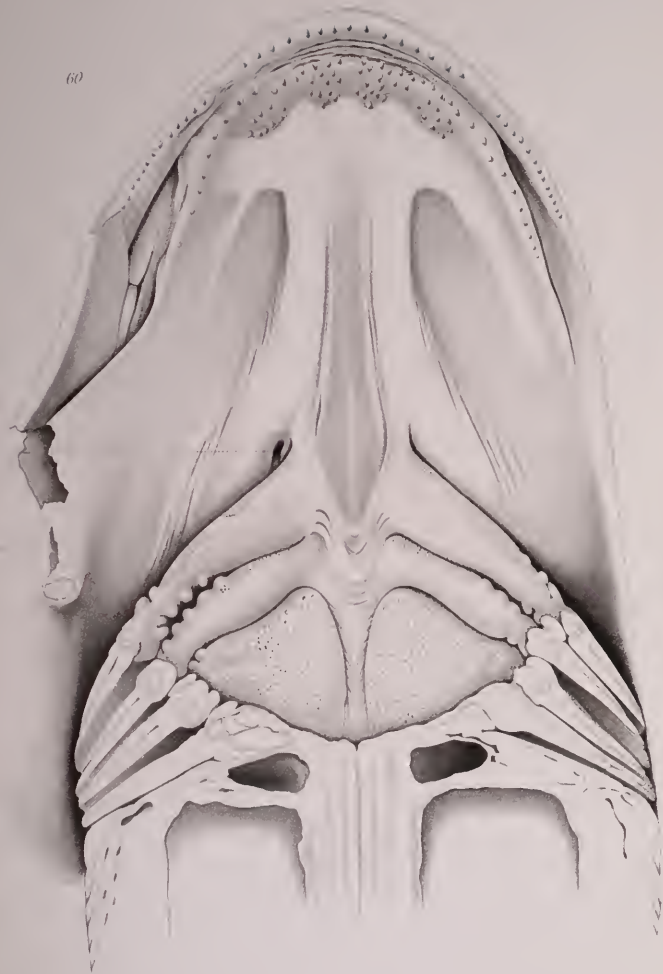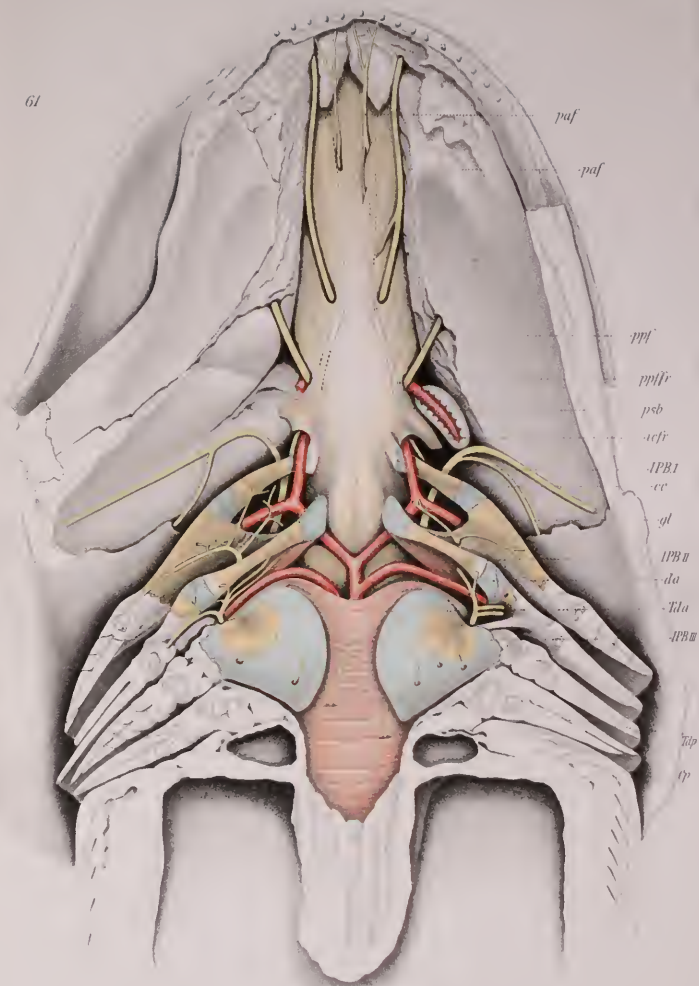
Pl. XXXI
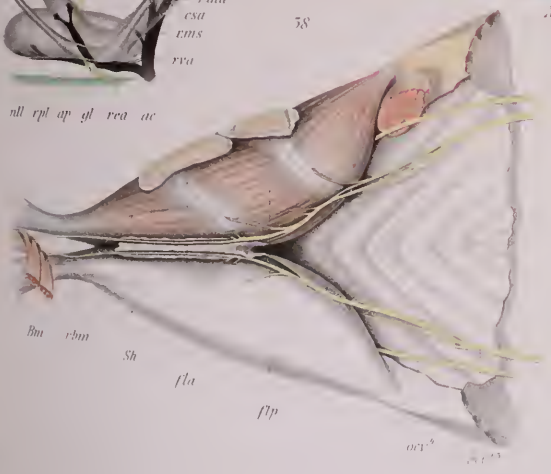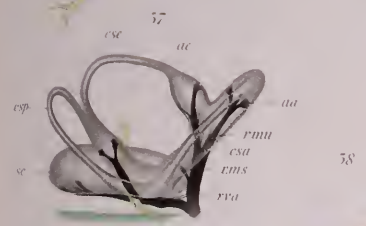