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THE SPERMATOGENESIS OF BATRACHOSEPS.

POLYMORPHOUS SPERMATOGONIA, AUXOCYTES, AND SPERMATOCYTES.

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I. Introductory.

Owing to a projected and extended voyage, this paper had to be brought to a sudden close, and much which I had intended to include in it had to be left out and deferred to a second part. This latter half will include the somatic mitosis of the polymorphous spermatogonia, the evolutions of the spermatids and their development into spermatozoa, and also a discussion of the literature. In this paper I have merely endeavored plainly and briefly to describe my own investigations, and time has hardly permitted me even to touch upon those made by others on the same subject.

The results of the present investigations, of whatever value they may be, are principally due to improved cytological and optical methods, especially to the new fixatives and to the achromatic light, all described in their proper places. Without them the chromioles would yet have remained a mystery, at least as far as my own investigations are concerned. The figures illustrating this paper have been corrected as many as four different times, and the first thirty figures made have been completely redrawn, in order to secure that accuracy of detail which can only be had after repeated failures.

It is proper to state here that the testes of Batrachoseps are very favorable for study, being small and containing large cells. But these advantages are more than offset by the scarcity of the material. While these batrachians are very common almost everywhere in California, their testes are only active at a time when it is almost impossible to procure any specimens of the species. Batrachoseps attenuatus is only adult in the months of June and July, at a time when, on account of the dry season, these animals have retired down in the ground, almost out of reach. In the summer of 1897 I had, however, the good fortune to find in the end of June three fully adult specimens at Monterey, Cal., in a damp meadow which had been kept cool by the fogs from the ocean and shaded by overhanging trees. The largest of the three specimens was made useless by an accident in sectioning the testes; the others, however, turned out most admirable preparations,

which were made the material for this memoir. After-
wards I was of course able to supplement them with less
favorable material from specimens collected at other times.
In size the adult testis is about five millimeters long by one
millimeter wide. It consists of only one single lobe, undivided
and of cylindrical shape. In the central parts of this lobe
are the ripe spermatozoa, while the other cells are found
arranged on either side in the direction of the long diameter
of the testis. The four testes were sectioned longitudinally,
thus affording a comprehensive view of the arrangement of the
different cells.

It is with much pleasure that I here acknowledge my indebt-
edness to my esteemed friend, Dr. W. J. V. Osterhout, of
the University of California, for many valuable suggestions and
for assistance in correcting MS. and proof.

II. METHODS OF INVESTIGATION.

Fixatives.

The first investigations were made on testes fixed with the
classical fixatives — Flemming's chromo-aceto-osmic mixture
and the platino-aceto-osmic mixture of Hermann, used in various
strengths, with or without admixture of water. Heidenhain's
corrosive-sublimate-acetic was also tried, both with and with-
out addition of formalin. Experiments were also made with a
number of other fixatives, such as mixtures of Flemming's and
Hermann's with corrosive-sublimate and palladium-chloride.
Vanadium-chloride, uranium-chloride, and osmium-chloride were
also experimented with, all of which, with the exception of the
last, proved of no value. I soon satisfied myself that any mix-
ture containing either platinum-chloride or osmic acid, or both,
would completely ruin several of the outer rows of cells, mak-
ing them unfit for microscopic research. As the testes of
Batrachoseps are so very small and possess only a few rows of
cells, none of the above-mentioned fixatives could thus be used.
Platinum-chloride is even more injurious than osmic acid ; while
the latter destroys the chromatin, the former ruins the finer
structure of the cytoplasm. Although by the employment of

these fixatives some of the interiorly situated cells gave fairly good images, yet it was readily seen that the cytoplasm of the cell had become greatly contracted and distorted, to such an extent as to represent no more the true structure of the cell. It is comparatively easy to fix chromosomes and spindles, more difficult to fix the cytoplasm and the linin. Osmium-chloride is in many respects a most valuable fixative, especially in solutions of from one-half to one-tenth per cent, but it always possesses the undesirable quality of blackening the tissue, though in a lesser degree than osmic acid. I have, however, no doubt that this chemical will be found very useful for fixing several kinds of tissue, especially in connection with potassium-bichromate, when it allows of intense staining and detailed differentiation. The fixative on which I finally decided as giving the most satisfactory results, as regards the fixation of the testes of Batrachoseps, is a mixture of iridium-chloride-acetic, according to the formula published in the *Zeitschrift f. wiss. Mikr.*, Bd. XIV, pp. 195–202. The time of fixation is from three to twelve hours, though I think that the best results were had with the shorter time. There is no perceptible shrinkage of the tissue, no blackening of the cells, and the outer rows of cells are as perfectly fixed as those in the center.

After fixation the testes were washed in tap water for about one hour, then passed through the alcohols. For clearing, bergamot oil was found most suitable, but it was followed by xylol, which latter was again displaced by bergamot before imbedding in paraffin. The sections were cut in paraffin of 54 Fahr. melting point, and from 4 to 6 μ thick, each cell being cut in from two to three parts. This latter is of importance because cells which are not cut into do not stain properly, making it impossible to study the finer structures of the cell. The sections were then fixed to the slide by the alcohol method as described in Bd. XIV, *Zeitschr. f. wiss. Mikr.* (1897), pp. 195–202.

Stains.

The majority of the sections were stained by the Benda iron-haematoxylin method, and after-stained with congo. Another

set was stained with congo, thionin, and ruthenium red, as will be described below.

The liquor-ferri-sulphurici-oxidati was used diluted about six times, and the slides were kept in the solution for about twenty-four hours. The haematoxylin solution was used concentrated and contained about 10% of alcohol, the solution being a year old. The sections were kept in the haematoxylin solution for from forty-eight to seventy-two hours, the longer time giving the best results. The differentiation was made with a 10% solution of glacial acetic acid in water, to which was added a small part of the liquor-ferri, sufficient to give it a very pale straw color. In from fifteen to twenty minutes the differentiation was finished. The slides were now rinsed in water and counter-stained with a watery solution of congo for one or two minutes, then as quickly as possible dehydrated in absolute alcohol, cleared with bergamot oil, and mounted in gum-thus in xylol. Several of the slides were stained over two and even three times before a sufficiently satisfactory differentiation was obtained. The use of congo as a counter-stain was decided on only after long experiments with numerous other anilin colors, and it proved to be the only stain which gave the desired differentiation in the highest degree. It was the only satisfactory stain for the differentiation of the spheres and their secretions.

Another combination of stains which proved useful is a triple stain of congo, thionin, and ruthenium red. The slides were first stained for a few seconds with a weak solution of congo in water, then for about ten minutes with thionin in water, and then differentiated with a watery solution of ruthenium red. This latter stain was made extremely weak and of a pale rosy tint ; still the differentiation was accomplished in a few minutes. The ruthenium washes out the thionin, and the differentiation should always be carefully watched under the microscope. This combination of stains proved especially useful for differentiating the chromoplasts, and also for the study of the outlines of the chromosomes, especially where the latter overlapped each other, as the outlines of the separate chromosomes could always be distinctly seen.

Optical Methods and Appliances.

No one who has not used an oil-immersion substage con-
denser can have any adequate idea of the value of such an
appliance.   In the study of the finer structures of the cell it is
of equal importance with the apochromatic objective, and its
value cannot be overestimated.   It is my opinion that a correct
and proper view of the structure of the protoplasm cannot be
had without the oil-immersion condenser, and that opinions
based on observations made without this condenser must neces-
sarily need to be reconsidered.   An achromatic, or better yet
an apochromatic, condenser should be used, and the immersion
oil should be slightly denser than the oil used on the objective,
in order to counteract the thickness of the slide.   This can
readily be accomplished by warming the oil for a short time,
or by using oil that has become thicker from ordinary exposure
to the air.   The oil-immersion condenser increases the sharp-
ness of the image and brings out details not otherwise visible.

The images were studied with artificial light alone, a filter
of cyanin and methylene blue being interposed between the
Welsbach incandescent gas burner and the substage condenser.
For a more detailed account of this light see *Zeitschr. f. wiss.
Mikr.*, Bd. XIV, pp. 444–447, 1897.   The use of this light
enables us to work continuously, independent of sun and
clouds, day or night, with the same strength of light and with
the same facility.   This light has also a great advantage in
not injuring the eyes, not even tiring them perceptibly after
ordinary work.   The light has also another advantage in that
it differentiates structures which are not differentiated by stain-
ing.   For instance, by the daylight and after staining with congo
no differentiation was had between the linin granula and the
cytoplasmic granula, but with the artificial light the linin gran-
ula were seen to be of a gray or brown color, while the cytoplasmic
granula were stained pale red.   A better image can be had with
this artificial light than with the best white-cloud light, and the
image is also perfectly steady, which it never is with even the best
cloud light.   The sections were studied with Zeiss Apochromats,
3 mm., Ap. 1, 40, and 2 mm., Ap. 1, 40; Oc. 12 and 18.

III. Constituents of the Cell.

General Divisions of Cell Structures.

The division of the cell structures adopted in this paper is almost the same as the one proposed in my paper on the plasmocytes of Batrachoseps, the only important change being that of the word centrosome to centriole. The principal reason for this change is that many investigators have not only used this word for different structures, but in some instances have even discarded it altogether. The confusion is really so great that it is in many instances impossible to know with certainty what organ or what part of the cell is referred to. W. Flemming, in his report, " Morphologie der Zelle," 1897, rejects the name centrosome and substitutes the word "Centralkörper." As this word cannot possibly be accepted by other investigators than Germans, and as Boveri has previously named this body the centriole, I can see no valid reason why we should drop a really very useful word, about the meaning of which there cannot be any misunderstanding, and which is so constructed that it can be adopted in every language used by biologists. Literal translations of new words often so conflict with words already existing that great confusion results. It seems, therefore, most appropriate that in composing new words they should be so constructed that they may with a slight change be used in other languages, or, better yet, be used without any change. Words of this kind are, of course, words of Greek and Latin derivation. I would also suggest that every biological paper be accompanied by definitions of the nomenclature, worded in such a manner that no misunderstanding will ensue. If such a method had been adopted, we would not now have had years' wrangling about, for instance, the single little word " centrosome."

In this paper I propose to use the following nomenclature for the divisions of the cell :

Cytosome — the purely cellular part of the cell, the cytoplasmic parts, all parts exterior to the nucleus, except those bodies which are known as archosomes or parts belonging to them. The cyto-

some thus contains such constituents as cell wall, cytomicrosomes, the two spheres which I here designate as granosphere and plasmosphere, metaplasmic secretions and metaplasmic granules, various rays and fibers, and spindles.

Karyosome, or *nucleus* — comprises the following parts : nuclear wall, or karyotheca, chromosomes and their constituents, linin, what is generally known as nucleoli of various kinds. All bodies which when the cell is at rest reside within the nuclear membrane.

Archosome — the centriole with its spheres, the somosphere and the centrosphere, all structures which may be counted as centrosomal structures. When there are more than two archosomes, I refer to the others as accessory archosomes. For the innermost dark-staining granules I have adopted Boveri's name, "centriole." The thin zone surrounding the centriole or centrioles is the somosphere, and the zone surrounding the somosphere is the centrosphere. The archosomes or archosomal structures have not with certainty been found in the higher plants.

The Cytosome.

By the cytosome I understand all that part of the cell which is situated exterior to the nucleus, excepting those parts which are undoubtedly of the same nature as the archosomes. The cytosome comprises thus the following parts : the cytoplasm proper, the plasmosphere, the hyalosphere, the granosphere, metaplasmic secretions, the cell wall, and finally some granules of undetermined nature, generally scattered among the cytomicrosomes, also the fibers, the spindle, and the mid-body.

The Cytoplasm. — During the greater part of the life history of the cell the cytoplasm proper is difficult to distinguish from the various granules comprising the spheres. Sometimes it is also difficult to distinguish it from the linin granules of the nucleus. The latter difficulty exists only when the nuclear membrane has been dissolved, as at that time the linin granules are scattered all through the cytoplasmic part of the cell. But even then the cytoplasmic granules may be distinguished from the linin granules both by their staining quality and by

the way they are arranged into threads or fibers. With the light-filter the linin granules appear darker than the pure cytoplasmic granules, and it is even possible under favorable circumstances to follow the course of the linin granules from their dispersion from the nucleus to their appearance in the cytoplasm.

While I have included the two spheres as a part of the cytoplasm, I nevertheless hold that they are of a somewhat different nature, with different functions, from the cytoplasm proper.

During the perfect resting stage of the large cell with polymorphous nucleus the cytoplasm proper is confined to a very thin stratum surrounding the deeply folded nucleus. At that time there is no distinction between the granules of the cytoplasm proper and the granules of the spheres (Fig. 1), and it appears as if the latter might later on be differentiated out of the former, though we can with equal reason assume that they are fundamentally different, but that they are intermixed, and that they cannot be distinguished one from the other. At a later period in the development of the cell such a distinction is possible, as the staining capacity is much greater in the granules of the spheres than in those of the cytoplasm proper (Figs. 30, 58, 60). During this early resting stage of the polymorphous cell the cytoplasm is never accumulated close to the cell wall, but merely forms a very thin zone around the nucleus (Fig. 1). As the cell grows, this zone increases in size and soon fills up all the available space between the nucleus and the cell wall, though it is always denser in the immediate vicinity of the nucleus (Figs. 3, 10, 12, 15, etc.). In the early stages of this class of cells the cytoplasm proper as well as the primitive spheres are composed principally of granules, but at a later stage, when the spheres are formed, the cytoplasm proper is generally distinguished by a fibrillar structure, while the spheres are almost exclusively granular (Figs. 34–37).

The Spheres.

By the spheres I understand that denser accumulation of cytoplasmic substance variously designated as spheres, archo-

plasm, aster, etc., generally containing the archosomes. The spheres when perfect are differentiated into an inner and an outer sphere, and at times also the outer sphere is further differentiated into two distinct zones (Figs. 8, 9, etc.). The word "differentiated" is used in the same sense as separated and is not intended to indicate that the two spheres are differentiated from the same kind of protoplasm. The inner one of the spheres is the most distinct and also the most permanent of the two. It is more constant as to form, and is characterized by a capacity to stain much deeper than any part of the outer sphere. It is also during a part of its life cycle well defined, having then the form of a concave disc, or a mulberry-shaped body, one side of which is strongly concave, while the other is more or less noticeably convex (Figs. 12, 29–31).

There is reason to believe that this inner sphere is always concave, but that the concavity of the sphere is only perceived when the sphere is seen from the side (Fig. 12), the concavity not being visible when viewed in the other direction. The comparison to a mulberry is yet more justified by the structure of the sphere. It is, when perfect, always composed of a number of alveoles of almost equal size, and so arranged that the wall of the sphere is just one alveole thick. The cavity of the sphere is not an empty cavity, but is more or less densely filled with granules, less distinctly arranged in alveoles. At a certain stage in the activity of the sphere and the archosome this inner more loosely constructed part of the sphere is drawn out, the archosome being at its top, forming a pointed cone of less staining capacity (Figs. 28, 35–37). The alveoles are non-permanent structures and formed by the peculiar arrangement of the granules composing the spheres. It appears as if these granules secreted some distinct substance, and that this substance was held together by the closely approached granules themselves, thus forming a bladder-like alveole surrounded by a membrane of granules. The development of the spheres will be described in another place; here it will suffice to state that the inner sphere is during mitosis gradually dispersed, part of it being undoubtedly used up in the formation of the central spindle. Similarly the outer sphere is

dispersed at an even earlier stage, the secretions of its granules, or perhaps even the granules themselves, supplying material for the new membrane which is formed between the two daughter-cells.

In a paper on the plasmocytes of Batrachoseps I have described and designated the spheres of the erythrocytes as granosphere, hyalosphere, and plasmosphere, these three spheres being of strictly cytoplasmic nature. A comparison with the spheres of our present cells satisfies me that the inner sphere is identical with the granosphere. For the outer sphere I use the name plasmosphere, though it is not absolutely settled to my satisfaction that they are in every way identical. But the similarity is considerable, even to the extent that we find between the plasmosphere and the granosphere at times another zone which probably is identical with the hyalosphere (Figs. 8, 16, 17, 34). In this paper I will, therefore, refer to the two spheres as the plasmosphere and the granosphere. While in the plasmocyte the non-staining sphere surrounding the granosphere has the form of a narrow, even band, the non-staining zone in our present cells is frequently aster-like, radiating through the plasmosphere (Figs. 6–8, 14). The three spheres during all their different stages of evolution possess a granulated alveolar structure, the cytoplasm proper taking the form of frequently granulated fibrilla, especially nearest the cell wall.

When I had almost finished this paper I found that Meves has proposed the name "idiozom" for the two spheres, or, as he defines it, "for the specific covering which surrounds the Centralkörper in the testes cells." The word is apparently selected under the supposition that the spheres are especially intended for the Centralkörper. That such is not the case I expect to show in this paper. Moreover, the new word does not distinguish between the two spheres, which, as I expect to demonstrate, are distinct and independent structures. If we are to retain one name for the two spheres, then the word "archoplasm" seems to me as good as any other. Neither of the two words expresses the true nature of the bodies which they are intended to designate. The names which I propose to

retain in this paper have the advantage of distinguishing between the two spheres, and have besides the priority. Upon the value of the latter I shall, however, not insist, and I am willing at any time to discard any words introduced by me or by any one else as soon as better ones are found, but not until then.

Position of the Spheres.

As will be seen from a perusal of Figs. 10–17, the spheres are only situated on the cell axis when they are in a stage of comparative rest, and at a time when the spheres and the nucleus appear to balance each other. If, on the other hand, we examine such figures as 42 and 45, we find that the spheres, especially the granosphere, have a different position relative to the central spindle and the nucleus. Instead of being situated on a line passing through both the central spindle and the nucleus, we find them situated on a line passing through the equator of the central spindle. From this we can formulate a rule that the position of the granosphere during the radiosomic process is dependent upon the position of the central spindle; and *vice versa*, that the position of the central spindle is dependent upon that of the granosphere. Whatever be the relative position of the nucleus on one side, and the granosphere and the central spindle on the other side, the central spindle will always be so situated that a line passing through its equator will also pass through the granosphere. The object of this relative position of spheres and central spindle is undoubtedly to enable the two opposite poles of the central spindle to draw an equal amount of nourishment or material from the granosphere.

As regards the mutual position of the two spheres, I expect to show that they are not directly dependent on each other, and that the position of the granosphere inside of the plasmosphere is probably regulated by convenience more than by dependence. If the granosphere were situated anywhere else it would not be able to furnish the central spindle with the material required for its development. The plasmosphere again, which furnishes material for the mantle fibers and for the new cell wall, must of a necessity be on the outside, in

order to be able to assume its proper position near the equator of the cell, where it is most required. Again, the development and the evolution of the cell require that as much as possible the various parts should be arranged concentrically.

Metaplasmic Secretions. — These secretions can be readily distinguished from the permanent elements of the cell by proper methods of staining. Among the methods which I have used there are only two which have proven of value. One is the Flemming triple stain, the other is the iron-haematoxylin stain, with an after-staining with congo. The latter method is much preferable, and it may almost be considered as a specific stain for the secretions from the spheres. It has already been stated that the ultimate visible structure of the spheres consists of granules and that they are arranged around alveoles. It is these alveoles which contain the metaplasmic secretions, and the only way to explain their presence is to assume that they are secreted from the granula of which the spheres are composed. The secretions appear only in the alveoles, and when these are scattered during mitosis they carry the secretions with them. Even with the highest optical powers no structure can be perceived in the secretion. During the metaphase the alveoles, together with their secretions, are found in the vicinity of the equator where the new cell membrane is to be formed. The secretions from the granosphere, and perhaps some of its granules, are used up in the formation of the central spindle. At least the granules lose, at this time, their intense staining capacity, diminish in size, and cannot be followed any longer with any certainty. Fibers and rays, both from the central spindle and from the spindle cones, are frequently found ending in alveoles filled with metaplasmic secretions, as if they were receiving nourishment from them (Figs. 41, 42, 45, 61).

The metaplasmic secretions teach us, among other things, that the two spheres are structures independent of each other, that one sphere is not a modification of, or a secretion from, the other, but that each is of a distinct nature. When the spheres reconstitute they do not do so together, but often in different places of the cell, later on to be joined together.

Cytoplasmic Membrane and Cell Wall. — The cell wall is un-
doubtedly the most constant structure of the cell. I have not
made this structure the subject of any particular study and can
only say that it appears to be composed of minute granules,
closely approached to each other and evidently of the same
nature as the granula composing the cytoplasm of the cyto-
some. The formation of the new cell wall between the two
new daughter-cells will be described under the general heading
of mitosis. Here I will only mention that the new wall is
formed by the aid of metaplasmic secretions from the outer
sphere. All through the evolution of the cell we find that
wherever large vacuoles are formed in the cell, these seem to
become surrounded by a thicker cytoplasm or incipient membrane.
This is, I think, especially the case when these vacuoles contain
some substance differing in quality from that surrounding them,
in which instances the cytoplasm appears to thicken into a
veritable membrane, greatly resembling a thin cell wall.

At a certain stage in the mitosis of the cell, when in the end
of the anaphase the new nucleus is beginning to increase in
size, a new membrane is formed surrounding the nucleus, but
at a considerable distance from it. This membrane is not a
nuclear membrane, but a true cytoplasmic membrane, which is
again dissolved, as soon as the new nucleus has reached its
final or desired size. This membrane only serves as an attach-
ment for the cone fibers, and by being pulled outwards causes
a large vacuole to be formed around the nucleus, thus giving
the nucleus ample room to expand and to grow (Figs. 59–68,
also Fig. 70). The nuclear membrane is formed later, imme-
diately around the chromosomes, often while the cytoplasmic
membrane is still in existence, as, for instance, in Fig. 70.
Later on this cytoplasmic membrane is dissolved. The process
of the formation of this false nuclear membrane, as well as of
the inner and thinner membranes around the vacuoles, and of
the cell wall itself, is, I think, one and the same, a condensing
of the cytoplasmic granules. In the formation of the new cell
wall the draught on the cytoplasm is so great that an extra
supply of cytoplasm and nutriment is required, which supply is
furnished by the plasmosphere and its metaplasmic secretions.

Paracellular Bodies. — I designate as paracellular bodies
numerous non-cellular bodies situated between the regular cells
of the testes. They appear to have been expelled from the
cells. Some of these bodies are lying free in the intercellular
space, others are more or less closely attached to the exterior
surface of the cells. These bodies are rarely found among the
polymorphous spermatogonia, or among the auxocytes, but are
quite numerous among the spermatocytes, the spermatids, and
the spermatozoa. At times we find only a few, at other times
we find them by hundreds. They may be divided in two
classes, one consisting of bodies of somewhat larger size and
which contain no granules. The other class comprises bodies
of smaller size, but which contain one or more darkly stainable
granules. The larger of these bodies are frequently attached
to the cells by thin threads (Figs. 67, 109). The smaller ones,
containing the granules, are sometimes attached, but more fre-
quently free (Figs. 85, 88). As regards the interior structure
of these bodies we find that those without any granules present
a striated appearance, as if their interior consisted of a fine
network. By the use of the congo-thionin-ruthenium method
these fibers can be stained bluish, while the other parts re-
main red.

The structure of the smaller bodies with the granules is
quite different. There is no sign of any fibrillar structures
nor of any network, but they are seen to contain from one to
five dark-staining granules, mostly arranged along the sides of
the wall of the main body. These bodies vary very consider-
ably in size, some being many times larger than the others.
If we consider the origin of these bodies, large or small, there
seems to be only one theory that is plausible, that they are
bodies expelled from the cells. A perusal of the figures re-
ferred to above makes this supposition also probable. We see
on the surface of many cells bodies exactly similar to those
which are free; at the same time we find in the interior of the
same cells bodies of a similar appearance. From my observa-
tions I judge that the bodies with the granules are expelled
accessory archosomes, while those without granules are ex-
pelled fragments of the spheres. The increase in size can be

due to a swelling up of the bodies as soon as they enter the intercellular liquid. Bodies of both kinds accumulate often in very large masses between the cells and are often found in complete dissolution. In another place I have suggested that the expulsion of the centrosomes is affected when there are too many archosomes in the cell, more than are necessary to accomplish the mitosis of the cell.

Karyosome or Nucleus.

General Remarks. — The constituents of the nucleus may, in a general way, be divided into three parts : *viz.*, chromatin, linin, and nuclear wall. But as the nuclear wall is probably only a thickening of the linin, just as the cellular wall is only a thickening of the cytoplasm, we may dispense with the third division and simply divide the nucleus in two distinct parts, chromatin and linin. In this general division we must include under chromatin such bodies as the chromoplast, directly to be described, while under linin we must arrange the other class of nucleoli, the linoplast, also to be further described below. But while it seems that the true nucleoli or linoplasts are principally of importance in furnishing or regulating the supply of linin, I must concede to the chromoplast a much more important function, that of regulating the formation of the chromosomes. It seems to me probable that the chromoplast has the same function to perform inside the nucleus as the archosome outside of the nucleus, and that while the archosome regulates the radiosomic process or the formation of the spindle and the final separation of the chromosomes, the chromoplast regulates the chromosomic process inside the nucleus.

The following elements of the nucleus are distinguished and will be described more in detail below : chromioles, chromomeres, chromosomes, endochromatic granula, parachromatic granula, chromoplast, chromoplasm, linin, linoplast, and nuclear membrane. These constituents are not of equal value and importance. The chromioles, the chromoplast, and the linin granula are the most permanent elements of the nucleus. All the other constituents of the nucleus, such as chromosomes, chromomeres, and linoplasts are only temporary and not

permanent organizations. As regards the nature of the para-
chromatic granules we do not know anything with certainty,
but it seems probable that they are of great importance.

The Chromioles. — The chromioles are the smallest visible
organized parts of the chromosomes. They undoubtedly con-
stitute the most important parts of the chromosomes, the fun-
damental elements which the other parts of the chromosomes
only serve to nourish and to preserve. If we view a perfectly
fixed and stained chromosome during any of the mitotic
phases, we find that it is not a homogeneous body, but one
that shows considerable differentiation in a regular manner.
We first observe that the outline or margin of the chromosome
is not an even one, but one which shows deep indentations of
even size and number. These indentations are so arranged
that the chromosome appears to be more or less beaded; that
is, a convexity on one side corresponds to a convexity on the
other side, and a concavity similarly corresponds to a concav-
ity. We moreover find that these beads are of a constant num-
ber, at least in chromosomes of average size. A chromosome
of Batrachoseps in the beginning of the metaphase of an auxo-
cyte contains just six such beads, the beads being identical with
chromomeres. A closer study of one of these chromomeres
shows that they are not of a homogeneous structure, but that
each one of them contains several interior round granules, sur-
rounded by an apparently homogeneous substance. These
granules, for which I propose the name of chromioles, are
of globular form and of equal size (Figs. 53, 54, 112,
120, etc.). I need not point out the necessity of having the
chromosomes properly stained. If too dark, the chromioles
will not be seen, but the whole chromosome will appear as a
solid homogeneous mass of chromatin. Even when the chro-
momeres have been so fused together that the margins of the
chromosomes are only slightly wavy, the chromioles are yet
distinct enough not only to be seen, but under favorable cir-
cumstances to be actually counted. A very good view is had
of the chromioles in the chromosomes, of which Fig. 112 gives
as correct a representation as it was possible to make. Of
course, instances where chromioles are as distinct as these

are comparatively rare. In most instances they can only be counted here and there in the same chromosome, the trouble generally being that the chromosome has not been properly differentiated. But wherever glimpses of the chromioles are had it will be seen that they are arranged in rows, one row on either side of the chromomere, and parallel to the longer axis of the chromosome. As is to be expected in a case like this, where we view an object of so small a magnitude that it lies on the limit of vision, it is a most difficult matter to count the chromioles and to be sure that the count is correct. I have made a number of countings, almost wherever I found a suitable opportunity, and the result is that I consider the number of chromioles constant in every chromosome of regular size. Thus in the metaphase-chromosome of the auxocytes and spermatocytes there are three chromomeres in each prong, or six chromomeres in all, making six chromomeres for the whole chromosome. Each chromomere contains six chromioles, three at each margin, which makes thirty-six chromioles for the whole chromosome. The regularity of the chromioles, both as regards number, size, form, and arrangement, precludes the possibility of their being artefacts, and we have no alternative but to assume that they are bodies of permanent structure and of the highest importance. The question now arises, to what extent can we trace the chromioles, backwards as well as forwards, in the development and evolution of the chromosome. This subject will be more particularly entered into when we discuss the development and evolution of the nucleus, and here it must suffice to give only a brief outline of the results of my observations. The chromioles can be readily observed in all the different stages through which the chromosomes pass, except in the confluent umbrella stage in which I have so far not been able to view them with any degree of satisfaction and clearness. In that stage there exist in the confluent mass besides the endochromatic bodies numerous dark staining granules, but they are irregularly scattered and stain darker than the chromioles in other places. But while the persistence of the chromioles in this stage has not been satisfactorily demonstrated, it is at least highly probable, as immediately when the

chromosomes begin to reappear from the confluent umbrella, the chromioles appear at once and as well defined as ever. We therefore must assume either that during the confluent stage the chromioles do not stain sufficiently to be distinct one from the other or from the chromoplasm surrounding them, or that those globules which are observed there are actually the chromioles, though less regularly arranged.

In all previous stages of chromosomic evolution the chromioles are distinct in almost every chromosome, and, as I have said, may in favorable instances be counted. Beginning with the resting stage of the nucleus of the polymorphous spermatogonia, we find that the chromioles are the only parts of the chromosomes which are distinctly visible or regularly organized. In this stage the chromioles are not united into chromomeres and chromosomes, but occur free in the nucleus and separated from each other and only connected by a thread of linin. They do not even appear to be surrounded by the usual covering of chromoplasm, but are, so to say, strung one after the other on linin strings, which latter apparently run in the same general direction (Figs. 1–3).

In a somewhat later stage several, or from two to three, chromioles are seen to congregate together and form the beginning of a chromomere, as, for instance, in Figs. 4, 5, etc. In a later stage the chromomeres are yet more distinct and they are then seen each to contain three chromioles, situated very close together and surrounded by a thin film of lighter staining chromoplasm (Figs. 12–14). When again at a later stage the chromomeres have been perfectly formed, we find that each chromomere contains six chromioles and the supposition lies near at hand that each individual chromiole has divided in two (Fig. 15). The chromioles are now so arranged that three and three are on each side of the chromomere, there appearing between them a lighter staining line which may be followed all through the leader or spireme segment. When this segment splits in the next stage the splitting is carried along this line, and the newly split half will thus only possess three chromioles in each split chromomere. But when at a later stage the chromomeres have formed into

their final number, then we find that each chromomere con-
tains six chromioles, just as before (Figs. 24, 48, etc.). As the
chromomeres fuse into each other, the chromioles also become
correspondingly more closely set and finally they appear in the
chromosome as two parallel strings imbedded in a common
sheath of chromoplasm. This leads us up to the very point
from which we started, the perfectly formed chromosome in
the metaphase. We can, if we wish, follow this process all
through the evolution of the polymorphous spermatogonia, the
auxocytes, the spermatocytes, and partly also into the sperma-
tids, though the latter are so minute that their finer structure
can be less satisfactorily studied. In order to test the existence
of chromioles in chromosomes of other animals, I have fixed
testes, by the iridium-chloride method, of a number of other
animals, especially of insects, and I am thus able to state that
in every instance where the chromosomes are of sufficient size
to allow of a closer study, I have been able to demonstrate
satisfactorily the existence of the chromioles. They are espe-
cially well defined in species of orthoptera (Stenopelmatus).
From the above observations I conclude that the chromioles
are permanent structures in the chromosomes, and that they
are the smallest visible individualized and organized parts of
the chromosomes, and further that the chromoplasm, the chro-
momeres, and the chromosomes are merely structures for the
conveyance of, the nourishing of, and the partition of, the
chromioles.

    There yet remains to say a few words about the division of
the chromioles. The proper increase of the chromioles is, of
course, an absolute necessity, provided we are correct in assum-
ing them to be the most important parts of the nucleus. As
the chromioles are too small to allow of any direct observation
as regards multiplication, all speculations on this subject are as
yet premature. The counting of the chromioles is a most diffi-
cult matter, as not even under the most favorable circumstances
can all the chromioles in the same chromosome be counted.
The best we can do is to approximate and average their num-
ber. I have stated that in the early anaphase we find at each
pole twelve chromosomes, each one containing about six more

or less distinct chromomeres, and that every such chromomere possesses about six chromioles. This makes 432 chromioles in all for the daughter-nucleus.

When the nucleus of the following cell, the spermatocyte, enters the metaphase we find the chromosomes in the shape of split *V*'s. The chromomeres in these are double and appear to contain each six chromioles, or seventy-two for each chromosome. As there are twelve chromosomes, each equatorial plate should contain 864 chromioles, which, after the equation-division of the chromosomes, would again give to each daughter-nucleus the same number as formerly, or 432 chromioles. From this we are justified in assuming that during the confluent umbrella stage of the nucleus the chromioles have been doubled. The easiest way to explain this increase is to assume that each chromiole has been divided in two, thus presumably preserving the quality while increasing the quantity. It is not improbable that one of the objects of the confluent umbrella stage is to allow the undisturbed division of the chromioles.

The Chromoplasm. — By the chromoplasm I understand the apparently homogeneous substance which directly surrounds the chromioles during all stages of their existence, except, perhaps, while they pass through the resting stage in the polymorphous spermatogonia (Figs. 1–3). In this stage the chromioles appear to lie free in a linin thread, at least there is no visual evidence of their being surrounded by any chromoplasm. That each chromiole is actually surrounded by a thin film of chromoplasm is, however, probable. As soon, however, as the chromomeres are beginning to form, then we can see that the individual chromioles are imbedded in a homogeneous substance for which I propose the name "chromoplasm." It is the chromoplasm which gives the chromosomes their general form, appearance, and color. The chromoplasm constitutes by far the greatest part of the chromosomes, and it appears to be the vehicle for conveying and nourishing the chromioles. As regards the want of chromoplasm in the polymorphous nuclei, we may assume either that the chromoplasm has been disintegrated or been used up as food for the chromioles, or we may suppose that the chromoplasm has become concentrated in

the body, which I will directly describe as the chromoplast. It is quite possible that both these processes have taken place, as the chromoplasm found in the chromoplast is not sufficient to account for the quantity found in the chromosomes at a later stage. That the chromoplast has something to do with the formation of the chromoplasm is more than probable, because at the time when the spireme segments are being formed, the chromoplasm is seen to be thicker nearer the chromoplast, while at the distal end from the chromoplast the chromoplasm is thin or very thin. It sometimes seems as if the chromoplasm had flowed from the chromoplast and been gradually distributed all along the spireme segment. It seems also probable that the chromoplasm is a prominent constituent in the chromoplast — at least the only visible differentiation between the chromoplasm in the spireme and the chromoplasm in the chromoplast is that the former stains less intensely than the latter. In most papers on cytology the chromosomes are figured with their margins drawn out into the linin network. I think this is probably an error caused by imperfect staining, as in all my best preparations I could distinctly see that the chromoplasm always possessed a rounded margin, and that the points consisted exclusively of linin which at times may be so stained that it cannot be distinguished from the chromoplasm.

The Chromomeres. — But little need be said about the chromomeres, as they will be again referred to in describing the evolution of the nucleus. The chromomeres begin to form as soon as the nucleus of the polymorphous cells enters the imperfect resting stage. The chromomeres are formed in the following manner : Two, and later three, chromioles which, during the perfect resting stage, were suspended singly in the linin, come together and are at the same time seen to be surrounded by a thin layer of chromoplasm, thus forming a small isolated body suspended in a linin network. The chromoplasm soon increases in quantity, and the chromomere is in this way increased in size. The number of chromioles present in each chromomere is not always the same in the early prophases, and some chromomeres may possess twice the number of chromioles as some others. Still the number is fairly constant, gen-

erally being three. There are more chromomeres in the early
spireme stages than in the later ones, and as these early ones
also are smaller, it appears as if the later chromomeres are the
results of the fusion of several smaller ones. In the perfected
bouquet stage there are, as a rule, about a dozen chromomeres
in each spireme segment (Figs. 15, 121). Those nearest the
chromoplasts are larger and possess more chromioles than
those more distant. When the chromomeres split there is
almost, without exception, six chromioles in each. With the
splitting of the spireme segment the chromomeres again lose
their identity to a considerable extent, appear smaller, and
are more numerous; the chromioles also are smaller. But
these chromomeres fuse again into larger chromomeres, and
when in the contraction stage the bretzel-shaped chromosome
is formed we find that it possesses twelve chromomeres, each
one with six chromioles (Fig. 121 E). The new or daughter-
chromosome, which results from the equation division of the
bretzel, contains only six chromomeres, each with six chromioles
(Fig. 121 l). From this time on the chromomeres gradually
lose their identity, and more and more fuse together until at
last, at the end of the anaphase, they have become so confluent
that no trace of their original form remains. But as soon as
the nucleus begins to reconstitute itself the chromomeres at
once reappear (Figs. 62, 118). The number of chromioles at
this time is uncertain. The following stage of growth of the
nucleus is characterized by a greater separation of the chromo-
meres (Fig. 119). The typical number of chromomeres in the
chromosomes of the spermatocyte is the same as in the chro-
mosomes of the auxocyte, or six in each. The exact number
of chromomeres which go to make up a chromosome sometimes
varies. Thus we now and then find chromosomes with only four
chromomeres instead of six, but we may then always expect to
find that some other chromosome possesses eight chromomeres,
and that in this way the proper number is made up. From the
above it will be seen that the chromomeres cannot be considered
as permanent organs of the nucleus or more in particular of the
chromosome, but that they are merely convenient forms of struc-
ture, the object and function of which is to facilitate the hand-

ling of and the disposition of the chromioles. The definition of a chromomere would thus be this: A small body of chromioles surrounded by or imbedded in a matrix of chromoplasm, the object of which is to facilitate the growth, nourishment, and multiplication of the chromioles.

Leaders. — As leaders I designate the threads or filaments of chromoplasm in which are suspended the chromioles in the earliest stages of nuclear activity. The chromoplasts which first lie free seem to attract a certain number of such threads, which radiate out in different directions from the chromoplasts. In the beginning the leaders are of a varying number, — how many is difficult to say, but decidedly more than twelve; but as the process goes on they diminish in number, and at the end of the process they are found to be only twelve, or just as many as the future chromosomes.

The exact process of this formation has not been properly followed, but it seems as if the chromioles actually passed through the chromoplast and were projected through it into the leaders. At least we find at that time in the chromoplasts granules which greatly resemble the chromioles in size and form, besides being of the same nature as regards their staining qualities. I have often found that the free, distal ends of the leaders were twelve in number, while the ends attached to the chromoplasts were more than twelve; and this fact I can only explain by assuming that the chromioles are pushed into the leaders from the chromoplasts, or, in other words, that the free ends of the leaders are finished first and that their ends, which are attached to the chromoplasts, are the last parts to be perfected. At the end of this process we find that there are twelve leaders which rest their free ends on the nuclear wall nearest the spheres, while their main parts are twisted and bent in the cavity of the nucleus. If this supposition is correct, then the process would be something like this. The chromoplasts attract chromioles from all sides and take them up in its plasma. They are then again expelled into twelve leaders, which latter are being fed with chromioles from the chromoplasts. The leaders attached to the chromoplasts would thus be of two kinds: one set, the genuine leaders,

which pass from the chromoplasts, and the other set merely strings of chromioles which pass into the chromoplasts, again to be expelled from them into the regular leaders. The formation of the chromomeres would, of course, take place in the regular leaders, or possibly even in the chromoplasts.
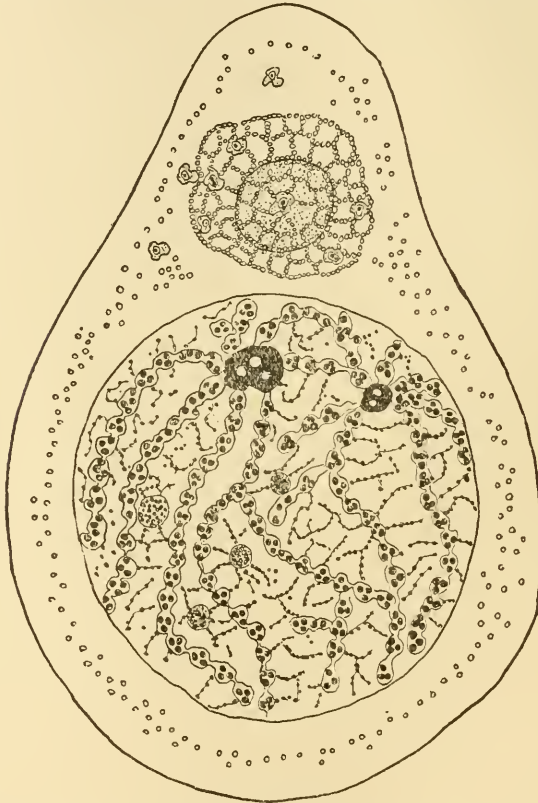
Auxocyte in the "imperfect resting stage," showing the formation of leaders consisting of round chromioles surrounded by a film of chromoplasm. The leaders start from two chromoplasts of unequal size, both containing endochromatic granules. The leaders are connected by a linosomic network. Four linoplasts. In the cytoplasm are seen the two spheres, the inner one, the granosphere, containing the archosome. There are eight accessory archosomes, some in the plasmosphere, others in the cytoplasm. The two spheres are of a foam-like structure. The cytoplasm is only partially indicated.

The Chromosomes. — The chromosomes are not in any sense permanent organs of the nucleus. They arise, disappear, and are re-formed as the case requires ; in fact, are mere convenient structures for the proper division and nourishment of the

chromioles.   A chromosome is built up of a certain constant
number of chromomeres without any other additional part than
a chromoplast.   A chromosome may thus be termed a string
of chromomeres attached to a chromoplast.   The formation of
chromosomes is of a necessity different in the different varie-
ties of cells.   In the polymorphous spermatogonia, where a
resting stage occurs in the nucleus, the chromosomes originate
from leaders or strings of chromioles, in the way that has been
partly described in the preceding paragraph.   This is the case
also in the auxocytes.   The leaders finally contract, their
chromomeres approach each other and finally fuse.   When
the required number of chromomeres have formed, the leader
splits lengthwise, and shortly afterwards the two halves sepa-
rate and spread out.   At the same time the chromoplasts
divide in as many parts as there are leaders, one part remain-
ing attached to each leader (Fig. 122).   The next step is a
further contraction of the leader, which again is followed by
a twisting of its free ends, thus forming the bretzel-shaped
chromosome.   The mitosis of the chromosomes will be treated
under the heading of mitosis.

In the spermatocytes the chromosomes appear from the
confluent umbrella stage in the form of staples, with strongly
marked chromomeres, and with the chromoplast attached to the
angle of the chromosome instead of to the end of one of its
arms, as in the auxocytes.   The chromosome in each cell
variety is characterized by a certain number of chromomeres;
in the auxocytes and spermatocytes they are six, though now
and then we find some chromosomes larger than others.   When
such is the case we at the same time find very small chromo-
somes with a smaller number of chromomeres, thus making the
number of chromomeres and chromioles the same for every
nucleus of the same kind of cell.   In the mitosis of the poly-
morphous cells we find the number of chromosomes to be
twenty-four, but in the auxocytes, as well as in the spermato-
cytes, the number of chromosomes is reduced to twelve.   The
reduction in number takes place in resting stages of the auxo-
cyte, and is due to the chromoplasts which project only twelve
leaders instead of twenty-four, as in the polymorphous cells.

In the polymorphous cells the chromosomes divide through common or somatic mitosis; in the following stages the auxocyte divides through heterotypic mitosis, while in the spermatocytes the mitosis is the homoeotypic one.

In the end of the anaphase of the two maturation cells the chromosomes enter an almost perfectly confluent stage, in which the individual chromosomes have lost their individuality, being fused into a single umbrella-like mass. From this mass the individual chromosomes reappear, but it seems almost incredible that the new chromosomes should be composed, each one of them respectively, of the same identical chromomeres and chromioles as before mitosis.

The changes which the chromosomes undergo in the maturation cells will be more particularly described under the chapter on mitosis. The chromosomes of the spermatocytes possess the peculiarity to take the congo stain after the haematoxylin, which causes them to appear reddish-black instead of pure black, as do the chromosomes of the auxocytes.

The chromosomes of the auxocytes are bretzel-shaped, while those of the spermatocytes are V-shaped before mitosis. The bretzel form is due to the fact that the ends of the chromosomes overlap each other instead of growing together.

The chromosomes in all these varieties of cells divide by equation division, and not by reduction division.

The Chromoplast and the Endochromatic Granules. — This body, which I consider to be of the greatest importance in the evolution of the nucleus, has been variously known as nucleolus, netknot, karyosome, etc., but as these names have also been applied to other structures in the nucleus, I consider myself justified in proposing for it a new and more distinct name, the "chromoplast," thus indicating at least one of its characteristic properties in connection with the chromosomes.

In the Batrachoseps testes the chromoplasts are most distinctly individualized in the resting stages of the nucleus, and in those stages in which the chromosomes have not yet reached their final and perfect form. They may, however, still be seen in the metaphase of the auxocytes, but after that stage is passed, they become less distinct, or may even so fuse with the chromo-

somes that they cannot always be distinguished from them.   In the resting stages, however, the chromoplast or chromoplasts, as there may be several, stand out quite distinctly and individualized and can then be studied to the best advantage.   This refers not only to the resting stages of the polymorphous nuclei, but also to those of the auxocytes, the spermatocytes,
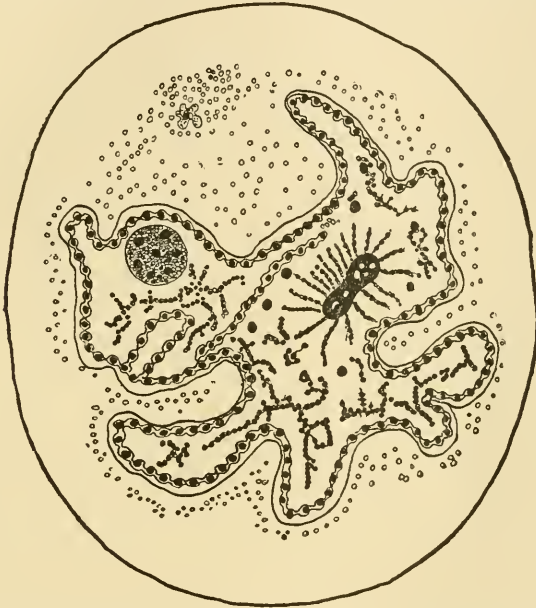
A polymorphous spermatogonium in the "perfect resting stage." The form of the nucleus allows the most perfect metabolism. Numerous chromioles are connected by a thread of chromoplasm. A network of linosomes is partially indicated, the individual granules being connected by linopodia. A large, oblong chromoplast with endochromatic granules. Eight parachromatic granules. A single archosome in the cytoplasm, the latter only partially indicated by small open circles. A single large, round linoplast, with seven endonucleolar granules.

and the spermatids.   In the two first-named cells, which also are the largest, they offer the best facilities for study.