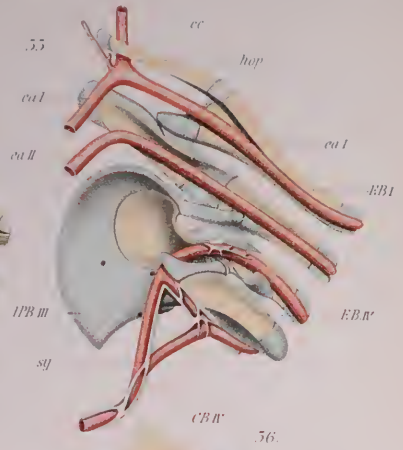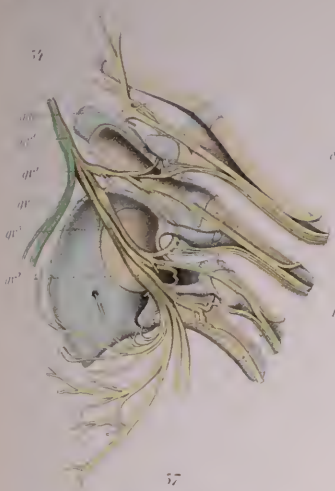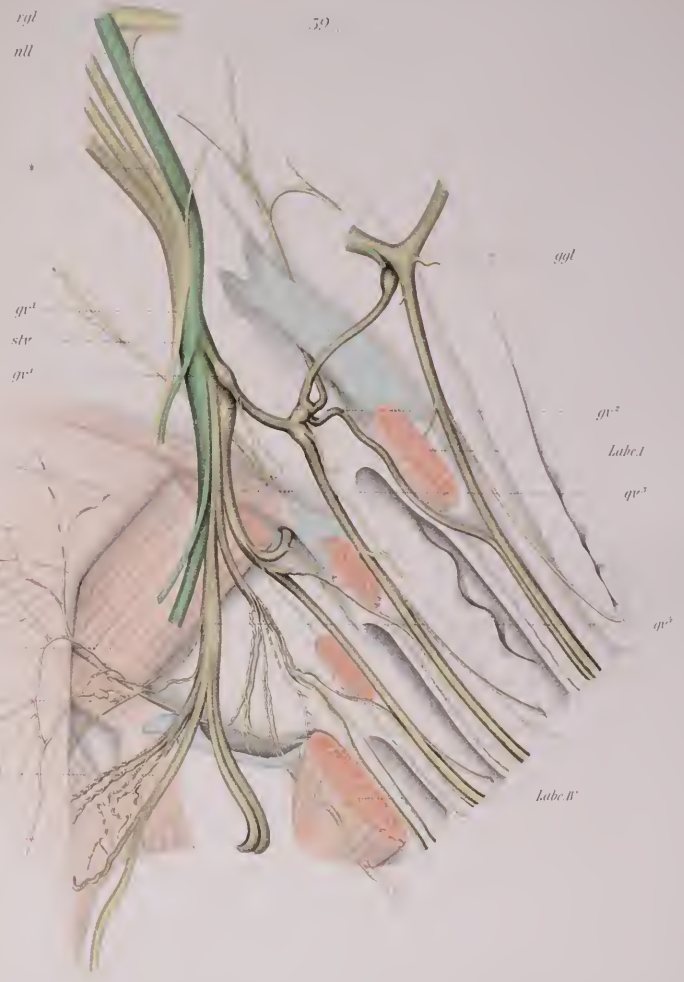
EXPLANATION OF PLATE XXXVI.