In the perfect resting stage of the polymorphous nucleus we find always one, but sometimes two or more chromoplasts, easily distinguished by their capacity for intense staining. When the iron-haematoxylin-congo stain is used the chromoplasts become stained, as a rule, most intensely black, while the true nucleoli, or linoplasts, take the congo and become red.   The chromoplasts are also characterized by possessing

in their interior several highly refractive bodies which I have termed "endochromatic granules." These granules never occur in the true nucleoli, which fact always enables us to distinguish between them and the chromoplasts, even in instances when the true nucleoli are stained darkly, as sometimes happens. The endochromatic granules never stain, but appear to be naturally of a yellowish color and always highly refractive. They vary in size, and are sometimes so small that their refractivity is not readily perceived, they appearing only as minute granules of an intensely dark color. But as they increase in size we begin to see in their center a light-colored, highly brilliant spot, which, in the larger granules, is correspondingly large and distinct. These refractive granules are almost invariably present, and they may be truly termed "landmarks," by which we can determine the position of the chromoplast.

Even in places where the chromoplast itself cannot be distinguished we can judge of its presence by one or more of these endochromatic granules, as, for instance, in the metaphase and anaphase of the auxocytes. It is the presence of these granules which enables us to follow with certainty the evolution of the chromoplasts, and to ascertain their presence in every stage of the nucleus. The number of granules in each chromoplast is in no way constant and seems to be of no great consequence. Some are perfectly round, others are angular, and their general appearance seems to indicate that they constitute secreted matter, or metabolic products, probably for the attraction and nourishment of the chromioles. While each granule is highly refractive in the center, its outline is, on the contrary, very dark, so dark indeed that it would almost seem as if it was surrounded by a shell of some particular substance. Whether that is the case, or whether the dark margin is only the effect of refraction, I have not been able to ascertain. In the small chromoplasts attached to the chromosomes in the metaphase we sometimes find one or two endochromatic granules, sometimes also none. In the confluent umbrella stage of the nucleus we generally find a larger number of granules which appear to have been newly secreted. They disappear at the end of the umbrella stage, though a few may remain even after the nucleus has

begun to reform and after it has fairly entered its stage of growth. For characteristic figures illustrating the endochromatic granules see Figs. 2, 9, 21, 26, 57–62, 121.

The chromoplasts are generally rounded in outline and well defined. When insufficiently differentiated and overstained they frequently appear to be star-shaped and very irregular, but this is merely an effect of the overstaining. While the form is always rounded, it is not always globular, but, on the contrary, oblong or beaded, especially in places where we expect a division of the chromoplast. In the earliest stages of the polymorphous nuclei the chromoplast is generally oblong or consists of two distinct beads (Figs. 1–3). It lies then isolated in a vacuole, only surrounded by linin threads radiating from it in every direction. At a later stage the chromoplast is seen to be in more or less intimate connection with the leaders on which the chromioles are suspended. To begin with this connection is very slight (Fig. 3), but later on it becomes more intimate, and leaders with chromioles are seen to start out in all directions, radiating from the chromoplast like the radii from the center of a circle. When there is more than one chromoplast, leaders connect with all.

Some chromoplasts, however, may be connected with more leaders than others (Figs. 8, 9). In the last resting stages, when all the leaders have formed, it will be seen that all of them connect with the chromoplasts in such a way that if, for instance, one of the chromoplasts is connected with only four leaders, the other is found to be connected with the balance, *i.e.*, eight ; there are always as many leaders as there will be chromosomes. The chromoplasts appear thus to attract the leaders, and my opinion is that in this manner the chromosomes are formed. We can follow the chromoplasts with certainty up to the end of the anaphase. In the bouquet stage, where the spireme segments begin to separate from each other, it will be seen that this separation is caused by a division of the chromoplasts into several parts. In the perfect bouquet stage we thus find that two or more spireme segments are held together by a single chromoplast (Figs. 14–16). The ultimate result of this division is undoubtedly to so divide the chromoplast

that one part will remain attached to each chromosome. This is also undoubtedly what takes place, as in the metaphase when the chromosomes are all perfectly formed and placed on the central spindle in the form of rings, we find that at the center of one side of each ring is a globular body, frequently
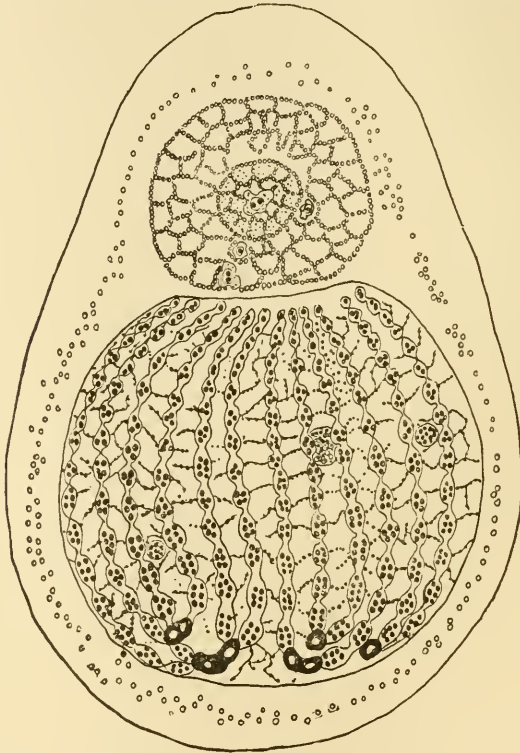
An auxocyte in the "bouquet stage." There are twelve leaders starting from five chromo-
plasts. The leaders consist of chromomeres containing chromioles suspended in a
film of chromoplasm. The spheres are of a foam-like structure. There are three acces-
sory archosomes and one archosome with two centrioles. The open space between the
inner granosphere and the outer plasmosphere represents the hyalosphere. The cytoplasm
is only partially indicated.

containing one or more endochromatic granules. These bodies attached to the chromosomes are, therefore, chromoplasts. In the confluent umbrella stage the endochromatic granules are the only indications of the presence of the chromoplasts. This is the case in the auxocytes. In the spermatocytes the chromoplasts are much more difficult to follow, but the greater

thickening of the chromosomes at the place where they are bent makes it probable that the chromoplast is situated at this point (Figs. 118, 119). In the mitosis of the spermatocyte I have not been able to affirm with certainty the presence of chromoplasts, except in the umbrella stage, where their presence is indicated by endochromatic granules. Again, in the resting stages of the spermatid the chromoplasts, generally one or two, are plainly definable, especially by the means of the congo-thionin-ruthenium red-staining method. In the resting stages of the auxocytes, which are especially favorable for study, a stream of chromoplasm is seen to project from the chromoplast to each leader, and each leader is more densely stained in the parts nearest the chromoplast. I do not, however, consider it with certainty established that a flow of chromoplasm actually takes place, as the phenomenon of deeper staining can also be explained by a greater attraction of chromoplasm in proportion as the chromoplast is approached. If this latter supposition is the true explanation, then we must also look for the source of the supply of chromoplasm elsewhere, possibly in the parachromatic granules.

Be this as it may, the observed facts warrant us in concluding that the chromoplast is of the utmost importance in the formation of the chromosomes, and that its function seems to be to attract the leaders, to segregate and define the chromosomes, and perhaps, in a general way, to supervise the formation and evolution of the chromosomic constituents of the nucleus.

Chromoplasts are probably present in all nuclei, and probably also in all chromosomes. They have been frequently confounded with true nucleoli, but their distinct nature has also been recognized by many investigators, and in all recent biological papers they are both described and figured as being of different nature from that of the so-called true nucleoli. In many instances, however, they have undoubtedly been overlooked, especially in nuclei in the bouquet stage, where they often are so small that they can only be distinguished through their endochromatic granules, the presence of which always indicates that the object in question is actually a chromoplast, and not a true nucleolus or linoplast.

Parachromatic Granules. — As parachromatic granules I designate a class of dark-staining granules which, during the resting stages of the polymorphous nucleus, are found in the vicinity of the chromoplast (Figs. 1–8). These granules stain in the same manner as the chromoplasts, and I have not been able to differentiate them by color. When the leaders are formed these granules are the first ones to join the leaders, and it suggests itself that possibly they furnish the necessary chromoplasm for the leaders. But as the aggregate of all these parachromatic granules does not equal the mass of the chromosomes we must suppose that, if the parachromatic granules furnish the chromoplasm, they cause it to be evolved and that they do not furnish it alone from the amount stored in them. The parachromatic granules are of various sizes and forms and vary also as regards number. With certainty they are only found in the polymorphous cells.

Linoplasts, or True Nucleoli. — The linoplasts are that kind of nucleoli which supply and nourish the linin during certain stages of the mitosis. When properly differentiated with congo they appear rather transparent and of a reddish-orange color. They are thus readily distinguished from the chromoplasts which take the iron-haematoxylin stain with great avidity. The number of linoplasts is variable; sometimes we find only one, sometimes again there are five or six. In the auxocytes the linoplasts are most numerous just before the stage in which the spireme segments are split, after which they generally disappear. Rarely one is left at the metaphase, and when this is the case it is thrown out into the cytoplasm and evidently dissolved. During the separated spireme stage the linoplasts are seen to dissolve, apparently giving off particles to the linin network (Figs. 12–17, especially 14 *c*). This is also the very period when the largest quantity of linin is required for the pulling apart of the two halves of the spireme segments. If we to this observation add the one that the linoplast consists of apparently the same kind of granula as the linin network, both as regards size, form, staining reaction, etc., we are, I think, justified in assuming that the linoplast actually does furnish the extra linin required for the pulling apart of the spireme leaders.

As regards the nature of the shell of darker granules surrounding the linoplasts, I have not any observations. This shell seems to exist only during the resting stages of the polymorphous nuclei and not in the maturation cells. Besides pure linin granules the linoplast undoubtedly also contains secretions with which the linin is nourished. The function of the linoplast appears thus to be not only to furnish stored-up linin, but also to nourish the general linin network. I hardly need to point out that the linoplast has been variously termed paranuclein, pyrenin, true nucleoli, Kernkörperchen, etc.

Linin. — The linin network is not difficult to differentiate, as with the congo-iron-haematoxylin method it stains reddish-yellow, while nearly all other structures take a black or at least a gray stain. When insufficiently differentiated it sometimes appears as if the chromatin of the chromosomes extends far out into the linin network; and in this way it is generally figured. With proper differentiation, however, it is seen that this is not the case, but that the chromatin is never extended into the linin, at least never as a fine thread (Figs. 14, 24, 119, etc.). As long as the nuclear membrane is unimpaired the linin always has the appearance of fine network, composed of granules of equal size and form. These granules occur either singly or in small groups, which latter may be mistaken for larger granular units. In Figs. 14 *c* and 26 *b* I have figured these granula in the way they appear under the most favorable optical conditions, though the individual granules are perhaps somewhat more rounded than they appear in the illustrations. We must distinguish between two distinct periods in the life cycle of the linin, one being the periods of rest, the other the periods of activity. There are two periods of rest. One is found in the resting stage of the nucleus before the leaders have properly formed, and the other occurs later in those stages of mitosis in which the nuclear membrane has been dissolved, and the linin been scattered away from the chromosomes. In the former of these stages the linin granula are more regular than in the latter (Figs. 1–3, etc., also Figs. 26, 37). After the nuclear membrane has been dissolved the linin network is carried away from the chromosomes, and

the granules are found both evenly distributed and arranged in larger heaps. The active stages of the linin are to be found during the prophases, during the formation of and the separation of the leaders. It is during this period that the linoplasts are dissolved in order to supply the necessary and extraordinary linin required for this process. The linin is then seen to consist of a network attached to the chromosomes or spireme segments, while part of it spreads out through the nucleus as irregular bridges between the chromatin segments. The linin possesses thus two distinct qualities, one being that of supporting the chromioles, chromosomes, or chromatin parts generally, the other being that of separating the two split spireme or leader halves from each other. As soon as this latter process is accomplished the linin is dispersed, at first all through the nucleus, and later on through the cytoplasmic part of the cell. The mass of the linin is composed principally of one kind of granules — the kind here always referred to as the linin granules. This general granule stains reddish or gray according to the process of differentiation. But in these granula we also find scattered isolated granules which stain only with the iron-haematoxylin stain (Fig. 26 *b*). Of the nature of these granula I have no knowledge, but it suggests itself to me that perhaps these denser appearing granula may serve as a support for the other kind, insuring an equal or at least a proper distribution.

It will be seen that during the period of activity the linin granule stains differently from what it does when in rest. This differentiation is, however, only brought out by the achromatic light-filter mentioned elsewhere. With the use of this filter we find that the linin granule during its activity stains bright and light red, while during the periods of rest it stains dark gray. This differentiation also allows us to follow the linin granules through the cytoplasm after the nuclear membrane has been dispersed. What finally becomes of these linin granules is uncertain. But I have no observations which would indicate that they reconcentrate themselves in the new daughter-nuclei. On the contrary, there is no sign of any accumulation of linin granules in the immediate vicinity of the

new nucleus, and the first appearance of the linin in the new nucleus is found close to the new chromosomes.

This new linin is, to begin with, of limited quantity, and it appears as if it were actually re-formed, probably from some linin granules with a generative function. In such case the majority of the linin granules are either absorbed by the cytoplasm or used up in the formation of the new nuclear membrane.

The Nuclear Membrane. — The nuclear membrane has already been referred to in connection with the cytoplasmic membrane and cell wall. The most favorable cell for the study of the nuclear membrane is the large auxocyte in the beginning of the chrysanthemum stage. In this stage the new nuclear membrane is being reconstituted. In case the nuclear membrane is formed by a thickening of or by an accumulation of cytoplasm, we should expect to find such cytoplasm in the immediate vicinity of the place where the new nucleus is to be formed. No such accumulation of cytoplasm exists at this place. On the contrary, a false, or rather an accessory cytoplasmic membrane has previously been formed around the nucleus, but at some distance from it (Figs. 62, 70). The object of this membrane is to enable a vacuole to form, in which the new nucleus can have ample space for development. While this membrane is yet in existence the nuclear membrane is formed around the new nucleus. As at this time there is no cytoplasm between the cytoplasmic membrane and the chromosomes, the new membrane must be formed of other matter than cytoplasm. It is probable that this other substance is linin, of which there is at this time a fair supply around the chromosomes.

As to the dissolution of the nuclear membrane it is most interesting to note that, at least in the auxocytes, the membrane is dissolved only after the chromosomes have formed. The spindle fibers and mantle fibers cannot, therefore, have anything to do with the formation of the chromosomes. The nuclear membrane always disintegrates first at those places where it is first touched by the fibers of the mantle. The central spindle has apparently nothing to do with the dissolution

of the nuclear membrane, which is fully dissolved before the fiber of the central spindle has reached its immediate vicinity.
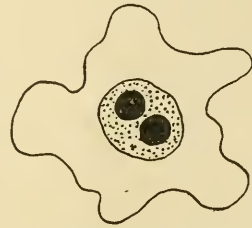
Phylogeny of the Nucleus. — It seems probable that the perfect resting stage of the nucleus, such as is seen in the polymorphous spermatogonia of the testes of Batrachoseps, represents a phylogenetically primitive nucleus, in which the necessity for the formation of chromosomes and chromomeres has not yet made itself felt. In the most primitive nucleus we should expect to find only a limited number of chromioles, which might be readily manipulated by the chromoplast without the assistance of chromomeres and chromosomes. But as the development of the species progressed and more characteristics were accumulated, we may presume that more chromioles were required, perhaps in order to propagate these characteristics. With this increase in the number of chromioles a more complicated system of mitosis became necessary ; hence the very complicated apparatus of spindles, etc., accompanying the mitosis of all higher cells. In the lowest forms of animal and plant life the chromioles were probably scattered in the cell itself, just as we now find them in the nucleus of Trachelocerca, a flagellate infusorian described by A. Grüber. In this instance there can be little doubt as to the nature of the granules and that they are real chromioles. The granules found in many bacteria resemble also greatly the chromioles of the higher cells ; and it may be possible that in these low organisms the chromioles are only suspended in the cytoplasm, without any surrounding nuclear wall.

In the blue-green algae, the Cyanophyceae, we have probably a similarly primitive nucleus, in which the chromomeres and the chromosomes have not yet been developed. In a later stage we should expect nuclei with a nuclear wall, but with the chromioles free as in the perfect resting cells in the testes of Batrachoseps. As is well known, many investigators deny the existence of nuclei in the Cyanophyceae and contend that the darker staining substance in the center of the cell is nothing but condensed cytoplasm. But this theory seems to me untenable in view of the fact that the Cyanophyceae are highly differentiated plants which certainly would require hereditary

granules for the transmission of characters. If, as I suppose, the chromioles constitute these granules, then it is probable that we have to identify the denser protoplasmic mass in the cell of the Cyanophyceae as being chromioles and not cyto-microsomes. In connection with this question it is interesting to note that the nucleus of leucocytes, both in batrachians and in man, contains only a few chromioles.

The Archosome.

The Archosome Proper: its Structure, Centrosphere, Somo-sphere, and Centrioles. — As the archosome I designate that particular structure which takes part in the radiosomic process of the cell, and which is situated at the very pole of the spindle, at least during certain stages of the mitosis. The archosome consists of the following parts : One or more interior granules — the centrioles of Boveri. Surrounding them we find a generally very thin zone or sphere — the somosphere. Both the centrioles and the somosphere stain deeply, the centrioles much more so than the somosphere, and it is sometimes difficult to distinguish one from the other. I believe the word "centrosome" might, with advantage, be retained to designate this inner part

An archosome consisting of an outer centrosphere, an inner somosphere, with two centrioles.

of the archosome, consisting of centrioles and somosphere. Next outside of the somosphere we find a larger, generally non-stainable, achromatic zone — the centrosphere. This zone is, however, not always achromatic, but takes the stain under certain conditions and stains then with plasma stains. Usually this centrosphere is figured as a round disc, with a perfectly circular outline, giving one the impression of being nothing but a vacuole, in the center of which is situated the centrosome proper. This description of the centrosphere is, however, not quite correct. While the centrosphere is frequently circular in outline, it is not always so ; indeed, I think that in the majority of instances it is not circular, but of an amoeboid form.

We see projections starting out just like the pseudopodia of an amoeba, and I have no doubt that these projections must actually be considered as true amoeboid projections, serving the very same purpose as do the pseudopodia of the amoeba, that is, as organs of locomotion and perhaps also of prehension. Unless we assume the existence of a special organ for the movement and propulsion of the archosomes and the accessory centrosomes, their movements and migrations from one part of the cell to the other are simply unaccountable. But if we assume, as our observations warrant us in doing, that the centrosphere is an organ for the propulsion of the archosome, then these movements become easily explainable. This theory is yet more affirmed by the fact that the centrosphere is amoeboid in almost every instance in which from its position we can consider it to be in activity, and in the act of moving from one place to another. On the contrary, when we have reaons to expect the archosome to be at rest, we also find that the centrosphere is globular or disc-shaped instead of amoeboid. This holds good also in regard to the accessory archosomes, though with them it is more difficult to determine when they are at rest and when in activity.

We are therefore, I think, justified in assuming that the archosome, as well as the accessory archosomes, propel themselves from one place to another by means of the amoeboid centrosphere, which sphere, when at rest, assumes a globular form, but when in activity shows an amoeboid margin, or pseudopodia.

The position of the archosome is variable, according to the stage of development of the cell. It may be said, as a rule, that the archosome is situated either in the granosphere or in the plasmosphere, when both these spheres are present. In the prophases of the auxocyte, in which the two spheres attain their highest development, the archosome is generally found in the granosphere. It then generally possesses two centrioles which are either surrounded by a common somosphere or are sufficiently apart to have a separate somosphere for each centriole.

The archosome seems to move around in the two cytoplasmic spheres in a most independent manner, sometimes being found

in the granosphere, sometimes again on the circumference of the sphere.   At other times it is found a considerable distance out in the plasmosphere.   It is at times impossible to distinguish the true archosome from the accessory centrosomes, as their structure is similar, though we might, with considerable certainty, assume that the body with two centrioles situated in or on the granosphere is the true archosome, but even about this we cannot always be certain.   Sometimes there is more than one such body in the granosphere, while, on the other hand, two-centrioled bodies are also found in the plasmosphere. Even when the central spindle is being formed, and at a time when the archosome is situated at the pole of the aster, we find in the cytoplasm bodies in no apparent way differing from the archosome.   At other times we find what appears to be the true archosome connected by a thread-like process with one or more accessory archosomes (Figs. 10–17, 27–33).   This thread-like connection is the remains of the somosphere which has been pulled out from the original somosphere surrounding the archosome from which the budding took place (Fig. 14 *a*). Such rings of somosphere are seen almost in every cell, often in considerable numbers.   Why the thread assumes the form of a ring is not quite apparent, but may be explained by supposing that it follows the inner walls of a vacuole.   This will, however, not explain all forms, as in many instances the thread on which the centrosomes are suspended circles around the granosphere, or seems otherwise to be entirely buried in the cytoplasm.   In other instances the granules connected by the thread are also suspended on the rays of some fiber cone or on some spindle cone.   The granules so suspended are not as a rule all of the same size and staining capacity, as some will stain intensely, while others adjoining hardly stain at all. Frequently this loss of staining capacity decreases as the granules are situated farther away from the archosome or accessory archosome from which they budded.   The same also often holds good as regards size, those farthest away being the smallest.   To this general rule there are many exceptions; small and large granules often alternate, and so do darkly stained ones, with those that are lighter.

As a result of my observations I have come to the following conclusions in regard to the nature, position, and functions of the archosome.

The archosome is a specialized accessory archosome, specialized for the purpose of conducting the radiosomic process of the cell. During the resting stages of the cell the archosome is generally, but not always, situated on the axis of the cell; during mitosis the archosome is situated at the pole of the central spindle. The archosome gives rise to the accessory archosomes by budding, and, *vice versa*, an accessory archosome may take the function of an archosome. As the archosome is, as a rule, found in the concave part of the granosphere, its peculiar and distinct qualities may depend on some particular food or stimulus furnished by that sphere.

As regards the functions of the archosome in connection with the spindle fibers, we find that the mantle fibers, as well as the central-spindle fibers, start out from the outer margin of the centrosphere and do not connect with the somosphere or with the centrioles. I have found this to be the case in every instance where I could see the pole of the spindle in a favorable manner. The fibers or rays are thus not inserted in the somosphere, but simply join the outer surface of the centrosphere. The only rays which are in actual contact with the somosphere are the contractile fibers, or those fibers which attach themselves to the chromosomes for the purpose of pulling them apart. The main part of the contractile fibers starts also from the exterior margin of the centrosphere, but each fiber is seen to be connected with the somosphere by a very thin thread of dark-staining substance (Figs. 110–113).

In the early resting stage of the polymorphous cells sometimes neither archosome nor accessory archosomes can be distinguished. Whether they are at such times situated in the nucleus or are not stainable I am unable to decide upon, but the former seems to me the most probable. In many such resting cells we find one or more darkly staining bodies which, however, are seldom situated in the axis of the cell. As soon, however, as the granosphere has assumed a definite form the two-centrioled archosome is seen to be situated in its center,

or rather in its concavity.   At the poles of the central spindle
we find always one and generally two archosomes.   Sometimes
the two are connected by a thin thread of somosphere.   Some-
times this thread is double, the two archosomes being situated
at the opposite ends of a dark-staining ring.   At the end of
the anaphase the archosomes have gradually diminished in size
and staining capacity, and are then only visible by the most
careful optical manipulation.   I have no doubt that in in-
stances where the archosome has not been figured in this
stage of mitosis, the failure to observe it is referable to insuffi-
cient optical means, and not to an actual absence of the archo-
some.   In the confluent umbrella stage the archosome is,
however, not to be distinguished.   But at this stage the apex
of the central spindle has also disappeared, to reappear later on
below the nucleus.   With its reappearance an archosome with
a single centriole is also seen at the pole of the fibers, and we
may with some reason presume that it is the same one that was
previously situated above the nucleus, but which has followed
the central spindle and been pulled through the ring-like
nucleus (Figs. 54–61, 63, 64, 69, 70).   As the granosphere is
reconstituted around or near this pole, it follows that the archo-
some will be found in or near the new granosphere.   But if
the same archosome will always perform the same function in
the new cell is doubtful; it frequently appears as if the place
of the archosome in the new spermatocyte was taken by some
accessory archosome, already at the pole of some spindle cone.

*The Accessory Archosomes, their Structure and Functions.
Expulsion of Superfluous Archosomes.* — As the accessory arch-
osomes have already been referred to in the preceding para-
graph, I will here only describe a few points not yet touched
upon.   We have seen that the accessory archosomes are quite
numerous, but of varying number.   In the earliest stages of
mitosis they are more numerous than in the later ones, and it
appears that they are in some manner used up.   At first they
circle around in the spheres apparently without any regularity;
later on they arrange themselves around the archosome at the
pole of the central spindle.   My observations are not conclu-
sive, but they tend to show that the accessory archosomes

either direct or actually furnish some substance to the contractile fibers. They are, during the metaphase and anaphase stages, found in close proximity to the points from which these fibers start, and in several instances I have seen them actually in contact with those fibers. It seems possible that an accessory archosome is first placed in position on the outer side of the centrosphere, and that it then gives rise to a contractile fiber by budding (Fig. 111). Again at other times (Fig. 110) we find these contractile fibers already formed, and yet in their vicinity a number of accessory archosomes, arranged in a ring around the archosome. Be this function of the accessory archosomes as it may, certain it is that they also possess another function of great importance, that of presiding over the fiber cones and the pulling of the cytoplasmic membrane away from the nucleus, while the latter is in a stage of growth (Figs. 65–71, 114–116). Thus at the end of the confluent umbrella stage we find them at first situated on the cytoplasmic membrane with fibers radiating out in several directions. They soon, however, rise from the membrane, carrying with them the fiber cones. As the ends of the cones remain attached to the membrane, the latter is naturally pulled away from the nucleus. There may be one or more accessory archosomes at the pole of each fiber cone. When the fiber cones have performed their function the accessory archosomes slide down along the fibers and congregate in the vicinity where the new granosphere is being reconstituted. This refers to the spermatocytes, as I have not found any fiber cones in the auxocytes. Towards the confluent umbrella stage in the auxocytes the accessory archosomes diminish in size, number, and staining capacity, just as does the archosome. They next reappear on the cytoplasmic membrane, but are not readily detected except on sections which pass obliquely or excentrically (Fig. 65).

A most interesting fact is that a large number of accessory archosomes are thrown out of the cell into the intercellular spaces, in which they sometimes remain free, sometimes remain attached to the outside of the cell membrane. (See the chapter on paracellular bodies.) The centrioles in the archosomes vary in size and number.

IV. Spindles and Spindle Fibers.

General Remarks.

The various fibers and rays which form during mitosis may be conveniently divided into six more or less distinct classes, as follows : central-spindle fibers, mantle fibers, polar fibers, contractile fibers, cone fibers, and the retractile fibers of the spindle cones. With the exception of the contractile fibers, which possibly may be of archosomal nature, all the others are decidedly of cytoplasmic origin ; none can be shown to be of nuclear origin. All these various kinds of fibers stain in about the same manner, readily taking the congo stain, with the exception of the contractile fibers, which retain the iron-haematoxylin longer than any of the others, and thus under proper treatment appear quite dark, even by their color indicating a different origin. Their structure is also from the beginning different, showing distinct beads. We will now consider each of these classes more in detail.

Mantle Fibers, Polar Fibers, and Central-Spindle Fibers.

As these fibers have the same origin and otherwise resemble each other they may most conveniently be considered together. The central-spindle fibers are the first ones to appear during the radiosomic process. As the two centrioles, with their somospheres, move apart, there appear between them two darkly staining, narrow threads which apparently help to push the archosomes apart. Between these two threads the lighter staining central-spindle fibers begin to appear, but only after the centrosphere has separated in two parts, one to each archosome (Figs. 37, 38). The central-spindle fibers thus originate from the outer edge of the centrosphere, just as do the polar fibers and the mantle fibers. But while the polar fibers and the mantle fibers are fed only from the cytoplasm proper, the central-spindle fibers receive, almost at once, material from the granosphere. At a very early stage we see numerous rays projecting from the archosome to the granosphere, and this radiation is so arranged and limited that, on the side

turned towards the granosphere, all rays which do not strike the granosphere must be counted as mantle fibers and polar fibers and not as belonging to the central-spindle fibers (Figs. 41–47). In order to supply this matter to the central spindle, the granosphere dissolves from the side that is turned towards the central spindle and not from the opposite side. As the
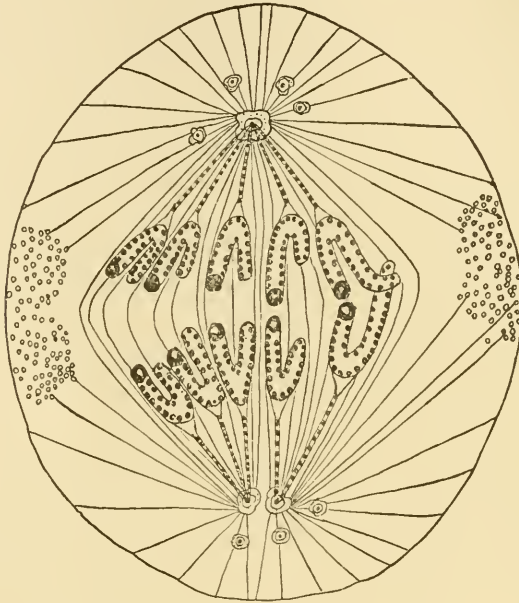
An auxocyte in the beginning of the anaphase. Only a few of the chromosomes are indicated. At each pole there are respectively one and two archosomes and three and four accessory archosomes. The chromosomes contain chromioles suspended in chromoplasm. At the apex of each chromosome there is seen a chromoplast with endochromatic granules. To the right and left in the cell are seen agglomerations of plasmosphere indicating the position of the new cell wall, which is to separate the two daughter-cells. The chromosomes are seen to be connected with the centriole by contractile fibers, the latter consisting of granules enclosed in a common sheath. The spindle fibers as well as the polar fibers start from the centrosphere.

central spindle grows in size, the granosphere is seen to diminish. The rays of the central spindle are also seen to end in the granules of the granosphere, and the whole appearance is such as to leave no doubt of the central spindle being fed principally on the granules and secretions of the granosphere. On the contrary, no such connection can at any time be seen between the polar and mantle fibers on one side and the grano-

sphere on the other.   These two classes of fibers are apparently only fed from the cytoplasm proper and to a limited extent also from the granules of the plasmosphere, and this latter probably only during the metaphase of the mitosis.   At that time many of the mantle fibers are seen to end in the granules of the plasmosphere (Figs. 48–56).   Also during the early anaphase such connection between the mantle fibers and the plasmosphere granules may be observed.   The polar fibers and mantle fibers sometimes reach the cell wall and connect directly with it, but generally the fibers end in a marginal layer of alveoles, which, however, is never as regular as that figured by Bütschli and his school.   These two classes of fibers at the end of mitosis resolve themselves into plasmosphere and cytoplasm proper, while the central-spindle fibers reconstitute themselves into granosphere, or remain for a long time comparatively unchanged as a spindle bridge between two cells.

There can be no doubt as to the continuity of the central-spindle fibers from one pole to the other.   In the later stages of the anaphase when the central spindle is being contracted we can follow without any difficulty the whole course of one or more fibers from one pole to the other (Figs. 57–62). These continuous fibers are much thicker than the early fibers of the central spindle, and it appears to me as if they originated by the fusion of several of the earlier fibers.   At this stage of the central spindle the various fibers constituting the same vary greatly as to thickness as well as to structure. While some are very thick, others again are as thin as they were in the earliest stages of the spindle.   Some of the fibers are beaded like the contractile fibers, others are smoother (Fig. 59) and show only the original structure of alternating granules.   The origin of the central spindle of the spermatocyte is less clear.   Here the mantle fibers appear first, being reconstructed fiber cones.   These mantle fibers meet and form a very wide spindle (Fig. 94) with very deeply sunken poles. At a later stage the central spindle is found inside of this wider mantle spindle, but as regards the process by which it is formed, I have no satisfactory observations upon which to base any theory.

Contractile Fibers.

This class of fibers is of sufficient importance and interest to be treated of separately. Their origin is different from that of other fibers, and they also appear, partially at least, to consist of a different kind of protoplasm. They are the only fibers which connect directly with the somosphere and which thus penetrate the centrosphere. As to the actual beginning of the contractile fibers, there are no satisfactory observations, and we do not know if the narrow thread in the centrosphere orginates previous to the part outside of the centrosphere, but I am inclined to think that this thin thread is formed after the balance of the fiber. The number of contractile fibers is the same as the number of the chromosomes, as there is a special fiber for each chromosome. These fibers show from the beginning a different structure from any of the other classes of fibers, being from their first appearance beaded (Figs. 41–54). Each fiber is composed of a thin outer sheath which is too small to allow of its structure being perceived. Inside of this sheath the protoplasm of the fiber is distinctly beaded in a manner that greatly reminds us of the cytoplasmic arrangement of the muscle fiber. These beads are not always of the same size, those in the middle of the fiber often being the largest. There is never more than one row of beads, which begins on the outer side of the centrosphere and extends to the immediate vicinity of the chromosome. Just before the fiber reaches the chromosome it divides into two tiny branches or arms, each arm connecting with different points of the chromosome. As soon as the chromosome has reached the equator the contractile fiber begins to contract, becoming thicker and shorter as well as darker staining. When the confluent umbrella stage is reached the fibers lose their intense staining capacity and finally disappear. As regards this disappearance there are several conjectures possible. Either the fibers are entirely absorbed and changed into cytoplasm, or they are condensed into accessory archosomes which reappear on the cytoplasmic membrane, at this time re-forming at a short distance from and around the nucleus. Or we may even suppose that they follow the central

spindle and are pulled through the nucleus, later to reappear as retractile fibers, emanating from the apex of the spindle cone. Against the latter assumption speaks the observation that these retractile fibers are not beaded. It seems more probable that the retractile fibers are new formations, and that the contractile fibers have condensed into accessory archosomes, now appearing on the cytoplasmic membrane around the nucleus. (See explanation of Fig. 112, p. 116.)

Fiber Cones and Retractile Fibers of the Spindle Cones.

As spindle cones and their retractile fibers, I designate structures which appear below the nucleus at the end of the anaphase and from which radiate numerous fibers towards the place where the new membrane, separating the two daughter-cells, is being formed. These fibers radiate from a small archosome at the apex of the central spindle and end partly in the granules and secretions of the plasmosphere, partly in the cell wall. The separation of the two daughter-cells seems entirely due to these retractile fibers (Figs. 59–61), which are the only ones so situated that they can accomplish such a separation. The plasmospheric granules situated in this immediate vicinity also indicate that they are used up in the construction of this membrane. The ultimate fate of these retractile fibers is a reconstitution into cytoplasm, and perhaps into plasmosphere. They disappear very soon, long before the fibers of the central spindle.

We have yet to consider the peculiar and unusual structures which I have designated as fiber cones, the origin of which is as follows. A new cytoplasmic membrane is formed exterior to the nucleus, and on this membrane are found a number of accessory archosomes. From these archosomes fibers radiate on the membrane in all directions. Later the archosome rises and pulls the membrane with it, and we then get a cone-like structure (Figs. 66–74) which in time pushes out the cell wall. Later on these cones again move inward, and at a yet later stage they dissolve into cytoplasm proper. I have already suggested that these cones help to form a cavity around the nucleus which enables the latter to increase in size and develop.

As far as I know, similar fiber cones have not been observed
in any other animal cells, though I have some reason to think
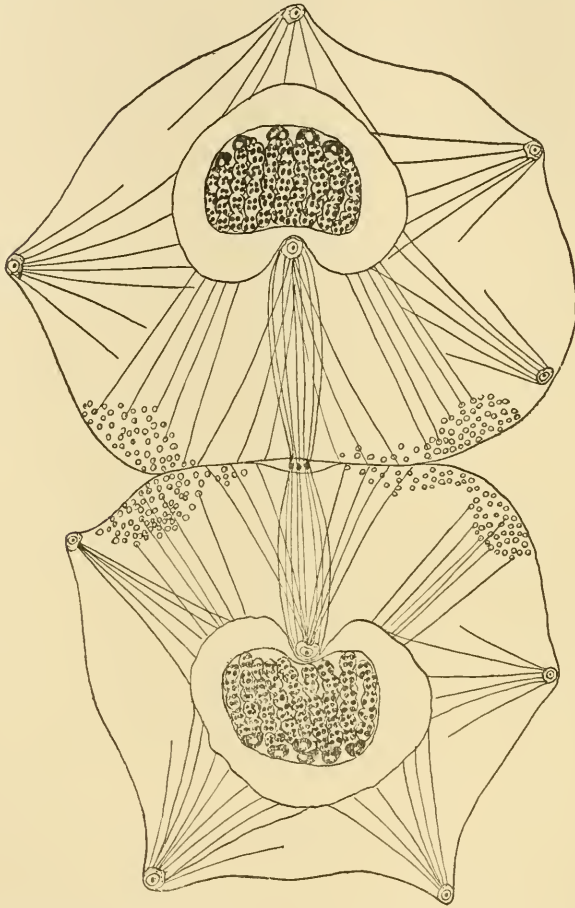
Two daughter-cells of an auxocyte connected by a spindle bridge. There are eight accessory
archosomes at the apex of as many fiber cones. Two archosomes are connected by a cen-
tral spindle. In the latter is seen a mid-body consisting of three condensation granules.
The chromosomes are being regenerated, and the chromoplasts appear at the angle of the
chromosomes instead of at the apex, as in the last cell stage. In one nucleus are seen
five, in the other six chromoplasts with endochromatic granules. Between the true
nuclear membrane and the false membrane is an open space caused by the false mem-
brane being pulled away by the fiber cones.

that the auxocytes of Batrachoseps are not the only cells which
possess them. Dr. W. J. V. Osterhout has kindly shown me
a preparation of the testes of Triton cristatus, in which I could

plainly recognize a couple of fiber cones, though they were much smaller than in the testes of Batrachoseps. These Triton testes had been fixed in Flemming's chromo-osmic-acetic mixture. The spindle cones, which Osterhout has described from the mitosis of the pollen cells of Equisetum, are exceedingly interesting, as they recall the fiber-cone structures in our present cells, even if their nature and origin be found to be entirely different. In the pollen cells the mitosis begins with spindle cones, while in the testes of Batrachoseps the mitosis ends with fiber cones.

Spindle Bridge and Mid-Body.

The spindle bridge is, as is now fully known, the remains of the central spindle. After this spindle has passed through the nucleus its fibers begin to diminish in number, several fibers apparently fusing into one. I judge that such is the case, because as the fibers decrease in number they increase in thickness without getting much shorter. At this stage the fibers also show a beaded structure in the same manner as the contractile fibers, though not quite so pronounced. This beaded structure is also found in the fibers of the fiber cones (Fig. 116), but not in any of the mantle fibers.

The spindle bridge remains a long time after the cells have otherwise separated, and in places we find not only one such bridge in the same cell but two, both starting from the same place but in different directions, and connecting several cells with each other (Fig. 32).

The object of the cell bridge is probably to prevent the cells from moving too far apart, and the formation of the mid-body is perhaps only a quick way to dispose of the cytoplasm of the cell, until it can be properly absorbed in the regular way by the spheres.

I have in another place suggested that the contemporaneous beginning of certain stages in the mitosis by all the cells in the same pocket may be due to some influence exerted or communicated by the spindle bridge. This body is the only visible connection between one or more cells in the same pocket. The spindle bridges are only found between cells which simultaneously begin the same stage of mitosis.

The mid-body is found on the central spindle at the end of the anaphase and is, as has been often described, formed in a vacuole between the two cell membranes. At the time when the mid-body appears, the spindle bridge is always greatly constricted in the middle and consists then of a lesser number of fibers than during its earlier stages. The mid-body appears to consist of a thickening or a concentration of the cytoplasm of the fiber at one certain point. Thus there is either one thick granule (Fig. 62) for each one of the thicker fibers, or there is one granule connecting several of the fibers (Fig. 63). A solid plate is never formed, and the individual granules of the midbody can always be recognized after proper differentiation. In Fig. 62 we see how some of the fibers have become beaded along their whole length, and we can easily understand that the individual granules of the mid-body can consist of a concentration of the granules of the fibers. If this is a correct explanation of the formation of the mid-body, then it is also likely that the nature of the mid-body is the same as that of the granular nodes of the individual fibers ; that is, a larger storehouse for the cytoplasm of the fibers from which the fibers may quickly draw a supply when, through the separation of the cells, the spindle bridge is suddenly extended. The mid-body would thus prevent the bursting of the spindle bridge at times of unusual strain. Again when the spindle is compressed on account of the pressure exerted by the retractile fibers, the cytoplasm of the fibers can be quickly concentrated in the midbody, there to be stored for further use. This theory of the mid-body is supported by the fact that in extended spindle bridges (Figs. 13, 17, 32, 109) the mid-body is always absent, evidently then having been used up by the extension of the spindle bridge.

V. Varieties of Cells.

Polymorphous Spermatogonia.

As polymorphous spermatogonia I designate the largest kind of spermatogonia which, during their resting stage, possess deeply folded or polymorphous nuclei. These polymorphous

nuclei occur only in the earliest resting stages, but as there are no other cells possessing similar nuclei, no difficulty will be encountered in recognizing the cells.   In testes from animals killed in June and July there are comparatively few polymorphous nuclei, frequently only one or two, seldom more than three or four, in each section of a pocket of cells.   The other cells in the pocket are mostly spermatogonia with round nuclei,
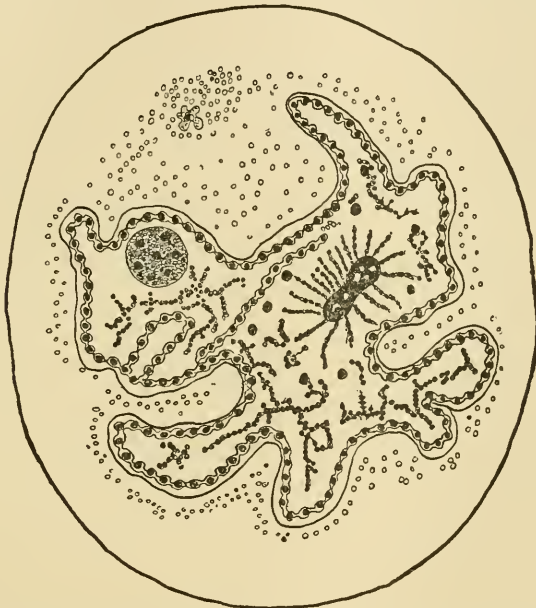
A polymorphous spermatogonium in the "perfect resting stage." The form of the nucleus allows the most perfect metabolism. Numerous chromioles are connected by a thread of chromoplasm. A network of linosomes is partially indicated, the individual granules being connected by linopodia. A large, oblong chromoplast with endochromatic granules. Eight parachromatic granules. A single archosome in the cytoplasm, the latter only partially indicated by small open circles. A single large, round linoplast, with seven endonucleolar granules.

the direct descendants of the polymorphous nuclei belonging to the imperfect resting stage and immediately preceding the prophases of mitosis.

The polymorphous nuclei are not only characterized by their form, but also by the appearance of the nuclear contents.   At first sight it appears as if the nucleus had not been properly fixed, but as if the chromatin had been disarranged and not taken the stain in a proper way.   But a more careful study of

the nucleus shows that this supposition is not correct. With inferior optical methods it seems as if the nuclear contents had been mixed up into an undefinable jumble of irregular granules or rather blotches of protoplasm. Nearly all illustrations of these nuclei appear as if they had been made under such an impression. This appearance may also have been due to improper fixing. In reality the nucleus is made up of numerous very small granules of different sizes, many of them arranged into regular rows, for which I have proposed the name "leaders." These leaders consist of a single row of granules connected or strung on a very thin thread of lighter staining substance. The granules are of two kinds, some darker, others lighter.

These darker granules are the chromioles described elsewhere, while the lighter ones are mostly linin granules, though it is probable that many of these lighter granules are also chromioles which have been bleached in the differentiation process.

Besides these granules we also find in the nucleus several larger bodies of a nucleolar nature, such as I have described under the name of "chromoplasts" and "linoplasts."

In the later stage of this kind of nucleus the leaders become more pronounced, and the chromioles aggregate into chromomeres of different sizes, while the chromoplasts become connected with the leaders in a more intimate manner than before. The leaders soon contract and change into chromosomes, which divide in accordance with the somatic process of mitosis.

In this paper I am not able, from want of sufficient material, to give a proper account of this mitosis, and a few words as to the process must suffice.

The mitosis is of the somatic type with twenty-four chromosomes. There are probably about four or five generations of cells in rapid succession. The chromosomes, which are at first of zigzag form, are thrown on the central spindle in the form of V's. There are one or two archosomes at each pole. The chromosomes of the spermatogonia are longer and more slender than those of any of the other cells, and there is thus no difficulty in recognizing these somatic mitoses, even when the cells have been so cut that the chromosomes cannot

be counted.   The daughter-cells resulting from these polymor-
phous spermatogonia are of oval form, which probably is due to
their great number and to the small space into which they are
crowded, and also to their rapid increase in size.   After a
period of growth these daughter-nuclei, as well as the whole
cell, resemble almost exactly the cells of the imperfect resting
stage of the polymorphous spermatogonia, and a description of
one kind would also fit the other.   The nucleus is round or
oval, with a very distinct membrane.

The largest part of the nucleus is taken up by an evenly
distributed but not quite regular network, consisting of very
small chromomeres or of isolated chromioles suspended in
and supported by a linin meshwork (Figs. 9, 19).   In this
meshwork, or rather in connection with it, we find from
two to five chromoplasts, rarely more or less.   In deeply
stained sections we also see several linoplasts of various sizes
but generally smaller than the chromoplasts.   The cytosome
or strictly cellular part is always smaller than the nucleus and
cone-shaped, with the base of the cone resting on the nucleus.
In this cone is seen a round sphere of about the size of a chro-
moplast.   It is always stained intenser than any other part of
the cytosome; it appears also to have a great affinity for the
congo stain.   Around this inner or granosphere is seen a much
lighter colored zone of more irregular form—the plasmosphere.
Surrounding these two spheres is generally seen a narrow zone
of fibrous cytoplasm proper.   Either in the center of or in the
immediate vicinity of the granosphere is seen a minute dark-
staining body—the archosome.   The archosome generally con-
tains two distinct centrioles, though often there are more than
two.   In rare instances we also find in the plasmosphere an
accessory archosome.

The mitosis of the polymorphous cells is less favorable for
observation than that of the two maturation cells, not only on
account of the greater number of chromosomes, but also on
account of the crowding of the cells, which prevents their
proper expansion.   The daughter-cells pass through a stage of
growth, at the end of which they enter upon the first matura-
tion mitosis.   The latter will here be referred to as auxocytes.

If we recapitulate the above, we find that the polymorphous spermatogonia pass through the following stages :

1. Perfect resting stage, in which the leaders are not yet connected with the chromoplasts.

2. Imperfect resting stage, in which the leaders are connected with the chromoplasts.

3. The various prophases of mitosis.

4. Metaphase.

5. Anaphase.

6. Separation of the daughter-cells.

7. A stage of growth, at the end of which the auxocytes have been formed and attained their resting stage.
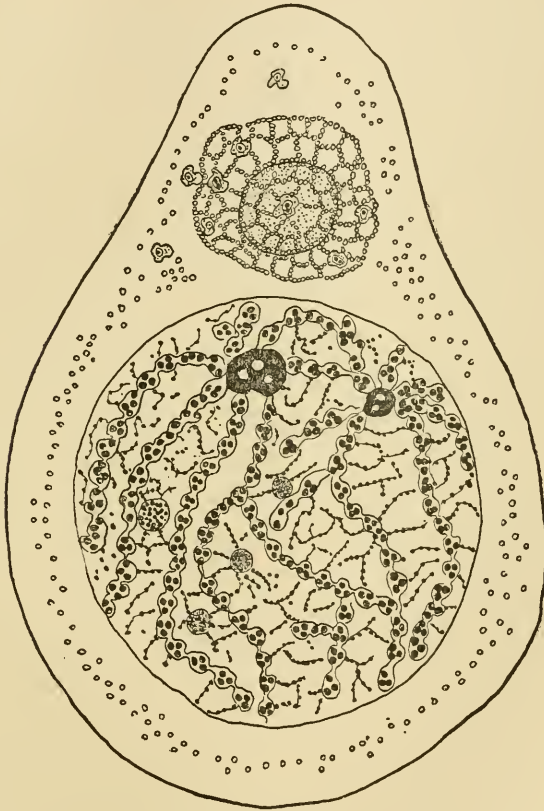
Auxocytes.

The auxocytes are the most interesting of the various cells in the testes of Batrachoseps, and a study of them is the principal object of this paper. The name "auxocyte" was first proposed by Arthur Bolles Lee for the large spermatogonia of Helix pomatia, though strangely enough he did not himself employ the name in his paper ('97) on this subject. As the name seems useful, and as the cells in question are highly characterized and exceedingly interesting, we can, I think, do no better than to adopt it for cells of this class. By adopting distinct names for the different varieties of the cells in the testes, much of the existing confusion as to nomenclature will be avoided. The auxocytes have previously been designated, by respective investigators, as small spermatogonia, spermatocytes of the first order, etc.; but as all names relating to size are less suitable, and as in this case the cells in question are not any smaller than the large spermatogonia, the name proposed by Bolles Lee is much to be preferred.

The auxocytes are characterized by a bouquet stage and by heterotypic mitosis. They are the only cells in the testes of Batrachoseps which pass through this kind of mitosis. They are the first cells to appear with twelve chromosomes, their mother-cells, the round nucleated spermatogonia appearing with twenty-four chromosomes.

The auxocytes give rise to spermatocytes, which divide by homoeotypic mitosis, the result of this latter mitosis being the spermatids.

The auxocytes pass through the following stages of development:

Auxocyte in the "imperfect resting stage," showing the formation of leaders consisting of round chromioles surrounded by a film of chromoplasm. The leaders start from two chromoplasts of unequal size, both containing endochromatic granules. The leaders are connected by a linosomic network. Four linoplasts. In the cytoplasm are seen the two spheres, the inner one, the granosphere, containing the archosome. There are eight accessory archosomes, some in the plasmosphere, others in the cytoplasm. The two spheres are of a foam-like structure. The cytoplasm is only partially indicated.

A. Resting Stages. — *A stage of growth*, during which the last daughter-cell of the previous generation increases in size and finally reaches the first stage of the auxocyte (Fig. 11).

B. Prophases. — *Bouquet with twisted spireme segments.*

In this stage the leaders have contracted and have become
arranged in a typical bouquet form, but the spireme segments
are larger than the diameter of the nucleus and accordingly
twisted. The chromoplasts are large and connect with more
than two leaders (Fig. 12).

Perfect Bouquet Stage. —The leaders have shortened and are
less bent and twisted; they are nearly all of the same size
and only slightly longer than the diameter of the nucleus
(Figs. 13, 14).

Bouquet with Split Spireme Segments. — The end of the
bouquet stages. The chromomeres have contracted into their
final number, or about twelve in each leader. Many of them
have split through the center, but have not yet separated.
The chromoplasts are generally only connected with one or
two leaders (Fig. 15).

Separated Spireme. — The leaders are not any more in the
bouquet form, but their respective halves have separated from
each other to a greater or lesser extent, and many of them
cross each other in various directions. More than two leaders
are rarely attached to the same chromoplast (Figs. 16–23).

Angular Spireme Segments. — The leaders have straightened
out, the chromomeres have fused, and the leaders diverge from
each other at sharp angles (Fig. 34).

Irregular Bretzel. — The leaders have contracted sufficiently
to be termed chromosomes. They are not yet free but con-
nected with each other by chromoplasts, in twos or threes
(Figs. 24, 33, 36–46).

Bretzel Metaphase. — The bretzel-shaped chromosomes are
in the equator of the central spindle (Figs. 47–53).

V-shaped Anaphase. — The chromosomes have been pulled
apart as *V*'s. (Figs. 54–56).

Confluent Umbrella Stage. — The chromosomes and the chro-
moplasts are confluent, forming an umbrella-shaped body
(Figs. 57–61), which later becomes ring-shaped.

Chrysanthemum Stage. —The chromosomes begin to reappear
and the spheres are being reconstituted. In this stage, as a
rule, the daughter-cells become separated, only remaining con-
nected by a spindle bridge.

The life cycle of the auxocyte comprises a stage of growth and a stage of mitosis. During the former both cell and nucleus increase in size. The stage of growth ceases as soon as the cell enters the bouquet stage with twisted spireme segments.

Spermatocytes.

The spermatocytes are the daughter-cells of the auxocytes. In size they are smaller than their mother-cells. They are characterized by the absence of the bouquet stage, by the homoeotypic mitosis, by twelve chromosomes, which are thrown on the central spindle in the shape of *V*'s. The chromosomes are borne in the form of staples or horseshoes and gradually contract to *V*'s. There is only one generation of spermatocytes. The mitosis is by equation division, in which they resemble the auxocytes. The daughter-cells of the spermatocytes are the spermatids. We can distinguish the following stages.

Chrysanthemum Stage, in which the chromosomes have the form of staples, arranged like the petals of a chrysanthemum flower (Figs. 62–70).

Checkerboard Stage, in which the chromomeres have separated and become more or less evenly distributed over the nucleus. Resembles a checkerboard (Figs. 71–82).

Contraction Stage, in which the chromosomes again contract into staple-shaped chromosomes (Figs. 83–86).

Angular Chromosomes. — In this stage the chromosomes become narrower, straighten out, and cross each other at various angles. The chromomeres also become so fused as to be scarcely distinguishable one from the other. This stage corresponds to the angular stage of the auxocytes.

Knotted Chromosomes. — In this stage the chromosomes are thrown in the center of the cell in a knot, in which the individual chromosomes are so mixed up as to be only recognized with difficulty (Figs. 88–91, 94–97).

V-Metaphase. — The perfect and split and *V*-shaped chromosomes are on the equatorial of the central spindle (Figs. 99–101).

V-Anaphase. — (Figs. 102, 103).

Confluent Umbrella Stage. — The chromosomes and chromo-
plasts have become confluent (Figs. 104–107).   Ring-shaped.

Chrysanthemum Stage. — A stage of the reappearance of the
chromosomes.   During this stage the spermatids are separated
(Fig. 108), their arrangement being as the petals in a flower.

The life cycle of the spermatocyte comprises thus a stage of
growth and a stage of mitosis.   The stage of growth comprises
the chrysanthemum stage and the checkerboard stage, while
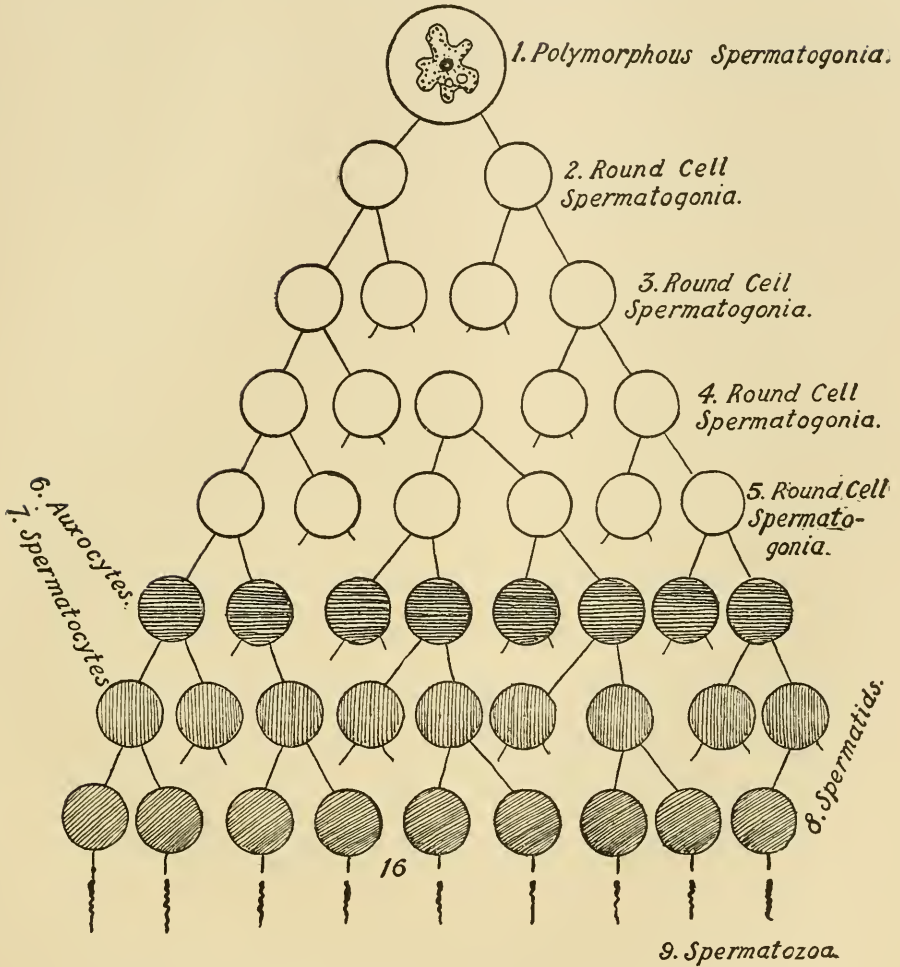the stages of mitosis proper are the stages 3–9.

Spermatids.

The spermatid is the daughter-cell of the spermatocyte.   It
is, even when full grown, considerably smaller than any of the
other cells.   A study of the development of the spermatid
into the spermatozoön will be the subject of a future paper.

VI. Mitosis.

A General Review of Mitosis.

The mitosis of the cells of the testes of Batrachoseps is the
result of *two independent parallel processes coöperating only at
certain points.*   For these two processes I propose the names
radiosomic and *chromosomic.*

The *radiosomic process* takes place in the cytoplasm of the
cell and does not directly affect the nucleus until the chromo-
somes have been formed; the object of this process is to pre-
pare material for the rays or fibers, to evolve the mantle fibers
and the contractile fibers themselves, to destroy the nuclear
membrane and to form the central spindle.   This radiosomic
process appears to be guided by the archosome and the acces-
sory archosomes.   The radiosomic process continues after the
anaphase and consists then in creating the vacuole around the
nucleus by the means of a cytoplasmic membrane elevated
by fiber cones.   The process ends with the formation of the
retractile fibers, the separation of the daughter-cells, and by
the dissolution of the fibers and the reconstitution of the
spheres.   The radiosomic process coöperates with the chromo-

1. Polymorphous Spermatogonia.

2. Round Cell Spermatogonia.

3. Round Cell Spermatogonia.

4. Round Cell Spermatogonia.

5. Round Cell Spermato-gonia.

6. Auxocytes.

7. Spermatocytes

8. Spermatids.

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9. Spermatozoa.

Explanation of the Diagram of the Various Generations of Cells in the Testes of Batra-choseps.

1. Polymorphous spermatogonia, with polymorphous nucleus, somatic mitosis with twenty-four chromosomes. The daughter-cells of these spermatogonia constitute the following generations 2–5.
2. Spermatogonia with round nucleus, somatic mitosis with twenty-four chromosomes. The daughter-cells of the polymorphous spermatogonia. There are several generations in rapid succession, and apparently all alike. These generations are as follows:
3. Spermatogonia with round nucleus similar to No. 2.
4. Spermatogonia with round nucleus similar to No. 2.
5. Spermatogonia with round nucleus similar to No. 2.
6. Auxocytes, the daughter-cells of the last generation of round nucleated spermatogonia. The change which caused a mitosis with twelve chromosomes takes place in the resting stage. The auxocytes are characterized by twelve chromosomes, a bouquet stage, and a heterotypic mitosis. Equation division. Fiber cones after the anaphase. One generation.
7. Spermatocytes with twelve chromosomes, the daughter-cells of the auxocytes. Characterized by the absence of a bouquet stage. Fiber cones with certainty only in the prophases, homoeotypic mitosis and equation division. One generation only. The daughter-cells of the spermatocytes are the spermatids.
8. Spermatids.
9. Spermatozoa.

somic process in arranging the chromosomes on the central
spindle and in pulling them apart. But the process takes no
active part in the formation of the chromosomes.

The *chromosomic process* again, as the name implies, refers
only to the nucleus and to the formation of chromomeres,
chromosomes, the splitting of the chromioles, and the manipu-
lation of the linin. The united object of the two processes is
to properly separate and divide the chromioles. The chromo-
somic process is presided over by the chromoplasts and the
linoplasts, just as the radiosomic process is presided over by
the archosome and the accessory archosomes.

These two processes are carried through about seven gen-
erations of cells, four of which belong to the polymorphous
spermatogonia, and one each to the auxocytes, the spermato-
cytes, and the spermatids. These five classes of cells divide
according to three distinct kinds of mitosis : the polymorphous
spermatogonia by somatic or regular mitosis (not by amitotic
division), the auxocytes by heterotypic mitosis, the spermato-
cytes by homoeotypic mitosis. As regards the mitosis of the
spermatids I am uncertain, not yet having properly studied it.
I have, however, seen mitotic figures among the spermatids,
hence my supposition that we may possibly have among the sper-
matids two generations. Only the prophases of the somatic
mitosis will be treated of in detail in this paper.

One of the most interesting parts of the chromosomic process
is the change of the mitosis with twenty-four chromosomes to
the mitosis with twelve chromosomes. This change seems
to take place in the imperfect resting stage of the auxocyte
and to be guided by the chromoplast. In the spermatogonia
the chromoplast projects finally twenty-four leaders, while in the
auxocytes the chromoplasts project finally only twelve leaders,
which latter change into chromosomes. As regards the reason
why this change is made and the particulars of how it is made,
we have no satisfactory observations upon which to base any
theories. Whether the reduction is the result of the chromo-
somic process alone, or the effect of a combination of the two
processes, is at present not quite clear ; there is, however, one
fact, that would seem to indicate that the radiosomic process,

to some extent at least, influences the formation of the leaders. I refer to the fact that at the very beginning of the bouquet stage those ends of the leaders which are not connected with these chromoplasts point to the granosphere, and they not only point to the sphere, but they actually cover a surface which is just as wide as the granosphere, this fact determining the bouquet form.   Just before the formation of the bouquet stage the chromoplasts are situated in that part of the nucleus which is nearest to the granosphere, but as the leaders are becoming more defined, the chromoplasts move away from the spheres to the opposite end of the nucleus, leaving the ends of the leaders resting on that part of the nuclear wall nearest the spheres. The ends of the leaders are so placed that at the point of contact with the membrane they are closer together than a short distance from it.   If there thus exists a real influence on the leaders from the spheres, this influence must be passing through the nuclear membrane, which at this time, and for some time to come, remains intact.   The reduction in the number of chromosomes is certainly not performed by any rays or fibers, as these have not yet penetrated the nuclear wall.

The Radiosomic Process, or the Evolution of the Spheres, Spindles, Fibers, Archosome, and Accessory Archosomes.

The radiosomic process begins in the polymorphous spermatogonia with the formation of the granosphere (Figs. 1–8). The cytoplasm proper is then in the form of a very thin shell on all sides surrounding the polymorphous nucleus.   In this cytoplasmic envelop a denser area appears (Fig. 2), which is at first homogeneous, but which later on differentiates into smaller isolated areas or vacuoles, surrounded by denser staining granules.   At the same time an outer zone is forming of much larger dimensions, but of less consistency.   The outer zone is the plasmosphere, and the inner one is the granosphere. While this has taken place in the spheres, the cytoplasm has spread over the larger part of the cell, having lost its thin shell-like form.   Already, with the first appearance of the granosphere, there appears also in the cytoplasm one or more

dark-staining bodies, one of which enters the granosphere and becomes the archosome, while the others remain as accessory archosomes. The archosome, as well as the accessory archosomes, divides. The two-centrioled archosome soon rises above the granosphere and carries with it a part of that sphere (Fig. 35). Metaplasmic secretions have also appeared among the granules of the two spheres. The central spindle is formed by the separation of the two centrioles and by the supplying of material from the granosphere to the rays formed between the two archosomes. The polar rays, or fibers, and the mantle fibers are formed at about the same time. The contractile fibers are formed, partly at least, of a different material and are from the beginning of different structure, being beaded and of a contractile nature. The relative position of the central spindle and the nucleus is alone dependent on the relative position of the granosphere and the central spindle. The central spindle is always so situated that a plane passing through its equator also passes through the granosphere. Numerous accessory archosomes have formed which probably assist in the formation of the contractile fibers. There is always one, sometimes two archosomes at each pole of the central spindle. Before the poles of the central spindle have reached the opposite sides of the nucleus, the nuclear membrane has been destroyed by rays of the mantle fibers (Figs. 41–47). Shortly afterwards the chromosomes are thrown on the central spindle and taken hold of by the contractile fibers. After the anaphase or mitosis is over, a cytoplasmic membrane is formed around the nucleus, and as this membrane is being pulled away by a set of fiber cones a vacuole is formed around the nucleus in which the nucleus has ample room to develop. These fiber cones are often numerous and as high as seventeen in a single cell. The cones are formed as follows. The accessory archosomes appear on the cytoplasmic membrane, and fibers are projected in various directions on the membrane. The archosomes then rise above the membrane, pulling with them the fibers, which, however, remain attached to the membrane with their distal ends. The cones rise so far as to project high above the regular circumference of the cell (Fig. 69). This formation

of fiber cones takes place only in the auxocytes. A new set of fibers appear radiating from the poles of the spindles after they have passed down through the ring-like nuclei of the auxocyte. The object of these fibers is to pull the daughter-cells apart. The latter part of the radiosomic process consists in the reconstitution of the spheres and the reassumption of its original position by the nucleus. With this the radiosomic process can be considered finished in the auxocyte. In the spermatocyte it commences in a different manner. Instead of a central spindle being formed by the separation of the two halves of the archosome, it is, at least in the majority of instances, formed by the junction of two opposite spindle cones.

The fibers of these cones dissolve the nuclear membrane and by approaching each other form the central spindle. This part of the radiosomic process I have, from want of sufficient material, been unable to study as carefully as that which takes place in the auxocytes. I do not deny that a central spindle may be formed in the same manner as in the auxocyte, but I have failed to find any evidence of such a formation. There is, however, undoubted evidence that a central spindle is actually formed by two opposite fiber cones which, receding from the cell wall, meet in such a way as to form the spindle. The other parts of the process are the same as that which takes place in the auxocytes.

To the radiosomic process must also be referred the formation of the new cell walls which separate the two daughter-cells and the pulling apart of the two new cells.

The new cell wall between the two daughter-cells is formed in the following way. Already during the metaphase (Fig. 53) the plasmosphere has scattered, and many of its granules and secretions have become located along the equator of the cell. As the anaphase progresses, this accumulation of plasmospheric granula becomes more prominent along the line of the future cell wall. The plasmospheric granula are never distributed evenly along the equator, but always in isolated rounded groups (Figs. 54–56). In the figures referred to, the plasmospheric fragments are stained deeper red than any other part

of the cytoplasm. Frequently the plasmosphere is seen on one side of the cell (Fig. 55), and not on the other, and it seems that this is rather the rule than the exception. The contraction of the cell wall commences at the place where the plasmospheric fragments touch the equator. Just previous to this contraction the plasmosphere has at that point divided in two in such a way that one-half of it lies immediately above the equator, while the other half lies under it, that is, one-half in each of the future daughter-cells (Figs. 55, 56). In the mean time the mantle fibers have become connected by their ends with the individual granules of the plasmospheric fragments, the other ends of the fibers being attached to the spindle pole. The contraction of the cell wall appears to be accomplished directly by the contraction of the mantle fibers. At a stage a little more advanced the ends of the mantle fibers, which were at first attached to the spindle poles, become attached to the cytoplasmic membrane around the nucleus (Fig. 61). Another set of fibers have also made their appearance in the vicinity of the mantle fibers. These fibers, for which I propose the name of retractile fibers, connect the archosome at the spindle pole, which has now passed through the nucleus, with individual granules of the plasmosphere (Figs. 59–61), as well as with purely cytoplasmic granules along the equator. While this contraction is taking place in the equator of the old cell wall, a change has also appeared in the equator of the central spindle. Instead of being comparatively dense and even, larger and smaller vacuoles have formed (Figs. 56, 57). These vacuoles are at first diamond-shaped, with their longer axis parallel to the spindle axis; but later on they are drawn out sideways, in the plane of the equator, while at the same time plasmospheric granules appear along their margins. The new cell walls appear to be secreted out from these granules along a double line of parallel walls of vacuoles. As soon as the two parallel cell walls have formed they are separated by the retractile fibers of the spindle cones. There are thus four processes coöperating in the formation of the new cell wall: the plasmospheric granules are placed along the equator of the cell; the central spindle is becoming vacuoled

in the equatorial plane, and the vacuoles are drawn out sideways ; a new membrane is secreted along the walls of the vacuoles from the plasmospheric granules, the two walls are pulled apart first by the mantle fibers, later on by the retractile fibers of the spindle cones.

A few minor points of this process are of sufficient interest to be noted. One of these is that in the beginning the contraction sinus (Figs. 56, 61) in the cell wall is rounded, while later on (Figs. 50, 60) it is very acute, thus indicating that, to begin with, there is only a contraction of the old cell wall, which, of course, is single, but that later on there is an actual pulling apart of two parallel walls. Another point is that the first contraction never takes place all around the cell at the same time, but always, or perhaps generally, along one side first. This may either be due to a want of sufficient plasmosphere, or to an effort to keep the cell more steady, or perhaps to both. It will be seen that the central spindle is the pivot upon which most all of this pressure is applied, and that the simultaneous passing of the central-spindle poles through the umbrella-shaped nuclei can be accounted for by the pressure exerted by the retractile fibers on the spindle poles, coupled with a contraction of the central-spindle fibers themselves.

The Chromosomic Process. The Formation of the Chromioles into Chromomeres and Chromosomes.

As has already been stated, this process is carried along independently of the radiosomic process, but parallel to it. It takes place in the nucleus principally before the nuclear membrane has been dissolved, and is, during all this time, not influenced by the action of the archosomes or by the accessory archosomes. It is undoubtedly presided over by the chromoplasts and the linoplasts. It begins in the perfect resting stage of the polymorphous spermatogonia with the formation of the leaders, and is thence carried on through the different varieties of cells without any cessation or perfect rest until the spermatozoa are formed. The whole chromosomic process can conveniently be divided into the following principal subdivisions:

Formation of twenty-four leaders in the polymorphous spermatogonia.

Somatic mitosis of the same.

Formation of twelve leaders in the auxocytes.

Longitudinal splitting of the spireme segments or leaders.
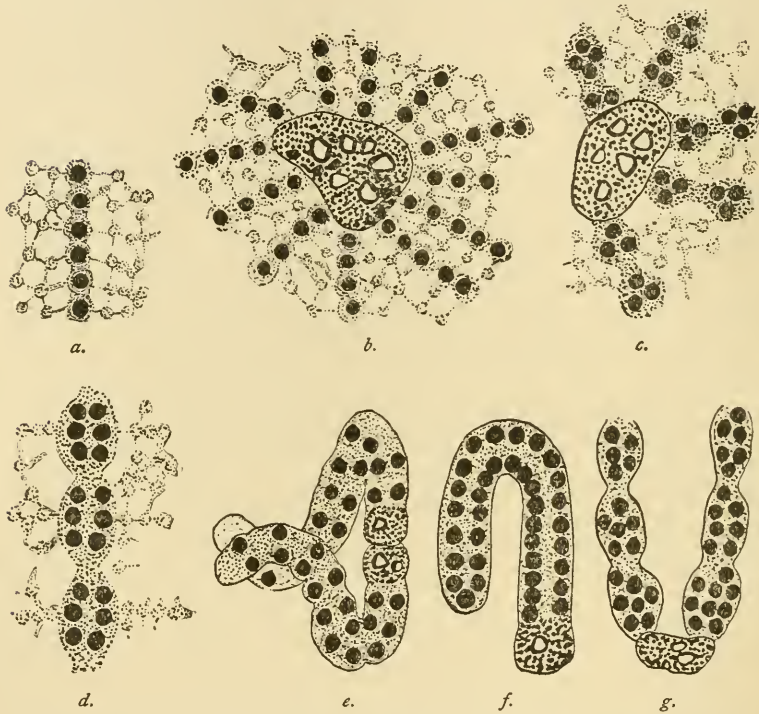
FIGS. *a.-g.* represent a broken series of leaders illustrating the formation of the leader and the chromosome. *a.* — Isolated row of chromioles surrounded by chromoplasm and suspended in a network of linosomes; *b.* — Chromoplast with twelve leaders of chromioles. From the imperfect resting stage of the polymorphous spermatogonium; *c.* — Chromoplast with five leaders. Each leader is made up of chromomeres, and each chromomere consists of three or more chromioles surrounded by chromoplasm. A network of linosomes between the chromomeres; *d.* — Three chromomeres, each with six chromioles surrounded by a chromoplasm and suspended in a network of linosomes; *e.* — A bretzel chromosome containing chromioles and two chromoplasts with endochromatic granules; *f.* — A chromosome from the metaphase. It contains thirty-six chromioles and a terminal chromoplast with an endochromatic granule; *g.* — Part of a chromosome from the spermatocyte.

Contraction of the separated halves of the leaders into chromosomes.

Equation division of the chromosomes by heterotypic mitosis.

Confluence of the chromosomes.

Reconstitution of the chromosomes and a period of growth.

Equation division of the chromosomes by homoeotypic mitosis.

Confluence of the chromosomes.

Reconstitution of the chromosomes and a period of growth.

Some of these stages are more composite than others and include several more or less distinct processes, all of which will now be considered together. For convenience' sake and for greater clearness I have for each one of them adopted distinct names, under which they will now be referred to. As these stages also mark the principal stages in the evolution of the nucleus, the same names will be used for the various stages in the evolution of the nucleus. It must be remarked that while the radiosomic process in a general way goes hand in hand with the chromosomic process, the various substages of each do not always meet in the same nodes. Thus, for instance, while generally the contraction of the cell wall begins before the confluent umbrella stage in the auxocyte, it may also be delayed until the end of this stage. This shows even more conclusively that the two processes are, to a great extent, independent of each other, and that they only meet in order to perform jointly the mitosis of the chromosomes.

A. *The Chromosomic Process in the Polymorphous Spermatogonia.*

Perfect Resting Stage. — In this stage the chromioles are spread over the polymorphous nucleus and generally separated one from the other, though they are connected by linin threads, forming winding lines all through the nucleus. These lines are not yet connected with the chromoplasts, which lie free in vacuoles, only surrounded by linin. There are from one to several linoplasts (Figs. 1–3).

Imperfect Resting Stage. — The threads of linin with the chromioles connect with the chromoplasts and form leaders. The chromioles begin more and more to approach each other, and to form small chromomeres with two or three chromioles in each (Figs. 8, 9).

The various phases of mitosis now follow according to the somatic process. There are twenty-four chromosomes on the central spindle, and the result of the mitosis is also twenty-four chromosomes carried to each daughter-nucleus. These become confluent, after which the nucleus enters a stage of growth. In this stage the appearance of the nucleus is very much the same as in the imperfect stage of rest, just before the beginning of the mitosis. This stage also marks the beginning of the auxocyte. There are several generations, but only one with polymorphous nuclei, the others having round nuclei.

B. *The Chromosomic Process in the Auxocytes.*

A Stage of Growth, during which the daughter-cell of the former mitosis increases in size and finally reaches an imperfect resting stage. In this stage the leaders are formed and their ends connected with the chromoplasts, of which there are two or more resulting from division of the original chromoplast. The chromioles are in groups of three, and each group is surrounded by a film of chromoplasm. The leaders are thus made up of numerous small chromomeres, connected by a linin string and suspended in a linin network. There are several linoplasts of various sizes (Figs. 10, 11). The two spheres are perfectly formed; a perfect archosome with two centrioles is generally present in the granosphere.

Bouquet Stage with Twisted Leaders. — In this stage the leaders have contracted to about one and one-third the diameter of the nucleus. They are bent and somewhat twisted, but their arrangement is still so regular as to present the appearance of a bouquet. One end of each leader in the bouquet is attached to the chromoplast, of which at this stage there is one or a few. There are many chromomeres, several linoplasts, and a perfect network of linin. The ends of the leaders, or spireme segments, point generally directly towards the spheres. This is, however, not always the case, as sometimes the narrow part of the bouquet points upwards or even in an opposite direction to the spheres. It appears, therefore, that the nucleus in the bouquet stages regularly revolves in such a way that the

spheres remain in the equator. This revolving of the nucleus is characteristic of all the bouquet stages (Fig. 12).

Perfect Bouquet Stage. — The leaders have contracted more, and their length is now only a trifle longer than the diameter of the nucleus. The chromoplasts have divided and the leaders have, to a great extent, separated. Seldom more than two leaders are connected by a chromoplast. The chromomeres during this stage diminish in number, two and two fusing together, so that each one finally has six chromioles instead of three, as previously. The divided chromoplasts are recognizable by the endochromatic granules (Figs. 13, 14).

Bouquet with Split Segments. — This is the last of the bouquet stages, in which the chromomeres are twelve in each leader. Each chromomere splits in two longitudinally. Generally, two leaders are connected by a dividing chromoplast. One or more linoplasts (Fig. 15).

Separated Segments. — The bouquet stage has been passed, the leaders have spread apart, and their free ends do not any more point to the spheres but stretch out in various directions. Not only the chromomeres, but the whole leader is divided, and the separated halves twist around each other, only remaining here and there connected through the non-division of certain chromomeres. Generally, only two leaders are connected by one dividing chromoplast. Several linoplasts (Figs. 16–23).

Angular Segments. — The separated leaders have now both contracted and straightened out in such a way as to form even and nearly straight rods which cross each other at regular intervals. The chromomeres are not as distinct as in previous stages. The rods which represent chromosomes are connected, as formerly, with chromoplasts. The linoplasts have all dissolved, and the linin network has become partly disarranged, its function evidently now having ceased for the time being. The exact process by which this straightening out of the chromosomes has been accomplished is difficult to explain, but the object is evidently to untwist the segments and to separate them from each other. This straightening out could not be accomplished without an almost perfect fusion of the chromomeres (Fig. 34).

Irregular Bretzel. — The leaders or spireme segments have contracted and formed bretzel-shaped chromosomes, two or more of which are connected by chromoplasts. The chromomeres are reconstituting into six larger chromomeres in every chromosome. The linin network is becoming more and more disintegrated, separating itself from the chromosomes and accumulating in a different part of the cell. During this stage the central spindle is forming, and at the end of the stage the nuclear membrane is being dissolved by the mantle fibers (Figs. 35–46).

Bretzel Metaphase. — The bretzel-shaped chromosomes have separated from each other, a part of a chromoplast remaining attached to each one of them, but no two are connected together. The nuclear membrane is entirely dissolved; the linin granules have mixed with the cytoplasm, and the chromosomes have been thrown on the central spindle and are at the end of this stage arranged in a ring on the equator of the spindle (Figs. 47–53).

V-shaped Anaphase. — The equation division of the chromosomes has taken place, and the daughter-chromosomes have been pulled towards the poles in the form of V's (Figs. 54–56) by the contractile fibers. The chromoplast remains stationary at the end of one of the arms of the chromosome.

Confluent Umbrella Stage. — The chromosomes have continued to contract and fuse together until they have so completely fused into an umbrella-shaped, ring-like mass that the individual chromosomes are no more definable. In this stage the endochromatic granules become distinct in the umbrella, indicating the presence of the chromoplasts (Figs. 57–61). One of the objects of this stage is to allow the chromoplasts to move from the end of the chromosome to its angle.

Chrysanthemum Stage. — In this stage the chromosomes begin to reappear, and they are at that time bunched together and the whole nucleus has the form of a chrysanthemum flower, the open part being towards the daughter-cell. The chromoplasts appear from the start at the angle of the chromosomic arms. The cytoplasmic membrane formed around the nucleus is being more and more pulled away, giving the nucleus

the opportunity for a stage of growth, enabling it to increase to about twice its former size. In this stage the daughter-cells become entirely separated, after which they are to be termed "spermatocytes." If we consider the spermatocyte to begin with the reappearance of the chromosomes, then this chrysanthemum stage should be counted as belonging to the spermatocyte and not to the auxocyte. If we date the appearance of the auxocyte from the stage of growth, then we ought also to date the appearance of the spermatocyte from the stage of growth of the nucleus.

C. *The Chromosomic Process in the Spermatocytes.*

The Chrysanthemum Stage, in which the chromosomes have the form of staples and horseshoes. This stage is similar to the one described as the last one of the previous cell generation (Figs. 62–70). Numerous fiber cones.

Checkerboard Stage. — The staple-shaped chromosomes of the previous stage have grown and become elongated, and the chromomeres have become so separated as to be spread over the nucleus almost as the squares on a checkerboard (Figs. 71–82). In the first half of this stage the fiber cones are disappearing, either completely or with the exception of two, which later join to form the new central spindle. The spheres are being reconstituted during this stage.

Contraction Stage. — The scattered chromomeres again approach each other and form strongly beaded chromosomes. The nuclear membrane is being dissolved by the fiber cones or by the fibers of the new central spindle (Figs. 83–86).

Angular Chromosomes. — In this stage the chromosomes straighten out and become narrower, cross each other at various angles, and the chromomeres become so fused that they can hardly be distinguished one from the other (Fig. 87).

Knotted Chromosomes. — The chromosomes have separated from each other to a lesser or greater extent and are thrown in a knot in the center of the cell. The chromosomes do not yet have the regular and finished form of *V*'s (Figs. 88–91, 94–97).

V-Metaphase. — The chromosomes are in the shape of perfect *V*'s and are as such thrown on the equator of the central

spindle. The V's are split and the mitosis is made by an equation division, the V's being exactly halved throughout their length (Figs. 99–101).

V-shaped Anaphase. — The chromosomes are at the poles in the form of contracting V's (Figs. 192, 193).

Confluent Umbrella Stage. — The chromosomes have become entirely confluent. This phase corresponds entirely to the confluent umbrella phase of the auxocytes. It possesses the same general characteristics as that phase, but there are no fiber cones formed (at least not to the same extent as in that phase, nor are they as plain or as pronounced, if they actually exist). The same kind of cytoplasmic membrane is formed around the nucleus which enters a reconstitution stage, just as in the auxocytes. From a want of sufficient material this stage has not been thoroughly studied (Figs. 104–108).

Transition Chrysanthemum Stage, in which the nucleus is reconstituted through a stage of growth, the chromosomes passing through a chrysanthemum stage into a checkerboard stage, as in the auxocyte. This is the last stage in the life cycle of the spermatocyte and also the first stage of the spermatid. As I expect to make the spermatids and their evolution into spermatozoa the subject of a special paper, I give here only a single figure of a perfectly formed spermatid (Fig. 109).

As regards the various stages of the chromosomic mitosis of the auxocytes, I will only offer a few remarks. A. Bolles Lee has recently described a stage in the mitosis of the spermatogonia of Helix pomatia, which he contends is new, and for which he proposes the name " phase de l'éparpillement." Lee supposes that the object of this stage is to prevent the two halves of the same chromosome from being placed side by side in the new nucleus. This " dispersion " stage is so similar to the one that I have here described as the "separated segments" that I have little hesitation in pronouncing them identical, and probably the object of the two phases is one and the same, not, indeed, to disperse the chromosomes, but to enable the two halves of a leader to separate sufficiently from each other during the contraction process. However, not having seen the

cells in this species of Helix, I can, of course, not express myself positively, but merely wish to call attention to the similarity and to the probable explanation.

In his review of *Zelltheilung* for 1897, Fr. Meves expresses his opinion that the ring-shaped nuclei described by Moore in the testes of the elasmobranchs are simply artefacts caused by reagents. In this I cannot agree with Meves, but must, on the other hand, express it as my decided opinion that the ring and umbrella stages represent actual phases of chromosomic evolution, equal in importance to any of the other mitotic figures.

The object of the ring and umbrella stages is, according to my idea, twofold. First, to allow the chromoplasts to rearrange themselves, that is, to proceed from the ends of the chromosomes of the auxocyte (where they are located at the time the chromosomes move apart) to the angle of the two prongs of the chromosomes, where they must be in the beginning of the new mitosis of the spermatocyte. Another object is to allow the chromioles an opportunity to divide and to be properly nourished during this division. It may also be possible that a rearrangement of the chromioles takes place, though we have no observations that would indicate that such is the case. I have some reason to think that such ring and umbrella stages are much more common than we suppose, and that they have often been allowed to remain undescribed on account of the belief of the investigator that they were mere artificial products of the fixatives.

That the first object of the umbrella stage, as stated above, is not the only one is evident from the fact that we also find an umbrella stage in the spermatocyte. As in this cell the chromosomes have from the beginning, or from the time they reappear, their chromoplasts situated at the angle where the arms of the chromosomes meet, it is evident that the same rearrangement of the chromoplasts cannot be an object in this class of cell. It may, however, be possible that the chromoplast must occupy yet another position in the spermatid, and that this is also accomplished in the umbrella stage of the spermatocyte. In the spermatid the chromoplasts appear to

have again reunited and to be situated very much as in the resting stages of the earlier cells, and it is more than probable that this reuniting takes place in the umbrella stage of the spermatocyte. My observations upon this point are only fragmentary and few, and the above is merely a suggestion to guide future observations.

I have already, in another place in this paper, referred to the fact that in the very earliest beginning of the bouquet stage the free ends of the leaders point to the two spheres, and that it is possible that one or both of the spheres have some influence over the ends of the chromosomes. These ends actually rest on the nuclear membrane, but do not penetrate it. For the formation of the leaders, see the part under the heading Leaders, pages 42, 43.

Equation or Reduction? — According to the foregoing descriptions there is no reduction in the testes of Batrachoseps, at least not in the sense of Weismann. The only reduction is in the number of the chromosomes, which are reduced from twenty-four in the polymorphous spermatogonia to twelve in the auxocytes. Both the heterotypic and the homoeotypic mitosis, as has been so conclusively shown by Flemming and Meves, accomplish the division of the chromosomes by equation and not by reduction. The splitting of the chromosomes is first accomplished, and later on, when the chromosomes have been placed on the spindle in the shape of bretzels, the separation takes place in such a way as to leave a part of the chromoplast attached to each daughter-chromosome, showing plainly that an equation has taken place. In Figs. 121 and 122 I have endeavored to illustrate this equation division. The chromoplasts are marked by a C, showing that they also are divided. As regards the homoeotypic mitosis there is even less room for a reduction. In Figs. 118 and 120 this mitosis is represented. These figures have been drawn from actual chromosomes and are not diagrammatic, like those represented in Figs. 121 and 122. As Meves has lately discussed this subject it will suffice to state that my own observations fully corroborate his.

Halting Stages in the Mitosis. — There are certain stages in the mitosis which are of much longer duration than others, as

may be seen from the fact that some cell pockets contain almost exclusively cells which are all in the same stage of development. As these different pockets with cells are found adjoining each other they give us a good guide by which we can correctly judge as to the proper succession of the mitotic stages. These stages will be enumerated below, beginning with the polymorphous spermatogonia and ending with the spermatocytes. It will be seen at once that the prophases last much longer than the middle and end phases.

In the polymorphous spermatogonia : the imperfect resting stage.

In the auxocytes : the imperfect resting stage, the bouquet stage with twisted spireme, the perfect bouquet stage, the separated segments. In other pockets we find all the succeeding stages mixed together, though in a successive order in the same pocket.

In the spermatocytes : the pockets contain all the different phases mixed together, though here also in a fairly regular succession from one end of the pocket to the other.

In the spermatids we find pockets which contain cells in only the checkerboard prophase. It thus appears that in certain phases all the cells in the same pocket enter the same phase of mitosis at the same time, and that these phases also last longer than the others. It is interesting to note that in these longer phases the cells remain mostly connected by spindle bridges, and the supposition readily suggests itself that these spindle bridges in some way actually regulate the starting time of the prophases.

## VII. Structures of the Protoplasm.

Fibers, Granula, or Alveoles.

As a perusal of the figures accompanying this paper shows, the protoplasm has everywhere been figured as granular, except in the confluent stages, where the dark staining prevents it from being properly studied.

As has been pointed out in the beginning of this paper, it is useless to attempt to study the finer structures of the proto-

plasm without adequate means. With a poor light and with an apochromat of N. A. 1, 30, the protoplasm appears different from what it does with better optical means. It is also my opinion that to the better light and to the better lenses should be added an oil-immersion condenser, as without this latter adjunct the structure of the protoplasm cannot be resolved. To speak of the ultimate organized structure of the protoplasm would, of course, be both presumptuous and premature, as even with our very best optical means we cannot hope to see anything smaller than the coarser structure, only a few grades more minute than the coarsest.

If we search among the cells of the Batrachoseps testes for the most favorable objects for the study of the protoplasmic
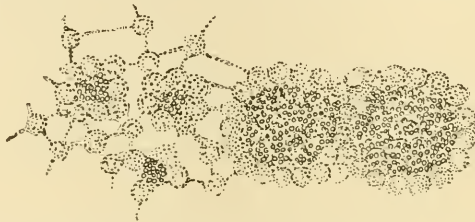
A diagrammatic representation of the structure of the granosphere. The dotted globules are cytoplasmic granules, and between them are seen metaplasmic secretions represented by small open rings. The globules are connected by linopodia, and form partly a foam structure, partly a network.

structure, we find that no cells can at all compare with the polymorphous spermatogonia. These are the only cells in the testes which can be said to be in a state approaching absolute rest, though even this resting stage is more apparent than real. As we have seen in these cells, the protoplasm is more minutely divided than in any of the other cell varieties. That this division is not the result of the fixative used seems probable. Every investigator who has studied these cells in detail figures the protoplasm as being much subdivided and generally here and there concentrated in blotches. As long as no great regularity could be perceived in these amorphous masses it was perfectly proper to consider the appearance due to improper fixing and staining. That such is the case can no more be conceded, as with proper means we can, even in this structure, see as much regularity as in any of the grosser structures of the

most highly organized cells. With proper differentiation by means of stains and differentiating light we find that the granules are not mixed promiscuously, but are placed alongside of each other in a most regular manner. There is no trace of any fibers nor of any alveolar or foam structure (Wabenbau). The chromioles are suspended singly on fine threads which are made up of other granules of a linin and chromoplasmic nature. Almost every one of these granules can be seen, and the majority can actually be counted.

If we again turn to the linin network, which also is evenly spread out between the chromioles, we also find that the individual threads and meshes of this network are composed of regular granules of uniform size and general appearance. This refers also to the linoplasts, which are principally composed of granules, as far as I can make out, of exactly the same size, form, and nature as the linin granules of the network. We have thus accounted for the two principal structures of the nucleus. The chromoplasts are generally so intensely stained that their finer structure is often obscured, but in favorable places we see that even they are composed of smaller granules of various sizes.

If we now turn to the cytoplasm proper, we find even more readily that it is composed of granules which at first appear to vary, but which, when more closely studied, are found to be uniform in size. This refers only to those which are stained and differentiated in the same manner. In places where we meet with larger globules a closer scrutiny will disclose the fact that these are only accumulations of the general granula of the cytoplasm. Of the cell wall I have made no special study. Of the cytoplasmic membrane forming around the new nucleus the structure is quite plain. It is composed of granules of the same size and color as the cytoplasm, so closely situated as to be practically continuous. In another place I have already referred to its probable derivation from the cytoplasm. With the nuclear membrane the case is very much the same, it being composed of granules resembling those of the linin network.

If we now examine into the nature of the fibrous structures so often appearing in the cytoplasm proper, especially outside

of the two spheres, we find that under the most favorable con-
ditions these fibers also can be resolved into continuous rows
of cytoplasmic granules.

These fibers frequently merge gradually into an alveolar or
foam structure, and in many cells such a foam structure is a
general one in the spheres (Figs. 10, 14, etc.). But even in this
foam structure we have no difficulty in perceiving that the
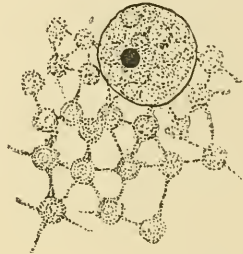walls of the alveoli contain granules.

When turning our attention to the two spheres we meet also
in them the alveolar structure with granules found elsewhere.
This is especially the case in the granosphere, as in it the gran-
ules are more densely stainable than in the plasmosphere. The
foam structure in the spheres is seen thus to contain granules
of uniform size, though here and there some of these granules
have joined to form larger composite ones.

The foam-like structure of spheres is, according to my opinion,
due to secretions from the individual granules. If we sup-
pose that such secretions are given out by a large number of
granules at the same time, and if we also presume that the
individual granules possess the power to adhere to each other,
the result will, I think, be a foam structure. The summary of
the above observations would then be this. Protoplasmic fibers
as well as foams are not the ultimate visible arrangement of the
protoplasm. All such fibers and foams in the Batrachoseps
testes can be further resolved into granules of uniform size,
which granules adhere to each other, forming granulated fibers
and foams. The fibers and foams are thus only secondary
structures and do not represent the ultimate visible structure
of the protoplasm.

If we extend this theory to the spindle fibers we find that all
observations go to confirm its correctness. The individual
spindle fibers are not only plainly composed of granules, but
these granules are often of different sizes and colors, indicating
that foreign granules have been taken up and suspended in the
thread of the fiber. That the formation of the spindle fibers is
really only a readjustment of the previous structure of the
cytoplasm is almost certain, but this readjustment cannot be
simply the result of a contraction and a drawing out of the

cytofoam, but rather the result of a real readjustment of the individual granules of the protoplasm (Figs. 62, 65). The beaded nature of the spindle fibers is probably due to the alternating of larger and smaller granules, as well as to the accumulation of several granules into larger heaps. The nodes of granules can thus be considered as storehouses of protoplasmic granules, which at any certain time may be given out at any particular point, or at all points at the same time, as the necessity either to contract or to expand may require.

In this connection it may be proper to consider the nature of the individual cytoplasmic granule and the manner by which it is able to adhere to other granules of the same nature. In the linin, as well as in the purely cytoplasmic network, the individual granules are either situated very close together or they are farther apart and only connected with each other by fine threads. The diagram illustrates the manner of adhesion of the linin granules. Every individual granule appears to have the prop-

A diagrammatic representation of the structure of linosomes and the linoplast. The individual linin granules are connected by means of linopodia. The linoplast contains linosomes as well as an endonucleolar body.

erty of sending out one or more of a kind of long pseudopodia, for which I propose the name *linopodia*. They differ from the pseudopodia of the amoeba by being always and throughout their length regular and even, resembling fine threads projecting from one granule to the other. Some granules have one or more of these linopodia which do not connect with any other granules, — which seems to show that the individual linopodium has actually been thrown out, and that it has not been drawn out through the moving apart of two or more granules from each other. By the aid of these linopodia the granules can adhere to each other, and can be drawn closer together or be moved farther apart as the case may require. When alveoles are to be formed, as, for instance, in the granosphere, then the linopodia are drawn in and the granules are drawn close together, thus enabling secretions to be retained in the alveole. A network again is formed by the moving apart of the granules and by an extension of the linopodia (Diagram, p. 78).