FIG. 60. Dorsal surface of mouth cavity and branchial chamber seen from below. The lower jaw, the lower half of the branchial arches, and the lower half of the oesophagus have been cut away. On the right side of the head the first branchial arch is pressed back slightly so as to show the ventral opening of the spiracular canal. × 1½.

FIG. 61. Same as above, the skin and underlying tissues removed so as to expose the ventral surface, of the cranium and the ventral surfaces of the dorsal halves of the branchial arches. The efferent arteries and the pharyngeal and pretrematic branches of the facialis, glossopharyngeus, and vagus nerves are seen. The pseudobranch on the right side of the head has been removed. × 1½.

Pl XXXVI



60

61

paf

paf

ppf

ppfTr

psb

acfr

IPB.I

cc

gl

IPB II

da
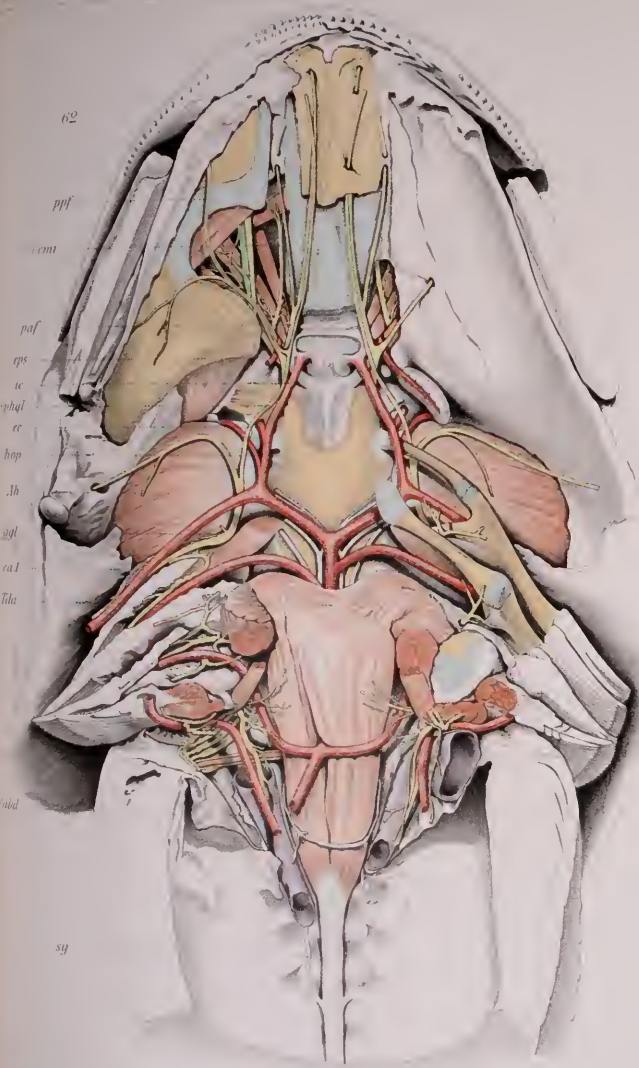
Tda

IPB III

Hyp

tp

spi

EXPLANATION OF PLATE XXXVII.