VIII. PERMANENCY OF THE STRUCTURES OF THE CELL.

The permanency of the various structures of the cell has already been referred to at various times in this paper, but a summary of all the observations may here be of interest. If we begin by considering the largest structures in the cell, the chromosomes, we find that they can in no possible manner be considered as permanent, even if we allow that some slight subdivision is unimportant. Not only do the chromosomes, during the confluent umbrella stage, become so fused together that there hardly remains a trace of their individuality, but we have at that time only one single confluent mass in which we only can perceive very faintly the chromiolic granules, and more distinctly the endochromatic granules; but of the original chromosomes there is at the end of this stage hardly a trace. I say hardly, because we might consider that the points which in some cases project from the umbrella actually represent the original chromosomes. But even this is not quite certain, because we find scattered all through the umbrella-shaped mass the endochromatic granules of the chromoplasts, which undoubtedly shows that a real and complete fusion has taken place, and that the points in question only represent the line along which this fusion has proceeded. On the other hand, in the resting stage of the polymorphous spermatogonia there are no structures which correspond to the chromosomes. The chromioles are, as we have seen, suspended on fine threads of linin, and are so scattered that not even the leaders can be recognized. Nor is there any "Anlage" even of chromosomes until the leaders have formed. The chromosomes can therefore not be permanent structures of the cell.

If we now turn to the chromomeres, we shall find that they also are only ephemeral structures and no more permanent than the chromosomes. Long before the chromosomes have become confluent, the chromomeres have lost their identity as chromomeres, the chromioles of which they were composed having become arranged in double rows in the chromosome. But while in this stage the chromosomes are of slightly greater permanency than the chromomeres, we find that in the resting

stage of the polymorphous spermatogonia the chromomeres appear a little before the chromosomes. But in the beginning of the resting stage there are no traces of any chromomeres. Their formation can be readily followed, and we have seen how they have originated by three chromioles being joined together and surrounded by a film of chromoplasm. Of the chromoplasts we can say but little, as their structure cannot be as closely followed as the chromosomes; but judging from the presence of the endochromatic granules, it is probable that they never fully lose their identity. There thus remains of the chromosomic structures only the chromioles yet to be considered. These can be readily recognized in all stages of nuclear development, from the perfect resting stage up to the confluent umbrella stage. In the latter they are rarely demonstrable, which we, however, have reason to suppose is due to their incapacity to stain deeply during this stage. When the new nucleus reappears the chromioles are found to have been doubled in number, each chromiole having probably been divided in two. This in itself contradicts any disappearance of the chromioles, but, on the contrary, indicates that the larger structures, the chromomeres and the chromosomes, must be considered as only temporary structures, originated in order to facilitate the evolution and the division of the chromioles.

Turning to the linin network, we find that it only exists as such during the prophases of the mitosis and in the resting stages of certain cells. After the equation division of the chromosomes, when the usefulness of the linin network has disappeared, the linin granules scatter all through the cell, and can yet be recognized on account of their staining capacity. When the nucleus is reconstituted and the function of the linin network is again required, then the network is re-formed, presumably from already existing linin granules.

What has here been said of the nucleus holds also good for the cytoplasm. We have seen how the two spheres are dissolved, and later reconstituted, apparently from some of the original granules or their offspring, which in the mean time have been used for the construction of fibers of various kinds.

As regards the archosomal structures, it appears that they

are somewhat more stable, though it is probable that they also
are subjected to changes through a readjustment or rearrange-
ment of their granula. However, our knowledge of these
minute bodies is so fragmentary that a satisfactory discussion
of this subject will for a long time to come be impossible.
All that we can now say with any degree of certainty is that
the chromosomes, the chromomeres, the linin network, the
linoplasts, the two spheres, and the nuclear membrane are
all ephemeral structures, their granula alone being permanent.
The most permanent visible organized structures of the cell
are the chromioles, and next to them the chromoplasts and the
archosomal structures, but even their permanency is only
approximately demonstrated.

IX. Summary.

I. The cell structures may conveniently be divided into
those belonging to the cytosome, to the karyosme or nucleus,
and to those of the archosome. Those of the cytosome are:
the cytoplasm proper, the plasmosphere, the hyalosphere, the
granosphere, the metaplasmic secretions, the cytoplasmic mem-
brane, and the various fibers and spindles, except the con-
tractile fibers.

The structures of the nucleus are the chromioles, the chro-
momeres, the leaders, the chromosomes, the chromoplasm, the
endochromatic granules, the linin, the linoplasts, the nuclear
membrane, and the chromoplast.

The structures of the archosome are the centrioles, the somo-
sphere, and the centrosphere, all of which combine to form
archosomes proper and accessory archosomes.

II. The spheres are composed of at least two, probably
three, distinct structures, independent of each other and con-
sisting of different granula. These structures are the plasmo-
sphere, the granosphere, and the hyalosphere, each one of
which has a different function or functions to perform. The
innermost of these spheres, the granosphere, furnishes material
for the central spindle, and also constitutes the main dwelling-
place for the archosome. The plasmosphere furnishes material

for the mantle fibers, for the retractile fibers, and for the new cell wall.   This material is both in the form of secretions and actual protoplasmic granula.   The form of the spheres is not permanent.   Each one of the spheres at times dissolves, again to reappear, the granules being the only permanent elements of the spheres.   The alveolated structure of the spheres is due to metaplasmic secretions from the granules composing the sphere.   The granules of the plasmosphere, and especially those of the granosphere, have a great affinity for congo, and by the use of this stain they may be recognized even when dispersed in the cytoplasm.

III.   The cytoplasmic membrane, or false nuclear wall, around the nucleus at the end of the anaphase, is formed by a condensation of cytoplasmic granules, and is not a true nuclear membrane.   It is dissolved as soon as its usefulness is past. Its purpose is to allow the formation of a large vacuole around the nucleus, in order that the latter may be able to enter a stage of growth without hindrance from the surrounding cytoplasm. This cytoplasmic membrane forms the base upon which are formed the fiber cones for the pulling away of the membrane.

IV.   The chromioles are the most minute, individualized, and the most permanent elements of the chromosomes which we can perceive with our present optical means.   They are subject to division and growth, but are probably otherwise constant.   There are generally six of these chromioles in every chromomere ; and as there are six chromomeres in every perfect chromosome, we have thirty-six chromioles in each chromosome of the metaphase of the auxocyte.   The formation of the chromosome commences with the formation of a chromomere with from three to six chromioles, according to the variety of the cell.   A number of such chromomeres are connected together to form a leader, the leader is finally contracted, splits, and divides, after which each half further contracts, and, after separation by equation division, forms a chromosome in the anaphase.   The chromomeres and chromosomes are only temporary structures, the purpose of which is to facilitate and to make possible the arrangement, the nourishment, and the division of the chromioles.   The chromioles, being the most

permanent granules of the chromosomes, with the exception of the chromoplasts, are possibly the carriers of heredity. The chromioles in each chromomere are kept together by a special chromoplasm, just as the centrioles are united by a somosphere.

V. The evolution of the chromosomes is directed by one or several bodies — the chromoplasts which collect the chromioles by the aid of the linin into leaders. The number of finished leaders is constant, being as many as there are to be chromosomes. The chromosomes are formed by contraction of the leaders and by the formation of chromomeres, each with a certain number of chromioles. The chromoplasts are characterized by the endochromatic granules, which probably constitute a food supply for the chromioles. The chromoplasts stain as the chromoplasm. They divide finally in as many parts as there are chromosomes, one part remaining attached to one end of each chromosome, thus constituting a landmark by which the location and position of the chromosome can be recognized, and by which its mitosis can be determined. In the confluent umbrella stage the chromosomes and the chromoplasts fuse completely.

VI. The linoplasts or true nucleoli supply the material and the nutriment for the linin network whenever an unusual supply is required, as, for instance, when the split halves of the leaders are to be spread apart. After this separation is effected the linoplasts disappear and the linin network is disarranged and its granules distributed and mixed with the cytoplasm of the cell. The linoplasts consist principally of the same kind of granula as the linin, with the addition of paralinin globules. The nuclear membrane is formed by a condensation of linin granules.

VII. The structure of the archosome and the accessory archosomes is the same, and the latter are derived from the former through budding. The archosome when not in activity is generally found in the granosphere, while the accessory archosomes are at the same time found in the plasmosphere. The archosome directs the formation of the spindle and of the fibers generally. The fiber cones are, however, presided over by the accessory archosomes. These latter bodies are also intimately

connected with the formation of the contractile fibers. The centrosphere is globular or rounded while at rest; when in activity it is amoeboid and its nature appears to be that of an organ of locomotion, by which the archosome and the accessory archosomes can move from one place to another in the cell.

VIII. The central-spindle fibers, the polar fibers, and the mantle fibers do not penetrate the outer or centrosphere of the archosome. The only fibers which penetrate this sphere are the contractile fibers; all others begin at the outer edge of the centrosphere. The contractile fibers show from the beginning a different structure, being strongly beaded. Their structure greatly recalls that of a striated muscle cell. The fibers of the fiber cones are formed for the purpose of pulling away the cytoplasmic membrane from the nucleus, in order to allow this latter body to pass through a stage of growth unhampered by the surrounding cytoplasm. These fiber cones are presided over by accessory archosomes. Another set of fibers, the retractile fibers, is formed for the purpose of pulling the two daughter-cells apart. This set seems to emanate partly from the archosome, partly from the accessory archosomes. The fibers of the central spindle and those of the fiber cones become also beaded shortly before they dissolve.

IX. The cell generations of the testes of Batrachoseps are as follows: Polymorphous spermatogonia, auxocytes, spermatocytes, spermatids. They are characterized as follows: Polymorphous spermatogonia, a large polymorphous nucleus during the resting stage, a perfect resting stage before mitosis; during that stage there are no chromomeres, no chromosomes, and no leaders; somatic mitosis with twenty-four chromosomes; there are three or four generations, but only the first possess polymorphous nucleus and the characteristics pertaining to it. The auxocytes are characterized thus: an imperfect resting stage before mitosis; the formation of twelve bretzel-shaped chromosomes which divide by heterotypic mitosis and by an equation division; a confluent umbrella stage of the chromosomes in which the chromoplasts and the chromosomes become entirely confluent. The growth of the nucleus is accompanied by the formation of a greater or lesser number of fiber cones.

The spermatocytes are characterized thus : the chromosomes
are thrown on the central spindle as split V's ; the central
spindle is often formed by the fusion of two opposite fiber
cones, in which case the accessory centrosomes assume the
functions of archosomes ; mitosis by the homoeotypic process
and by equation division ; the chromosomes are twelve in num-
ber ; only one generation.

X. The mitosis of these genetic cells is the result of two
parallel independent processes, for which I propose the names
" radiosomic " and " chromosomic." The radiosomic process
has for its purpose the formation and evolution of the spindles
and fibers (necessary for the dissolution of the nuclear mem-
brane and for the separation and equation of the chromosomes)
and the reconstitution of the cytosome. The radiosomic process
is directed by the archosomes and the accessory archosomes.
The chromosomic process has for its object to form the leaders
through the arrangement and formation of chromomeres, the
arrangement of the chromioles into chromomeres, the contrac-
tion of the leaders into chromosomes, the splitting of the
leaders, and the separation of the split halves by the aid of
the linin and the linoplasts. The chromosomic process is con-
ducted by the chromoplasts. The coöperation of the two
processes commences possibly with the arrangement of the
leaders to form a bouquet, at which time the spheres appear to
attract the ends of the leaders. But the first time when the
two processes coöperate with a certainty is when the nuclear
membrane is dissolved by the mantle fibers. The coöperation
of the two processes has for object the equation division and
perfect separation of the chromosomes, and indirectly the divi-
sion of the chromioles. The archosomes and accessory archo-
somes take no part in the chromosomic process.

XI. The perfect resting stage of the nucleus of the poly-
morphous spermatogonium belongs to a phylogenetically very
primitive type of nucleus. In this type the chromomeres and
chromosomes have not yet become a necessity, the chromioles
simply being suspended on chromoplasmic and linin threads.

XII. The confluent umbrella stage of the nucleus is not an
artefact, but an actual stage in the development and growth of

the nucleus.   The object of this stage is to enable the chromo-
plasts to move from one part of the chromosome to another.
Thus in the auxocyte it moves from the end of the chromo-
some to the angle of the chromosome (Fig. 122).   In the
umbrella stage, between the spermatocyte and the spermatid,
the chromoplasts again fuse together, forming one or more
bodies, just as they did in the imperfect resting stage.

XIII.   The central spindle is formed in a different manner in
each of the two maturation cells.   In the auxocytes it is formed
from fibers starting from the centrosphere of the archosome,
the first formation appearing between the two halves of the
original archosome in the way in which central spindles are gen-
erally formed.   But in the spermatocytes the central spindle is
formed by the approach of two opposite fiber cones.   The man-
ner of formation of the central spindle observed in the auxocyte
has not been seen in the spermatocytes, though it is not impos-
sible that it exists there also.

XIV.   The ultimate visible structures of the protoplasm are
the individual granules.   These granules have the power of
adhering together by the means of " linopodia," or small straight
arms of protoplasm of the same nature as themselves (Dia-
grams 14 and 15).   Through the secretion of metaplasmic
products these granules are pushed apart from each other and
form thus alveoles and vacuoles filled with these secretions.
The original granules can so arrange themselves as to form
fibers, foams, or reticulum.   The alveolar or foam structure is
simply an aggregation of alveoles formed in the manner just
described.

XV.   The plasmosphere, granosphere, hyalosphere, chromo-
meres, chromosomes, spindles, fibers, and linin network are all
ephemeral structures temporarily formed for the accomplish-
ment of mitosis, which latter process ultimately concerns the
chromioles.   In the life cycle of every one of the testes cells
there is always to be found a period during which the one or
the other of these structures does not exist as such, their gran-
ula alone remaining, though scattered among other structures.
The chromioles, the chromoplasts, the archosome, and the cell
wall are the most permanent structures of the cell.   But it is

possible that even these structures are ephemeral ones, and that ultimately they also may be proven to be aggregations of more permanent granules.

X. NOMENCLATURE.

Accessory Archosomes. — All archosome-like bodies found in the cytoplasm, and which have the same structure as the true archosome, that is, consist of centriole, somosphere, and centrosphere. Siderophile granules of A. Bolles Lee. The accessory archosomes differ only from the archosome in function, the latter presiding over the formation of the spindle. They originate from the archosome (Fig. 69).

Alveoli. — Rounded or variously shaped vacuoles, surrounded by granules. They contain secretions of various kinds, according to the structure in which they are found.

Angular Segments. — The spireme segments have contracted and straightened out, and have become of uniform thickness throughout (Fig. 34).

Archosome or Spindle Archosome. — The perfectly developed archosome which guides the formation of the central spindle. It generally dwells in the granosphere when it is not situated at the pole of the spindle. It is composed of an outer centrosphere, an inner somosphere, and one or more interior centrioles. It is not an integral part of the granosphere. The archosome gives origin to the accessory archosomes by budding. The granosphere and the plasmosphere are not parts of the archosome. The word " archosome " was first proposed by me in my paper on the Plasmocytes of Batrachoseps.

Auxocytes. — This name was first proposed by A. Bolles Lee. The first maturation cells, the last generation of daughter-cells of the polymorphous spermatogonia. Only one generation. The mitosis is heterotypic with twelve chromosomes, and is characterized by the bouquet stage. Mitosis by equation division. The nucleus is never polymorphous.

Bouquet Stage. — The spireme leaders have contracted and formed twelve segments of about equal size, one end of which is attached to the chromoplast, the other being free, and ending in the vicinity of the spheres, thus forming a figure resembling

a bouquet.   This stage contains the three substages mentioned below (Figs. 12–15).

Bouquet Stage with Twisted Segments. — The spireme segments are about one-third longer than the diameter of the nucleus.   Many small chromomeres (Fig. 12).

Bouquet with Split Segments. — The chromomeres are distinctly split, but are not yet separated (Fig. 15).

Bretzel Stage. — The segments have the form of bretzels or twisted rings.   These bretzel-shaped chromosomes may be more or less regular, and their ends may only overlap each other, or they may be actually grown together (Fig. 25).

Central Spindle. — The primary spindle by which the archosomes are united, and which form the center of the mitotic figure.   It does not include the contractile fibers attached to the chromosomes, nor the mantle fibers.   The spindle which connects the two poles (Hermann, Figs. 49–56).

Centriole. — The innermost dark-staining granule or granules, situated in the center of the archosome, and also in the accessory archosome.   It does not include the somosphere.

Centrosome. — On account of the many and various definitions given by respective investigators, this name has recently been discarded by several investigators, among them by W. Flemming, who substitutes the word *Centralkörper.*   But while we are told that this word expresses the same thing as the word "centriole," we are yet at a loss to know if it includes the somosphere, or the somosphere and centrosphere.   When the word "centrosome" is used in this paper, it is always left open and undecided whether we have before us an archosome or an accessory archosome.   I use this word only to indicate a centriole surrounded by its somosphere.

Centrosphere. — The more or less hyaline and indifferently stainable zone surrounding the somosphere and centriole, the outer sphere of the archosome, as well as of the accessory archosomes.   It is sometimes amoeboid, sometimes again circular or globular, with a perfectly even outline.   It is principally an organ of locomotion.

Checkerboard Stage. — The second prophase of the spermatocyte.   The chromomeres have separated and scattered over

the nucleus, making it appear as a checkerboard. This is the principal stage of growth of the nucleus (Fig. 77).

Chromatin, the darkly staining substance of the chromosomes, applied without reference to any of the particular structures.

Chromioles. — The smallest visible organized parts of the chromosomes. Possibly the bearers of heredity. They are of constant form, size, and number in each typical and perfect chromosome. In the perfect resting stage of the auxoyctes, the chromioles are scattered free in the nucleus, and not collected in chromioles or chromosomes. There are typically thirty-six chromioles in every chromosome.

Chromomeres. — Small aggregations of chromioles, surrounded by a film of chromoplasm. There are six chromomeres in each perfect chromosome.

Chromoplasm. — The dark-staining plasma, which surrounds the chromioles, and which unites them into chromomeres and chromosomes. Also found in the chromoplasts.

Chromoplasts. — One or more rounded and well-defined bodies, found in the nucleus, either free or attached to the leaders and the chromosomes. Chromoplasts guide the formation of the chromosomes, just as the archosomes guide the formation of the spindles. Variously named karyosome, net-knot, Netz-Knoten, nucleolus, etc.

Chromosomes. — The contracted leaders, with six chromomeres. There are twenty-four of these in the polymorphous spermatogonia, and twelve in the two maturation cells.

Chromosomic Process. — One of the two independent processes, the formation of the chromomeres and the chromosomes from the chromioles and chromoplasm; this process is presided over by the chromoplast.

Chrysanthemum Stage. — The first prophase of the spermatocyte. The chromosomes have begun to reappear, forming a figure resembling a chrysanthemum flower (Fig. 67).

Contractile Fibers. — Fibers directly connecting the chromosomes with the somosphere and the centriole, and which thus penetrate the centrosphere. There are as many contractile fibers as there are chromosomes (Figs. 111–113).

Contraction Stage. — The third prophase of the spermatocyte. The chromosomes have again contracted and assumed forms resembling staples or horseshoes (Fig. 83).

Cyto-Microsomes. — The most minute visible granules of the cytosome, the granula of which the spheres and most of the fibers are constructed.

Cytoplasm. — All the protoplasm in the cytosome. The protoplasm of the cell proper. Does not refer to the protoplasm of the archosome and the nucleus.

Cytosome. — The part of the cell outside of the nucleus and the archosome. The two spheres are essential parts of the cytosome.

Endochromatic Granules. — Highly refractive granules found in the chromoplasts and in the confluent stage of the nucleus. Probably food supply or stimulant for the chromioles.

Fiber Cones. — Cones of fibers projecting from the accessory archosomes, and which make their appearance at the end of the anaphase. They sometimes elevate the cell membrane, forming large cones. The base of the cone is at first attached to the cytoplasmic membrane around the nucleus (Fig. 114).

Granosphere. — The inner strongly granulated sphere, sometimes called the attraction sphere. It stains more intensely than the other sphere, and it furnishes material for the central spindle. It is the favorite dwelling-place for the archosomes.

Heterotypic Mitosis. — Mitosis, in which the chromosomes are thrown on the central spindle in the shape of bretzels or rings. The mitosis is by equation division. The mitosis of the auxocytes.

Homoeotypic Mitosis. — The chromosomes are thrown on the spindle in the form of *V*'s. The mitosis of the spermatocytes.

Imperfect Resting Stage. — In this stage of the nucleus the leaders have formed, but there are no finished chromomeres, nor any chromosomes. This stage follows the last described stage. Is found in the polymorphous spermatogonia and in the auxocytes.

Leaders or Spireme Segments. — Strings of chromoplasm and linin on which the chromioles are suspended, singly to begin

with, later on in twos and threes. There are as many leaders as there are to be chromosomes. The leaders condense into chromosomes. The leaders are connected with each other only by the chromoplasts.

Linin Granules or Granula. — The smallest visible granules composing the linin network and threads. Also found free in the nucleus during the resting stage and after the prophases.

Linin Network. — The congo-staining network supporting all the chromatin structures during the prophases.

Linoplast. — One or more round bodies in the nucleus, staining like the linin and the cytoplasm. They supply the material for the linin network, when this is in rapid increase. Generally called true nucleolus.

Linopodia. — The thread-like or bar-like projections from the individual granules of the cytoplasm, and from the linin and other granules of a similar nature. By these linopodia, the individual protoplasmic granules are able to adhere to each other and to form network or foams. These linopodia are retractile, very much like the pseudopodia of the amoeba, but they are more regular and even throughout their length.

Mantle Fibers. — All fibers of the mitotic figure, which radiate from the outer margin of the centrosphere, and which surround the central spindle. The polar fibers and the contractile fibers are not included in the mantle fibers.

Metaplasmic Secretions. — The various secretions confined in vacuoles of the spheres.

Mid-Body. — A number of darkly staining granules, situated on the fibers of the central spindle, at a place where the two daughter-cells separate. They are probably caused by a concentration of the cytoplasmic granules of the fibers, separated and only suspended by thin threads of linin (Fig. 64).

Paracellular Bodies. — Bodies of various sizes and structure found between the cells. They are probably expelled centrosomes and particles of the spheres. Some are free, others are attached to the cells by threads of protoplasm.

Parachromatic Granules. — Granules found in the nucleus during the resting stage and in the immediate vicinity of the

chromoplasts.   Stain as the chromatin with the iron-haematoxylin.   Their nature is not known.

Paralinin Granules. — Larger, deeper staining granules of unknown nature mixed in among the linin granules of the linin network.

Paranucleolar Granules. — Dark-staining granules, forming a shell around the linoplast or true nucleolus.

Paraplasmic Granula. — Granules of undetermined quality found in the cytoplasm.   They are often difficult to distinguish from the centrosomes.

Perfect Bouquet Stage. — The spireme segments are only slightly longer than the diameter of the nucleus.   The segments are more parallel (Fig. 14).

Plasmosphere. — The outer, generally lighter staining of the two spheres of the cytosome.   It surrounds the inner or granosphere, but is sometimes scattered.   It furnishes material for the mantle fibers and for the nuclear membrane.

Polar Fibers. — All fibers radiating from the outer margin of the centrosphere, and which extend in a direction opposite to that of the mantle fibers.   Of the same general nature as the mantle fibers.

Polymorphous Spermatogonia. — The largest spermatogonia with polymorphous nuclei during the resting stage, becoming less and less polymorphous, as the leaders are being formed.   They divide by somatic mitosis, and possess twenty-four chromosomes.   There are three or four generations, but only the first one of these contains polymorphous nuclei.

Prophases. — All mitotic stages between the imperfect resting stage and the perfect metaphase.   The phases in which the chromomeres and the chromosomes are being formed, and their structure finished (Figs. 12–44).

Radiosomic Process. — The evolution of the spheres, spindles, and fibers ; one of the two independent processes by which the mitosis of the cell is accomplished.   This process is presided over by the archosomes and the accessory archosomes.

Retractile Fibers. — A set of fibers radiating from the poles of the central spindle, when the poles have descended through the ring-like nuclei of the daughter-cells.   They end on or

near the new membrane forming between the two cells, their
function being to separate the two cells (Figs. 68–70).

Ring Stage. — The last of the bretzel stage in which the
chromosomes are ring-shaped (Fig. 25, *n.p.q.*).

Separated Segments. — The chromomeres and the segments
have separated, and the latter have twisted around each other,
and often cross each other in various directions (Figs. 16, 17).

Somatic Mitosis. — Mitosis, with twenty-four chromosomes,
dividing in the same way as the somatic cells. The mitosis of
the polymorphous spermatogonia.

Somosphere. — The thin, dark-staining zone nearest surround-
ing the centriole, and situated interior to the centrosphere. It
has sometimes the form of a narrow, even band or thread.

Spermatids. — The daughter-cells resulting from the mitosis
of the spermatocytes. Possibly two generations, the last of
which change directly into spermatozoa.

Spermatocytes. — The second maturation cells. The daughter-
cells of the auxocytes. Mitosis homoeotypic and by equation
division, with twelve chromosomes. One generation only.
Chromosomes are placed on the spindle in the form of *V*'s.

Spindle Cones. — The cones formed of the retractile fibers
around the poles of the central spindle, when the latter has
been pulled through the ring-like nuclei of the daughter-cells
(Fig. 114).

Spireme Segments or Leaders. — Strings of chromoplasm on
which are suspended the chromioles. There are as many
spireme segments as there are to be chromosomes. The spi-
reme segments do not form a single continuous thread, but are
individually separated.

Umbrella Stage. — The chromosomes in the amphiaster or
anaphase have become confluent, and formed an umbrella-like
body, in which the individual chromosomes cannot be distin-
guished as such (Figs. 58–61).

V-stage, in which the chromosomes have the shape of *V*'s,
the apex of which is attached to the spindle. Includes a meta-
phase and an anaphase (Fig. 120).

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XII. EXPLANATION OF THE FIGURES.

PLATES I–XIV, FIGS. I–122.

General Remarks. — All the figures have been drawn to the same scale projection on the working table, by the aid of a Grünow drawing camera and with a Zeiss Apochromat, 3 mm., Ap. 1, 40, Oc. 18. The details were studied with a Zeiss Apochromat, 2 mm., Ap. 1, 40, Oc. 12, the magnification thus being in both instances about 1500. An achromatic oil-immersion substage condenser was used for all detail work, and it was always supplemented by an achromatic light-filter as described under the heading of methods. A few of the figures, as stated in the text, have been drawn from Oc. 18, and with 2 mm. Objective. The staining, with the exception of a few, has been the same for all the sections: iron-haematoxylin, with after-staining with congo. A few preparations were stained with congo-thionin-ruthenium red. The sections were cut about 5 μ thick and affixed to the slides by the alcohol method. All the figures are from the testes cells of Batrachoseps attenuatus Esch. Fixative exclusively iridium-chloride-acetic.

The figures have principally been arranged according to the serial development of the nucleus, and not according to the development of the spheres and the spindle. In the later stages of the spermatocytes the figures have been partly arranged according to the development of the spindle. The figures in the text are strongly diagrammatic.

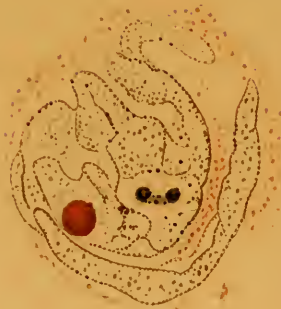*Large Polymorphous Spermatogonia (Figs. 1–9).*

FIG. 1. Large polymorphous spermatogonium, perfect resting stage. The large black body is the chromoplast surrounded by filaments of linin stained red. The chromoplast contains several highly refractive endochromatic granules. The large red body is the linoplast. The small granules, of uniform size and dark color, are the chromioles. The lighter stained granules are partly chromioles, partly linin granules, all of which are suspended in the linin network. A number of parachromatic granules are seen around the chromoplast. The cytoplasm has the form of a thin hollow shell surrounding the much folded nucleus. Two centrosomes in the cytoplasm. Remains of, or the beginning of, a granosphere at the top of the cytoplasmic shell. The dark-staining granules which form a shell around the linoplast are the paranucleolar granules.

FIG. 2. Large polymorphous spermatogonium, perfect resting stage; the chromoplast is larger and divided in two nearly equal parts, and is surrounded by an aster of linin threads of even length; at the tips of the threads are seen more distinct linin granules. The granosphere is being reconstructed at the upper end of the nucleus, but in the cytoplasm. No centrosomes visible. A large linoplast with paranucleolar granules.
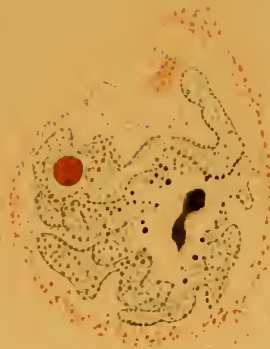
FIG. 3. Polymorphous spermatogonium in a more advanced stage than Figs. 1 and 2, but still in the resting stage. The chromioles, which are strung on threads of linin and chromoplasm, are being drawn towards the chromoplast, the leaders thus beginning to form. Two linoplasts of unequal size stained red. The nucleus is less polymorphous than in Figs. 1 and 2. The cytoplasmic granules are more scattered, forming a more extended and less ring-shaped cytosome. Centrosome in the granosphere.

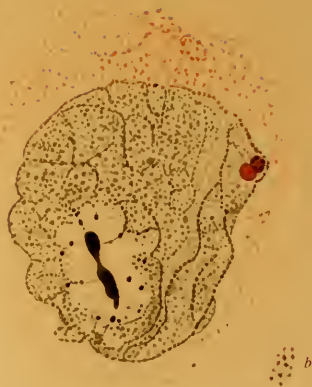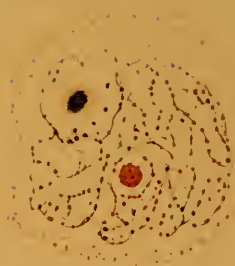FIG. 3 *b*. Detail of the granular reticulum or network of the linin.
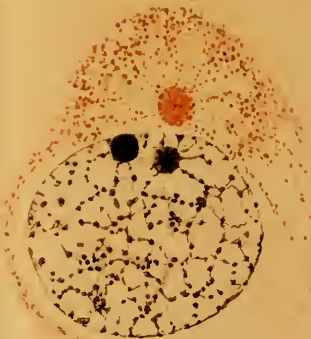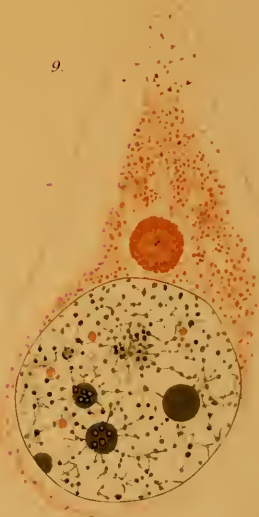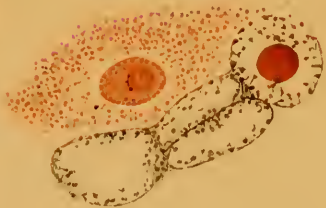
Pl.1.
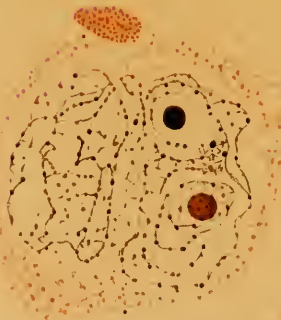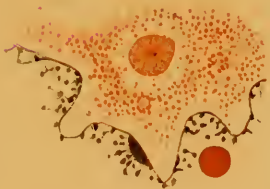

Spermatogenesis of Batrachoseps.

Pl. II.

Spermatogenesis of Batrachoseps.

Pl. III.
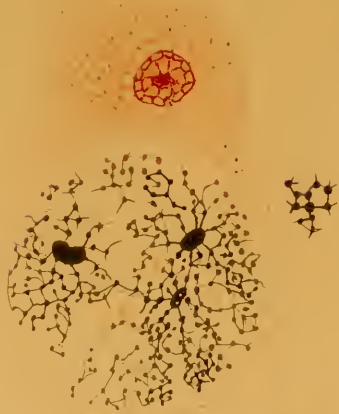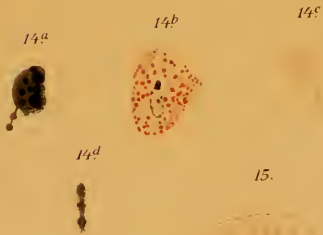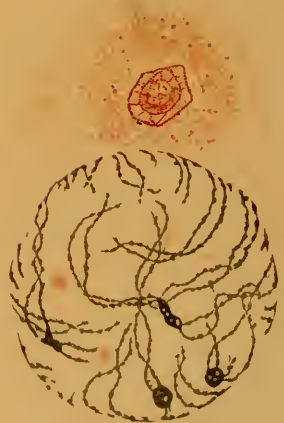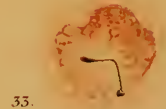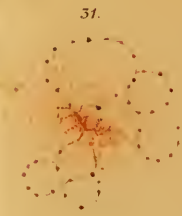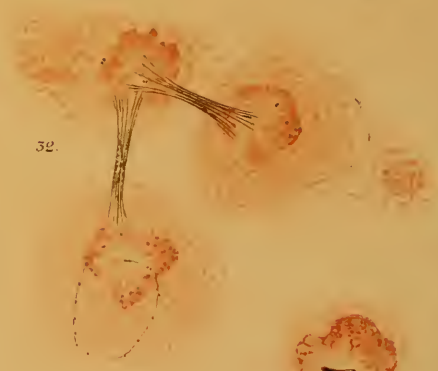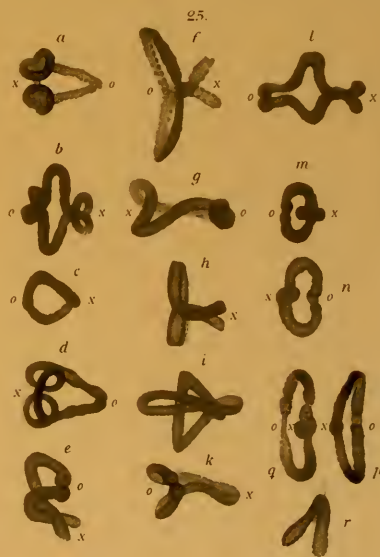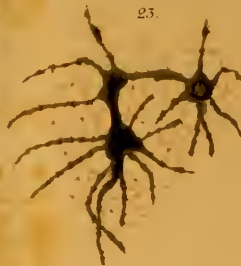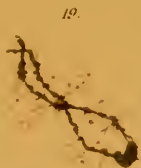
Spermatogenesis of Batrachoseps.

FIGS. 4 and 5 represent two successive sections of one polymorphous sper-
matogonium in the resting stage. The nucleus is more contracted, with fewer
folds. Two linoplasts. The chromioles are being drawn into the leaders and
united into chromomeres. The spheres are being reconstituted, the granular
zone being the granosphere. An archosome is seen in Fig. 5.

FIGS. 6 and 7. Two detail figures of polymorphous spermatogonia with fully
reconstituted spheres, each with centrosomes. The nuclei are more advanced
than in Figs. 4 and 5. In Fig. 7 are seen several accessory archosomes, each
surrounded by a centrosphere. In each cell is a large linoplast. The nuclear
network is merely sketched in and not carried out in detail.

FIG. 8. A large polymorphous spermatogonium in a more advanced stage of
development, in the imperfect resting stage. The nucleus has changed from poly-
morphous to globular. The largest dark body is the chromoplast, the smaller
one is probably a linoplast. Many distinct leaders have been formed, and are
now connected by a network of chromioles and chromomeres. The spheres have
been almost perfectly reconstituted, consisting now of an inner granosphere and
an outer plasmosphere, an archosome with two centrioles, and several accessory
archosomes, the latter scattered in the cytoplasm.

FIG. 9. A large polymorphous spermatogonium, but in which the nucleus has
lost its folded or polymorphous nature, being now at the end of the resting stage
and just entering the prophases of mitosis. Between this cell and the one figured
in 10 there is not only a whole somatic mitosis, but at least three or four genera-
tions of round nucleated spermatogonia, all dividing by somatic mitosis, and with
twenty-four chromosomes each. The last of these generations gives rise to smaller
oblong cells, which pass through a stage of growth and then constitute the first
or imperfect resting stage of the auxocytes. In Fig. 9 there is seen a large lino-
plast, three chromoplasts, with endochromatic granules. Leaders are seen to
emanate from the chromoplasts. The spheres are not quite reconstituted. In the
center is an archosome with two centrioles. Several accessory archosomes in the
cytoplasm. The plasmosphere is starlike, the granosphere rounded and cup-shaped.

*Auxocytes: Spermatogonia with Round Nucleus, Heterotypic Mitosis, and Twelve
Chromosomes (Figs. 10–61).*

FIG. 10. Auxocyte in the imperfect resting stage. Three darkly stained chro-
moplasts with endochromatic granules. Leaders are centering towards the
chromoplasts and connected with them. The spheres are reconstituted. The
granosphere is cup-shaped ; the plasmosphere is indistinct and hardly to be defined
from the cytoplasm. An archosome with two centrioles at the outer edge of the
granosphere ; several accessory archosomes in the plasmosphere, all being con-
nected by a thread and by rings of somosphere. The darker granules in the
nucleus are chromomeres, containing each from one to three chromioles. Only a
small part of the nuclear contents is sketched.

FIG. 11. Auxocyte in the imperfect resting stage more advanced than the last
figure. Two chromoplasts. The spheres are reconstituted, the inner one
stained red being the granosphere. In its center is an archosome with two
centrioles. Numerous accessory archosomes in the cytoplasm, some of them
connected by threads of somosphere. The detail figure alongside shows the chro-
matin network, each chromomere consisting of several chromioles. The leaders
are projecting from the chromoplasts.

FIG. 12. Auxocyte in the beginning of the bouquet stage, the bouquet with twisted spireme segments. The segments are considerably longer than the nucleus and much twisted. They are all with one end attached to the chromoplast. Two linoplasts stained red. The linin network is also stained red. The granosphere is cup-shaped; at its upper margin is an archosome with two centrioles. Several accessory archosomes in the plasmosphere.

FIG. 13. Auxocyte in the perfect bouquet stage. The twelve segments have shortened and straightened out; some are connected, two and two, by a chromoplast, others are isolated. There are two dissolving linoplasts, intimately connected with the linin network. The spheres not yet fully reconstituted, but in a state of activity. A cell bridge connects two adjoining cells at the point of reconstitution. An archosome in the granosphere and accessory archosomes both in the spheres and in the cytoplasm.

FIG. 14. Auxocyte in the perfect bouquet stage, but a little further advanced than the last. The segments are more contracted, and some are seen to be attached to chromoplasts, recognizable by their endochromatic granules. One linoplast stained red. The spheres are being reconstituted. A centrally situated archosome with two centrioles. The granosphere is concave, with the concave side upwards, facing the reader. The radiations are from the cytoplasm and plasmosphere combined, the latter being in an active state of reconstitution. The letters *a–d* indicate detail figures drawn to a larger scale in order to be more distinct, but with the same objective and ocular. Figs. 14 *a* and 14 *b* are from a deeper focus or plane.

FIG. 14 *a*. An accessory archosome with a somosphere with centrioles, the same one as figured in 14 *b*.

FIG. 14 *b*. The granosphere focussed deeper than in Fig. 14. The centrosomes connected by a thread of somosphere.

FIG. 14 *c*. Detail figure of linin network showing the linin granules and the connection of the network with a linoplast.

FIG. 14 *d*. Detail of three chromomeres showing the interior chromioles. Drawn on a somewhat larger scale than Fig. 14.

FIG. 15. Auxocyte in bouquet stage with split spireme segments. Chromomeres about twelve in each segment; most of them are split, but not yet divided. About six chromioles in each chromomere. The outer sphere not yet fully reconstituted, the granosphere being small, with a centrally located archosome with two centrioles. An accessory archosome in the plasmosphere. A red-stained linoplast in the nucleus. Several split chromoplasts connected with the spireme segments.

FIG. 16. Auxocyte in the separated and crossed spireme stage. The divided and partly separated segments are not any more parallel, but cross each other in various directions. Some are attached to chromoplasts. Four linoplasts. The plasmosphere contains numerous rounded alveoles, containing secretions around which are seen the individual granules of the plasma. The granosphere is large and deeply stained. A centrally located archosome with two centrioles. Three accessory archosomes in the plasmosphere, each with several centrioles surrounded by somosphere. The granosphere is cup-shaped, with the concave part turned upwards.

FIG. 17. Auxocyte, separated spireme stage. Four chromoplasts with endochromatic granules. Four linoplasts. The segments are twisted and separated

from each other, and all have been greatly lengthened out. The outer or plasmo-
sphere is in a state of evolution, showing starlike radiations emanating from the
vicinity of the granosphere. Many metaplasmic secreted granules in the plas-
mosphere. The granosphere is angular in outline, cup-shaped, with a denser
marginal wall consisting of closely packed granules of the plasma of the sphere.
A centrally located archosome with two separated centrioles. Two accessory
archosomes at the edge of the granosphere. Numerous paraplasmic granules of
unknown nature in the cytoplasm, stained darker and connected by rings. Faint
trace of a cell bridge emanating from the granosphere or from its immediate
vicinity.

FIGS. 18–22. Auxocytes, all in the twisted and separated spireme stage.
The dark nodes are the chromoplasts, from which start the separated spireme seg-
ments. The two segments which twist around each other are the two halves of
one original spireme segment. The segment having split, the two halves have
separated, and becoming considerably elongated have twisted around each other
in various ways. In Fig. 21 we see one dividing chromoplast which supported
originally four undivided spireme segments, which latter have separated and
twisted around each other and also have become much elongated. The small
dark bodies are the chromomeres, containing each several chromioles imbedded
in a chromoplasm and surrounded by an irregular network of linin, here and
there stained gray by the iron-haematoxylin. It will be observed that in no
instance are the distal ends (those not connected with the chromoplasts) of the
spireme segments grown together, but simply cross each other. These segments
will soon have contracted, after which we will find them as represented in Figs.
23 and 24.

FIGS. 23 and 24. Auxocytes. Separated spireme segments which have yet
more contracted. In Fig. 23 the segments are twisted around each other. The
darkly stained nodes are the chromoplasts which hold the segments together. In
Fig. 24 we have a more advanced stage, such as is found in the beginning of the
angular spireme (Fig. 34). Only about one-half of the chromosomes have been
represented in Fig. 24, the other half having been cut away by the knife. It will
be observed that there are six original segments attached to the chromoplast, and
that each one of them has become divided and contracted. Each such pair
marked *a, b, c, d, e, f,* etc., will form a bretzel-shaped chromosome, similar to those
represented in Fig. 25, *a, b, c, d, e, f,* etc. The linin network has not yet separated
from the segments.

FIG. 25. Auxocytes. A series of perfectly developed chromosomes from the
metaphase and the anaphase. The figures are copies of selected chromosomes
and intended to represent the most common forms assumed by them. At a place
marked " *o* " is seen the chromoplast adhering to the chromosome, while the free
ends are marked " *x.*" In many instances it is difficult, and in others it is impos-
sible, to determine which is the free and which is the chromoplastic end, as, for
instance, in "*i.*" As a rule, the separation begins at the chromoplastic node
" *o.*" At " *r* " is seen a separated chromosome from the anaphase, the other half
having been pulled to the opposite pole. The darker globules in the chromo-
somes are the chromioles, of which there are thirty-six in each chromosome. In
some of the chromosomes are seen traces of chromomeres.

FIG. 26, *a, b, c, d,* four chromoplasts with parts of leaders, from resting stages
of polymorphous spermatogonia and auxocytes. In their interior are seen endo-

chromatic granules. The linin is stained red, the chromioles are blue, but the chromoplasm is not differentiated. Congo-thionin-ruthenium red.

Fig. 26½. Part of the linin network, from an auxocyte in the bretzel stage. The linin network is disarranged and has separated from the chromosomes. The small brown granules are the linin granula; the darker granules, of which there are comparatively few, are paralinin granules of unknown nature. The granules are connected by threads of the same apparent nature as the granules, and which may be considered as projections from the granules.

Fig. 27. Auxocyte, in the bouquet stage; detail of the plasmosphere with two large accessory archosomes, each of which consists of a slightly amoeboid centro-sphere, a somosphere, and several interior centrioles of unequal size. The accessory archosomes are connected by a thin ring of somosphere, on which are also suspended granules of either archosomic or paraplastic nature.

Fig. 28. Auxocyte in the irregular bretzel stage. Detail of the cytoplasmic end of the cell, showing the two spheres with accessory archosomes. The inner part of the granosphere has been drawn out by an archosome, the outer concave shell of the sphere being viewed sideways.

Fig. 29. Auxocyte in the perfect bouquet stage. Detail showing the two spheres and part of the surrounding cytoplasm. The granosphere is cup-shaped, and differentiated into two parts, the outer one of which is in the form of a deeper stained ring, at the edge of which is seen an archosome with a faintly differen-tiated centrosphere. The somosphere and centrioles are not differentiated from each other; the plasmosphere is well defined from the cytoplasm proper. The alveoles of the granosphere are seen to be surrounded by the individual granules of the spheres.

Fig. 30. Auxocyte in the perfect bouquet stage. Detail of the spheres and of the free ends of some of the spireme segments, the chromomeres not yet being split. The chromomeres and the chromioles are slightly exaggerated as regards size, but other details are in exact proportion. The granosphere and the plasmo-sphere are both plainly alveolated, each alveole being surrounded by a single row of granules. An archosome with two centrioles in the granosphere. Three accessory archosomes in the plasmosphere. Three rows of alveoles in the plasmosphere.

Fig. 31. Auxocyte in the bouquet stage. Detail of the two spheres. The granosphere is cup-shaped, consisting of one row of alveoles. The plasmosphere is less regular, with one or two rows of alveoles. At least five accessory archo-somes connected by rings of somosphere. On these rings are also suspended among the centrosomes paraplastic granules of undetermined nature.

Fig. 32. Auxocytes in the bouquet stage. Detail figures of three adjoining cells. Two spindle bridges connect the three cells in the vicinity of the spheres. The spheres are not fully reconstituted, showing the chromosomic evolution to be more advanced than the radiosomic one. The accessory archosomes are connected by rings. The dark red spheres are the granospheres. The archo-somes are seen at the points where the spindle bridges join the spheres. Some of the accessory archosomes have the same structure as the archosomes.

Fig. 33. Auxocyte in the bouquet stage; detail of the sphere not yet fully re-constituted. The archosome is connected with an accessory archosome by a fine somospheric filament very sharply defined; four centrioles in the upper somo-sphere and at least two in the lower one. The latter centrosome is probably the

offshoot of the upper one. Alveoles are being formed in the plasmosphere probably by secretion of interalveolar and metaplasmic matter.

Fig. 34. Auxocyte in the angular spireme stage. The separated segments have first contracted and then straightened out, having been angularly bent at the nodes, and the chromomeres have so approached that they are hardly to be distinguished from each other. Several chromoplasts are seen attached to the segments at the nodes, where they are joined together. Some chromioles are distinct in the segments. The granosphere is strongly stained and is seen to consist of one row of alveoles and several central ones. The plasmosphere and part of the cytoplasm are alveolate with distinct granules. A large archosome in the granosphere and an accessory archosome in the plasmosphere. There are no linoplasts left in the cell.

Fig. 35. Auxocyte in bretzel stage, the chromosomes having the form of bretzels and rings. Only a few of the chromosomes are figured. The cytoplasm is alveolate with distinct granules. The granosphere is cone-shaped, with the denser part cup-shaped as usual. Near the apex of the cone lies the archosome with at least two centrioles. An accessory archosome at the upper left end of the granosphere.

Fig. 36. Auxocyte in bretzel stage. Only a few of the chromosomes have been figured; one of them has been cut by the knife. The linin has separated from the chromosomes, and scattered to the opposite part of the nucleus. The cup-shaped granosphere is turned sideways, and appears as a crescent with one row of alveoles. The cone-like structure belongs probably exclusively to the granosphere. At its apex lies the archosome with two centrioles. Four separate accessory archosomes in the plasmosphere, the latter being strongly alveolated.

Fig. 37. Auxocyte in bretzel stage. Only a few of the chromosomes are in the field. Several of them are connected in pairs with chromoplasts. One of the ring-like chromosomes is free. Several chromosomes show distinct chromioles. The linin network is scattered and not any more connected with the chromosomes. The plasmosphere is in dissolution; the granosphere is elongated. At the apex of the latter is situated the archosome, which appears to have been divided preparatory to the radiosomic process. The somospheres connected by a thin ring; there are three accessory archosomes.

Fig. 38. Auxocyte in bretzel stage. The section is cut so that only the cytoplasmic pole is seen. The archosome has left the granosphere and become divided. A small spindle is formed between the somospheres. The centrosphere is elongated; from its outer margin radiate numerous mantle fibers. The plasmosphere is greatly shattered, and the granosphere is only partly connected with the mantle fibers. There are numerous accessory archosomes both in the cytoplasm and in the spheres. At the upper right-hand corner are seen the remains of a spindle bridge.

Fig. 38½. Auxocyte in bretzel stage. Most of the chromosomes are halved by the knife. The linin network is scattered and retracted from the chromosomes. The nuclear membrane is yet intact. The archosome is entirely divided and a small central spindle has formed outside of the granosphere. The latter is being used up as material by the fibers. The centrosphere is stained, and the various fibers are seen to emanate from its outer margin. The plasmosphere is scattered, and parts of it are seen in the cytoplasm. Three groups of accessory archosomes, some with three and four centrioles.

Fig. 39. Auxocyte in bretzel stage. The nuclear membrane is mostly dissolved. The spindle is viewed from one of its poles, showing the mantle fibers to emanate from the outer margin of the centrosphere. The granosphere is partly used up, and the plasmosphere is mostly scattered in the cytoplasm. There are numerous accessory archosomes, some of which are connected by filaments of somosphere. A row of accessory archosomes is seen around the granosphere, ' all connected by a filament of somosphere. Many of the chromosomes show darkly stained chromioles. Outside of the plasmosphere the cytoplasm is fibrous, the fibers consisting of closely packed granules.

Fig. 40. Auxocyte in bretzel stage. The chromosomes are rather heavy and contracted. The spindle is seen from one of the poles, and is in the figure not clearly definable. The granosphere is being used up by the spindle fibers. Much of the plasmosphere is being scattered ; some of it is seen far down to the right outside of the nucleus. The darker stained granules in the cytoplasm are the remains of the metaplasmic secretions of the plasmosphere. There are several accessory archosomes stained more or less deeply, some of them connected by a thread of somosphere. The linin network is scattered.

Fig. 41. Auxocyte in bretzel stage. Several of the chromosomes are halved by the knife. The central spindle is well advanced. A set of contractile fibers has formed from both archosomes, but has not yet reached the chromosomes. A large accessory archosome at the upper end of the central spindle below the granosphere is situated in a much deeper plane than the archosome and is not in a direct line between the archosome and the granosphere. The plasmosphere is scattered in the cytoplasm. The part of the nuclear membrane nearest the mantle fibers is dissolved.

Fig. 42. Auxocyte in bretzel stage. This figure, together with the previous one and several following, is arranged according to the development of the central spindle. The chromosomes in all these figures are in about the same stage of development. In the present figure the spindle is upright ; its axis connects with the axis of the cell. The granosphere has hardly begun to dissolve, its concave side is upwards. In the upper archosome are two centrioles, in the lower one is only one centriole. The contractile fibers are not deeply stained and are thus less well definable. Eight accessory archosomes, each with a centrosphere and with from one to three centrioles. The plasmosphere is scattered, the remains are seen in two isolated groups at the left margin of the nucleus. The nuclear wall nearest the spindle is dissolving.

Fig. 43. Auxocyte in bretzel stage. This is an abnormal cell as regards the position of the granosphere and the central spindle, the granosphere being generally so situated as to be equidistant from both the poles of the spindle. At the upper margin are seen remains of the plasmosphere. An accessory archosome at the periphery of the granosphere. The contractile fibers are well differentiated, especially around the left pole of the central spindle. The nuclear membrane is dissolved nearest the central spindle.

Fig. 44. Auxocyte in bretzel stage. The nuclear membrane is less dissolved than in the last figure. The granosphere is being used up. The contractile fibers are well differentiated and deeply stained ; none of them has as yet reached the chromosomes. An accessory centrosome at the edge of the granosphere. The mantle fibers possess several denser nodes. The linin network has entirely separated from the chromosomes. The contractile fibers show a strongly granulated cytoplasm.

Pl. IV.

Spermatogenesis of Batrachoseps
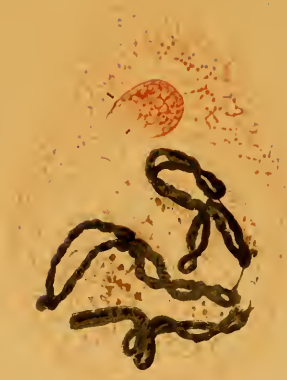
FIG. 45. Auxocyte in bretzel stage. Only a few fragments of chromosomes are seen in the section. The central spindle is upright, its position depending on the position of the granosphere. At the upper pole are seen two archosomes, at the lower pole only one. The contractile fibers are strongly granular. A few small groups of accessory archosomes are seen to the left, the individual archosomes being connected by threads of somosphere. Many of the individual granules of the granosphere are seen to be in direct connection with the mantle fibers. At the lower right-hand margin of the cell are seen parts of the scattered plasmosphere.

FIG. 46. Auxocyte in bretzel stage. The granosphere is very much used up and in direct connection with the spindle fibers. The chromosomes are thrown in a bundle, all, however, being perfectly formed bretzels. At the poles of the spindle are seen several accessory archosomes surrounding the archosome. What I take to be the beginning of contractile fibers are seen to start out from the accessory archosomes. The linin network has left the chromosomes, and is broken up into globules, each one of which consists of several granules. At the lower margin of the cell are seen parts of the plasmosphere.

FIG. 47. Auxocyte in bretzel stage, a transition stage between the last and the following figure in the metaphase. The last of the prophases. The central spindle is almost perfect. The remains of the two spheres are seen at the left in the cell. The contractile fibers are well advanced and several of them have reached the chromosomes. The latter are in the form of bretzels and rings. Numerous accessory archosomes as well as paraplasmic granules, difficult to distinguish from each other. The nuclear membrane is entirely dissolved and the linin granula are scattered through the cytoplasm. The alveoli of the cytoplasm are very large and have assumed the forms of large vacuoles.

FIG. 48. Auxocyte in a stage immediately preceding the metaphase. The chromosomes have not yet been drawn into the equator of the central spindle. One chromosome which is in its proper position has begun to divide. Two superfluous or unused linoplasts are seen in the cytoplasm. An archosome and numerous accessory archosomes are seen at each pole of the central spindle. Four groups of plasmosphere are scattered. The contractile fibers are distinctly beaded. In some of the chromomeres the chromioles are distinct; there are about six chromioles in a chromomere.

FIG. 49. Auxocyte in a stage immediately preceding the metaphase. An archosome with several accessory archosomes at each pole. Some of the chromosomes are not yet in their proper position on the central spindle. There are five or more groups of scattered plasmosphere and secretions.

FIG. 50. Auxocyte, metaphase. There are two archosomes at each pole, also numerous accessory archosomes. Three groups of scattered plasmosphere and its secretions. Several of the mantle fibers connect directly with metaplasmic granules of the plasmosphere. The group to the left has assumed its final position near the equator of the cell.

FIG. 51. Auxocyte, metaphase. Only a few of the chromosomes are figured. One archosome at each pole, but several accessory archosomes. A number of linin granules as well as paraplasmic granules are seen in the cytoplasm. The contractile fibers are well defined, and one of them in the upper right-hand corner has been torn by the knife and has been bent outwards, a fact illustrating the independent nature of the contractile fiber.

Fig. 52. Auxocyte, metaphase. The section is cut at an angle with the cell axis and consequently the upper pole is seen slightly from above, while the lower pole is so viewed that the archosomes are not distinctly seen. The circle of dots around the upper pole are the starting points of the contractile fibers, which are seen to be connected with the somosphere and the centriole by a fine bar of dark-staining plasma. Several accessory archosomes in the cytoplasm. The red spots in the cytoplasm are the scattered remnants of the plasmosphere. The chromosomes show here and there distinctly the chromioles and the chromomeres. This cell is unusually small for an auxocyte.

Fig. 53. Auxocyte, metaphase. The chromosomes are regularly placed on the central spindle, all being in about the same stage of development. The spindle is halved and presents its inner concave surface to view. Chromioles are seen in all the chromosomes and chromomeres. Most of the chromosomes have commenced to separate. The red blotches are parts of the plasmosphere. The contractile fibers are all well defined, and some of them are seen to connect with the somosphere by a fine bar of darkly staining plasma. At the upper pole is seen one archosome, while at the lower pole there are two. The upper archosome is connected with the contractile fibers in that part of the section which is not figured here. This archosome is darker and refractive, the two centrosomes being of a dull color and not refractive. The cytoplasm contains no accessory archosomes, but numerous dark-staining granules, which perhaps may be interpreted as linin granules. Many granules are connected by rings or threads. See also Fig. 51.

Fig. 54. Auxocyte, metaphase. Most of the chromosomes have separated, and some have begun to contract. The contractile fibers have also contracted; they are strongly beaded. Chromioles are seen in the chromomeres. The archosomes are large and distinct. A few accessory archosomes in the cytoplasm. In the central spindle is seen a separated chromatin granule. This figure is a composite one as regards the poles. A few chromosomes found in the following section were added to the lower pole. The accessory archosomes were also added from that section.

Fig. 55. Auxocyte, anaphase. A stage succeeding that shown in Fig. 54. The chromosomes are drawn much nearer the poles. Chromioles are seen plainly in many places. An archosome at each pole. The contractile fibers are disappearing. The plasmosphere is accumulated at the left side of the equator. The central spindle is beginning to dissolve, and shows irregular vacuoles along a line where the coming new cell wall is to appear. A chromatin fragment in the central spindle. Numerous paraplasmic and linin granules in the equator of the central spindle.

Fig. 56. Auxocyte, anaphase. The chromosomes are further advanced, approaching the confluent umbrella stage. On account of improper washing out, this figure does not show the details as well as the preceding and following figures. The cell is lengthening out and the central-spindle poles have been pulled down through the chromosomes. A few chromosomes are not yet separated from each other, but connected by chromoplasm.
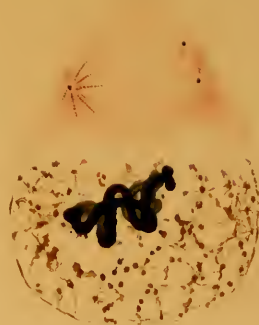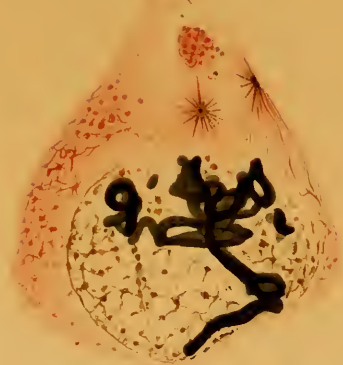
Fig. 57. Auxocyte, anaphase. The chromosomes are entering the confluent umbrella stage. The individual chromomeres are yet distinguishable, but the endochromatic granules of the chromoplasts have already come into plain view. The archosomes have diminished in size, now appearing as very faint points.

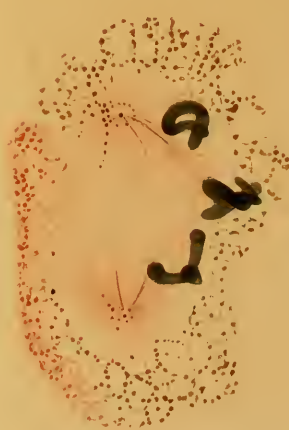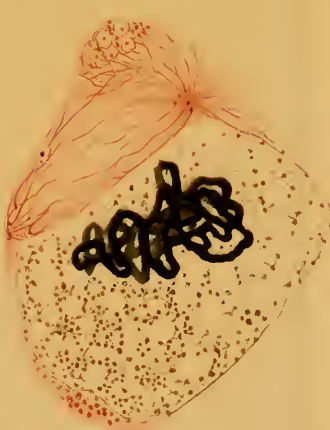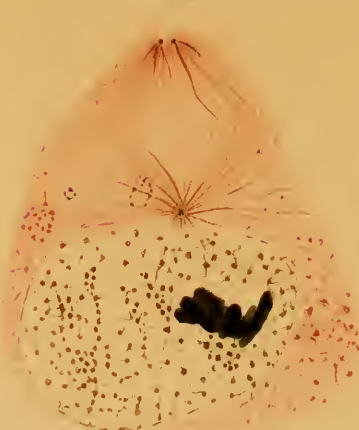Spermatogenesis of Batrachoseps.

Pl. VI.
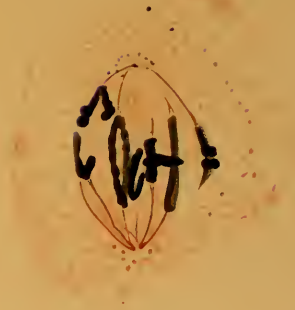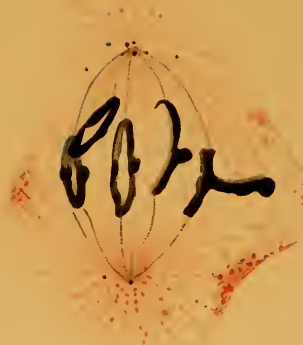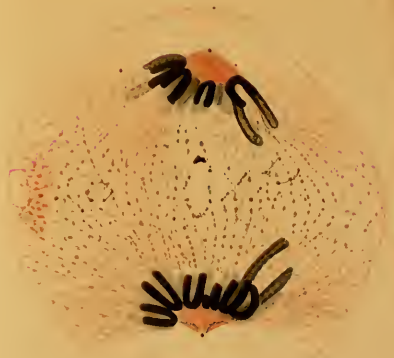
Spermatogenesis of Batrachoseps.

The plasmospheric granules are seen near the equator of the spindle.   The future cell wall is outlined, and several mantle fibers are seen connected with the plasmospheric granules in the vicinity of the contraction in the equator.

Fɪɢ. 58. Auxocyte, anaphase. The chromosomes in the umbrella stage. The chromosomes have lost their individuality, and only here and there does a projecting point indicate their former outline. The distribution of the endochromatic granules of the chromoplasts indicates that the confluence is perfect. At the upper pole is seen the apex of the central spindle, with the remains of the contractile fibers under the form of four granules, corresponding to as many accessory archosomes. The archosome is seen at the very apex of the central spindle. The red blotches are part of the scattered plasmosphere. The alveoli of the central spindle are widened along the equator and along a line where the new cell wall is to appear.

Fɪɢ. 59. Auxocyte, metaphase. The confluent umbrella stage in which the confluence is perfect. In the umbrella are seen vacuoles and endochromatic granules. A cytoplasmic or false nuclear membrane is formed around the nucleus. Numerous polar fibers and mantle fibers connect the cytoplasmic membrane with the cell wall. The plasmosphere, now fragmented, appears as four agglomerations along the new cell wall separating the spermatocytes. The spermatocytes are more separated than in Fig. 61, but this separation is only apparent as the cell in Fig. 61 is seen from the side, while the present one is viewed from the front. The spindle is much contracted in the middle, and the muscular nature of some of the fibers is indicated by the beading. A few accessory archosomes are seen below each nucleus. The poles of the central spindle are not visible.

Fɪɢ. 60. Auxocyte, anaphase, ring-like, confluent umbrella. The two new spermatocytes are almost separated. The cytoplasmic membrane around the nucleus is being pulled away from the umbrella, and numerous fibers are seen to connect with the membrane. Numerous fibers from the poles of the spindle connect with the cell wall, separating the two spermatocytes. Several accessory archosomes are seen on the cytoplasmic membrane.

Fɪɢ. 61. Auxocyte, metaphase, and confluent umbrella stage. The central spindle has contracted in the middle, and has been pulled through the umbrella. A large vacuole has appeared around each nucleus, the cytoplasmic membrane around the nucleus is being pulled away allowing the nucleus to expand. On the membrane are several accessory archosomes, from which start out fibers singly and in bundles. The fragments of the plasmosphere are in the equator at points where the new cell wall is being formed. The granules of this sphere are seen to be connected with fibers both from the poles and from the cytoplasmic membrane. This and several of the following figures are from different slides from the previous ones, the tissue having been stained much more intensely by the congo. The fibers of the central spindle are all strongly granular and beaded like muscle fibers. The contraction from the old cell and the formation of the new cell wall is seen to proceed from one side only, which process appears to be normal.

Spermatocytes or Spermatogonia of the Second Maturation Stage.

Fɪɢ. 62. Spermatocytes. Two cells not yet entirely separated. The chromosomes in the beginning of the chrysanthemum stage, emerging from the confluent

stage. Darkly stained bars indicate the new chromosomes in the confluent mass, all pointing in the direction of the cell axis. The spindle shows several contractile fibers ending on the cytoplasmic membrane which has receded from the chromosomes. On this membrane are seen several accessory archosomes, from some of which radiate fibers. From one of these archosomes proceed the remains of retractile fiber cones. The reddish blotches are the remains of the plasmosphere. Some of the accessory archosomes are furnished with distinct centrospheres. In the chromosomes are seen a few endochromatic granules, characteristic of the chromoplasts. The cell wall is made up of cytoplasmic granules.

FIG. 63. Spermatocyte, in the chrysanthemum stage, having entered the prophase in which the individual chromosomes are being reconstituted. Only a small section of the nucleus is seen. Only one cell is figured. The cytoplasmic or false membrane has been pulled back in order to allow the growth of the nucleus. The central spindle is fully retracted and the archosome has assumed its position at the apex of the fibers. Several accessory archosomes pulling fiber cones towards the cell wall. Numerous accessory archosomes attached to cone fibers, others are seen on the cytoplasmic membrane. Many of the accessory archosomes are seen to have a centrosphere.

FIG. 64. Spermatocyte. Spindle bridge ; remains of the central spindle which has greatly contracted, many of its fibers showing muscular beading. One of the dark granules is probably the archosome, it being situated where the granosphere is being reconstituted. The cells which were connected by this bridge were in about the same stage as the cell figured at 63.

FIG. 65. Spermatocyte in the chrysanthemum stage, more advanced than Figs. 63 and 64. The chromomeres and chromosomes are plainly indicated and partly individualized. The section passed obliquely to the central spindle, showing the cytoplasmic or false nuclear membrane with three or more accessory archosomes, from which radiate several fiber cones. This is not a free cell, but one which was connected with another cell by a spindle bridge.

FIG. 66. Spermatocyte in the chrysanthemum stage. The cells are not yet separated, but connected by a cell wall and a spindle bridge. Several fiber cones are formed on the cytoplasmic membrane, pulling the latter away from the nucleus. The accessory archosomes are not as distinctly stained as in the other cells, which is due to the greater washing out of the iron stain. A mid-body in the center of the spindle bridge. The retractile fibers are emanating from the apex of the upper spindle pole.

FIG. 67. Spermatocyte in the chrysanthemum stage ; the cells are not yet separated. The central spindle is greatly contracted, the fibers at each pole are being retracted and condensed into a reconstituting granosphere. The fiber cones have advanced towards the cell wall, the accessory archosomes actually resting on the cell wall. Several paracellular bodies are seen on the cell wall, some being connected with the cell by fine threads. Retractile fibers emanating from the poles of the central spindle. The accessory archosomes at the poles of the fiber cones are too much washed out to be distinct. From two to three fragments of the plasmosphere in each cell, easily identified by their deeper stain.

FIG. 68. Spermatocytes in the chrysanthemum stage. The two cells are more separated than in the preceding figures. The fiber cones have reached the cell wall, and some of them have receded from the cytoplasmic membrane. The
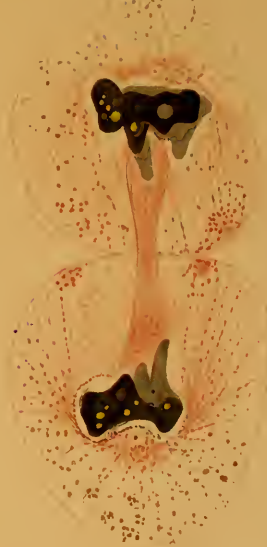
57. 58. 59. 60. 61. 62. 63. 64.

Spermatogenesis of Batrachoseps.
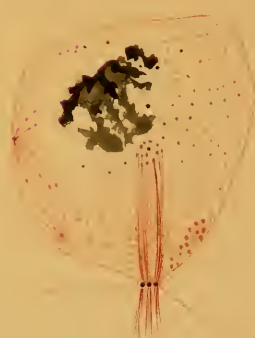
granospheres are being reconstituted at the poles of the central spindle. In the upper cell are seen three fragments of plasmosphere near the cell wall. The pole of the upper central spindle rests on the cytoplasmic membrane, while the pole of the other cell ends in the cytoplasm. A linin network is forming between the chromosomes in the lower nucleus. Archosomes and accessory archosomes are too much washed out to be distinctly visible. The mid-body consists of a double row of granules.

Fig. 69. Spermatocyte in the chrysanthemum stage. The linin network is appearing among the chromosomes. The fiber cones are pushing out the cell walls in both cells. The accessory archosomes at the poles of the cones are well defined; some are also seen on the cone fibers. The fragments of the plasmosphere are seen in both cells, stained dark red. There are two archosomes at the pole of the central spindle of the lower cell. At a deeper level the false nuclear membrane was distinctly seen in each cell.

Fig. 70. Spermatocytes emerging from the chrysanthemum stage and entering the second prophase. The two cells are only connected by a spindle bridge. The chromosomes are separated, though some are yet connected by chromoplasts, which latter are now located at the angle where the two prongs of the chromosome meet. The cytoplasmic membrane is yet seen around the nucleus, the latter having filled out the vacuole. But a true nuclear membrane or karyotheca has also been formed directly around the chromosomes, showing that the two membranes are of different origin. Accessory archosomes are seen on the cytoplasmic or false nuclear membrane; some are also seen on the cone fibers. Two fragments of plasmosphere in the lower cell along the new cell wall. The granosphere is being reconstituted, especially around the pole of the central spindle in the upper cell. The chromosomes in the lower cell are further advanced than those in the upper cell.

Fig. 71. Spermatocyte in the checkerboard stage, the chromomeres being much separated. The fiber cones are yet pushing out the cell wall, though some of them have evidently begun to dissolve. The new nuclear membrane has formed around each nucleus. A large plasmosphere in each cell. Accessory archosomes at the pole of each fiber cone. A mid-body on the spindle bridge.

Fig. 72. Spermatocytes connected by a spindle bridge on which is seen a mid-body. The fiber cones are disappearing. The two spheres are reconstituting separately, later on to be united. Many accessory archosomes on the fibers as well as in the cytoplasm.

Fig. 73. Spermatocytes in the checkerboard stage, though further advanced than the last figured cell. The fiber cones, however, are less degenerated, and the granosphere is less advanced. A comparison of the two figures, 72 and 73, shows that here, as elsewhere, the chromosomic process and the radiosomic process do not run quite parallel, but that one may be in advance of the other. Thus in 73 the nucleus is further advanced than the nucleus of 72, but the cytosome of 72 is further advanced than the one of 73.

Fig. 74. Spermatocyte, free and in the checkerboard stage. The fiber cones are yet faintly traceable. Several rings of somosphere with accessory archosomes. An unusually small cell.

Figs. 75 and 76. Spermatocytes, free, in the checkerboard stage. These two figures represent two sections of the same cell. The numerous fiber cones have not yet begun to recede. Several rings of somosphere with accessory archosomes.

The chromomeres are seen to be of different sizes and to contain a variable number of from nine to three chromioles each.

FIG. 77. Spermatocyte, free and with chromosomes in the checkerboard stage. The chromomeres are well separated and contain about three to nine chromioles each. The spheres are being reconstituted together, the inner one being the granosphere and the outer one the plasmosphere. An archosome with two centrioles on the plasmosphere. The fiber cones have not yet disintegrated, the upper one with a distinct accessory archosome at the apex.

FIG. 78. Spermatocyte, free and with the chromosomes in the chrysanthemum stage. The chromomeres are much separated, though the staple-shaped form of one of the chromosomes is distinct. The spheres are reconstituting in the upper left corner of the cell. Accessory archosomes connected by a ring of somosphere. The remains of the fiber cones are recognizable in the four projecting corners of the cell. A linin network is stained reddish.

FIG. 79. Spermatocyte, free, the chromosomes in the beginning of the contraction stage or the third prophase. All traces of fiber cones have disappeared. The spheres are being reconstituted, the darkly stained one being the granosphere. Numerous accessory archosomes in the plasmosphere.

FIG. 80. Spermatocyte, free and the chromosomes in the beginning of the contraction stage. The two spheres are being reconstituted. Numerous accessory archosomes on the plasmosphere. Figs. 80–82 are in very much the same stage of development, but representing a serial development and contraction of the chromosomes. In Figs. 81 and 82 the plasmosphere is reconstituted in the opposite end of the cell from the granosphere. This is frequently the case in the spermatocyte, and saves the redistribution of the sphere to the equatorial of the new spindle.

FIGS. 81 and 82. See Fig. 80.

FIG. 83. Spermatocyte. Contraction stage in which the chromosomes are again assuming their staple form, and in which the linin network is separating from the chromosomes. This stage corresponds to the bretzel stage of the auxocytes. Figs. 83 and 84 are in nearly the same stage. The plasmosphere is reconstituted in the opposite part of the cell from the granosphere. Numerous accessory archosomes around the granosphere. The similarity of all is so great that it cannot be decided which of them is the archosome. Chromomeres show the interior chromioles.

FIG. 84. See Fig. 83.

FIG. 85. Spermatocyte in the beginning of the angular chromosomes, the fourth prophase. The chromosomes have become narrower and the margins are more even. This stage corresponds to the angular segments of the auxocytes, though there are some important differences. The chromoplasts, for instance, are very prominent in the auxocyte, while in the spermatocyte they are only now and then to be distinguished from the chromosomes. Only a few of the chromosomes are figured. At the lower margin of the cell are seen the spheres, but it is doubtful if the round mass is anything but the plasmosphere. The linin network is disintegrated and retracted from the chromosomes. Several of the superfluous archosomes have been expelled from the cell and are now seen attached to the exterior of the cell wall. They have also swelled up and increased perhaps fivefold in size, but to what extent their inner structure has become modified by the swelling up is not clear. It seems, however, most probable that the somosphere

Spermatogenesis of Batrachoseps.

Pl. IX

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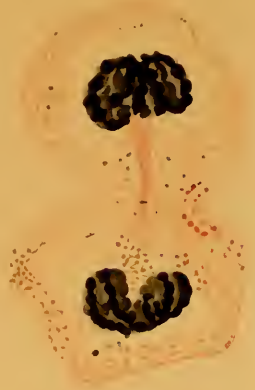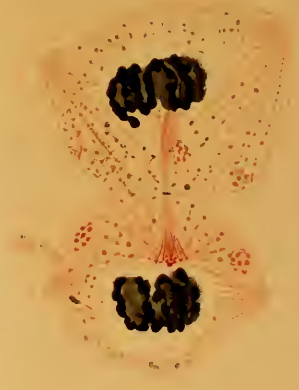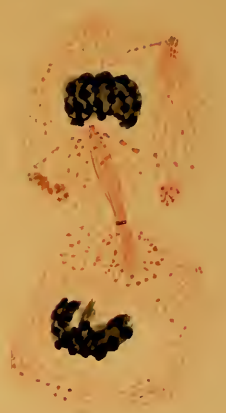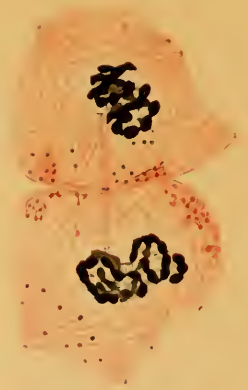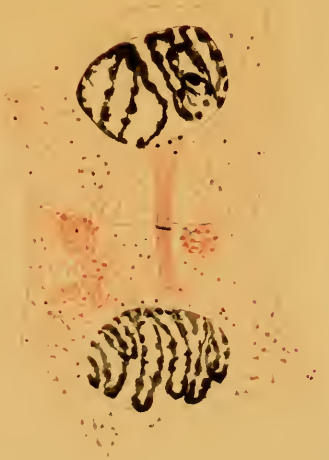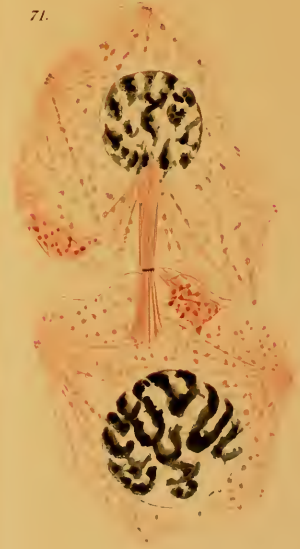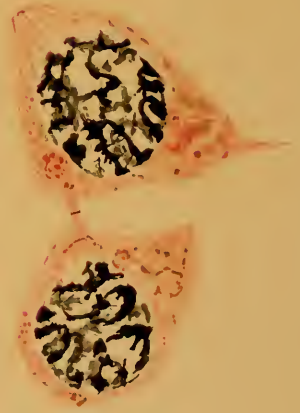
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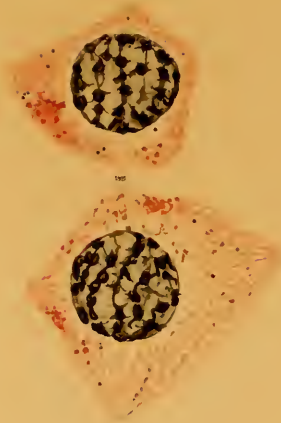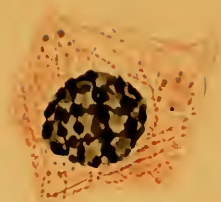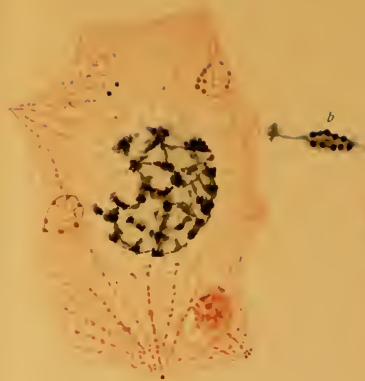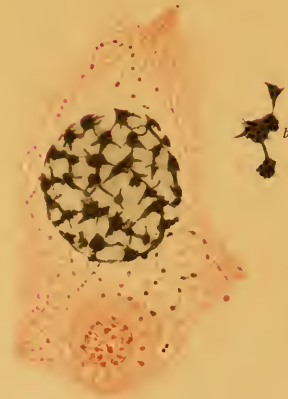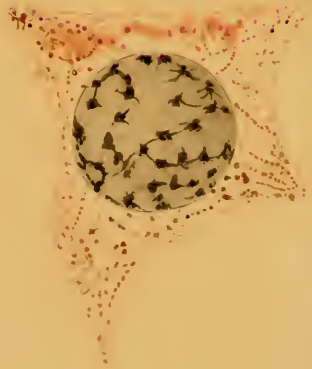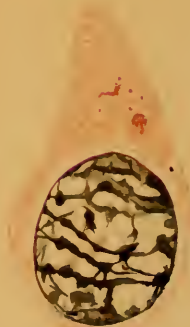
has filled out the centrosphere, and that the centrioles have separated from each other.

Fig. 86. Spermatocyte in the angular chromosome stage. This and the previous figure are very much in the same stage as regards the nucleus. The spheres are not distinct and there are no expelled archosomes. Three accessory archosomes at the upper part of the cell.

Fig. 87. Spermatocyte in the beginning of the knotted chromosome stage. The chromosomes are separated and, have not yet accumulated in the center of the cell. Numerous accessory archosomes, each one surrounded by an amoeboid centrosphere. A very small granosphere. Chromioles are seen in the chromosomes.

Fig. 88. Spermatocyte in the knotted chromosome stage. A distinct granosphere, a scattered plasmosphere, and numerous accessory archosomes in the cytoplasm. Some of the archosomes are being expelled from the cell. Chromioles are seen in the chromosomes.

Fig. 89. Spermatocyte in the knotted chromosome stage. Several fiber cones are yet seen, and it is probable that two of them will form the central spindle. Numerous accessory archosomes on the fibers of the cones, some of the fibers evidently dissolving the nuclear wall.

Fig. 90. Spermatocyte in the knotted chromosome stage. The nuclear membrane is completely dissolved by two fiber cones. The individual chromosomes are so tightly knotted that they are difficult to segregate one from the other. The chromosomes are only approximately correctly figured. These two fiber cones are probably the beginning of the central spindle. A sphere is seen in the upper apex of the cell; many accessory archosomes and an archosome with an amoeboid centrosphere.

Fig. 91. Spermatocyte in the knotted chromosome stage. Only a few chromosomes are seen in the section. The nuclear membrane has been dissolved by the fiber cone emanating from the upper apex. The linin network is scattered and the granules are mixing with the cytoplasm. Accessory archosomes on the fibers and one at the lower pole of the cell. Each one is furnished with an amoeboid centrosphere. Some of the chromioles in the chromosomes are distinct.

Fig. 92. Spermatocyte in the *V*-stage; the chromosomes are in the center of the cell. Only one pole of the central spindle is developed, there being no trace of the other pole. The nuclear membrane is dissolved. A plasmosphere to the left of the future equator. A dividing archosome in the pole of the spindle.

Fig. 93. Spermatocyte in the beginning of the *V*-stage, the chromosomes being in the center of the cell. A central spindle is being formed out of two old fiber cones. At the apex of each cone is an accessory archosome which is now assuming the function of an archosome.

Fig. 94. Spermatocyte. Chromosomes in the knotted stage, but the central spindle has already formed out of two opposing fiber cones. This is the characteristic form of the spindle in this stage, the two poles being greatly depressed. An archosome at each pole. There is no distinction between the mantle fibers and the central spindle fibers. I found several cells like this one, but no intermediate stages with Fig. 93.

Fig. 95. Spermatocyte. In the *V*-stage, not yet in the metaphase. The upper pole with several accessory archosomes, one of which is the spindle archosome. The lower pole has probably been cut off by the knife.

FIG. 96. Spermatocyte in the beginning of the *V*-stage. Half of the spindle has been cut off. There are three accessory archosomes at the upper pole, one of which probably has the function of a spindle archosome. The plasmosphere is scattered in the equator. The archosomes at the pole have a remarkable similarity to the expelled archosomes figured elsewhere (Fig. 85).

FIG. 97. Spermatocyte in the beginning of the *V*-stage, or the end of the knotted chromosomes. The central spindle is finished, but the chromosomes are not yet in position. The plasmosphere is in the equator. The accessory archosomes lie in a ring around the poles of the spindle.

FIG. 98. Spermatocyte in the *V*-stage just before the beginning of the metaphase. The chromosomes in the form of perfect *V*'s, which, however, are not yet distributed along the spindle. The mantle fibers are connected with the granules of the plasmosphere. An archosome at each pole, also a large accessory archosome with an amoeboid centrosphere. Contractile fibers are forming and projecting from the centrosphere of the archosomes.

FIG. 99. Spermatocyte in the perfect *V*-metaphase. The contractile fibers are formed and connect with the chromosomes. An archosome at the upper pole; the lower pole is cut off. A trace of the plasmosphere to the left of the equator. Only a few of the chromosomes are figured.

FIG. 100. Spermatocyte in the perfect *V*-metaphase. Only a few of the chromosomes are figured. The chromosomes are splitting at the bottom or angle of the *V*. An archosome at each pole surrounded by a pale centrosphere. The contractile fibers are plainly beaded. Blotches of plasmosphere stained pink. Many chromioles are seen in the chromosomes.

FIG. 101. Spermatocyte in the perfect metaphase; some of the chromosomes are separating, while others are not yet in their proper position on the central spindle. Several larger groups of plasmospheres along the equator. An archosome at each pole, surrounded by several accessory archosomes, each one with developed centrosphere.

FIG. 102. Spermatocyte in the beginning of the anaphase. An archosome at each pole, and a few accessory archosomes in the cytoplasm. Chromomeres and chromioles visible in the chromosomes.

FIG. 103. Spermatocyte in the perfect *V*-anaphase. The chromosomes have separated, the chromomeres have mostly disappeared, but the chromioles are yet distinct, and arranged in two parallel rows in each chromosome. The plasmosphere is scattered along the equator of the contracting spindle. The mantle fibers are seen to be connected with the plasmospheric granules. An archosome at each pole.

FIG. 104. Spermatocyte in the beginning of the confluent stage of the anaphase. The contractile fibers have shortened and the chromosomes have become partly confluent. The chromomeres have disappeared, but the chromioles can yet be distinguished here and there. Parts of the plasmosphere along the equator.

FIG. 105. Spermatocyte in the beginning of the confluent stage of the anaphase. The cell has already begun to divide and a new membrane is being secreted along the vacuolated equator by the plasmospheric granules. The polar cones of the central spindle have so shortened as to be hardly distinct. The archosomes are reduced in size and barely visible. A few of the chromioles are distinct in the chromosomes.

Pl. X.

Spermatogenesis of Batrachoseps.

Spermatogenesis of Batrachoseps.

Pl XII
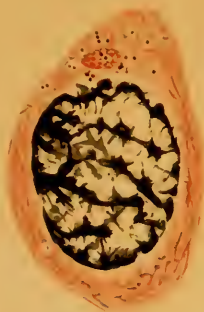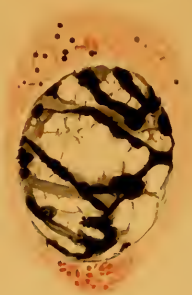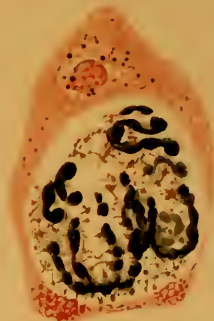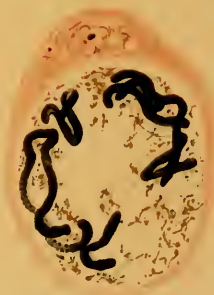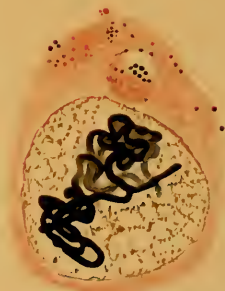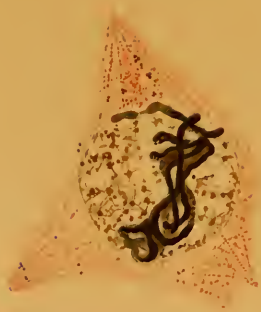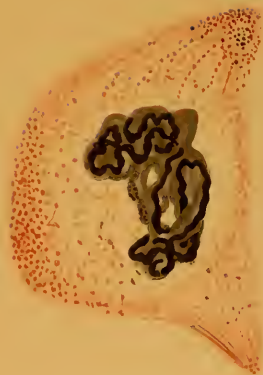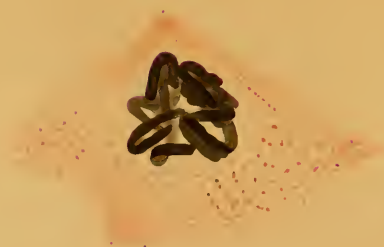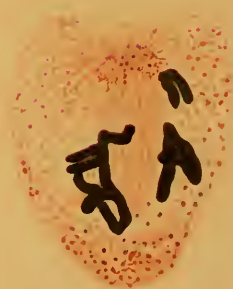
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Spermatogenesis of Batrachoseps
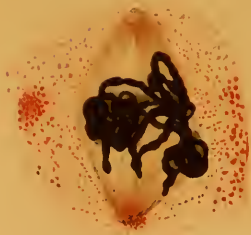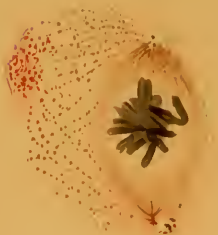
FIG. 106. Spermatocyte in the confluent stage, but the confluence is not yet perfect. The polar cones are mostly gone and the archosomes so faint as to be indistinct. The new cell membrane has formed along the equator of the central spindle. The latter has contracted, several of its fibers being beaded. Many plasmospheric granules along the new membrane, especially on the left side.

FIG. 107. Spermatocyte in the perfectly confluent umbrella stage. The cells are almost separated and are only connected with each other by the central spindle. The spindle is greatly contracted, showing some beaded fibers. No distinct archosomes, and the polar cones have so descended into the nucleus as to be no more visible. A false nuclear membrane is being formed around the nucleus. The pole of the lower spindle is just above the nucleus; at the apex is a small archosome. In the upper cell is a large accessory archosome.

FIG. 108. Spermatocyte in the end of the confluent stage, the chromosomes just beginning to reappear in the chrysanthemum stage. The cytoplasmic membrane is fully formed around the nucleus, and is now being pulled away by the centrosomes. The granospheres are being reconstituted around the poles of the spindle. Parts of the plasmospheres are seen along the new cell walls. A midbody on the central spindle, which latter has been greatly contracted. The poles of the central spindle are connected with the cytoplasmic membrane by a few fibers.

FIG. 109. Spermatid. In a rather advanced stage of development, the nucleus having assumed its full size. The two spheres have been reconstituted. An archosome is seen to the left of the granosphere. The nucleus is in the checkerboard stage. Several paracellular bodies are attached to the exterior of the cell. These are probably expelled particles of the spheres, which have become inflated. They show in their interior a fibrous structure. There are two distinct chromoplasts in the nucleus, recognizable by their endochromatic granules. The remains of a spindle bridge at the upper pole of the cell.

FIG. 110. Auxocyte. Detail figure of one of the central spindle poles, showing the formation of the contractile fibers and their connection with the somosphere of the archosome. In the center of the field is seen a large archosome consisting of a darkly stained inner centriole and somosphere, surrounding which is a large unstained centrosphere. Twelve fine bars connect the centriole with the contractile fibers which begin on the outer margin of the centrosphere. On the upper ends of the centrosphere only the ends of the contractile fibers are seen. Several accessory archosomes in a ring around the archosome. The red, granular plasma, divided in three groups, is the remains of the plasmosphere. Each accessory archosome possesses a centrosphere. The contractile fibers are strongly beaded.

FIG. 111. Auxocyte. Detail figure of a pole of the central spindle, showing the archosome and its connection with the central spindle fibers and the contractile fibers. The archosome is large and contains two centrioles, each surrounded by a somosphere. Several accessory archosomes, one of which appears to be the starting point of a contractile fiber. The contractile fibers are strongly beaded. Zeiss Apo. 2 mm., Apert. 1, 40, Oc. 18.

FIG. 112. Auxocyte. Detail figure of an archosome; the contractile fibers start from the outer margin of the centrosphere. The chromosomes are in the anaphase, with the contractile fibers greatly contracted. They are strongly beaded, the beads being situated between a covering of granulated fibers, evi-

dently forming a casing to the granules. The beading of the contractile fibers is probably of the same nature and origin as the mid-body. That is, the beads may serve as storage reserves of plasma to be used when the contractile fiber is lengthened or shortened. When suddenly lengthened on account of strain the plasma is probably supplied by the beads, and vice versa; when the contractile fiber requires to be suddenly shortened its superfluous plasma is quickly accumulated in the beads. Chromioles are plainly visible in the chromosomes, especially in the one to the left. Zeiss Apo. 2 mm., Apert. 1, 40, Oc. 18.

FIG. 113. Auxocyte. Detail figure of an archosome and six of its contractile fibers, showing them to start from the outer edge of the centrosphere. Some of the fibers connect with the inner centriole by a fine bar of somosphere. The contractile fibers are strongly beaded, somewhat of the nature of a muscle fiber. The beads are situated in zigzag fashion and covered by a sheathing of fibrous nature. There are three strongly stained accessory archosomes at the pole. The chromosomes are only indicated. The figure is drawn to a larger scale.

FIG. 114. Spermatocyte. Detail figure of a fiber cone showing the connection of the fibers with the accessory archosomes. Several of the latter possess amoeboid centrospheres. The nucleus is in the checkerboard stage.

FIG. 115. Spermatocyte. Detail figure of a fiber cone. The outer edge of the cone forms also the outer cell wall. An accessory archosome with several centrioles is near the apex of the cone. This cone is less advanced in dissolution than the one figured in Fig. 114, though it is from the same cell.

FIG. 116. Spermatocyte. Detail figure of a fiber cone in dissolution, showing the granulated and beaded structure of the fibers. At the apex is an accessory archosome. The outer edge of the cone is closely pressed against the cell wall.

FIG. 117. Spermatocyte. Detail of a fiber cone in dissolution. The accessory archosomes have left the apex of the cone and are now congregating around the reconstituting granosphere. Each centrosome possesses an amoeboid centrosphere. Some of the centrosomes are yet attached to the cone fibers. The outer lining is the cell wall.

FIG. 118. Spermatocyte. A nucleus in the chrysanthemum stage, showing the staple-shaped chromosomes in a stage of growth. The nuclear membrane is formed in the immediate vicinity of the chromosomes. The chromomeres and some of the chromioles are distinct. The linin network is stained red.

FIG. 119. Spermatocyte. A nucleus and two detail figures of chromosomes. The details are drawn on a slightly larger scale, but with the same magnification and objective. The chromosomes are further advanced than in Fig. 118. The chromomeres are separating. Some of them contain eight or ten, others only three to four chromioles.

FIG. 120. The homoeotypic mitosis by equation division of the spermatocyte from the perfectly split V to the confluent umbrella stage. a to f show the V-shaped chromosomes as they are thrown on the central spindle; some are seen in front view, others in side view. The fibers connecting with the chromosomes are contractile fibers. g to h show the chromosomes in the act of separation, being pulled apart by the contractile fibers. At i is seen one of the daughter-chromosomes in which the chromioles are very distinct. i to k, chromosomes after the halves have separated and the daughter-chromosomes have formed. l, m, chromosomes in the confluent umbrella stage; in l no endochromatic granules are seen on account of too dense staining. In m many endochromatic granules.

Spermatogenesis of Batrachoseps
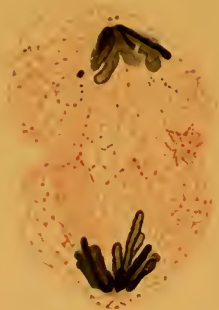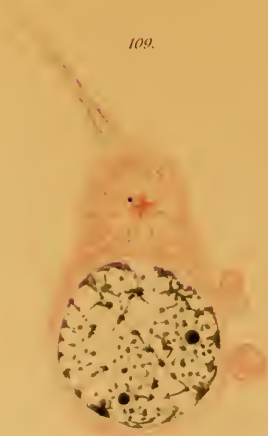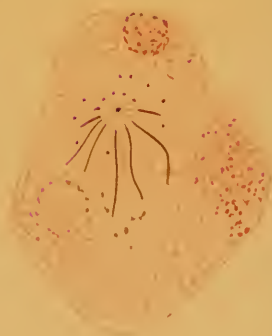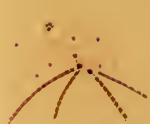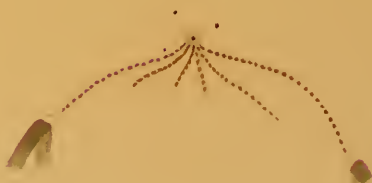
All the above chromosomes are copies from actual chromosomes and not diagrammatic.