FIG. 62. Same as Figs. 60 and 61, but a deeper dissection. The pharyngeal bones on the right side of the head and the constrictor pharyngei have been entirely removed. On the left side of the head the first two infrapharyngobranchials and a part of the third are left in place. The parasphenoid has been partly removed, exposing a part of the base of the skull. A part of the eye-muscle canal is seen filled with membranous and fatty tissues. The pterygo-palatine arch on the right side of the head is partly cut away so as to show the muscles and nerves of the orbit. × 1½.

FIG. 63. Same as above, but still deeper. The median portions of all the visceral arches with their associated muscles, the retractor arcuum branchialium included, have been entirely removed. The larger part of the base of the chondrocranium has also been removed. The ventral surfaces of the vertebral column and the trunk muscles, of the hypophysis cerebri, the saccus vasculosus, and the eyeballs and their associated nerves and muscles, are thus exposed. × 1½. -

62

ppf

cm

paf
eps
ic
ephgl
ec
hop
Ah
ggl
ca.l
Tda
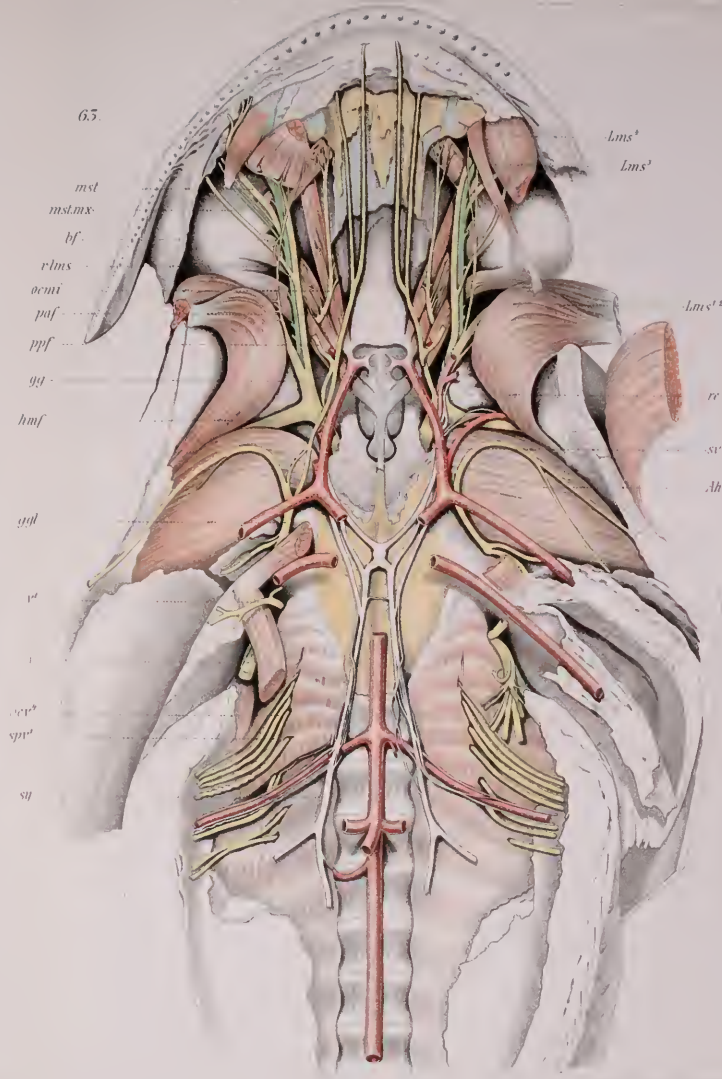
abd

sg

63.