FIGS. 121 and 122. The heterotypic mitosis by equation division. A diagrammatic representation of the development of leaders of an auxocyte into chromosomes, their splitting and equation, in a progressive series from *a* to *l*. *a*, two leaders connected by a divided chromoplast, the latter marked *c*. Numerous chromomeres on the leaders, each with several chromioles. *b*, the same two leaders, the chromomeres having contracted into a smaller number, each chromomere having more chromioles, which latter have now been arranged in two parallel rows in each chromomere. This is the beginning of the stage in which the leader splits. *c*, the same two leaders, but which have now split into four leaders, each one of which will become a single chromosome in the daughter-nucleus. The two halves of one leader form the bretzel-shaped chromosome. Observe that the chromomeres have again separated and become smaller. *d*, the same two leaders, the chromomeres having contracted anew, the whole leader having shortened. *e*, the same two leaders, which have yet more shortened, and the chromomeres have contracted into a smaller number; the two chromoplasts are seen at the junction of the two leaders. *f*, a single leader which has become separated through the division of the chromoplast. It now forms a perfect bretzel-shaped chromosome, consisting of two prongs connected by a dividing chromoplast. The two prongs of the leader have crossed each other and thus formed the bretzel. *g* to *k* are various forms of bretzel chromosomes, some of which have their free ends overlapping, while two of them have the ends actually grown together. *l*, a chromosome which has just undergone equation division. The parting has taken place through the chromoplast; *c c*, the two ends which were grown together, are yet united by two fine threads of chromoplasm. *m*, two chromosomes in the confluent umbrella stage, with several endochromatic granules.

XIII. STAINS, FIXATIVES, OPTICAL APPARATUS, ETC.

Congo, No. 1209, The Substantive Colors Co. Actien Gesellschaft für Anilin-Fabrication.

Thionin, Cogit & Co., Paris.

Rutheniumroth, Dr. G. Grübler & Co., 200, Leipzig.

Iridium Chloride, Merck & Co., Darmstadt, Germany.

Apochromat No. 412, 2 mm., Apert. 1. 40, Homog. Immers. Carl Zeiss, Jena.

Apochromat No. 262, 3 mm., Apert. 1. 40, Homog. Immers. Carl Zeiss, Jena.

All the chemicals and optical apparatus furnished by Charles C. Riedy, San Francisco, Cal.

Spermatogenesis of Batrachoseps

PROFESSOR COLLETT ON THE MORPHOLOGY OF THE CRANIUM AND THE AURICULAR OPENINGS IN THE NORTH–EUROPEAN SPECIES OF THE FAMILY STRIGIDÆ.

R. W. SHUFELDT, M.D.

ONE of the most extensive and valuable contributions to the subject of the anatomy of owls, and their classification, was given to science by Prof. Robert Collett of the Zoölogical Museum of Christiania, Norway, in 1881. This brochure was read by him before the Scientific Society of that city on December 9, and published in its *Proceedings* for the same year. This memoir printed nearly forty pages octavo, and is illustrated by three folding lithographic plates, giving thirty-five figures of the skulls and ear-parts of various species of the family considered, the whole being entitled *Craniets og Öreaabningernes Bygning hos de nordeuropæiske Arter af Familien Strigidæ*. Appearing, as it did, in the Norwegian language, the usefulness of this very excellent piece of work was to a great extent limited, and many comparative anatomists all over the world could not readily employ it or avail themselves of its results, both of which uses it so amply merited, for the very reason that it was in Norwegian, a language rarely mastered by naturalists at large. With the view of obviating all this, and bringing the investigations of Professor Collett in the osteology and taxonomy of the *Strigidæ* before the English-speaking world, I have long planned to have his memoir in these fields translated, but, from one cause or another, the task had to be postponed. Events of a particularly unfortunate nature stood in my path during the entire summer of 1895 and late into the following autumn, but with the opening of the year 1896, circumstances became far more propitious for the resumption of all my work in comparative

morphology, and among the first tasks undertaken by me was the translation of this worthy contribution to avian anatomy by my esteemed colleague in Christiania. This has been rendered possible through the kind assistance of my young Norwegian friend, Miss Alfhild Dagny Lowum, now my wife, who, with great patience, made the literal part of this translation. With this before me, Professor Collett's memoir was given its English version in the language of science. My private osteological cabinets contain the skeletons of many species of owls, and the crania of these I have compared with his researches as the labor of translation progressed, giving such additional information as was thus obtained in footnotes, and signed by my own initials. I have also seen fit to rearrange his figures, redrawing some of them for text-figures, and retaining those for lithography that especially demanded that kind of reproduction.

The present contribution, then, consists in a full and complete English translation of Collett's memoir, supplemented by footnotes of my own, and a reproduction of all the figures given in the original Norwegian work. And to this I have added some of the more recent opinions of recognized authorities upon the subject, relative to the systematic position of the *Strigidæ*, thus bringing the whole up to date and rendering the entire memoir available to students of comparative morphology everywhere; it being, as it were, a comprehensive treatise upon the value of certain structures to be found in the head and cranium in owls in the classification of that family, together with their relations to other groups of birds, as those relations are understood at the present time.

Professor Collett says :

"The ten North-European species of the family *Strigidæ*, all of which belong to the subfamily *Buboninæ* (the other subfamily, which is represented by *Strix flammea*, does not occur in Scandinavia), can be arrayed in six (6) groups based upon the morphology of the cranium and upon the structure of the external ear-openings and their dermal appendages."

It is clear from the following table that only the first of these groups, which includes *Surnia funerea, Glaucidium passerinum,*

Order, *Striges* : Family, *Buboninæ.*

A. Dermal auricular flaps absent.

1. Cranium symmetrical.

1. Group. Auricular openings symmetrical.

1. Sect. Process developed on jugal.
   a. Supraorbital processes styliform . . . . 1. *Surnia funerea* (Linn.) 1776.
   b. Supraorbital processes broad and short . . . 2. {*Glaucidium passerinum* (Linn.) 1776.

2. Sect. Jugal linear . . . . 3. *Nyctea scandiaca* (Linn.) 1766.

2. Group. Auricular opening largest upon the right side . . . . 4. *Bubo ignavus* (Forst.) 1817.

B. Dermal auricular flaps present.

I. Cranium symmetrical.

3. Group. Auricular openings asymmetrical; resembling a gill-slit; aural skin-flaps of equal size, as are also the auricular openings.
   a. Frontals narrowly sculpt. . . . . 5. *Asio accipitrinus* (Pall.) 1771.
   b. Frontals broadly sculpt. . . . . 6. *Asio otus* (Linn.) 1766.

4. Group. Auricular openings asymmetrical; reniform; the skin-flap largest upon the right side . . . . 7. *Syrnium aluco* (Linn.) 1766.

II. Cranium asymmetrical.

5. Group. Cranium asymmetrical upon the right side; ear-openings reniform in outline; and the skin-flap largest upon the right side.
   a. Os squamosum slightly asymmetrical . . . 8. *Syrnium uralense* (Pall.) 1776.
   b. Os squamosum markedly asymmetrical . . . 9. {*Syrnium lapponicum* (Thunb.) 1789.

6. Group. Bilateral asymmetry of the cranium; auricular openings wide; equal in size; dermal flap similar to cranial asymmetry . . . . 10. *Nyctala tengmalmi* (Gmel.) 1788.

and *Nyctea scandiaca* (all lacking ear-flaps), have perfectly symmetrical auricular openings and crania. In all the other groups, including seven species, the auricular apertures are unequal in size or asymmetrical in other ways. On the two last groups, which include *Syrnium uralense, Syrnium lapponicum,* and *Nyctala tengmalmi,* the asymmetry is so pronounced that even the cranium is more or less involved.

This asymmetry of the auricular openings, their dermal flaps, or the cranium, commonly exhibits itself as an anomalism of the right side of the head, so the opposite or left side in these must be regarded as the normal one.

This anomalous condition in the majority of our species, so far as the auricular openings and their dermal flaps are concerned, consists in these strictures being larger and of greater width on the right than on the left side. Where this condition also exists in the cranium, it is again the right side which exhibits the anomalous development. It is only in *Nyctala tengmalmi* wherein we find that both sides present the condition referred to, and perhaps the most so upon the left side. In the two species of *Asio,* where the irregularity is confined to the dermal parts of the aural apertures, the right side must again be regarded as the normal side. *It would appear that inasmuch as the internal ear and the brain cavity are perfectly symmetrical, neither of these parts enter into this anomalous state of affairs.*

The six groups into which the North-European species fall can be briefly characterized as follows :

GROUP I. *Dermal ear-flaps absent.   Cranium and auricular openings symmetrical.*

a. Jugal with an elevated osseous apophysis.

1. SURNIA FUNEREA (Linn.). 2. GLAUCIDIUM PASSERINUM (Linn.).

Auricular openings of medium size or small. Osseous crests on *os squamosum conspicuously individualized;* viewed anteriorly they come into plain sight at the posterior aspect of either orbit. Posterior periphery of either orbit sharp where formed by the frontal bone. The greatest

vertical height of the cranium is posterior to the orbits. Vomer rudimentary. *Infraoccipital foramen* present, and large in the case of *G. passerinum.* The supraorbital processes in *S. funerea* long and styliform. Crania lack the superficial median furrow upon their superior aspects.

b. Jugal linear.

3. Nyctea scandiaca (Linn.).

Auricular openings of medium size and placed inferiorly. Osseous crest of *os squamosum* comparatively small, and *non-united superiorly ;* viewing the skull from in front, they are almost entirely concealed by the orbits. Vomer rudimentary. Supraoccipital foramen present. Median furrow on superior aspect of cranium present.

To this first group, then, which includes species without dermal ear-flaps, and where no asymmetry is present in any part of the head, belong *Surnia funerea, Glaucidium passerinum,* and *Nyctea scandiaca.*

Of these three, the two first-named species constitute a subgroup of themselves, since in certain of their cranial characters which they exhibit in common and in which they differ from the Norwegian species, they must doubtless be considered, systematically speaking, as allied to each other. Both *Surnia funerea* and *Glaucidium passerinum* develop, superiorly, upon the jugal bone an elevated, oblong process of some length, while this bone in all the remaining North-European species is linear. Further, the superior aspect of the skull is flat and entirely lacking in a median furrow, which latter in all the other species is present. Within this group the crania can be distinguished in the two species one from the other, in addition to the difference in size, by the feebler development of the mandibles in *Glaucidium passerinum,* which in its case, as compared with the cranium proper, are shorter than are the mandibles in any other species. Further, the supraoccipital foramen in this species is unusually large, both relatively and absolutely ; in fact, larger than it is in any other form. In addition thereto, *Surnia funerea* has the supraorbital processes long and spiculiform, approaching in this respect the diurnal *Raptores.*

Nyctea scandiaca, constituting the second subgroup, has

another type of cranial structure. In it the orbits are notably large, and the mandibular part strong. The jugal bone is linear, and the supermedial furrow of the cranium is well marked. The auricular openings in most of their characters agree with those forms already described, in being relatively small or of medium size, and in lacking any external dermal conch, or ear-flap.

GROUP II. *Aural skin-flaps absent. Cranium symmetrical.*
Auricular apertures largest upon the right side.

4. BUBO IGNAVUS (Forst.).

Ear-openings of medium size; nearly of the same dimensions. Osseous crest of the *os squamosum* comparatively small, *completely free above;* viewed anteriorly, they are almost concealed by the orbits. Posterior periphery of either orbit sharp where formed by the frontal bone. Cranium has a supermedial furrow present; its greatest height is posterior to the orbits. Jugal linear. Vomer present. Supraoccipital foramen present.

To this, the second group, belongs *Bubo ignavus*, which is further characterized by possessing a symmetrical cranium, and the lack of dermal ear-flaps, but in this species the first evidence of asymmetry exhibits itself in an insignificant difference in the size of the two ear-openings. Here the cranium has a structure most like *Nyctea scandiaca*, and has, as in that species, powerfully developed mandibles, a conspicuous median furrow on the supero-external aspect of the cranium, broad and prominently outstanding orbital wings, markedly capacious orbits, and feebly developed osseous crests on either *os squamosum*.

Owing to the feeble development of these processes we may infer that the sense of hearing in this owl is comparatively less acute than it is in any other North-European species. The similarity in the structure of the cranium in these two species is, upon the whole, so close that doubtless they would have both been relegated to the same group had not the right ear-opening in *B. ignavus* been a little larger than the left, a state of affairs that I have not been able to demonstrate with certainty in the case of *Nyctea*.

GROUP III. *Ear-flaps present. Cranium symmetrical. Ear-openings asymmetrical, resembling a gill-slit, but the aural skin-flaps of equal size.*

5. ASIO ACCIPITRINUS (Pall.).   6. ASIO OTUS (Linn.).

Auricular fissures or slits carried high up. The entrance to the ear is on the right side below, and on the left side above an outstretched fold of the skin. Osseous crest of the *os squamosum*, upon either side, meets with the frontal bone above, without any intervening notch. Viewed from in front they are wholly visible beyond the orbits. Frontal bones obliquely sculptured at the posterior margins of the orbits ; the sculpturing being most marked in *A. otus*. External supermedial furrow of the cranium present ; greatest vertical depth of cranium lies in the postero-orbital plane. Jugal linear. Vomer present. Supraoccipital foramen generally found.

The third group contains the two species of *Asio, viz.*: *A. accipitrinus* and *A. otus*. In the case of these also is the cranium symmetrical, while the auricular openings and their dermal appendages exhibit in their structure a very remarkable asymmetry. The apertures to the ears are so markedly wide that they remind one of gill-slits, inasmuch as they extend from the nether side of the mandible, upon either side, up to a point near the middle of the forehead, where they are separated only by a small interval of space. Upon either side of this long slit-like aperture there is a raised fold of skin, the two resembling a pair of lids, of which the supero-anterior one is the real ear-flap. Crossing the aperture is an elevated fold of skin, and it is in its neighborhood that the asymmetry is especially observable. To its left side, and above it, we find the entrance to the ear, while on the right side of the head it lies below this fold. So far as the cranium itself is concerned it does not exhibit any special asymmetry. The osseous crests of the squamosal bones are peculiar in form, inasmuch as they ascend up to the frontal, upon either side, without any intervening notch, whereby they are endowed with an unusual amount of superficial surface, and with great depth ; this, taken in connection with the unusual development of the external ear-openings, accounts for the sense of hearing having attained to its greatest acuteness in this group. The

difference in the cranium of the two species is not very marked, it being confined principally to the oblique osseous plane formed by the frontal, upon either side, that extends to form the posterior periphery of the orbit. This is greater in *A. otus* than in *A. accipitrinus.*

Group IV. *Ear-flaps present. Cranium symmetrical. Auricular openings reniform in outline, and the ear-flap largest upon the right side.*

7. Syrnium aluco (Linn.).

Auricular openings wide, carried high up, and with broad ear-flaps. Osseous crest on *os squamosum* completely free above. Viewed anteriorly, they are almost concealed by the orbit upon either side. Posterior periphery of orbit, where formed by the frontal, rounded. Median furrow present upon superior aspect of cranium; and the greatest depth of the latter is at a point just over the center of the orbit. Jugal linear. Vomer rudimentary. Supraoccipital foramen present.

In *Syrnium aluco*, which belongs to the fourth group, the cranium, ear-openings, and ear-flaps are constructed upon a very different type as compared with the other species. We find the cranium still symmetrical ; but the auricular openings, which are slit high up, and are reniform in outline, are larger upon the right side. The same obtains with the broad, almost door-shaped ear-flaps, the form of which is somewhat different upon either side of the head.

To this group also belong the other two North-European species — *S. uralense* and *S. lapponicum*. In them the cranium is alike in form, and the auricular openings and the ear-flaps are as they occur in *S. aluco*. In these species, however, the asymmetry is extended even to include the cranium, while in *S. aluco* the cranium is perfectly symmetrical. *S. aluco* has the margins of the frontals evenly rounded where they go to form the posterior peripheries of the orbits, and the hinder part of the cranium is prettily dome-shaped, with a conspicuous, nail-like, anteriorly projecting process upon either *os squamosum*. The thinner part of the interorbital septum, which until now has been comparatively thick, is beginning

to be less extensive, inasmuch as the orbits themselves are, comparatively, not very large.

GROUP V.   *Ear-flaps present.   Cranium asymmetrical upon right side.   Auricular openings reniform in outline, and, as in the case of the ear-flaps, largest upon the right side.*

a. Crania with slight asymmetry.

8. SYRNIUM URALENSE (Pall.).

Auricular openings wide, carried high up, with broad ear-flaps, with the difference between the two flaps not as marked. Osseous crest on *os squamosum* completely free above, more conspicuously bent forwards upon the right than upon the left side, though without coming in contact with the posterior surface of the *alisphenoids* in either case. Frontal bones rounded at the posterior margins of the orbits, with a deep concavity behind either supraorbital process. Longitudinal median furrow present upon the superior aspect of the cranium, and the greatest height of the latter is situated somewhat posterior to the middle of the orbital cavity. Interorbital septum quite thick. Jugal linear. Vomer rudimentary. Supra-occipital foramen absent.

b. Cranium decidedly asymmetrical.

9. SYRNIUM LAPPONICUM (Hunt.).

Ear-openings wide, slit high up, and with broad ear-flaps. Asymmetry slight. Osseous crest on *os squamosum* normal on left side, conspicuously individualized. Osseous crest on right side has its supero-external angle inclined forwards and coössified with the posterior margin of the alisphe-noid, which is strongly developed laterally. Frontal bones completely rounded off at the posterior borders of the orbits, and with a profound notch posterior to the supraorbital processes. Cranium with a longitudinal median furrow on its superior aspect, and the greatest depth of the former is posterior to the orbits. Interorbital septum short and thick ; orbital diameter comparatively small. Jugal linear. Vomer rudimentary. Supra-occipital foramen absent.

The fifth group, which, as above noted, is closely related to the fourth, and contains the two other North-European species of *Syrnium*, is characterized by having the cranium as well as the auricular openings and ear-flaps asymmetrical. So far as the two last-named parts are concerned, they have a structure

similar to what occurs in *S. aluco*, but the difference between
the two sides is, strangely enough, upon the whole, less decided
than in the last-named species, although always noticeable.   It
is here likewise that it is the right side wherein the auricular
openings and ear-flaps are the larger.   The asymmetry of the
cranium is similar in either species, owing to a peculiar struc-
ture of the right osseous crest of the *os squamosum*, which is
the case in a more marked degree in *S. lapponicum* than it
is in *S. uralense*.   In the last-named species the asymmetry is
but feebly pronounced, although always present ; the osseous
crest of the *os squamosum* is somewhat more produced for-
wards upon the right side, but nevertheless does not quite
come in contact with the hinder border of the alisphenoid.
On the other hand, this is the case in *S. lapponicum;* the
osseous crest is more lofty and wider, and extends its superior
margin quite to the above-named bone, with which it coösifies.
At the same time the entire right side of the head is more
drawn out laterally, and exhibits a greater vertical compres-
sion than it does upon the left side, and this is especially to
be observed when the cranium is viewed upon its anterior
aspect.   In both these species the orbital cavity is compara-
tively small, and the interorbital septum low and thick, which
is especially the case in *S. lapponicum*.

In the last-named species the cranium is, upon the whole,
smaller as compared with the beak than it is in any other
species, and this, notwithstanding the fact that the beak is
not very powerfully developed, as it is, for example, in *Bubo*
and *Nyctea*.

Group VI.   *Ear-flaps present.   Cranium decidedly asymmet-
rical.   Auricular openings wide, uniform in size, and
together with the ear-flaps exhibiting an asymmetry agree-
able with that of the cranium.*

10. Nyctala tengmalmi (Gmel.).

Auricular openings very wide, with crescent-formed ear-flaps ; Asym-
metry of aural entrance agreeing with that of the cranium.   Osseous crest
of *os squamosum* large, deep, and distorted upon both sides ; especially

lofty upon the right side, and situated far posterior to the orbit ; on the left side greatly compressed, and comes in contact with the hinder margin of the orbit above. Anteriorly, both osseous crests are produced as long, forward-projecting processes ; that on the right side meets the posterior border of the orbital crest of alisphenoid, and on the left is deflected, so as to come in contact with the mandible. Medio-longitudinal furrow exists upon superior aspect of cranium ; the skull's greatest depth being post-orbital. Jugal linear. Vomer rudimentary. Supraoccipital foramen present.

In the last group, which is created to contain *Nyctala teng-malmi,* the cranium sees its greatest asymmetry, as this asymmetry occurs in the left as well as in the right side. It is here also that the *os squamosum* with its osseous crest exhibits its maximum amount of anomalous development. On the right side the osseous crest is lofty, approaching the same plane above in which lies the superior contour line of the head. On the left side it is as decidedly drawn downwards, and with the tongue-like process on the osseous crest likewise so markedly inclined in the same direction that the apex of the latter is in direct contact with the lower jaw — a condition which is per-haps unique for this genus in the class *Aves.*

I have already availed myself of the opportunity to point out the cranial asymmetry in *N. tengmalmi* in an article that has since appeared in the *Proceedings of the Zoölogical Society of London* for 1871, p. 739, entitled "On the Asymmetry of the Skull in Strix tengmalmi" (it having been sent in June of the same year) ; and also in *Vid. Selsk. Forh. Chria.,* p. 68, 1872. Auricular openings are broad and wide, and have a depth equal to that of the head ; both these and the ear-flaps, which are not very broad, are of the same dimensions upon either side, other-wise they present no asymmetry other than that which pertains to the cranium itself. Apart from the asymmetrical structures seen in the latter, it is perhaps most like that part of the skel-eton in *Syrnium aluco,* and has, as in that species, a notably wide interorbital septum, part of which is transparent, and a uniform convexity of its posterior aspect.

The three resident species occurring in south and middle Europe, *viz., Strix flammea* (Linn.), 1766, *Athene noctua* (Retz), 1800, and *Scops giu* (Scop), 1769, together with *Asio capensis* (Smith), 1835, which occurs as a straggler in the

countries about the Mediterranean, are not found in Norway.
The two first-named species have been found as far north as
Jutland and the southern part of Skaane, but are, like *Scops
giu,* principally south and middle European forms.   *Asio
capensis* belongs to the Ethiopian region.   I have not had the
opportunity to examine the heads of any of these in the flesh ;
and, as the dermal structures of the auricular openings cannot
with entire safety be described from dried or steamed heads,
the following remarks will have reference only to the cranial
characters in these species.   *Strix flammea,* which is the type
of the first subfamily of Striges, belongs, as is well known, to a
special group, different from the other six groups of North-
European *Buboninæ* just described.   Its principal characters
are a symmetrical cranium, and a broad ear-flap, which is much
larger than the comparatively small (symmetrical ?) ear-open-
ings.   The cranium, which appears to be entirely lacking in
asymmetry, is peculiarly lengthened, with a long and slender
mandibular portion, and with a marked development of the
diplöic tissue in several of the cranial bones, to an extent not
approached by any of the other North-European species.

As in *Syrnium lapponicum,* the forehead slopes conspicu-
ously downwards, but here, to such a high degree that the
line becomes almost concave.   The medio-longitudinal furrow
on the top of the cranium is deeper than it is in any of the
other groups, and the forehead is raised almost pyramidically
above the orbits upon either side of this deep median furrow.
No distinct fossa stands between the crest upon the *os squa-
mosum* and the parietal bone, as is seen to be the case in most
of the other groups of *Buboninæ,* and this crest has, upon
the whole, no extraordinary or very pronounced development ;
but the cranium is especially remarkable on account of the
great thickness of the interorbital septum, which here presents
no point where it is thin, as is the case in all the other species.
The orbits, relatively speaking, are notably incapacious ; the
lacrymals are disproportionately large and swollen, and are
found beneath the frontal, upon either side, and not as in the
subfamily *Buboninæ,* partly (or often quite) beneath the supe-
rior mandible.

Finally, it may be said that the mandible is markedly widened beneath the ramal vacuity; that the *pars planæ* of the mesethmoid are thick and swollen at their apices, and widely spread out ; that the palatine bones are narrow, and the vomer well developed and mesially swelled ; and, lastly, the large orbital wings of the alisphenoid are so long that they almost reach the linear *os jugale.*

Taking into consideration the depression of the cranium, its small orbits, and the marked development of the diplöic tissue in all the bones, *Strix flammea*, of all the North-European species of owls, stands nearest to *Syrnium lapponicum*, though in the latter the interorbital septum is very far from being as thick as in *Strix flammea*, and the lateral processes of the ethmoid are not swollen at all.

Upon the whole, then, when taking into account the structure of the cranium in *Strix flammea*, although this exhibits no asymmetry, this form occupies an isolated place among the owls, and these peculiarities of its cranium, when taken in connection with the characters obtained from the structure of the *sternum* and *furcula*, doubtless contribute towards sustaining the opinion of placing this form in a separate subfamily.

As far as the auricular openings in *Strix flammea* are concerned, they are small or of medium size, and probably symmetrical (or very little asymmetrical). The ear-flaps, which are both superiorly and inferiorly squarely truncated, and are about as broad as they are high, give them, upon the whole, a resemblance to the right ear-flap in the *Syrnium* group ; on the other hand, these ear-flaps are larger than the apertures they are intended to cover, and consequently they overlay the auricular margins. Further, we observe the characteristic long and broad fold of skin, overgrown with stiff feathers (the veil), which stretches, as an oblong semi-arc, from the base of the superior mandible above the eyes, down behind the ear-opening upon either side, from thence quite out to the symphysis of the lower jaw. This fold of skin corresponds to the vertical fold that surrounds, posteriorly, the slit-like ear-opening in the genus *Asio.*

That *Athene noctua* is doubtless especially closely related to the genus *Surnia* can plainly be discerned from the structure of the cranium alone.

It appears that Kaup, in a paper (*Trans. Zoöl. Soc.*, Vol. IV) to which I have not had access, has shown that *Athene noctua* presents an asymmetry (although slight) in the development of these dermal structures.[1] This asymmetry cannot, with certainty, be pointed out in a dry head that I possess, but in any case must have been very slight.   They are of medium size, or relatively of about the same size as in *Surnia funerea*, with the flap absent.   The cranium itself, which is symmetrical, agrees in its main characters with the cranium in the last-named species.   The jugal bone is furnished with an elevated process; the osseous crest of the *os squamosum* resembles that structure as it occurs in *Surnia funerea;* but the greatest resemblance to this species is especially exhibited in the slender, spine-like supraorbital process, which goes far towards making clear the affinity these forms have with the diurnal birds of prey (of the genus *Astur*); this also extends to the structure of the cranium in these two species.

In this particular the genus *Athene* comes nearer to the genus *Surnia* than to *Glaucidium;*[2] on the other hand, it is unlike both in possessing an evident median furrow.

Scops giu has also relatively small or medium-sized auricular

[1] Kaup's figure appears in A History of North-American Birds (Baird, Brewer, and Ridgway), vol. iii, p. 97.   Boston, 1874.

In this connection compare the pterylography of the so-called "Burrowing Owls" of the American continent, reference being made to one of them in my memoir entitled "Notes on the Anatomy of Speotyto cunicularia hypogoea" (*Journ. Morph.*, vol. iii, No. 1, June, 1889, pp. 115-125, Pl. VII), also in a very excellent paper by Mr. Hubert Lyman Clark, entitled "The Pterylography of certain American Goat-suckers and Owls," *Proc. U. S. Nat. Mus.*, vol. xvii, 1894, pp. 551-572.   Many figures in text.   The comparison of the feather-tracts of *Speotyto* and *Athene* would be especially interesting. — R. W. S.

[2] The supraorbital processes are also spine-like and well developed in *Speotyto*, as may be seen in my figures of the skeleton of that species (*Bull. U. S. Geog. and Geol. Surv. of the Terr. Dept. of the Interior*, vol. vi, No. 1, Washington, February, 1881, Pls. I–III), but they are quite rudimentary in *Micropallas whitneyi*, one of the smallest owls in the world, and belonging to a genus related to *Glaucidium*.   *Speotyto, Glaucidium*, and *Micropallas* all possess the elevated process upon the jugal. — R. W. S.

openings that are lacking in dermal coverts.   The structure of its cranium makes it clear that it belongs to a different type as compared with the other North-European species, even if it does appear in one of the first two of the above-arrayed groups.   The cranium, which is symmetrical, has the osseous crest of the *os squamosum* terminating in a pointed process, that almost comes in contact with the hinder margin of the alisphenoid, and to which it is united by ligament.   Therefore the structure of this crest corresponds to what we find in the right side of the cranium in *Syrnium lapponicum.*   The aural entrance is contracted, quite slit-like, on account of the fusion of the crests with the posterior orbital margin.

As to the remaining characters it may be said that the cranium's greatest depth is found to be at a point situated unusually far forwards, almost in the region of the supraoccipital processes ; that the jugal is linear ; vomer, rudimentary ; and the orbital cavities notably large.

That these characters of the crania, and the structure of the external auricular openings, can be shown to be present to a great extent in all the species of the same genus, is probable, and the few examinations that I have had the opportunity to make of the heads or crania of non-European species have sustained this.   Of the genus *Nyctala*, in which form the asymmetry of the cranium is most evident, there is, at this time, but a single species known that belongs to the nearctic region, viz. — *N. acadica* (Gmel.), 1788, with the exception of the circumpolar *N. tengmalmi.*   It is quite probable that the asymmetry seen in the former is also similarly exhibited in the last-named species.   In a note in the *Proc. Acad. Phila.*, 1870 (p. 73), Mr. Hale Streets invites attention to the fact that a pair of crania in the collection of the Academy, which were thought to belong to *Nyctala acadica*, exhibited an asymmetry in their cranial structure which from the description corresponds with that seen in *N. tengmalmi.*

The second species exhibiting asymmetry in its cranium, *Syrnium lapponicum*, is represented in the nearctic region by a related species, *S. cinereum* (Gmel.), 1788, which was described one year earlier than *S. lapponicum.*   That this species,

characterized in the main only by its darker plumage, will present about the same structure in the cranium as the palæo-arctic *S. lapponicum*, is evident.[1]

This is further shown in two figures, which are given in Baird, Brewer, and Ridgway, *A History of North-American Birds*, Vol. III, pp. 99, 100.

Finally, *Syrnium uralense*, the third species having an asymmetrical cranium, is represented in the eastern part of the palæo-arctic region (Japan) by a similar race or subspecies, *S. rufescens* (Temm. and Schl.), 1850,[2] which is smaller and darker in plumage than the type species, but nothing is known of the structure of its cranium.

It is not improbable that there are still other species of the genus *Syrnium* that will furnish examples of cranial asymmetry. Of this genus, Sharpe has in his *Catalogue of Birds, British Museum*, Vol. II, 1875, described twenty-seven species, besides various subspecies, to which are to be added two others, old species that of late years have been transferred into this genus.

1. SURNIA FUNEREA (Linn.), 1766.

(Plate XV, Figs. 1–3.)

Both the auricular openings and the cranium are symmetrical, flap being absent in the former.

The skin-like auricular openings are of medium size, symmetrical, and comparatively lowly situated, inasmuch as their upper edges barely ascend above the middle of the eye, the lower reaching down to about the mandible. They are evenly rounded above and below, perpendicular, and in an adult female specimen (collected in West Aker, Christiania, Nov. 12, 1881) measure 12 mm. in height and 9 mm. in breadth. There is no evidence whatever of the presence of any dermal flaps, or of any raised dermal folds about the margins.

[1] "One point of note is to be observed, however, and that is, in some species of *Syrnium* the skull is symmetrical, while in some others asymmetrical distortion to a moderate degree is observable. Of the first condition *S. nebulosum* is an example, and of the latter, *S. cinereum* furnishes us an instance." (Shufeldt in MSS., March 20, 1896.) — R. W. S.

[2] "Referred to as *Strix rufescens* in the text, and *S. fuscescens* on the plate."

The *nostrils* are of medium size, situated low down comparatively; their height is 3 mm. and very little more than their width, which is 2 mm. A tuberous *cere* is situated above the nostrils.

The *cranium*, which is quite symmetrical, attains its greatest height posterior to the orbits.

The *beak* is short and very much curved; the mandibles, not taking into account their horny sheath, measured from the frontal bones, will enter 2.6 times into the total length of the cranium. The *superior surface of the cranium* is even and smooth, without any median furrow, a character which, among the North-European species, it possesses in common with *Glaucidium passerinum.*[1]

The *supraorbital process* is, in the older individuals, especially long, and is seen to be a narrow, stiletto-formed, osseous process, that is directed obliquely backwards.

The *forehead*, posterior to the supraorbital processes, is broad, of the same breadth as is that region in front of those apophyses; that is to say, the frontal borders are very nearly parallel.

Frontal bones are sharp where they go to form the posterior periphery of either orbit. The *interorbital septum* has its thinner, semitransparent part especially extensive; in the middle of the *alisphenoid* there is a vacuity caused by non-ossification[2] that in size about equals the *foramen opticum*. The osseous crest of the *os squamosum* is completely free superiorly and juts out sharply from the cranium. It is broadest in the middle, where it develops an apophysis, directed forwards, that conceals the posterior extremity of the quadrate, when the cranium is viewed upon its lateral aspect. Regarding the cranium anteriorly, the osseous crests are seen in plain view standing out beyond the orbital wings (that is, the osseous crests of the alisphenoids).

[1] While making this translation I have before me three skulls of adult specimens of *Surnia ulula caparoch*, and three skulls of adult specimens of *Micropallas whitneyi*. The median furrow is entirely absent in the former, but there is a slight indication of one in the skulls of *Micropallas*. — R. W. S.

[2] This is referred to in the following words, *viz.*: " et hudagtigt parti," which, being literally translated, means " a membraneous part." — R. W. S.

Supraoccipital (*squama occipitis*) has a small, round *supra-occipital foramen*, the size of which varies in different individuals (diameter ½–1½ mm.).

The *jugal bone* is broadest in the middle, inasmuch as its superior border develops a rather long, low, but distinct process. The *pterygoid bones* are slender, becoming pointed in front, where they offer but small articular facets, for articulation with the palatines.

The *palatine bones* are notably broad; *pars plana* of the *mesethmoid* comparatively short, not coming out beyond the external margin of the palatine bone, upon either side, when the cranium is viewed upon its basal aspect. Vomer rudimentary, and present as a slender osseous spine, or (in younger individuals) completely unossified.[1]

2. GLAUCIDIUM PASSERINUM (Linn.), 1766.

(Plate XV, Figs. 4–6.)

The auricular openings, which are without flaps, are, as well as the cranium, symmetrical.

The dermal parts of the auricular openings are small, symmetrical, and, comparatively speaking, placed high up, inasmuch as their inferior extremities do not descend further down than the lower border of the eye, the superior extremities ascending to a point opposite the center of that organ, upon either side. They are oval in form, with the longitudinal axis, in each case, obliquely directed backwards. In an adult female specimen (collected at Lillehammer, Dec. 9, 1876) the longest diameter measured 6 mm., and the transverse diameter 4.5 mm. The flaps were absent, and the skin did not create an elevated fold about the borders.

The *nostrils* are small, with a comparatively broad space between them, and are directed forwards, inasmuch as they are situated in the fore part of the much swollen cere. Their vertical diameters slightly exceed their transverse ones.

The *cranium*, which is symmetrical, agrees, upon the whole,

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[1] Literally translated this reads "fuldkommen hudagtigt," meaning "fully membraneous." — R. W. S.

with the corresponding characters as they occur in the cranium of *Surnia funerea;* its greatest height, as in that species, lies posterior to the orbits.

The *beak* is short, thick, and very much hooked; the mandibles, not taking their horny sheath into account, measured from the frontal bones, will enter almost two times (2.8–2.9) into the total length of the cranium; thus it is shorter than in any other North-European species.

The surface of the cranium is smooth, without any median furrow; the forehead, posterior to the supraorbital processes, is very slightly convex, its surface being broad behind them, but progressively narrower in front of them.   The *frontals* form sharp borders at the posterior parts of the orbits; the thickness [*fortykkelse*] of the forehead in the neighborhood of the supraorbital processes is less than it is in any other species.

The thin, semitransparent part of the interorbital septum is extensive; in the middle of the alisphenoid there is an unossified point, that is occasionally as large as the optic foramen.

The osseous crest of the squamosal agrees with what we found in *Surnia funerea*, and though standing well outwards, its process is less produced.   When we view the cranium upon its lateral aspect, then the crest conceals from view the posterior extremity of the quadrate bone, and, viewed from the front, this is seen outside the orbital wings (that is, the osseous crests on the alisphenoids).

The *jugal* is, as in *Surnia funerea*, broadest in the middle, inasmuch as its superior border develops in that region a longish, low apophysis; in all the other North-European species the jugal is linear.

Supraoccipital (squama occipitis) has an oblong supraoccipital foramen, that is relatively, as well as absolutely, larger than in any other inland species (vertical diameter 3 mm., transverse diameter 2½ mm.).[1]

The *pterygoid bones* are somewhat broader at their anterior ends, and are there furnished with better developed articular facets for the palatines than they are in *Surnia funerea*.

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[1] *Micropallas* also presents this foramen, and in this species it attains a size equal to one-fourth the size of the foramen magnum. — R. W. S.

The *palatine bones* are quite broad ; *pars planæ* of the *meseth-moid* comparatively short, and are concealed by the palatines when the skull is viewed upon its basal aspect.

Vomer rudimentary, being developed only as an almost invisible osseous spicula, or, as in the younger individuals, completely unossified.

The *maxillo-palatines* are notably small, and well separated in the median line.[1]

3. NYCTEA SCANDIACA (Linn.), 1766.

(See Figs. 1 and 2.)

The auricular openings, which are without flaps, are, as well as the cranium, symmetrical.

The dermal parts of the auricular openings are of medium size, symmetrical, and situated comparatively low down, as their lower extremities do not pass the superior borders of the mandible, upon either side, and the superior tips ascend only about to the middle of the eye. They are evenly rounded off both above and below, with their longer axes somewhat oblique. In an adult male specimen (collected in Ringebo, Oct. 19, 1876) the vertical diameter is 20 mm., and the transverse diameter 11 mm. There is no evidence of any flap, but the skin on the anterior borders forms, in either case, a somewhat raised fold. The *nostrils* are quite large, roundish, and placed high. Vertical diameter is 6 mm., and the transverse diameter the same. Posterior to the nostrils, the cere is very little swollen. The *cranium*, which is symmetrical, possesses especially large orbital cavities, and its greatest height is posterior to the latter.

The *beak* is of medium length and comparatively strong. The *mandibles*, not including their horny theca, measured from the frontal bones, will enter slightly more than twice (2.1) into the total length of the cranium. On its superior aspect the

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[1] These ossifications are also comparatively small in *Micropallas whitneyi*, and owing to the relatively as well as absolutely shorter superior mandible in this pygmy species, these spongy masses of bone are brought closer together medially ; indeed, they come very near being in contact. — R. W. S.

cranium has a longitudinal median furrow, which is especially well marked in the region of the base of the superior mandible, and between the parietal bones.

The supraorbital processes are situated comparatively far forwards (anterior to the middle of the orbits).

The forehead, posterior to the supraorbital processes, is somewhat contracted, narrower in fact than it is anterior to these apophyses, and the orbital borders are here about parallel to each other. The *frontal bones* are sharp where they form the posterior borders of the orbits; the forehead is especially thick in the region of the supraorbital processes.

The thinner, semi-transparent part of the *interorbital septum* is extensive; in the middle of either *alisphenoid* there is an unossified area, and in the cranium of one of the specimens at hand there are, more anteriorly, in the direction of the ethmoid two other minute areas of a similar character.

FIG. 1. — Left lateral view of the skull of *Nyctea scandiaca* (Linn.); two-thirds natural size.
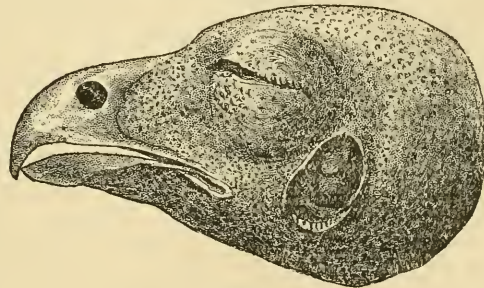
FIG. 2. — Left lateral view of the plucked head of a specimen of *Nyctea scandiaca* (Linn.); two-thirds natural size. (Both figures by Shufeldt, after Collett.)

The osseous crest of the squamosal bone is, comparatively speaking, more feebly developed than it is in the other groups. Superiorly, it terminates in a pointed process, that is directed somewhat anteriorly, which, when the cranium is viewed upon its lateral aspect, conceals from view the distal end of the *os quadratum*.

The broad orbital wings (that is, the osseous crests of the

alisphenoids) hide those structures upon the *os squamosum* of either side, when the skull is looked at from in front.

The *supraoccipital* (*squama occipitis*) possesses an exceedingly small, round *supraoccipital foramen* (the diameter being about 1 mm.).

The *pterygoid bones* are narrow both anteriorly and posteriorly, while their upper and lower margins are sharp.

Their facets for the palatines are triangular in outline.

The *palatine bones* are of about medium width; the *pars plana*, upon either side of the mesethmoid, long and strong, and, when viewed from beneath, are seen to extend laterally well out beyond the external borders of the palatines.

Vomer rudimentary, or else unossified (that is, in all the five specimens examined up to the present time, all of which are probably subadult individuals). In older or adult birds, ossification, to some slight extent, may take place. Mesially, the *maxillo-palatines* are not widely separated.

4. Bubo ignavus (Forst., 1817).

(See Fig. 3.)

The auricular openings, which are without flaps, slightly asymmetrical; the cranium is symmetrical.

The dermal parts of the auricular openings are rather large and subequal in size, inasmuch as the right one is somewhat larger than the left. They are oval in outline; a male specimen (collected at Western Aker, Sept. 23, 1875) has its right ear-opening measuring 30 mm. in height, and with a width of 19 mm., the left aperture possessing a height of 26 mm. and a width of 16 mm. There are no ear-flaps, but the anterior border upon either side develops a slightly elevated fold of skin.

The nostrils are subcircular in outline, and, as in the genus *Nyctea*, situated rather superiorly. The *cranium*, which in all its main characters agrees with the cranium in *Nyctea scandiaca*, is large and coarsely constructed, being symmetrical, and with comparatively large orbital cavities; posterior to which latter we find the greatest height of the cranium to be situated.

The *beak* is somewhat long and strong, the *mandibles*, when not covered by their horny theca, measured from the frontal bones, will enter a little more than twice into the total length of the cranium.   The superior surface of the cranium exhibits a median furrow, which, as in *Nyctea*, is deepest in the frontal region at the mandibular base and between the parietals. The *orbits* are markedly capacious, the diameter in each being twice as long as the postorbital part of the cranium.   The crest of the alisphenoid (posterior orbital process) is conspicuously broad and prominent above, and powerfully developed.
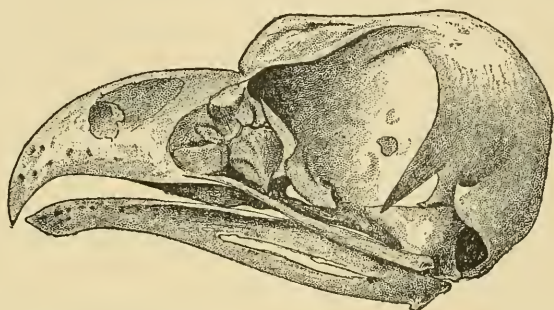
Fig. 3. — Left lateral view of skull of *Bubo ignavus;* two-thirds natural size.
(Shufeldt, after Collett.)

The forehead is, as in *Nyctea scandiaca*, somewhat contracted posterior to the supraorbital processes, and the latter are situated comparatively far forwards (anterior to the middle of the orbits).

The *frontals*, where they enter into the posterior peripheries of the orbits, are sharp ; the thickening of the frontal region in the vicinity of the supraorbital processes is especially well marked.   The thinner (transparent) part of the *interorbital septum* is less extensive than it is in *Nyctea scandiaca*, from the fact that the alisphenoid is thicker in front of the optic foramen than it is in that species, which is likewise the case with the mesethmoid element.   The osseous crest of the *os squamosum* is, comparatively speaking, feebly developed, but, upon the whole, somewhat stronger than it is in *Nyctea;* it is, as in that genus, completely free superiorly, and develops in the margin there a semi-anteriorly directed sharp-pointed

process, which, when the cranium is viewed laterally, conceals the distal extremity of the quadrate. Viewed from in front, they are almost completely hidden behind the broad orbital wings (that is, the osseous crests of the alisphenoids).

The *supraoccipital* (*squama occipitis*) has a very minute and circular supraoccipital foramen (diameter barely 1 mm.). The *pterygoid bones* are, as in *Nyctea*, narrowed both before and behind, and develop cultrate margins both superiorly and inferiorly; the articular facets for the palatines are triangular in form. The palatines themselves are comparatively slender; the *pars plana*, upon either side of the *mesethmoid*, long and stout, and extends far out beyond the external border of the palatine bone, when we view the skull upon its basal aspect.

Vomer rudimentary, and somewhat enlarged mesially. Inferiorly, the *maxillo-palatines* are separated but by a small interval of space.

5. ASIO ACCIPITRINUS (Pall.), 1771.

(Plate XV, Figs. 7 and 8. — Plate XVI, Figs. 9–11.)

Auricular openings and their flaps asymmetrical; cranium symmetrical.

The dermal parts of the auricular openings are asymmetrical, remarkably long, being almost level with the top of the head, and resemble gill-slits. They extend from the frontal region in a curved direction (*halvbue*) down to the nether side of the lower jaw, where they reach or even pass beyond the angle of the gape (the superior angles of these slits are separated at the vertex of the head by a very narrow interval). Their vertical height in an adult specimen (collected at Krogskoven, Oct. 16, 1876) is about 35 mm.

The auricular opening, both in front and behind, is bounded by a raised fold of skin, that extends the entire length of the aperture, performing the function of the ear-flap, although the true ear-flap is here, as well as in the other species possessing it, composed of the anterior, which is at the same time the larger fold of skin. This, the true ear-flap, is crescentic in outline (narrowing upwards and downwards), and broadest in the middle (about 12 mm. wide). While, on the whole, this ear-flap

is symmetrical for the two sides, the tensor muscle that closes them, and which transversely divides either auricular opening at about its middle, exhibits a peculiar asymmetry. This muscle arises symmetrically on the right as well as on the left side, near the center of the lid's inner surface, while its attachment, upon either side, to the cranium posteriorly is thoroughly asymmetrical. On the right side the tensor muscle is attached posteriorly, at a point about in the middle of the osseous crest of the *os squamosum*, or rather so high up that the entrance to the ear upon this side must necessarily be below the transverse fold formed by the muscle, and that part of the entrance above the muscle consequently closed. On the left side the muscle is directed more obliquely downwards and makes an inferior attachment posterior to the mandible, at the lowest extremity of the osseous crest of the *os squamosum;* consequently the aural entrance is found above the muscle, while the lower part of it is closed over. The muscle's attachment to the ear-flap, as already described above, is seen to be upon the inner surface of the latter, near its center, after first having passed and received support from the short but distinct process which occurs upon the posterior aspect of the osseous crest of the alisphenoid.