mst
mst.mx
bf
r.lms
ocmi
paf
ppf
gg
hmf

ggl

rᵈ
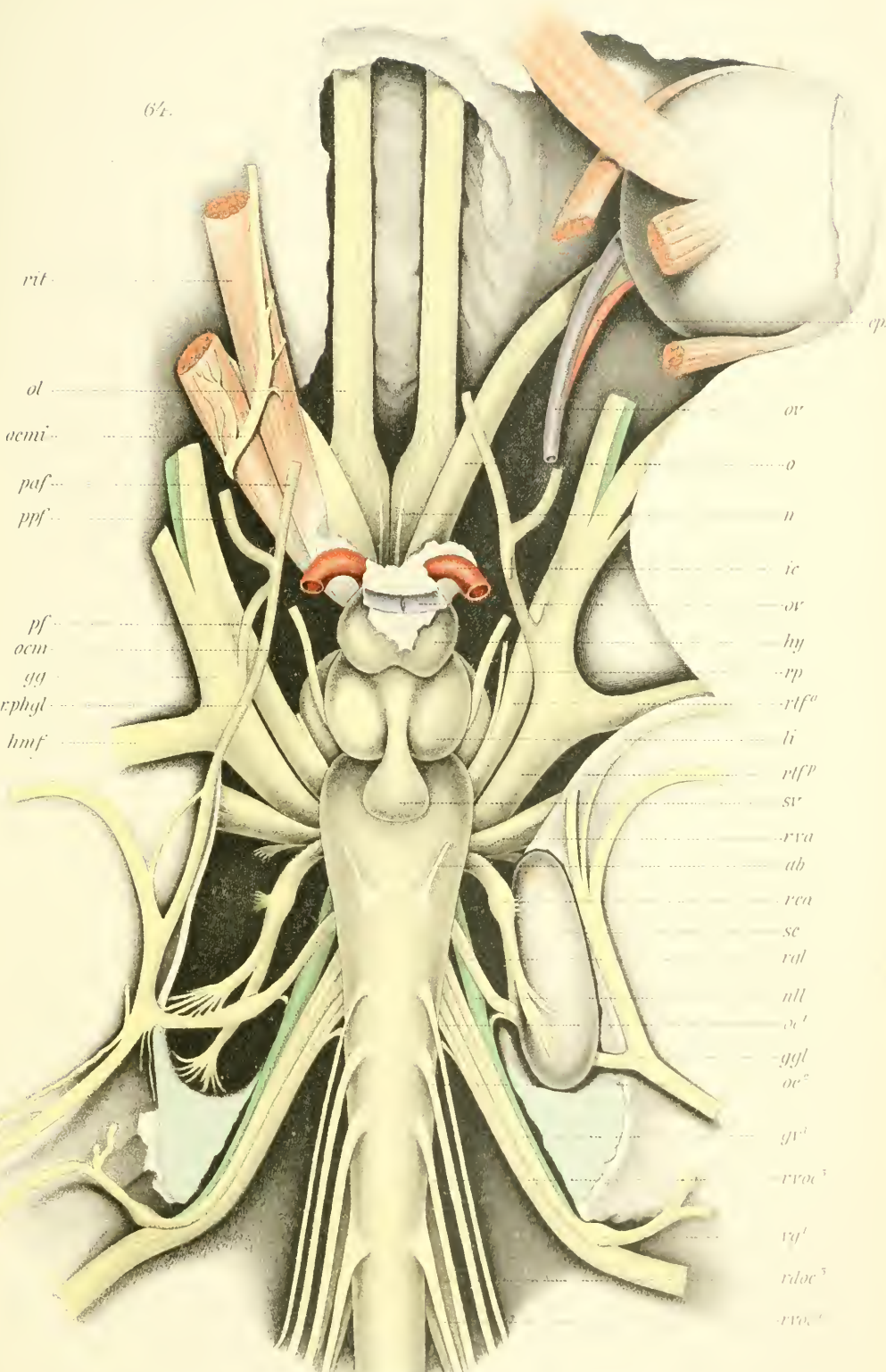
i

cᵉrᵛ
spr²

su

Lms⁴
Lms³

Lms⁴⁻²

re

sv

Ah

EXPLANATION OF PLATE XXXVIII.

FIG. 64. Same as Fig. 63, but still deeper. The base of the skull has been entirely cut away so as to show the base of the brain and the roots of the nerves as far back as the fourth occipital nerve. The sympathetic nerves have all been removed. × 4.

Pl. XXXVIII.



64.

rit

ol

ocmi

paf

ppf

pf

ocm

gg

r.phgl

hmf

eps

ov

o

n

ic

ov

hy

rp

rtf°

li

rtfᴾ

sv

rva

ab

rea

sc

rql

nll

oc¹

ggl

oc²

gv⁴

rvoc³

vq⁴

rdoc³

rvoc⁴

# THE ORIGIN OF THE CLEAVAGE CENTROSOMES.

KATHARINE FOOT, Evanston, Illinois.

DURING the past two years the weight of authority has greatly increased in favor of the conclusion that the cleavage centrosomes are of spermatic origin.

These centrosomes are asserted to be the daughter centrosomes of the so-called male centrosome, and this is assumed to originate from the middlepiece of the spermatozoön, — to be of the very substance of which the middlepiece was formed.

The phenomena of fertilization in the egg of *Allolobophora foetida* do not sustain this view; they suggest that this centrosome has the same origin as the so-called egg centrosome, and that both are cytoplasmic elements, of like origin and constitution.

The sperm attraction sphere and the fertilization cone have several points in common; both structures appear to be dependent, not alone upon the entrance of the spermatozoön, but also upon a definite stage of maturation reached by the egg; for I have never found a cone after the first polar body is constricted off, nor a sperm attraction sphere before the anaphase of the first spindle, no matter how far the spermatozoön may have penetrated into the egg; both appear to be formed of substances not confined to the structures themselves, but belonging to the entire cytoplasm.[1]