In addition to the tensor muscle proper, there is a feebler one lower down, which, upon either side, passes from the mandibular border to the lid's inner surface, in the direction of the gape of the mouth.

The nostrils are oblong, elevated, close together, and comparatively large, besides being longer than they are high (length 6 mm.; vertical diameter 4 mm.).

That part of the beak posterior to the nostrils, and covered by the cere, is somewhat raised and of oblong form. The cranium, which does not exhibit any notable asymmetry, is, if viewed upon its superior aspect, seen to be strikingly pointed, or almost triangular, inasmuch as such parts of the margins of the orbits formed by the frontals are obliquely sculptured; therefore the postorbital region of the cranium is more contracted, and lacks the rounding off which takes place in the other groups. The greatest vertical height of the cranium is

posterior to the orbital cavities. The beak is comparatively feebly developed, being powerfully deflected from the frontal region, obliquely downward, in addition to being quite short. The mandibles, omitting their horny sheath, and measured from the frontal bones, will enter almost 2.2 times into the total length of the cranium. The cranium's superior surface exhibits a median furrow that is especially well marked in the frontal region, posterior to the supraorbital processes.

The *orbital cavities* are relatively small, due to the fact that their posterior borders, where formed by the frontal bones, are obliquely truncated; and, moreover, the alisphenoids are short and thick. The osseous crest of the alisphenoid (*proc. orbit. poster.*), as we pass upwards, is seen to become rapidly narrower, it having its greatest width about its center; upon the broadest portion there is, on the external border, a process directed backwards and outwards, and which affords attachment for the superior portion of the tensor muscle that passes to the ear-flaps. This process is symmetrical upon either side, although the muscle referred to is asymmetrical at its distal extremity. The frontal region is comparatively narrow, particularly so posterior to the supraorbital processes, where, indeed, it is narrower than is the part in front of them; while the orbital borders are nearly parallel to each other. The frontal bones of the posterior orbital peripheries slope obliquely away, being abruptly truncated above and below, and have a height that is somewhat less than the length of a pterygoid bone. Inasmuch as both the alisphenoid and mesethmoid are thickened bones, the interorbital septum is necessarily so, the only exception to this being a place just above the sphenoidal rostrum, where the septum is seen to be thin (semitransparent).

The osseous crest of the *os squamosum* is not especially well individualized superiorly (by an evident and deep groove separating it from the alisphenoid), but is continuous in the upward direction without any intervening cleft, or glenoid fossa, quite up to the frontal; as the crest thus becomes somewhat long, and is likewise rather deep, and possesses a semi-anteriorly directed border, it forms a fossa, opening anteriorly, which is larger than the similar cavity seen in the other species having

a symmetrical cranium.   Therefore they are enabled to appreciate the least evident vibrations of sound far easier than the others, and this, moreover, becomes even still easier inasmuch as they possess, too, such conspicuously large dermal parts to their auricular openings.

The external border of this osseous crest is quite even, and no process is developed upon it, and, as mentioned above, it is but slightly deflected forwards, so that it does not come quite opposite to the quadrate bone when the skull is viewed upon lateral aspect.   Regarding the cranium from in front, the osseous crests, in their entirety, project, lateral-wise, beyond the orbital wings (that is, the osseous crests of the alisphenoids).

Supraoccipital (*squama occipitis*) has in some specimens a very small supraoccipital foramen, while in others the bone is not perforated at all.

The pterygoid bones are, especially in front of the basipterygoid processes (*proc. pteryg. oss. sphenoid.*), broad and flat, their borders being even and their distal ends presenting extensive and compressed articulatory facets for the palatines.

The palatines are of medium width ; the *pars plana* upon either side of the mesethmoid is widely spread out and, if the cranium is viewed from beneath, is seen to come out beyond the externo-lateral margins of the palatines.   Vomer present ; usually, but not always, somewhat mesially enlarged.

The maxillo-palatines are large and come almost in contact in the median plane.

6. Asio otus (Linn.), 1766.

(Plate XVI, Fig. 12.)

Auricular openings and their flaps asymmetrical ; cranium symmetrical.

The dermal parts of the auricular openings agree exactly with what was found in *A. accipitrinus,* and the transverse fold (tensor muscle) presents precisely the same asymmetry in its posterior attachment as in that species.   On the other hand, the ear-flap, as well as the corresponding posterior fold of integument, is perhaps somewhat a little higher, and consequently the entrance to the ear is larger.   This really insignificant

departure agrees with such other differences as are to be found
in the structure of the crania of the two species under consid-
eration.   The flap is 13 mm. wide, and the posterior integu-
mental fold about 8 mm.; the height of the ear-slit in an adult
male specimen (collected at Hamar, May 23, 1880) is, as in
A. accipitrinus, about 35 mm.   The nostrils, the structure of
the beak, and its relation to the total length of the cranium
are identically the same as in *A. accipitrinus*.   The cranium
likewise, in the main, agrees with the cranium of *A. accipi-
trinus*.

Its pointed, triangular form is here even better marked,
inasmuch as the obliquely sculptured part of either frontal is
more extensive and reaches further backwards.   The greatest
vertical height of the cranium is found in the posterior orbital
region.

The superior surface exhibits the same median furrow as in
A. accipitrinus.   The orbits are comparatively even of less
diameter than they are in *A. accipitrinus*, due to the fact that
their obliquely sculptured portions at their posterior borders,
which are contributed by the frontal bones, are, in the present
species, more extensive than in *A. accipitrinus*.

The longitudinal diameter of the postorbital part of the cra-
nium is here almost as great as is the diameter of the orbit; in
A. accipitrinus it is considerably less.

The osseous crest of the alisphenoid (*proc. orbit. post.*) is
notably small and narrow ; while the apophysis upon its poste-
rior surface (at least in the specimens examined by me up to
the present time) is either rudimentary or absent.   The frontal
region is somewhat broader than it is in *A. accipitrinus;* its
width is greater posterior to the supraorbital processes than it
is in front of them, while the reverse of this is the case in
A. accipitrinus.

The frontals form at the posterior borders of the orbits, as
in *A. accipitrinus*, a slanting surface, which is abruptly trun-
cated both above and below; but this surface is deeper than in
the species named, inasmuch as its height is equal to the length
of a pterygoid bone.   The interorbital septum is transversely
thick, as in *A. accipitrinus*, and, as in the species named,

exhibits only a single, limited, thin (semitransparent) area just above the sphenoidal rostrum.

The structure of the osseous crest of the *os squamosum* corresponds exactly with what was found in *A. accipitrinus*, but, inasmuch as the truncated portion of the frontals posterior to the orbits is more extensive than it is in that species, and the big orbital wings (on *os alisphenoides*) being, as a consequence, situated further forwards, the distance between the osseous crest and the orbit is greater, and the fossa thus created, more capacious, particularly above, than *A. accipitrinus*.

The supraoccipital (*squama occipitis*) is pierced by an extremely minute supraoccipital foramen, which, as in the species just mentioned, is situated at the base of a little oblong fossa, and in some individuals it doubtless will be found to be absent.

The pterygoid bones seem to be somewhat narrower than they are in *A. accipitrinus* and offer a less extensive articular surface for the palatines than in that species. The palatine bones are perhaps a little broader than in *A. accipitrinus; pars plana* of the mesethmoid barely passes beyond the external border of the palatine, upon either side, when the cranium is viewed upon its nether aspect. Vomer present, and developed as in *A. accipitrinus*. The *maxillo-palatines* are large and come near being in contact in the median plane of the skull.[1]

7. SYRNIUM ALUCO (Linn.), 1766.

(Plate XVII, Figs. 13, 14. — Plate XVIII, Figs. 17–20.)

Ear-openings, as well as the ear-flaps, asymmetrical ; the cranium symmetrical.

The dermal parts of the auricular openings are of subequal size, and they possess asymmetrical flaps. These openings are, upon the whole, wide ; their borders giving them a reniform, or oblong bean-shaped, outline ; on the right side, where the

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[1] While this translation was being made, I have had before me a complete skeleton of *Asio wilsonianus* (*Asio* Brisson ; *Strix otus* Linn.), collected by me at Fort Fetterman, Wyoming, in April, 1881, and I find the characters it presents agree, in so far as the skull is concerned, with the corresponding ones so correctly given above by Professor Collett for *Asio otus*. — R. W. S.

aperture is the larger, it has, in an adult female specimen (collected at Aker, Nov. 10, 1876), a height of 25 mm. and a width of 12–13 mm. ; the entrance is smaller upon the left side, and has a height of 22 mm. and a width of 11–12 mm.   Comparatively speaking, they are situated rather high up, inasmuch as their lower extremities do not fall below the inferior arc of the eyeball, upon either side.   Superiorly, the right opening passes above the eyeball ; the left being situated a little lower down than this.   The distal border of an ear-opening is bounded by a thickened integument, approaching in its nature a low, free fold of skin.   The ear-flaps are also asymmetrical.   On the right side, where the aperture is the larger, it is broad, being squarely truncated both superiorly and inferiorly, and has an average width of about 12 mm. ; on the left side the flap narrows as it ascends, but below is carried out as an irregular, inferiorly directed point ; in this locality the flap sees its greatest breadth, being about 11–12 mm.   The nostrils are markedly small, elevated, and almost circular ; their diameter, in either case, being about 2 mm.   The cranium, which is symmetrical, is comparatively large, and the posterior region is prettily dome-shaped ; its greatest vertical height being comparatively far forwards, that is to say, about over the middle of the orbits. The beak is of medium size, with flattened sides ; the mandibles, measured from the frontal bones, not taking into consideration their horny covering, will enter 2.3 times into the total length of the cranium.   The superior aspect of the cranium has a longitudinal, median, shallow furrow that becomes more distinct in the frontal region between the supraorbital processes.[1]

Posterior to the supraorbital processes the frontal region is notably broad, and considerably (sometimes almost double the width) broader than the forehead is in front of the processes. The latter diminishes rapidly in width as one passes anteriorly, and, when the cranium is viewed from above, it is seen that

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[1] At this writing I have at hand the skeleton of a specimen of *Syrnium nebulosum*, a " bird of the year," collected by me at New Orleans, La., in July, 1883. In it the furrow, as usually seen in owls on top of the cranium, is replaced by a sharp, linear, deep-seated crease that is distinctly carried from the base of the superior mandible to the supraoccipital prominence behind. — R. W. S.

here the lateral borders are quite concave. Where the frontals enter into the formation of the posterior peripheries of the orbits their borders are rounded off ; and, if we view the skull from behind, the supraorbital processes are concealed from sight by the vertex of the cranium.

The thinner (transparent) parts of the wall of the inter-orbital septum are quite extensive. The osseous crest on either squamosal bone is of medium size only. Above, it is completely free, and there develops a curved process, directed forwards and upwards, which, when the cranium is viewed lateral-wise, is seen to be opposite to the posterior border of the quadrate. Regarded from in front, it is only their outside edges that become visible to the outer side of the large orbital wings (that is, the osseous crests on the alisphenoids). An extremely minute supraoccipital foramen pierces the supraoc-cipital bone (*squama occipitis*), the diameter of which in the subadult individuals is equal to about 1 mm., while in the older birds it is barely more than ½ mm.

Anterior to the basipterygoidal processes (*proc. pteryg. oss. sphenoid.*) either pterygoid is compressed from above down-wards, having a uniform width, and with its cultrate edge directed anteriorly. The palatine bones are quite broad ; the lateral processes of the *os ethmoides* pass outwards so as to be about opposite the external margins of the palatines, when we regard the cranium from beneath. Vomer is rudimentary, as only its posterior moiety ossifies as a minute spicula of bone ; ossification not being extended to its anterior end. It is very likely that in still younger individuals it does not ossify at all. The maxillo-palatines are of unusually large size and come very near being in contact in the middle line, below.

8. Syrnium uralense (Pall.), 1776.

(Plate XVII, Figs. 15, 16; Text-fig. 4.)

Auricular openings and their flaps asymmetrical, the cranium slightly asymmetrical.

The dermal parts of the auricular openings are of subequal size, with a somewhat asymmetrical lid ; upon the whole,

these structures agree with the corresponding ones as they occur in *S. aluco*, only being relatively a little smaller. The auricular openings may be said to be quite wide, and have a reniform outline. On the right side, where the opening is the larger, it has, in an adult specimen (collected at Löjten, Nov. 1, 1881), a vertical height of 26–28 mm. and a width of about 14 mm. The aperture upon the left side is somewhat smaller, the height being 23 mm., the width about 14 mm. Comparatively speaking, they are placed pretty well up on the side of the head, inasmuch as on the right side the lower end descends to a point slightly below the inferior arc of the globe of the eye; superiorly, they are carried up, upon either side, to a point in the same plane with the top of the eyeball.

The hinder border of either auricular opening is, as in *S. aluco*, bounded by a thickened fold of integument, inferiorly. An asymmetry of the ear-flaps, also agreeing with what we find in *S. aluco*, is present; on the right side, where the aperture is the larger, it is broad ; both above and below it is transversely truncated, and, upon the whole, has a considerable breadth, which corresponds in size to that of the ear-opening. On the left side the flap is somewhat irregularly drawn downwards, and terminates in a shorter projection, though it still agrees with what we find in *S. aluco*. The nostrils are of medium size, oval, and with their breadth and height about equal (nearly 4 mm.). The cranium, which is almost but not completely symmetrical, is, in its main characters, like the cranium of *S. aluco*, although the mandibular portion is more powerfully developed as compared with the cranium, and the orbits are comparatively smaller. The asymmetry, to which reference has been made, and which is almost imperceptible, is due to the fact that the osseous crest of the squamosal bone is inclined slightly more forwards upon the right side than it is upon the left, thus foreshadowing the very decided asymmetry which will be found to be present in these structures in *S. lapponicum*. The greatest vertical height of the cranium is at a point posterior to the orbital cavities. The bill is moderately long, though not of a powerful build ; it will enter twice into the total length of the cranium, measur-

ing from the frontal bones, and not including the horny theca that covers it.   Upon the superior aspect of the cranium there is a very distinct median furrow, which, in the three specimens examined, is best marked at the cranial vertex in the interparietal region.

The orbital cavities are comparatively smaller than they are in *S. aluco*, inasmuch as the *os alisphenoides* is here shorter and thicker.   Taken upon the whole, the orbital diameter is of about the same length in the two species.   The big orbital wings (the osseous crests of the alisphenoids) are in both these owls comparatively broad and large.   The frontal region, posterior to the supraorbital processes, is conspicuously wide, and even much wider than it is in front of them, where it gradually narrows anteriorly, but not in as marked a degree as it does in *S. aluco*.   The frontal bone is, upon either side, thoroughly rounded off where it forms the hinder border of the orbit, but it is seen to slope obliquely away in the direction of the large orbital wing, so that we are enabled to see the supraorbital processes when the cranium is viewed upon direct posterior aspect.

The *interorbital septum* is quite thick transversely, inasmuch as both *os alisphenoides* and *os ethmoides* enjoy a similar condition throughout all their parts ; as in *Asio* there is only a localized area, situated above the rostrum of the sphenoid, where the septum is thin and semitransparent. The osseous crest of the *os squamosum*
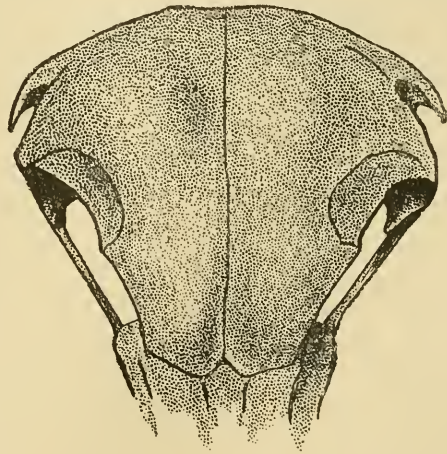
FIG. 4. — Cranium of *Syrnium uralense*, seen from above, and showing slight asymmetry of the crests of the *os squamosum;* compare this with same view of cranium of *S. lapponicum.* (Adapted by the author from Collett.)

agrees entirely with what was found in *S. aluco*, being an outstanding, nail-like, and superiorly free process ; but at the same time it exhibits in the slight forward inclination of its anterior

border of the right side, as has already been pointed out above, a disposition to approach the asymmetry which is present in *S. lapponicum.*

The difference seen in the two sides is better marked in the crania of some specimens than it is in others, and can in the adult individual vary between 1 and 2 mm., this difference being interesting rather than remarkable, inasmuch as it is just here that in *S. lapponicum* such decided asymmetry is present. This difference can best be appreciated when the cranium is viewed from above (see Fig. 4), although even then it is seen to be very slight, though it is invariably present.

The *occipitale superius* (*squama occipitis*) has no supraoccipital foramen.

The *pterygoid bones* are, as in *S. aluco,* broad in front of the basipterygoid processes, and quite compressed.

The *palatine bones* are, comparatively speaking, narrower than in *S. aluco; pars planæ* on *os ethmoides* reach a little beyond the outer margin of either palatine, when the cranium is viewed from below.

Vomer is almost rudimentary, inasmuch as it is present only as a thin osseous spicula posteriorly, and non-ossified in front.

Maxillo-palatines are very large, and meet in the line below.

9. Syrnium lapponicum (Thunb.), 1789.

(Plate XIX, Fig. 27; Plate XX, Figs. 28–30. Text-figs. 5–7.)

Auricular openings ; ear-flaps ; and cranium asymmetrical.

The dermal parts of the auricular openings, structurally, about agree with the two other species of *Syrnium* and present a similar asymmetry. Upon the whole, they are quite large, being subequal in size, although this asymmetry is apparently less than in *S. aluco.*

Upon the right side, where they are of the greater size, the ear-opening has in an adult female specimen (collected in East Aker, Nov. 17, 1881) a vertical height of 30 mm. and a width of 15 mm.; on the left side the height is 28 mm. and the width 14 mm. They may be said to be placed high up on the side of the head, being situated just back of the

eyes, and consequently their upper and lower ends (more particularly upon the right side) extend slightly past the highest and lowest points in the eyeball.

The distal border in either auricular opening is surrounded by a thick and somewhat raised fold of integument similar to what is found in the other species. The flaps are large and somewhat asymmetrical, though in a less degree than in *S. aluco*. The flap is larger on the right side, and has a length that somewhat exceeds that of the ear-opening, being about 35 mm. The width is 17 mm., and this flap is less obliquely truncated than it is upon the left side ; its lower end is, transversely, quite straight. The ear-flap is, upon the left side, greatly narrowed above and broadest below, where it forms a long, produced, and deflected extremity. The length and breadth of this flap do not materially differ from the same measurements given for the right side.

Across either auricular opening there is stretched a fold of skin or tensor muscle [1] that is attached at a point somewhat above the middle of the lid ; on the right side this arises from the tuberous and comparatively inferiorly situated superior border of the osseous crest of the alisphenoid (or the large orbital wing) ; on the left side this tubercle is found higher up and less prominent, and the tensor muscle passes this, its origin being found upon the posterior aspect of the osseous crest of the squamosal bone, at its superior extremity. Owing to the formation of the cranium, the entrance to the ear, or the canal leading from the same to the parts within, has a different direction upon the two sides. On the right side this canal passes almost directly into the cranium immediately beneath the tensor muscle of the ear-flap ; on the left it passes obliquely downwards beneath the muscle.

The *nostrils* are comparatively large, elevated, somewhat oblong ; their longitudinal diameter is 7 mm., the height 5 mm.

The *cranium*, which is asymmetrical, resembles in its struc-

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[1] I presume Professor Collett means the "tensor muscle," the same having the *appearance* of a "fold of skin." It reads in the original "*Strækker sig en Hudfold eller Lukkemuskel.*" To be sure, the muscle is included within a fold of skin. — R. W. S.

ture the cranium in *Syrnium uralense*, but in it the mandibular parts are more powerfully developed ; the parietal region is more pyramidal in contour, and with comparatively smaller orbits than in that species. The cranium sees its greatest height opposite the posterior margins of the orbits. The asymmetry is chiefly due to the somewhat distorted develop-
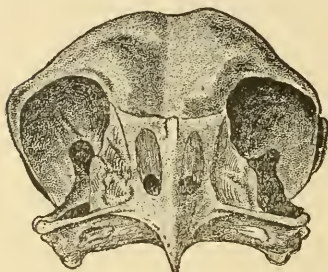
FIG. 5. — *Syrnium lapponicum* (Thunb.). Skull seen from in front; mandible attached; two-thirds natural size. (Shufeldt after Collett.)

ment of the osseous crest of the right *os squamosum*. The beak is laterally compressed, but is not especially strong; the mandibular portion has a greater length than in any other inland species, and does not quite enter twice (1.9) into the total length of the cranium, not taking into account its horny, integumental theca. The median furrow upon the superior aspect of the cranium is quite well marked its entire length, being especially so in the frontal region, posterior to the supraorbital processes.

The orbit has a comparatively less diameter than in *S. uralense*, inasmuch as *os alisphenoides* is notably short and thick, which is likewise the case with that part of either frontal which forms the margin of the orbit superiorly. The supraorbital processes are

FIG. 6. — *Syrnium lapponicum* (Thunb.). Skull seen from behind; mandible attached; two-thirds natural size. (Shufeldt after Collett.)

situated far back, so much so that their apices, upon either side, reach to a point upon the orbital crest of the *os alisphenoides*. Further, either one of these processes is very broad, and develops as a long frontal extension, which is connected by a membrane with the tubercle of the orbital wing, superiorly, and in this manner contributes towards the formation of the hinder roof of the orbital cavity.

The osseous crest of the alisphenoid (*processus orbitalis posterior*) is on the right side somewhat asymmetrical, as is

also the *os squamosum*, inasmuch as it is here drawn out in a greater degree laterally, and also more depressed than upon the opposite side, in such a manner that the crest becomes broader above, on the right side, and more tuberously swollen than upon the left side.

The orbits thus become a trifle wider, but at the same time lower, than upon the left side. Upon the posterior aspect of the apex of the osseous crest there is to be found a process that is not especially conspicuous.

The frontal region, posterior to the supraorbital processes, is profoundly concaved,[1] but, upon the whole, particularly broad, and much broader than the surface in front of these processes, which, relatively speaking, is also of considerable width. Either frontal bone slopes obliquely downwards from the middle of the cranium towards the hinder border of the orbit, and is very deep posterior to the supraorbital processes. If the cranium is seen upon direct posterior view, the supraorbital process of the right side, including its upper border, is in view beyond the limiting profile line of the frontal, while the left one is hidden behind it. The interorbital septum is notably thick and of limited area, and this thickness is to be found over its entire extent, and it is only just above the rostrum of the sphenoid that there is to be found a thinner place, which only in a very limited degree is semitransparent.

The osseous crest of the squamosal bone is normal upon the left side, having the same structure as in *S. aluco* and *S. uralense*, forming there a supero-anteriorly directed process, that is, above, perfectly free. This crest is particularly lofty upon the right side, where it extends upwards and forwards as a prominently curved apophysis that reaches to the upper border of the *os alisphenoides;* this latter is decidedly massive, and, as has before been remarked, stands out from the side of the skull in a most abnormal manner. The two processes do not fuse at their point of contact, but are simply joined there by membrane. The fossa formed by the osseous crest, the entrance to which is in front, thus becomes more capacious, and

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[1] I take this to refer to the superior orbital borders, they being roundly concaved in most species of *Syrnium*, just behind the supraorbital processes. — R. W. S.

especially more lofty than upon the left side.   Regarding the
cranium upon its anterior aspect, it will be seen that the promi-
nent orbital wing completely conceals from view the osseous
crest of the right squamosal, while upon the left side the ex-
ternal border comes into sight beyond the orbital wing.   Upon
the whole, the asymmetry of the cranium in this species can
best be appreciated by viewing it from above ; while thus seen,
one can also more easily compare the powerfully anteriorly
directed superior bor-
der of the osseous crest
of the right side with
its retreating border
upon the left.   (See
Fig. 7.)

Supraoccipital (*squa-
ma occipitis*) lacks, as
in *S. uralense*, a supra-
occipital foramen.

The pterygoid bones
are more slender than
in *S. aluco* and *S.
uralense*, and possess
smaller articular facets
for articulation with
the palatines.   Com-
paratively speaking,

Fig. 7. — Cranium of *Syrnium lapponicum* seen from above,
and showing the degree of asymmetry of the squamosal
crests ; compare this with Fig. 4, given above.   (Adapted
by the author from Collett.)

the palatine bones are especially slender ; the lateral processes
of the ethmoid are well developed, and have an unusually high
point of origin, being upon a level with the olfactory foramen,
or slightly below the superior border of the lachrymal bone,
upon either side; they are widely spread out, and when the
skull is seen from below, nearly the whole of either one of
them can be seen beyond the external border of the palatine
of the same side.

The vomer is, as in other species of this genus, nearly rudi-
mentary, as it is only represented posteriorly by a minute
osseous spicula, being otherwise unossified (in subadult indi-
viduals, perhaps entirely so).

The maxillo-palatines are unusually large, and are in contact in the mesial plane, below.

10. Nyctala tengmalmi (Gmel.), 1788.

(Plate XIX, Figs. 21–26.)

Auricular openings and flaps asymmetrical ; the cranium profoundly so.

The dermal parts of the auricular openings are nearly of equal size upon the two sides, but in other respects exhibit an asymmetry that agrees with the asymmetry assumed on the part of the cranium itself; they are very large, occupying as they do the whole side of the head, upon either side ; they are somewhat semilinear or oval in outline, but less pointed above and below than in *Asio*, and therefore not as gill-slit-like as in that genus. Their vertical height in an adult specimen (collected at Hamar, Sept. 20, 1876) is about 28 mm., the greatest inner width being about 12 mm.; but they frequently have a greater width than this.

Either of these openings extends from beneath the mandible or lower jaw (close to the mandibular commissure) up to the side of the frontal region, where the space separating the one from the fellow of the opposite side is comparatively wide, being but a little less than the height of either ear-opening. The ear-flap that closes the aural aperture in front is of a semilunar outline, but is not very broad, as at a point opposite the center of the eyeball it barely exceeds 6 mm. ; in the frontal region, and below the eye, it is a little broader. Posteriorly the opening is closed by a very well developed integumental fold resembling an ear-flap, which, with a breadth coequal with that of the true ear-flap, extends the entire length of the auricular opening, and both above and below indistinguishably merges into the ear-flap proper. Thus the entire aural aperture is surrounded by a continuous, free, and rather high dermal fold. The contour assumed by this posterior integumental fold is that given it by the form of the cranium; thus it is high on the right side, and comparatively as low on the left, where the osseous crest of the *os squamosum* holds such an abnormally low position.

The nostrils are small and oblong; their height, which is hardly 3 mm., is greater than their width; they are subvertical in position, with their openings somewhat anteriorly directed. Posterior to the nostrils, the cere constitutes the swelled part, and this, superiorly, develops two tubercle-like elevations.

The cranium, which is profoundly asymmetrical, is comparatively large, with orbits of about medium size.

The asymmetry is present upon both sides, but the left is the more abnormally so. In its structure generally it agrees, perhaps, in so far as the inland species are concerned, most nearly with the cranium of *Syrnium aluco*, although it widely departs even from it. Its greatest vertical height is at a point posterior to the orbits. Longitudinally the superior aspect of the cranium presents for its entire length a feeble median furrow, which is best marked in the frontal region, between the supraorbital processes. A rather well marked cranio-facial hinge stands between the not very powerfully developed superior mandible and the frontal bones; this mandibular portion is comparatively short, for, upon being measured from the frontalia, it will enter 2.6 times into the total length of the cranium, provided its horny theca is not taken into account. Posterior to the supraorbital processes the frontal region is rather broad; the area anterior to the processes rapidly narrows as we pass forwards, and it has quite concave borders.

The asymmetry. — The bones that take part in the asymmetry of the cranium are principally the *os squamosum*, and in a lesser degree the adjacent parts of the frontal, the parietal, and the alisphenoid.[1]

The frontal bones are smoothly and very completely bounded off where they enter into the posterior peripheries of the orbits,

[1] The internal configuration of these bones can only be examined with certainty in the very young. If the individual has arrived at maturity, even if the downy plumage (the first feathers, which are moulted shortly after the bird becomes full grown) is still worn, the sutures among the separate bones have already disappeared. Although I have made every effort to obtain the young just taken from the nest, I have not succeeded in securing them, having only obtained a pair of indifferent specimens of nestlings, and in these obliteration of the sutures had already partly taken place. So perhaps the above description of the defining of the separate bones in certain instances can be corrected or supplemented.

and have the appearance of being almost quite symmetrical. Thus, their orbital portions do not seem to offer any difference upon the two sides, as both descend about an equal distance upon either posterior orbital wall; on the other hand, the lateral portion on the right side, where it articulates with the anteriormost apex of the parietal bone, and the here abnormally developed and much uptilted crest of the *os squamosum*, is more elevated than on the left side.   Otherwise the difference is very slight.

The osseous crests on the *os squamosum*, where the asymmetry is most evident, are, upon the whole, so abnormally large, deep, and conspicuously outstanding, that they, almost in their entirety, can be seen beyond the orbits, if the cranium be viewed from in front.   As in the genus *Asio*, they are not distinguished from any of the bones with which they articulate above by any distinct groove or depression, but enter into the uninterrupted lateral contour of the cranium, where it is seen from in front.   Both sides are distorted, the left side being the more so.   On the right side, the osseous crest is extended upwards to a height quite coequal with that of the superior border of the orbit, but it abruptly terminates here, as it comes in contact with the frontal, at a considerable distance (7.2 mm.) posterior to the orbit, and in this manner crowding far backwards the apex of the parietal bone.   In the middle the osseous crest is produced in such a way as to form an anterio-inferiorly directed and rounded process, which with its point impinges upon the hinder border of the orbital crest of the *os alisphenoides*.   The combined heights of the osseous crests upon this side are equal to 20 mm.   On the left side the osseous crest is abnormally vertically compressed; it commences at a point above, at about opposite the middle of the orbit, and close to the latter's hinder border, thus being farther forward anteriorly than it is upon the right side, as the crest is here supported by the superior extremity of the orbital crest upon *os alisphenoides*.   Here also, from a point a little below its middle, the crest is produced in a long, inferiorly directed process; but its apex is found as low down as the mandible, where, with a feeble, yet with an easily distinguished articulatory facet, it

meets the jaw, as well as the *os quadratum* and the *os jugale*, at their point of articulation. It is in this way that the cranium itself comes in contact with the lower jaw, a phenomenon which is certainly without parallel in the class *Aves*, outside of this genus. Owing to the unusual development of this osseous crest, the fossa, in which the aural entrance is found, is of considerable width, particularly upon the right side. On the other hand, the entrance to the ear itself is normal upon both sides, and quite symmetrical; and, as the asymmetry is thus mainly confined to the external osseous crest and its neighboring structures, while the *os squamosum*, internally, is normally developed, upon either side, it follows as a result that the inner walls of the brain casket are symmetrical, and the brain itself does not appear to offer anything anomalous in so far as its superficies are concerned.

The *parietal bone* upon the left side, on account of the lowly situated osseous crest of the *os squamosum*, is quite pointedly produced anteriorly, though it extends forwards quite to the hinder margin of the orbit; on the right side, where the osseous crest is situated higher up and at the same time placed farther backwards, it is less pointed, though crowded farther to the rear, and as a consequence does not reach to the posterior border of the orbit. The *alisphenoid* is, upon the right side, larger and posteriorly broader than it is upon the left side; in other respects the orbital crest does not present any asymmetry, that is, beyond the fact that its superior border is extended a little higher up on the right side than it is upon the left.

The *interorbital septum* is, anteriorly, quite thin and translucent; the *os ethmoides* has a comparatively thick wall.

The *supraoccipital* (*squama occipitis*) is pierced by a small *supraoccipital foramen* (diameter ¾ mm.).

The *pterygoid bones* are slender, being somewhat compressed from above downwards, thus causing their cultrate edges to turn obliquely outwards and downwards.

The *palatine bones* are very broad; the *pars planæ* upon the mesethmoid are quite short, and do not extend beyond the external palatine borders, when the cranium is viewed upon its basal aspect.

Vomer is present, but is very slender; in subadult individuals it is probably unossified (that is, *hudagtigt*, or skin-like).

The *maxillo-palatines* are, mesially, almost in contact upon their inferior side.

[Conclusion of the translation.]

Opinions upon the Position of the Strigidæ in the System.

Huxley, in his celebrated paper "On the Classification of Birds," published in the *Proceedings of the Zoölogical Society of London* in 1867, says that his Aetomorphæ is a division which is equivalent "to the 'Raptores' of Cuvier — an eminently natural assemblage, and yet one the members of which, as the preceding enumeration of their characters shows,[1] vary in most important particulars."

"They appear to me to fall naturally into four well-defined primary groups — the *Strigidæ*, the *Cathartidæ*, the *Gypætidæ*, and the *Gypogeranidæ*. But this arrangement is so different from that ordinarily adopted that I shall proceed to justify it by enumerating the principal circumstances in which the members of the several divisions agree with one another and differ from the rest."

This is first followed by a fairly complete *résumé* of the osteological and other characters of the owls; but as many important skeletal strigine characters have, since that paper was published, been described by ornithotomists, I will complete Professor Huxley's opinion by what he thought of the systematic position of the *Caprimulgidæ*, which he believed "come near *Trogon*, and more remotely approach *Podargus* and the Owls" (p. 469).

This is important, for as early as 1867 so keen an observer as Huxley saw the affinity between the goat-suckers and the owls. In his admirable article "Ornithology," in the ninth edition of the *Encyclopædia Britannica* (Vol. XVIII, p. 47), Professor Newton says that "it has so long been the custom to

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[1] It has not been thought necessary to give these characters here; they are surely not of a nature to convince one that a typical hawk, an American vulture, and an owl all belong to the same group; for example, *Accipiter* + *Cathartes* + *Strix*! — R. W. S.

place the owls next to the diurnal birds of prey that any attempt to remove them from that position cannot fail to incur criticism. Yet when we disregard their carnivorous habits, and certain modifications which may possibly be thereby induced, we find almost nothing of value to indicate relationship between them. That the *Striges* stand quite independently of the *Accipitres* as above limited can hardly be doubted, and, while the *Psittaci*, or parrots, would on some grounds appear to be the nearest allies of the *Accipitres*, the nearest relations of the owls must be looked for in the multifarious group *Picariæ*. Here we have the singular *Steatornis*, which, long confounded with the *Caprimulgidæ*, has at last been recognized as an independent form, and one cannot but think that it has branched off from a common ancestor with the owls." But the same eminent authority, in the volume just quoted, under the article " Owl," further says, on page 89, that " the owls form a very natural assemblage, and one about the limits of which no doubt has for a long time existed. Placed by nearly all systematists for many years as a family of the order *Accipitres* (or whatever may have been the equivalent term used by the particular taxonomers), there has been of late a disposition to regard them as forming a group of higher rank. On many accounts it is plain that they differ from the ordinary diurnal birds of prey more than the latter do among themselves ; and, though in some respects owls have a superficial likeness to the goatsuckers, and a resemblance more deeply seated to the Guacharo, even the last has not been made out to have any strong affinity to them." [1]

" A good deal is therefore to be said for the opinion which would regard the owls as forming an independent order, or, at any rate, suborder, *Striges*. Whatever be the position assigned to the group, its subdivision has always been a fruitful matter of discussion, owing to the great resemblance obtaining among all its members, and the existence of safe characters for its division has only lately been at all generally recognized."

[1] Nevertheless, Professor Newton believes, at least, that *Steatornis* " has branched off from a common ancestor with the owls." (Compare first quotation above.) — R. W. S.

Upon consulting the plates and text of so distinguished an authority as Professor Max Fürbringer, "Untersuchungen zur Morphologie und Systematik der Vögel," we are to note that there the *Caprimulgi* and *Striges* are considered as arising from a common ancestral stock, the suborder *Coraciiformes* of the order CORACORNITHES, and this last-named division is quite apart from the order PELARGONITHES, which contains the Accipitres.

In 1892 a no less careful examiner of the structure of birds than Prof. Hans Gadow published in the *Proceedings of the Zoölogical Society* of that year a very excellent article, in many respects, upon the "Classification of Birds," and in the scheme set forth in that work Gadow placed the STRIGES in a group by themselves, standing between the parrots and the goat-suckers, and far removed from the Accipitres. Huxley, Newton, Fürbringer, and Gadow must have especial weight attached to their opinions in the matter of the classification of *Aves*, for each and all of them carefully looked into and compared the anatomical structure of the members of this puzzling division of the Vertebrata. Many of the taxonomers of birds have not done this, and consequently are often guilty of classifying these forms upon such characters as strike their eye after a superficial examination of the general characters presented on the part of "series of museum skins."

In as yet unpublished MSS. the present writer has said : " Regarding the owls as a whole, they are to be considered as forming a group of nocturnal birds of markedly raptorial habits. Some of the species, however, are largely diurnal in their ways. They are not especially related to the *Accipitres*, but are, on the other hand, remotely allied with the *Caprimulgi*. What we now know of the structure of such forms as *Steatornis* and *Podargus* sufficiently indicates this much."

This opinion is based upon an examination of the anatomy of the last two forms mentioned ; upon the osteology of all the species of North-American owls, *Accipitres*, *Caprimulgi*, and a host of forms suspected of having alliance with these groups.

In 1894 Mr. Hubert Lyman Clark published in the *Pro-*

ceedings of the United States National Museum (Vol. XVII, pp. 551–572 (many cuts)) a very able paper entitled "The Pterylography of Certain American Goatsuckers and Owls," in which all the principal North-American forms were examined. At the end of this memoir Mr. Clark said: "The conclusion, then, to which this study of their pterylography has brought me is that the Caprimulgi are related to Striges, and not very distantly either — probably a branch from the early part of the Strigine stem" (p. 572).

My own opinions have been based upon a study of *all* the characters of the groups we have under consideration; this, to a considerable extent, was the case likewise with Huxley; certainly so with Fürbringer and Gadow, while Professor Newton gave the external characters and the skeleton the greatest weight. This being the case, the results arrived at by Mr. Clark very aptly fill in a gap that long stood greatly in need of the very kind of treatment he has bestowed upon it.

As to what relations may exist between the owls and the parrots, I am, just now, not prepared to give a decided opinion; certain it is, however, that we have both "owl-parrots" (*Stringops*) and parrots in Australia that are sufficiently "rapacious" to make good enough use of their claws and hooked beaks to prey upon living sheep, and that display quite as much taste for the habit as a *Bubo* does when he kills and devours a hare. — R. W. S.

EXPLANATION OF PLATE XV.

(All the figures of the plates are by Professor Collett.)

FIG. 1.   Skull of *Surnia funerea ;* anterior aspect, with mandible attached ; natural size.

FIG. 2.   Skull of *Surnia funerea ;* left lateral aspect, with mandible attached ; natural size.

FIG. 3.   Head of *Surnia funerea ;* feathers removed and showing ear-opening ; natural size.

FIG. 4.   Skull of *Glaucidium passerinum ;* anterior aspect, with mandible attached ; natural size.

FIG. 5.   Skull of *Glaucidium passerinum ;* left lateral aspect, with mandible attached ; natural size.

FIG. 6.   Head of *Glaucidium passerinum ;* feathers removed and showing ear-opening ; natural size.

FIG. 7.   Skull of *Asio accipitrinus ;* anterior aspect, with mandible attached ; natural size.

FIG. 8.   Skull of *Asio accipitrinus ;* superior aspect, with mandible attached ; natural size.
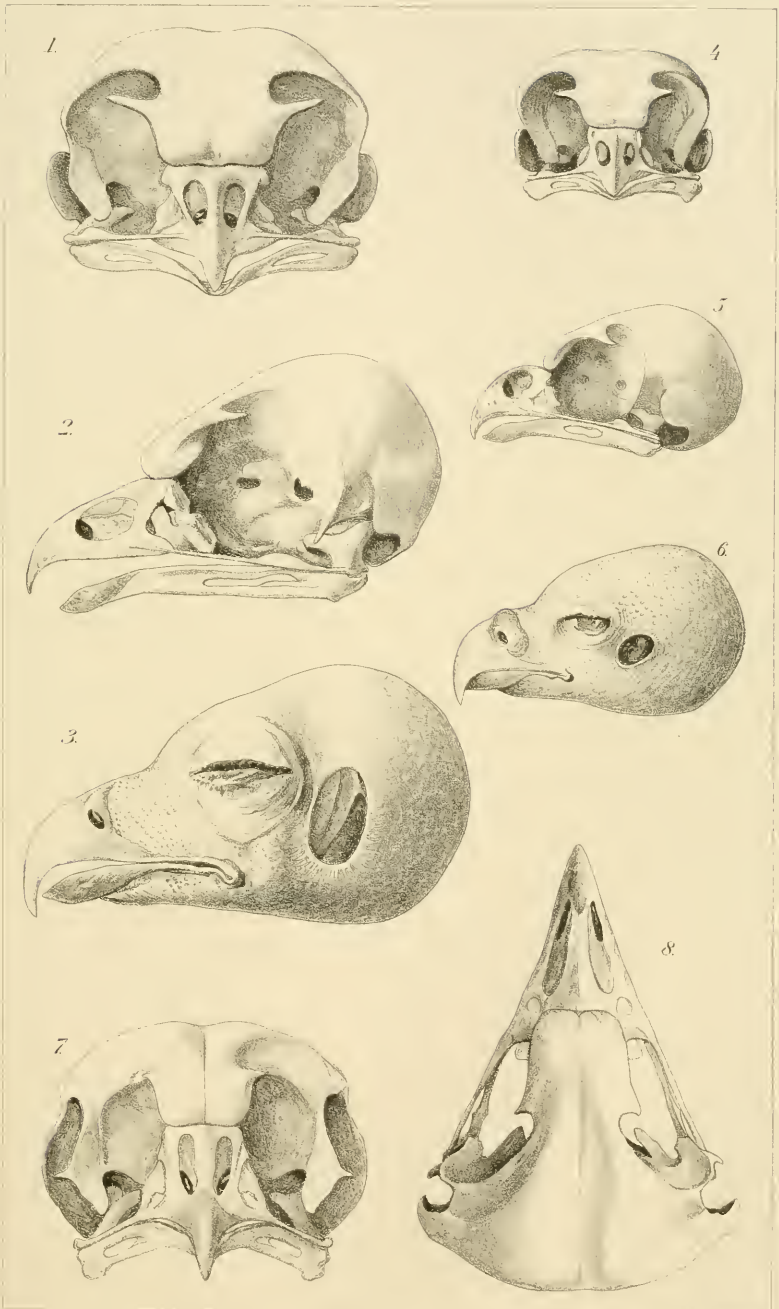
EXPLANATION OF PLATE XVI.

FIG. 9. Skull of *Asio accipitrinus ;* right lateral aspect, with mandible attached ; natural size.

FIG. 10. Head of *Asio accipitrinus ;* left lateral view, with feathers removed and showing ear-opening ; natural size.

FIG. 11. Head of *Asio accipitrinus ;* right lateral view, with feathers removed and showing ear-opening ; natural size.

FIG. 12. Skull of *Asio otus ;* left lateral aspect, with mandible attached ; natural size.

Pl. XVI.

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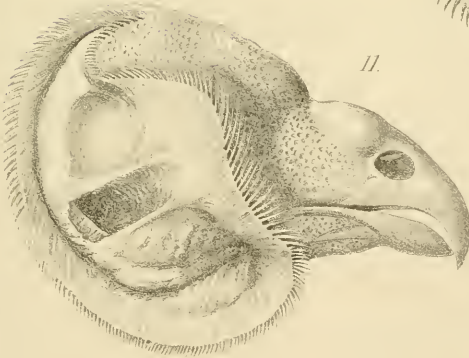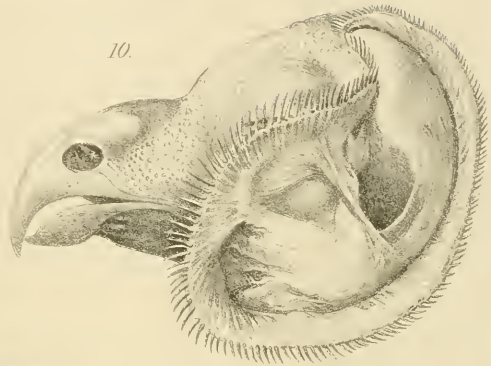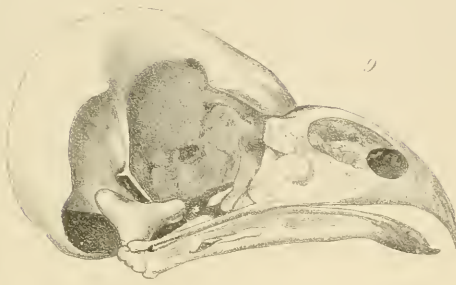
EXPLANATION OF PLATE XVII.

Fɪɢ. 13.   Skull of *Syrnium aluco ;* left lateral aspect, with mandible attached ; natural size.

Fɪɢ. 14.   Skull of *Syrnium aluco ;* anterior aspect, with mandible attached ; natural size.

Fɪɢ. 15.   Skull of *Syrnium uralense ;* anterior aspect, with mandible attached ; natural size.

Fɪɢ. 16.   Skull of *Syrnium uralense ;* left lateral aspect, with mandible attached; natural size.

Pl. XVII.
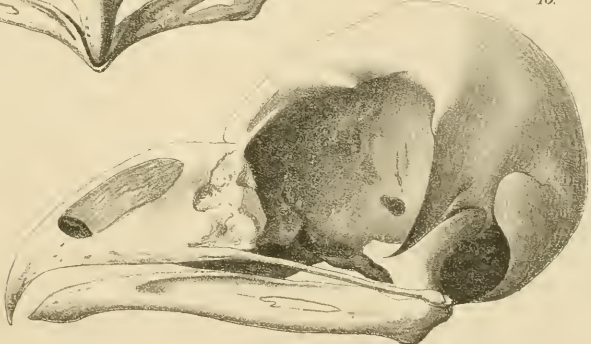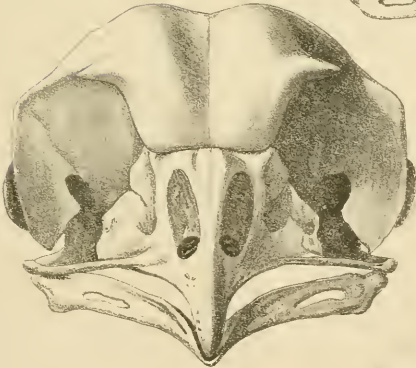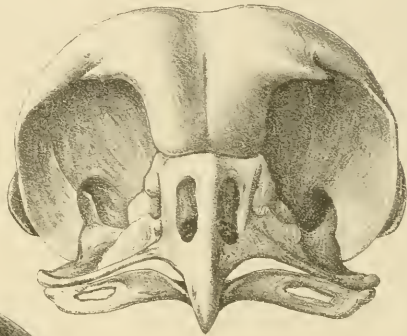
EXPLANATION OF PLATE XVIII.

FIG. 17.   Head of *Syrnium aluco;* right lateral view, with feathers removed and showing ear-opening ; natural size.

FIG. 18.   Head of *Syrnium aluco;* left lateral view, with feathers removed and showing ear-opening; natural size.

FIG. 19.   Skull of *Syrnium aluco;* basal aspect, with mandible attached ; natural size.