These observations suggest that both the cone and the attraction sphere are expressions of a definite effect produced upon the cytoplasm by the entrance of the sperm, the phenom-

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[1] In the summer of 1895 I differentiated from the cytoplasmic network a substance (archoplasm) which is present in the attraction spheres, in the fertilization cone, in the spindle, and throughout the cytoplasm (see *Journ. of Morph.*, vol. XII, Plate I). Recently I have differentiated the microsomes and centrosomes from the cytoplasm and archoplasm (Figs. 2, 3, 4, 7, 9, 10). These microsomes do not appear to be merely thickenings of the cytoplasmic threads; they appear to be morphological elements, many of them scattered throughout the cytoplasm in a relatively independent manner, others imbedded in the cytoplasmic network.

ena differing in that the anterior end of the head of the spermatozoön produces a cone, whereas it is the middlepiece that produces the attraction sphere.

Before the first polar body is formed we find a cone the moment any part of the head of the spermatozoön penetrates the egg, and the extent of the cone appears to depend upon the length of head which has entered : if a relatively short piece of the head has entered, we find a relatively small cone. The sperm attraction sphere, on the contrary, does not appear until the middlepiece is within the egg, and in other forms this fact has been cited as evidence for the assumption that the centrosome of the sperm attraction sphere is of the substance of which the middlepiece of the spermatozoön was formed.

The more important observations adduced to prove that the male centrosome is formed from the middlepiece are the following : First, the attraction sphere does not appear until after the spermatozoön has entered the egg. Secondly, it appears at or near the point formerly occupied by the middlepiece of the spermatozoön. Thirdly, the middlepiece and the centrosome have been shown to select the same stains. Might not the first and second observations be cited with equal pertinence to prove that the spermatozoön produces merely a physiological *effect* upon the cytoplasm ? The third observation has a more definite bearing upon the point in question. In the egg of *Allolobophora foetida*, however, I have differentiated by two methods the middlepiece of the spermatozoön from the male centrosome. This differential staining might be explained by the assumption that the middlepiece undergoes a physiological change on entering the egg ; but as the head gives no such evidence of physiological change, are we justified in assuming without further evidence that this takes place in the middlepiece ?

The centrosomes of the egg of *Allolobophora foetida* lend support to the view that the centrosome is a mechanical center — the *expression*, rather than the cause, of cell activity. The egg attraction sphere is present during the two maturation divisions, but after the second polar body is formed and the female pronucleus begins to develop, it totally disappears (Fig.

5). The sperm attraction sphere is present until the head of the spermatozoön begins to develop into the male pronucleus, when it also totally disappears (Fig. 6). Both spheres are absent during a relatively long period (*i.e.*, while the young pronuclei are developing) ; and when the pronuclei have attained their maximum size and are in contact, two attraction spheres appear again in the cytoplasm, and the cleavage spindle is formed.

*Method.* — The middlepiece of the spermatozoön was first differentiated from the centrosomes by iron haematoxylin followed by erythrosin. The same results were obtained by staining with a mixture of orange and methyl green. (Saturated aqueous solution of orange two parts, in eight parts water. Saturated aqueous solution of methyl green one part, in four parts water. Wash in absolute alcohol.) This stain was the outcome of a series of experiments with the anilins, undertaken with the aim of supporting the method of double staining (Figs. 8–10) by one containing fewer possibilities of error. This method demands relatively no technical manipulation, and practically the same results were obtained whether the sections remained in the stain fifteen minutes or twenty-four hours. The stain was used after several fixatives, with the same result, but all the figures for this paper were drawn from chromo-acetic preparations.

An examination of the figures will show that a variety of structures select the methyl green, — *viz.*, the nucleoli, microsomes, centrosomes, chromosomes, and sperm granules, — and that the orange is selected by the chromatin of the vesicles and resting nucleus, the cytoplasmic network, and the archoplasm (which is present in the attraction spheres, fertilization cone, spindle, and throughout the cytoplasm).

This method of staining sharply differentiates the very young nucleoli of the vesicles found at the telophase of the second maturation spindle, and destined to become the female pronucleus. These vesicles when first formed are eleven in number, thus corresponding with the chromosomes. As they progress towards the formation of the female pronucleus, their walls break and fuse with each other, thus forming the chro-

matic network of the pronucleus, while their nucleoli grow and
fuse with one another. Fig. 5 shows several small vesicles
and a few relatively large vesicles, the result of the fusing of
two or more small ones. Just before the vesicles appear, the
chromosomes select the methyl green stain, but as soon as
the vesicles are formed we have a tiny, green nucleolus sur-
rounded by *yellow* chromatin, this clearly suggesting that the
nucleolus is a substance which a moment before was contained
in the chromosomes. The chromatin which at first surrounds
each vesicle continues to select the orange stain until it has
again assumed the form of chromosomes. Fig. 6 shows the
male pronucleus forming in exactly the same manner as the
female pronucleus, the head of the spermatozoön breaking up
into vesicles similar to those of the young female pronucleus.

Vesicles entirely similar to those found at the telophase of
the second maturation spindle are found also at the telophase
of the first maturation spindle, though I have never seen any
evidence of their development into a resting nucleus.

*Sperm Granules, Fig. 2.* — The sperm granules are not con-
stant structures. In eggs found during the height of the
breeding season (when they are less likely to present ab-
normalities), the sperm granules are either not present at all
or are relatively insignificant in both size and number, and
in such cases they are often found near the posterior end
of the head of the spermatozoön. Near the close of the
breeding season, however, when few normal eggs are found
(sometimes only one in a cocoon that contains fifty eggs, show-
ing various degrees of degeneration), — at this season nearly
all the eggs having one or more fertilization cones contain
relatively many and large sperm granules. These granules
appear to be formed at the expense of some of the surrounding
substance, and such preparations as are represented by Fig. 2
suggest that the substance sacrificed to the formation of these
bodies is archoplasm, for there is relatively very little archo-
plasm at the side of the cone occupied by the sperm granules.

The suggestion that these granules are metamorphosed
archoplasm raises several questions that I am entirely incom-
petent to answer. Wherein do they differ from apparently

similar, and also inconstant, large and small granules (or bodies) found outside and sometimes within the first and second maturation spindles (Fig. 4), outside and sometimes within the attraction spheres (Figs. 3, 4, 9, 10), and, again, often scattered throughout the cytoplasm? Furthermore, as no exact distinction can be made as to size, how do these bodies differ from the microsomes? (Figs. 2, 3, 4, 7, 9, 10.) Can these sperm granules and large granules throughout the cytoplasm — apparently indicative of incipient degeneration — be at first merely an expression of abnormal activity in the cell? These questions must be left to the more experienced student to answer.

I am greatly indebted to Dr. Whitman for criticism of my work, and for other courtesies in connection with this paper.

EXPLANATION OF PLATE XXXIX.

*Zeiss hom. immer. 2 mm. 140 ap.    Abbe Camera.*

FIGS. 1–7.   Orange, methyl green.   See Method, p. 811.
FIGS. 8–10.   Iron haematoxylin followed by erythrosin.
FIGS. 1, 2, 3, 4, 8, × about 687.
FIGS. 5, 6, 7, 9, 10, × about 500.

FIG. 1.   Spermatozoön.   (Middlepiece yellow.)

FIG. 2.   Longitudinal section through fertilization cone.   (Only such micro-somes represented as appear to be independent of the cytoplasmic threads.)

FIG. 3.   Sperm rod and sperm attraction sphere.   (Microsomes and centrosome green.)

FIG. 4.   Longitudinal section through second maturation spindle.

FIG. 5.   Section of egg.   (Telophase of the second maturation spindle.   Vesicles forming female pronucleus.   Egg attraction sphere has nearly disappeared.   Micro-somes not represented.)

FIG. 6.   Vesicles forming male pronucleus.   (Male attraction sphere has dis-appeared.   Microsomes not represented.)

FIG. 7.   Pronuclei in contact.   (Attraction spheres present.)

FIG. 8.   Spermatozoön.   (Middlepiece red.)

FIG. 9.   Male attraction sphere.   (Microsomes and centrosome black.)

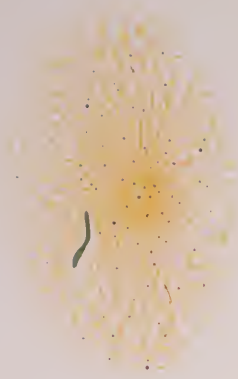FIG. 10.   Egg attraction sphere.   (Microsomes and centrosome black.)
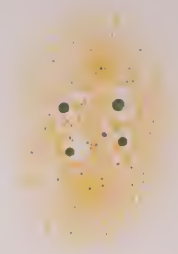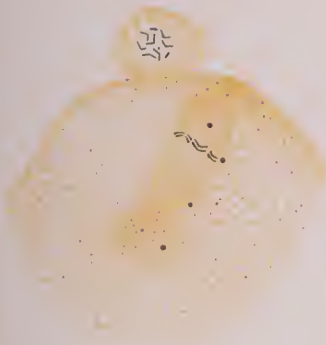
Pl. XXXIX.



1.

2.

3.

4.

5.

6.

7.

8.

9.

10.