FIG. 20.   Skull of *Syrnium aluco;* superior aspect, with mandible attached ; natural size.
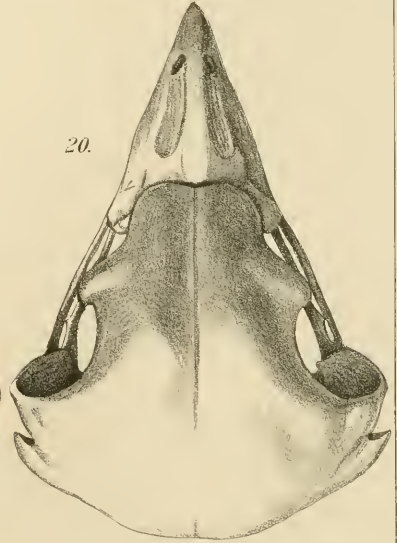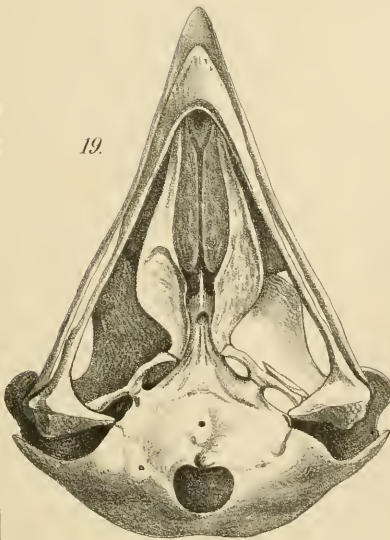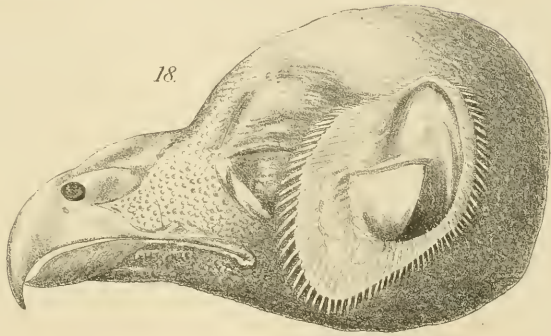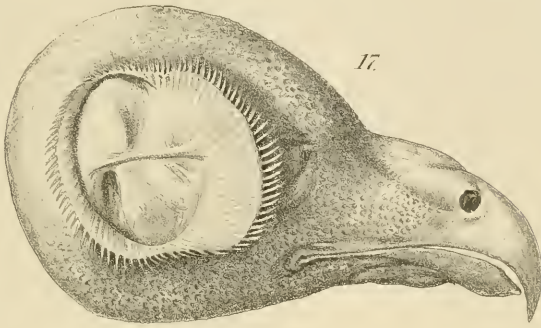
EXPLANATION OF PLATE XIX.

FIG. 21. Skull of *Nyctala tengmalmi ;* anterior aspect, with mandible attached ; natural size.

FIG. 22. Skull of *Nyctala tengmalmi ;* posterior aspect, with mandible attached; natural size.

FIG. 23. Skull of *Nyctala tengmalmi ;* right lateral aspect, with mandible attached; natural size.

FIG. 24. Skull of *Nyctala tengmalmi ;* left lateral aspect, with mandible attached; natural size.

FIG. 25. Head of *Nyctala tengmalmi ;* right lateral aspect, with feathers removed and showing ear-opening; natural size.

FIG. 26. Head of *Nyctala tengmalmi ;* left lateral aspect, with feathers removed and showing ear-opening; natural size.

FIG. 27. Skull of *Syrnium lapponicum ;* left lateral view, with mandible attached ; natural size.

Pl. XIX.
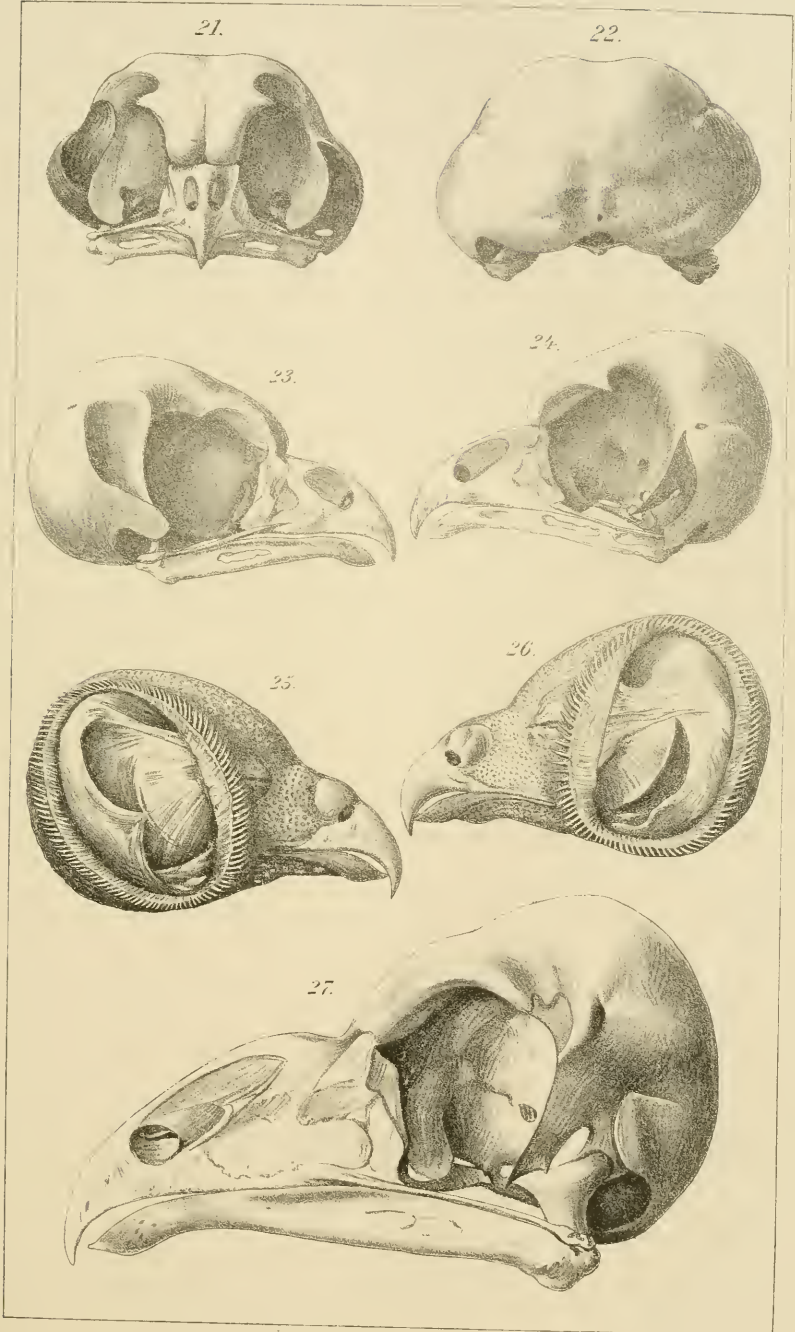
EXPLANATION OF PLATE XX.

FIG. 28. Head of *Syrnium lapponicum ;* left lateral aspect, with feathers removed and showing ear-opening; natural size.

FIG. 29. Skull of *Syrnium lapponicum ;* right lateral aspect, with mandible attached ; natural size.

FIG. 30. Head of *Syrnium lapponicum ;* right lateral aspect, with feathers removed and showing ear-opening; natural size.

Pl. XX.
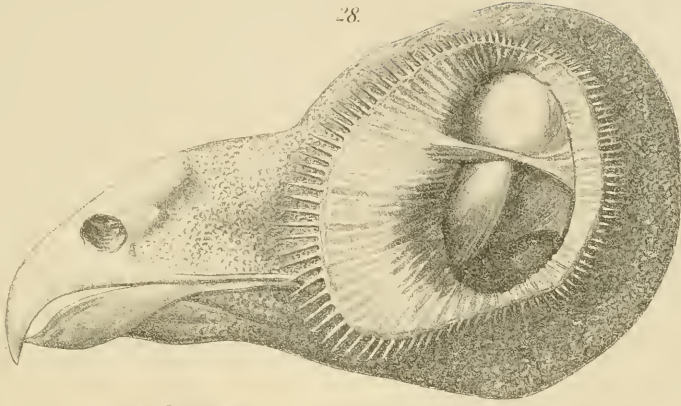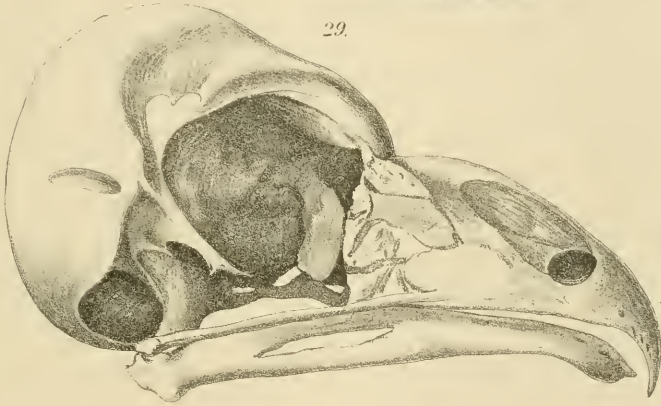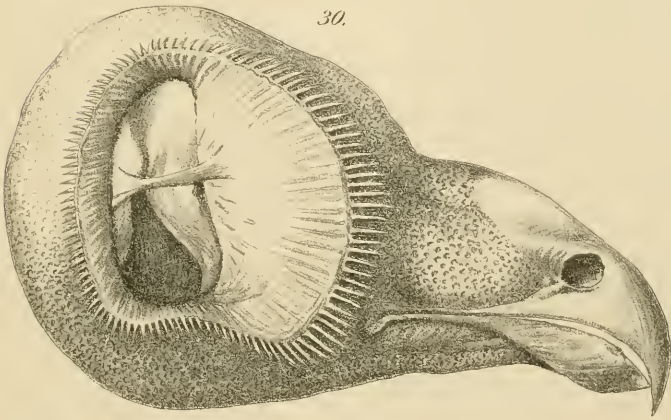
JOURNAL

OF

MORPHOLOGY.

NOTES ON AEOLOSOMA TENEBRARUM.

EDITH M. BRACE.

Aeolosoma tenebrarum, a fresh-water oligochaete belonging to the Aphaneura, has been supposed to hold a unique position among annelids, having been described as having a brain but no ventral nerve cord.

Vejdovsky, Maggi, and Beddard have given the more detailed accounts of its structure. Vejdovsky[1] found a few cells supposed to represent a rudimentary nerve cord which was not connected with the bilobed brain : " Bei *A. tenebrarum* treten auf der Bauchseite zerstreute Elemente hervor, die auf eine nervöse Natur hinweisen." And again : " Man erkennt in der Centrallinie der Bauchseite, eine kurze Strecke' hinter der Pharyngealregion, eigenthümliche, aber sehr undeutliche Zellen- und Fasserstränge, die jedoch mit dem Gehirnganglion nicht zusammenhängen." From the plates it is impossible to tell just what cells are referred to, but as they were found in the median line, they could not have been a part of the ventral cord.

Maggi[2] has described a brain, ventral cord, and lateral nerves for Aeolosoma : " Un cordone schiacciato che si estende lungo

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[1] Vejdovsky, F. System und Morphologie der Oligochaeta. 1884.
[2] Maggi, L. Intorno al genere Aeolosoma. 1865.

tutto la linea mediana ventrale dell' animale mandando laterala-
mente degli esili fili nervosi, ed un ganglio cefalico consti-
tuiscono per quel che potei scogere, il sistema nervoso degli
Aeolosoma." This description has not generally been credited.
He gives no illustrations of the nervous system, and his drawing
showing the mouth at the end of the prostomium, with the space
inside the prostomium designated as the buccal cavity, leaves
no place for a brain and is incorrect, as well as the statement
that the nerve cord extends along the median ventral line.

Beddard [1] says of *A. tenebrarum :* "This species alone shows
any traces of a ventral cord, which is very short and is not
connected with the brain."

A further study of the subject was undertaken in the Zoö-
logical Laboratory of the University of Chicago, at the sug-
gestion of Professor C. O. Whitman, to whom I am indebted
for the supervision of my work.

Material for study was found among the water plants in the
park ponds of Chicago, where it was especially abundant around
the water hyacinth and *Victoria regia.*

The worm is not an active swimmer, but prefers to lie among
the algae or to crawl between the meshes of a decaying leaf.
It is white and semi-transparent, and the integument is studded
with innumerable green oil drops contained in gland cells
which have their large nuclei flattened against the cell wall,
similar to the gland cells of the Turbellaria. A delicate chiti-
nous covering may be seen after treatment with reagents. The
worms feed upon algae or bits of decayed leaves and have a
tendency to collect on the sunny side of the aquarium. They
vary in length from 3 to 10 mm.

A number of worms will frequently get together, twist them-
selves into a ball, and remain so for a long time. It has been
suggested that this was connected with conjugation, but that
is improbable ; they are presumably feeding upon each other,
as one worm is usually found partly eaten, if the ball is pulled
apart. They have great powers of regeneration ; in one case,
where the head had been eaten away to the first pair of setae,
a new head was regenerated in about three days.

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[1] Beddard, F. E.   A Monograph of the Order Oligochaeta.   1895.

The head segment, which is broader than the following segments, is separated from them by a constriction, and seen from above, it appears to have a ciliated pit on each side like those of the Turbellaria.   The mouth is on the ventral side and is overhung by the prostomium, which is ciliated on its lower surface, and serves as a tactile organ.   There is no proboscis.

Intersegmental septa were not found, but the segmentation is defined by the nephridia and the setae.   Each segment has one pair of nephridia and four bundles of setae, placed dorso- and ventro-laterally.   There is also a segmental arrangement of single, nucleated muscle fibers which extend from the alimentary canal to the body wall between the setae sacs.

The alimentary canal comprises a circular mouth opening into a bell-shaped pharynx, followed by a narrow oesophagus, which extends through two segments and leads to a broad stomach with glandular walls, which extends through the sixth segment where it narrows into a straight intestine.   As the worms are transparent the movements of the cilia lining the alimentary canal may be seen in the living specimen.

The muscular system is comparatively simple.   There is one layer of longitudinal and one layer of circular muscle fibers just beneath the epidermal wall, and single nucleated fibers are connected with the setae and hold the various organs in place. These single nucleated fibers are especially numerous in the head and resemble the muscle plates of the Turbellaria.

The worms were under observation from October until July, and during that time they were constantly reproducing by fission, with sometimes as many as three zoöids developing at once.   Back of the seventh setigerous segment there is a fission zone in which all the tissues of the epidermal wall are greatly thickened, especially on the ventral side, where they nearly obliterate the body cavity.   The new brain arises as a dorsal thickening of the epidermis.   No sexual reproduction was observed.

Methods. — At the slightest irritation the worms will coil in a circle, throw off the contents of the gland cells, and contract so violently that the tissues are injured for study.   To

secure good specimens for sectioning they were mounted on a
slide and held in place by a cover-glass that pressed on them
slightly. They were then placed on ice for a few moments
until chilled and unable to contract any portion of the body,
when they were treated with the fixing fluid. The cold also
prevented abnormal activity of the glands, so that very perfect
preparations were obtained, although the worms will go to
pieces if left on the ice too long.

The fixing fluid after the second formula of vom Rath (picric
+ acetic + osmic + platinum-chloride) was found most effect-
ive in demonstrating the nervous system. Specimens were
left in this for fifteen minutes, washed in alcohol, and placed
in a 20 per cent solution of tannin in acetic acid for periods
of time varying from twenty-four hours to four days, or else
they were stained in section with safranin or iron-haematoxylin
after vom Rath.

Paraffin was used for imbedding, and sections were cut from
3–20 μ thick. Sections from 10–15 μ thick were found most
favorable for study.

Nervous System. — The brain lies in front of the mouth in
close contact with, and partly imbedded in, the epidermal wall
(Pl. XXI, Figs. 1, 6–8), the lower part projecting more or less
into the cavity of the prostomium. Its ventral and lateral sur-
face, so far as free from the epidermis, is covered by a delicate
nucleated membrane which may be seen in section. It has a
slightly bilobed appearance, as seen from above, each half
having a rounded anterior margin and a large posterior lobe,
the latter composed entirely of nerve cells (Pl. XXI, Figs. 2, 3).
Closely packed nerve cells with large granular nuclei cover the
whole dorsal surface, and are from three to four cells deep in
the anterior and posterior lobes, but only one layer deep in
the middle region where the anterior lobes meet (Pl. XXI,
Figs. 8, 10).

A pair of nerves composed of fibers partly from the brain
and partly from the oesophageal commissure, runs forward
from the brain into the prostomium, and another pair runs
back from the angle between the posterior lobes and the com-
missures (Pl. XXI, Figs. 1, 2, 5, 6, 9).

Commissure. — Immediately after leaving the brain the oeso-phageal commissure passes into an accessory ganglion, from which a nerve runs forward into the prostomium (Pl. XXI, Fig. 1). It then passes downward and backward, in close con-nection with the epidermal wall, to the ventral side, where it expands into a second ganglion before passing into the ventral cord (Pl. XXI, Fig. 1). The fibers of the commissure form a broad band which is clearly distinguishable, but it is often difficult to determine whether the cells along its course belong to it or to the epidermis.

Ventral Cord. — The two parts of the ventral cord are sepa-rated by about one-fifth of the diameter of the body and com-municate with each other by fibrous commissures, forming the ladder type of nervous system (Pl. XXI, Figs. 1, 12).

There is one pair of ganglia in each segment, and each ganglion is deeply bilobed, the anterior lobe being somewhat smaller, while the posterior lobe extends out farther in the body wall. The fibrous portion forms the greater part of the ganglion, and is covered by cells one layer deep (Pl. XXI, Fig. 11). In the posterior segments the ganglia are crowded together more closely than in the anterior segments (Pl. XXI, Fig. 1).

Lateral Nerves. — Four distinct lateral nerves are given off from each ganglion, two from the anterior and two from the posterior half (Pl. XXI, Figs. 1, 11).

This whole system of brain, ventral nerve cord, commissures, and nerves is connected throughout with the epidermal wall, no portion of it being entirely free in the body cavity. The cells of the ventral ganglia, as well as those of the brain, are often so closely connected with the epidermis that it is hard to find the boundary line between them. The nuclei of the gan-glion cells are of about the same size as those of the epidermis, but stain a little more deeply.

Ciliated Pits. — Vejdovsky[1] states that in Aeolosoma we find the only instance of an oligochaete possessing a pair of lateral ciliated pits, and he compares them with the ciliated pits of the Turbellaria. From the dorsal side the appearance is very similar to these organs in the Turbellaria, but frontal

[1] Vejdovsky, F. Thierische Organismen der Brunnenwässer von Prag. 1882.

sections of the ventral side show that they are not pits at all,
but the terminations of deep ciliated grooves which curve for-
ward and outward from the mouth to the edge of the pro-
stomium (Pl. XXI, Fig. 13).

The mouth is circular, bordered posteriorly and laterally with
a thick swollen lip, which may be greatly extended, and which
is continued as the posterior wall of the ciliated furrows.   The
cilia of the grooves, and those around the mouth, are excep-
tionally long.   Sense organs are as numerous along either
side of the furrows as on the prostomium.

Vejdovsky describes a nerve connecting the lateral pits with
the brain.   I find muscle fibers here, but no nerve, and from
the nature of the structure should not expect to find one.

Sense Organs. — There are many large pear-shaped cells,
that have the appearance of sensory cells, lying in all parts
of the prostomium and disposed through the body segments
(Pl. XXI, Fig. 14).   The cytoplasm of these cells is finely gran-
ular and deep-staining, the nucleus is of medium size, coarsely
granular, and usually eccentric, taking its position at the base
of the cell.   Between the nucleus and the opposite end of the
cell there is a large, sharply outlined, clear space containing
a refractive body with peculiar granulations at its periphery
(Fig. 14) which may represent an otolith.   These cells are
sometimes isolated, but are often collected into small groups
(Fig. 15), as seen to best advantage in the prostomium.   They
suggest sense organs of some kind.   They have no pigment.

At the anterior end of the prostomium there is a group of
about fifteen of these large compound organs, crowded together
so closely that their sides are somewhat flattened against each
other (Fig. 16).   Back of these there are smaller compound
sense organs, some distance apart, arranged in rows across the
ventral surface of the prostomium, and there are large sense
organs along both sides of the ciliated furrows leading to the
mouth (Fig. 16).   The smaller compound sense organs are
also found on the ventral side of the segments back of the
mouth.   All of these sense organs lie immediately under the
epidermis, so that they project slightly into the body cavity.

Aeolosoma undoubtedly possesses the essential annelidan

characteristics, although Vejdovsky favored classifying it with the Turbellaria on account of the similarities which he found between the ciliated pits, muscle plates, and gland cells of these forms, together with the structure of the brain and the supposed lack of a ventral cord.

The course of the large nerves running back from the brain has not yet been traced for an annelid; they present an anomalous feature which is most interesting from its suggesting a possible transitional form of nervous system between unsegmented and segmented worms. The position of the brain in the first segment, the continuity of the entire nervous system with the epidermis, and the wide separation of the halves of the ventral cord are primitive characteristics which would be consistent with such a form.

REFERENCE LETTERS.

ag. accessory ganglion.

b. brain.

c. connecting commissures of the ventral nerve cord.

cl. cluster of large sense organs in the end of the prostomium.

d. second ganglion of the oesophageal commissure.

ep. epidermal cells.

f. lateral ciliated furrows leading to the mouth.

g. gland cells.

l. lip bordering the mouth and the ciliated furrows.

ln. lateral nerves.

m. mouth.

mb. membrane lining the body cavity and covering the free, lower surface of the brain.

mf. muscle fibers attached to the brain and connecting it with the epidermis of the ventral side.

n. nucleus.

$n._{1\text{-}3}$ first, second, and third pairs of cephalic nerves.

nm. nuclei of the lining membrane.

o. refractive body.

oc. oesophageal commissure.

P. posterior ganglionic lobes.

v. vesicle containing a refractive body.

vc. ventral nerve cord.

EXPLANATION OF PLATE XXI.

FIG. 1. Frontal view of the central nervous system, reconstructed from sections. (× 210.)

FIG. 2. Ventral view of the brain. (× 1200.)

FIG. 3. Dorsal view of the brain, showing its bilobed form. (× 1200.)

FIG. 4. Frontal section near the middle of the brain, showing the first pair of nerves. (× 800.)

FIG. 5. Frontal section next above Fig. 4. (× 800.)

FIG. 6. Sagittal section of the brain in the plane of the anterior nerve and the posterior lobe. (× 800.)

FIG. 7. Sagittal section of the brain a little nearer the middle than Fig. 6. (× 800.)

FIG. 8. Sagittal section near the middle of the brain, showing a single layer of cells on the dorsal side. (× 800.)

FIG. 9. Oblique section of the brain, showing the posterior lobe and the roots of the first and second cephalic nerves. (× 800.)

FIG. 10. Cross-section of the brain in the plane of the commissure. (× 800.)

FIG. 11. Frontal section through a segment of the ventral nerve cord, showing the roots of the lateral nerves and of the connecting commissures. (× 1200.)

FIG. 12. Cross-section of the ventral nerve cord in the thoracic region, showing the ganglia connected by a commissure. (× 800.)

FIG. 13. Drawing showing the mouth with the furrows leading to it, and the prostomium, ciliated on the ventral side. (× 1200.)

FIG. 14. Pear-shaped sensory cell, with vesicle containing a refractive body. (× 1200.)

FIG. 15. Sense organs composed of several cells similar to those of Fig. 14. (× 1200.)

FIG. 16. Partial diagram showing the position of the compound sense organs on the ventral surface of the prostomium. (× 1200.)

Pl. XXI.
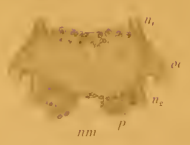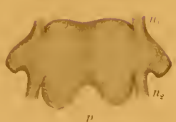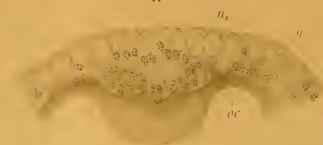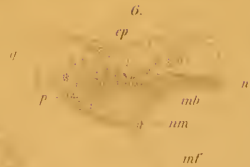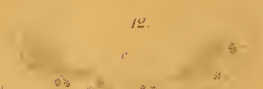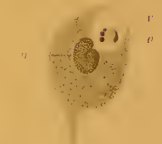
MORPHOLOGY OF THE MYXINOIDEI.

I. *SKELETON AND MUSCULATURE.*[1]

HOWARD AYERS AND C. M. JACKSON.

CONTENTS. PAGE

INTRODUCTION.

IT is now sixty-four years since Johannes Müller published the first part of his " Anatomie der Myxinoiden." Since that time almost nothing has been added to our knowledge of the anatomy of these interesting and important forms of ancestral

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vertebrates.   Not a few papers have dealt with the comparative anatomy of the myxinoid fishes ; but the authors of these papers, almost without exception, have drawn their anatomical knowledge from Müller's monograph, instead of going to nature for facts, the assumption being that Müller saw everything worth knowing and saw it right.   As illustrating the condition of our knowledge of myxinoid anatomy at the present time we shall cite the statements with reference to these animals in Parker and Haswell's *Text-Book of Zoölogy*, which is perhaps the most excellent zoölogical text-book in any language up to date.

On page 115, under the head of the "Distinctive Characters of the Craniata," it is stated that "the pharynx is of moderate dimensions and is perforated by not more than seven pairs of gills."   In 1894 · Dr. Ayers called attention to the general neglect, of which this is an example, of the fact that Bdellostoma dombeyi has from 6 to 14 pairs of gills, and that consequently in any statement of the general characters of the Craniata such an important and evidently ancestral character must be given prominent notice.   Instead of this it has been generally ignored.

In the same paragraph it is stated that there are at least ten pairs of cranial nerves, whereas in Bdellostoma we can show that there is no trace of the three eye-muscle nerves.   In the description of " Class 1 — Cyclostomata " it is stated that these animals are "distinguished from all other Craniata by the possession of a suctorial mouth devoid of functional jaws, by a single olfactory organ."   In 1890 Dr. Ayers proved conclusively that the Petromyzontes had a *pair* of nasal organs, and again, in 1894, that the myxinoids were no exception to the general rule among the Craniata, since Bdellostoma and Myxine possess a paired nasal organ.   Dr. Ayers also showed conclusively that the myxinoid mouth is not *suctorial* but *raptorial*.

With reference to the absence of functional jaws, we refer to the following pages and plates, in which we believe we have demonstrated that in these fishes the lower jaw is present and functional, while the upper jaw is rudimentary and fused with the cranial cartilages.   With reference to the inaccuracies and

omissions contained in Müller's and all subsequent descriptions of the cranial skeleton, we refer to the account which we give in the following pages.

On page 120 our authors write: "The whole branchial basket lies external to the gill pouches and branchial arteries, not, like typical visceral arches, in the walls of the pharynx." We are now prepared to show that the skeleton of the branchial apparatus in myxinoids occupies both positions, and that no such morphological distinction can be made between the internal and external branchial apparatus of the cyclostome fishes.

On page 128, under head of distinctive characters of the Cyclostomata, Parker and Haswell repeat some of the statements already noted and add the following : "The Cyclostomata are Craniata in which the mouth lies at the bottom of a sucker-like buccal funnel, and 'has no jaws.' A buccal funnel is present only in the Petromyzontes, for in the Myxinoids the mouth is terminal.

" Horny teeth are borne on the interior of the buccal funnel and on the large tongue." This statement is true, as regards the buccal funnel, only of the Petromyzontes, since the funnel is entirely wanting in the myxinoids. The statement that the teeth are also borne on the tongue brings us to a very interesting psychological phenomenon.

Ever since Johannes Müller described the so-called tongue of Bdellostoma, every writer on myxinoid anatomy, as well as every anatomist who has personally studied the anatomy of these forms, has accepted without question or modification this homology, based solely on the authority of the eminent German anatomist. Yet, strange to say, among all other fishes a tongue is held to be absent, and, stranger still, Müller's own account of the organ effectually disproves the homology he sought to establish. For a further consideration of this structure we refer to the body of this paper.

On page 128 they say that "there is no exoskeleton." This statement must certainly be modified, for the teeth are true exoskeletal structures.

In Bdellostoma the adult kidney is certainly sometimes both Pronephros and Mesonephros, not simply Mesonephros.

Parker and Haswell's statement that the genital products make their exit by the genital pores is not true of the myxinoids, for their eggs and spermatozoa both leave the body through the *abdominal pore.*

On page 129 it is stated that "the slime glands of Myxine contain peculiar thread cells, containing a much coiled thread which unwinds either before or after the discharge of the cells from the gland."

Under normal conditions the thread cell never unwinds until it is extruded from the glands; for its unwinding is due to the absorption of water by the thread and is a hygroscopic function entirely. The thread filament constitutes almost the entire mass of the oval spool.

The statement that "the branchial basket in the myxinoids is reduced to a vestige," that "it is quite rudimentary, being represented in Bdellostoma only by a small, irregular cartilage in the walls of the oesophago-cutaneous duct, and in Myxine by a smaller cartilage on the right side supporting the common external gill tube," is, as we shall see further on, due to the failure of Müller and subsequent anatomists to find the major part of the branchial skeleton. Every gill in Bdellostoma is provided with a branchial cartilage.

On page 130 the statement that "the neural canal is over-arched merely by fibrous tissue; there is no trace even of the rudimentary neural arches of the lamprey," is far from the truth, since we have discovered that Bdellostoma possesses a true neural arch as a part of its axial skeleton.

As regards Johannes Müller's descriptions of the muscles of Bdellostoma, we find them inadequate in many cases, since he failed to carefully trace out the forms of the muscles; and in other cases he failed to see the true homologies, and thus necessitated the changing of several names.

The erroneous statements in myxinoid anatomy which we have pointed out above are certainly sufficient to render necessary a careful reëxamination of the whole subject, and upon such a work Dr. Ayers has been engaged since 1892.

During the past year Mr. C. M. Jackson has taken part in a final review and careful redissection of Bdellostoma, with

partial dissections of Myxine and Petromyzon, and the results which we conjointly publish are vouched for alike by both of us. Mr. Jackson has made a complete, extremely careful, and independent study of the myxinoid skeleton, and Dr. Ayers has likewise restudied the musculature. We have checked each other's work on the skeleton, and while we do not presume to have exhausted the subject, we think we have not overlooked any very important anatomical details. The following papers on myxinoid anatomy are now ready for the press :

"On the Morphology of the Eye of Bdellostoma dombeyi."

"Ovigenesis in Myxinoids."

"On the Structure of the Slime Glands and Skin of Bdellostoma dombeyi."

Preservation of Material.

The Bdellostoma material used in the present investigation was killed and hardened in 10% formalin, and preserved partly in alcohol and partly in formalin, as well as in the following mixture of the two : 95% alcohol, 6 parts ; 2% formalin, 4 parts. The formalin has been found to be exceedingly valuable in preserving specimens for study of the cartilaginous skeleton. The effect of formalin is to give the cartilage a pink or reddish tinge, which gives an excellent differentiation and brings out many points of structural detail which might be overlooked in alcoholic material. Occasionally, however, non-cartilaginous connective tissue takes on the same tinge, so that in all doubtful cases a histological examination is necessary. In all other respects as well, the formalin material is far superior to alcoholic material.

The Skeleton of Bdellostoma.

The skeleton includes all those condensations of the mesoderm which are specially developed to support or protect the softer tissues of the body. The skeletal parts which are within the body make up the endoskeleton, while those in the external integument constitute the exoskeleton. In the latter case the mesoderm enters into intimate relations to ectodermic structures.

Endoskeleton.

The endoskeleton, excepting the notochord, is mainly membranous. Here and there it is reinforced by cartilaginous rods, bars, rings, and plates, which lie within the membranous skeleton, and may be regarded simply as chondrifications along certain lines of stress. No bone or calcified tissue of any kind is present anywhere in the skeleton.

The *notochord* (Pl. XXII, Figs. 1, 2, 4–6 ; Pl. XXIII, Fig. 14, *nt*) is an elastic cylindrical rod extending nearly the entire length of the body (14–26 inches). It lies in the median dorsal line, deeply imbedded between the lateral trunk muscles of the right and left sides. Anteriorly it tapers to a point and ends between the parachordal cartilages. Posteriorly its termination lies in a groove upon the dorsal surface of the median ventral cartilage, and its end lies a short distance in front of the posterior end of the spinal cord. The shape of the notochord in cross-section varies somewhat in different regions. Though approximately circular, it is usually somewhat flattened or slightly concave above, where it is in contact with the neural tube. In the extreme anterior region it is laterally compressed and somewhat triangular in cross-section.

The *notochordal tissue* (Pl. XXII, Fig. 3, *nt*) is made up of cells containing very large vacuoles (*vc*) filled with a clear, homogeneous liquid, around which the protoplasm forms an extremely thin layer. The chordal tissue has a honeycomb structure which produces the characteristic reticular appearance seen in sections. The cells are somewhat flattened, with angular walls, and have their long axes placed radially, as shown in Figs. 1 and 2 of Pl. XXII. The nuclei are prominent, and generally found lying in the protoplasmic layer toward the ventral side. In some cases, however, especially toward the outer surface of the notochord, nuclei are found near the center of the cells, and apparently connected with the cell wall by protoplasmic strands. The striking resemblance between chordal tissue and certain vegetable tissues has often been noted.

The central axis of the notochord is marked by a dense white fibrous *central core* (Figs. 1, 2, *fc*) which extends throughout almost its entire length. The form and size vary somewhat in different regions. In the head region the core is triradiate in cross-section (Fig. 1). In the gill region it is flattened dorso-ventrally, while farther back in the body region it becomes more irregular, and relatively much larger. The central core is made up of dense, coarse fibers, closely packed, and running for the most part longitudinally. The fibers run out among, and are continuous with, the walls of the chordal cells. In the extreme anterior region of the notochord, and in some places near the posterior end, the fibrous core fades out and passes gradually into ordinary chordal tissue. The core contains no traces of nerves or blood vessels.

Surrounding the chordal tissue we find a cellular sheath having the appearance of an epithelial layer (Fig. 3, *ex*). In the anterior notochordal region, where the cells of this sheath are larger and more definitely marked, they form a one-celled layer, the cells being somewhat cubical above the notochord and columnar below. Toward the posterior region the layer becomes two or three cells deep, and the individual cells are smaller and flattened. The nuclei are large, and vacuoles are often found in the ends of the cells lying next to the chordal tissue. This layer is directly connected with the chordal cells, and is not separable as a distinct layer. All stages are found between the solid, undifferentiated cells of the external cellular sheath, and the deep chordal cells within walls enclosing large vacuoles.

The *notochordal sheath* proper (Pl. XXII, Figs. 1–4, *sh*) is a thick, strong investment immediately surrounding the notochord. It is fibrous rather than laminated in structure, at least for the most part. The fibers are chiefly circular in direction, but vary somewhat in different regions. They are usually so closely packed as to give a homogeneous appearance to the sheath. No nuclei, nerves or blood vessels are present. In most places the notochordal sheath seems to be made up of different layers, whose fibers vary somewhat in direction. Three layers may usually be distinguished, especially in the

anterior region (Fig. 3, sh_1, sh_2, sh_3).   The fibers of the inner and outer of these three layers have the same general direction, and are usually parallel, while those of the middle layer are often interlaced in the most complex fashion (see Fig. 3, sh_2).   The relative and actual thickness of these layers varies considerably in different regions, but the inner layer is always the thinnest.   In some places all three layers are fused into one, the boundaries being indistinguishable.

The external boundary of the notochordal sheath is always formed by a thin, dense membrane, which stains deeply.   This is the *elastica externa* (Fig. 3, *mle*).   Though usually homogeneous in appearance, in some places it shows a distinctly fibrous structure.   Whether the *elastica externa* is really a part of the notochordal sheath proper, or a derivative of the surrounding skeletogenous layer, is a matter of doubt.   The structure and appearance of the layer indicate the former. It may be remarked that although both the central core and the notochordal sheath are doubtless derived from the chordal tissue (the latter from the cellular sheath), it is evident that neither is a *cuticular* product, in the ordinary sense of the term, although it is always so described.

An interesting histological variation is found in the anterior region, where the notochord lies imbedded between the parachordal cartilages.   The *elastica externa*, which separates the notochord from the surrounding cartilage, becomes irregular in outline, and the different sheath layers fuse into one.   The cellular sheath of the chordal tissue becomes irregular, while the chordal tissue itself is very gradually replaced by *cartilaginous* tissue.   Thus near the anterior end of the notochord we find *the space inside the notochordal sheath entirely filled by cartilage* (Fig. 4, *nt*)   Just behind the tip of the notochord, which projects in the median line in front of the parachordal cartilages (Pl. XXIII, Fig. 7), the notochordal sheath disappears, and we find that the tip of the notochord is a conical *cartilaginous structure*, surrounded only by the skeletogenous layer. The cartilage replacing the chordal tissue has an appearance somewhat different from that of the surrounding parts.   Near the posterior end of the notochord we find in places a similar

development of *cartilage within the notochord*, but not so marked as in the anterior region.

The *skeletogenous layer* (Plate XXII, Figs. 1–4, *sk*) lies immediately external to the *elastica externa*. It surrounds the notochord, and above, on each side, it divides. One layer continues in contact with the *elastica externa*, the other rises and arches to form the *neural tube*. This tube forms a continuous semicylindrical canal just above the notochord, with lateral foramina for the exit of the spinal nerves. Within this neural tube there is also a fibrous layer closely investing the spinal cord (*sp*) and another which covers the so-called "fatty tissue" (*F*) which more or less completely fills the dorsal part of the tube. The skeletogenous layer is composed of strong fibrous connective tissue, well supplied with blood vessels and nerves. It is directly continuous with the intermuscular septa, as shown in Figs. 1 and 2, *ims*. Above, it is continuous with the median septum, separating the myotomes of the right and left halves of the body (*mds*). Below, on each side, it is continuous with the *fascia superficialis interna*, which surrounds and supports the body cavity. In the skeletogenous layer, especially near the inferior lateral angles of the neural tube, we occasionally find incipient patches of cartilage which are interesting as the first traces of the cartilaginous structures more fully developed in the head and tail region.

Skeleton of the Head Region.

(Pl. XXII, Figs. 5, 6; Pl. XXIII, Fig. 7.)

The skull, as a whole, may be described as forming two tubes, a smaller dorsal and a larger ventral. The smaller dorsal tube includes the cranium, posteriorly, and the nasal tube, anteriorly. The larger ventral tube is composed of a framework of cartilages surrounding the mouth and pharynx. The mouth is surrounded by a system of cartilages supporting the tentacles. For some distance behind the mouth the side walls of the ventral tube are unsupported by cartilage, but posteriorly the tube is surrounded by the complicated framework

of cartilages known as the "pharyngeal basket"; while in the gill region the series of small gill bars represents the remarkable "branchial basket" of Petromyzon.

The membranous cranium (Pl. XXII, Figs. 4–6, *cr*) is a direct continuation of the skeletogenous layer surrounding the spinal cord (*cf.* Figs. 1 and 4). The "fatty tissue" is also continued into the cranial cavity as a thick layer of loosely interwoven elastic fibers (*F'*). The cranium is somewhat flattened dorso-ventrally.

The anterior wall has a median dorso-ventral ridge which projects backward between the olfactory lobes of the brain. The floor is concave within, both laterally and longitudinally. The roof projects upward in a median longitudinal septum (Fig. 4, *mds*) between the myotomes of the right and left halves of the body. The cranium is perforated laterally for the exit of all the cranial nerves, except the olfactory, which perforate the anterior wall. The cranial walls have no cartilaginous support, excepting the floor, which is supported by the parachordal cartilages and the trabeculae.

The parachordal cartilages (Pl. XXII, Fig. 4; Pl. XXIII, Fig. 7, *pc*) are a pair of cartilages which support the posterior portion of the cranial floor (*os basilare* of J. Müller). The parachordals are composed chiefly of a pair of roughly prismatic cartilaginous bars lying in the skeletogenous layer of the membranous cranium. The posterior halves of the parachordals are situated one on each side of the notochord. They are nearly parallel, converging slightly toward the median line, from behind forward. The anterior halves of the parachordals diverge, and extend antero-laterad to fuse with the posterior ends of the trabeculae. The diverging anterior halves of the parachordal bars form the posterior boundary of the large hypophysial fontanelle (see Fig. 7). In the angle formed by these diverging bars the cartilaginous anterior tip of the notochord projects in the median line, as previously noted. In the posterior region the parachordal cartilages fuse together in the median line under the notochord, so that the latter appears to lie in an open groove, as shown in cross-section in Fig. 4. In some places, however, the parachordal cartilages fuse together both above

and below the notochord, forming a complete cartilaginous sheath. The form and extent of this sheath varies considerably in different specimens.

The parachordal cartilage on each side is expanded laterally into a thin sheet of cartilage forming the *auditory capsule* (Pl. XXII, Fig. 4; Pl. XXIII, Fig. 7 *A*). These capsules are two somewhat kidney-shaped structures, with their long axes lying parallel to the parachordals. Their shape in cross-section is shown in Fig. 4. The ventral walls are continuous with the inferior external angles of the parachordals. In the anterior and posterior regions of the capsules the dorsal walls are continuous similarly with the superior external angles of the parachordals; in the middle region, however, the dorsal walls of the capsules are separated from the parachordals by the auditory foramina (Pl. XXII, Fig. 4; Pl. XXIII, Fig. 7, *f*). These are two large elliptical foramina extending the greater part of the length of the auditory capsule. The foramina are closed by the membranous cranial wall, which is perforated by small openings for the exit of the auditory nerves. The posterior third of the auditory capsules is continuous, externally, with the upper ends of the hyoid arches. Anteriorly the walls of the auditory capsules, together with the anterior ends of the parachordals, are fused with the posterior ends of the trabeculae.

The *trabeculae* (Pl. XXII, Figs. 5, 6; Pl. XXIII, Fig. 7, *Tr.*, *tr*) are a pair of latero-inferior bars which form a frame for the support for the anterior half of the membranous cranium. Posteriorly they are comparatively thick and strong. At their posterior ends they have three attachments : (1) an internal, with the anterior ends of the parachordal cartilages; (2) a median, with the anterior wall of the auditory capsule; and (3) an external, with the posterior process from the pterygo-quadrate cartilage. Anteriorly, a short distance behind the anterior end of the membranous cranium, the anterior horns of the trabeculae extend downward, forward, and inward, as slender bars (*tr*) which merge into the median hypophysial plate. At the point of junction of the anterior horn (*tr*) with the main bar of the trabecula (*Tr*) there is given off externally,

on each side, a short, thick lateral process which connects the
trabecula with the palatine bar (*Pl*). The space enclosed by
the trabeculae (including the hypophysial plate) and the an-
terior halves of the parachordals we shall call the hypophysial
fontanelle. The floor of the membranous cranium occupying
this fontanelle forms the roof of the hypophysial canal. This
canal is a short tube which opens posteriorly into the pharynx,
anteriorly and superiorly into the nasal tube and nasal cap-
sule. The floor of the hypophysial canal, which is a part
of the roof of the pharynx, is supported by the median
unpaired hypophysial plate (Pl. XXII, Fig. 6; Pl. XXIII,
Fig. 7, *Hp*). It is a flattened cartilaginous plate, very thin
posteriorly, but thicker anteriorly. At the posterior end the
plate spreads out into two lateral processes, which are some-
times bilobed at the tips. These processes, together with the
posterior end of the body of the plate, support the posterior
margin of the valvular septum between the hypophysial and
pharyngeal canals. At the junction of the posterior third with
the anterior two-thirds of the hypophysial plate, it widens out
laterally on each side to fuse with the corresponding anterior
ends of the trabeculae, as previously described. So that the
trabeculae, with their hypophysial expansion and the para-
chordals, completely encircle the hypophysial canal. Near the
anterior end the hypophysial plate divides into two antero-
lateral processes (Fig. 7, *k*) which are closely attached by liga-
ments to the inner sides of the palatine bars, a short distance
behind their connecting commissure (*cm*).

The *olfactory capsule* as distinct from the entire olfactory
chamber (Pl. XXII, Figs. 5, 6, *oc*) is a saccular structure di-
rectly in front of, and in size corresponding to, the membra-
nous cranium, whereas the whole chamber is tubular in form.
Anteriorly and inferiorly the capsule is open, communicating
with the nasal tube and hypophysial canal. The posterior
wall is formed by the anterior wall of the cranium, and is per-
forated near the dorsal margin by a row of foramina on each
side which transmit the large branches of the olfactory nerves.
The lateral and dorsal walls of the olfactory capsules are sup-
ported by a framework of cartilage which may be described as

consisting of nine longitudinal bars and two transverse connecting bars, an anterior and a posterior.

Of the nine longitudinal bars, seven are dorsal and two lateral. The seven dorsal bars are slender parallel rods, separated by spaces wider than the width of a bar. These bars support the roof of the olfactory capsule and correspond to the seven folds of mucous membrane which hang from the roof inside the olfactory capsule. The lateral bars are two somewhat irregular flattened cartilaginous plates which support the lateral walls of the olfactory capsule. The exact shape of these cartilages, which we shall call the *lateral plates*, varies in different specimens. Anteriorly a process is given off from the inferior angle, which runs forward and connects with the end of the last nasal arch. The anterior superior angle of the plate is connected with the end of the anterior connecting bar. The anterior portion of the lateral plate extends in a longitudinal direction. Posteriorly, however, the plate curves around, extending downward and inward toward the median line under the olfactory capsule. The posterior ends of the two plates do not fuse, but end in the inferior wall of the capsule near each other. A short distance from the posterior ends of the lateral plate a slender process is given off posteriorly, which is closely connected with the corresponding trabecula near the point of attachment to the palatine bar. A case of actual fusion between the lateral plate process and the trabecula has not been observed, however.

The posterior connecting bar is a narrow strip placed transversely across the roof of the olfactory capsule. It connects the posterior extremities of the nine longitudinal bars. The anterior connecting bar is a similar band, transversely placed, and giving attachment posteriorly to the anterior ends of the nine longitudinal bars. Anteriorly the anterior connecting bar gives off three processes which connect it with the last nasal arch. Thus, by means of these three connecting processes, together with the anterior processes from the lateral plates, the space between the last nasal arch and the anterior margin of the olfactory capsule is divided into four parts — two dorsal, somewhat elliptical, and two lateral, nearly circular.

The *nasal tube* (Plate XXII, Figs. 5, 6, *NT*) is a direct anterior continuation of the olfactory capsule. It is slightly funnel-shaped, being widest at the anterior end, and narrowest a short distance in front of the posterior end. The length of the nasal tube is greater than that of the olfactory capsule and cranium combined. The nasal tube and the olfactory capsule have a combined length about twice that of the cranium. Anteriorly the nasal tube opens to the exterior. Posteriorly it communicates with the olfactory capsule and the hypophysial tube, as previously described. The membranous wall of the tube is supported dorsally and laterally (and to some extent ventrally) by a series of cartilaginous nasal arches. When viewed from the sides, or from above, these arches appear as rings ; but when viewed from below, it is seen that the ventral fourth of the ring is lacking. The nasal arches are usually nine in number, but sometimes eight or ten. The anterior four, and the posterior two arches have cartilaginous connecting processes ; the remaining arches are independent. The anterior arch is the largest and widest. It is inclined sharply forward, its plane making with the horizontal plane an angle of about 45°. The anterior, or first arch is connected with the second by a median dorsal connecting process. Inferiorly, the first four arches end on each side in a longitudinal connecting bar. This bar ends in two terminal processes. The anterior process extends downward, inward, and forward, curving around and running forward parallel to the corresponding process from the opposite side. The two processes end near each other in the anterior inferior margin of the nasal tube, but do not connect, either with the labial cartilages or with each other. (In one case the two processes were found fused together, so that a complete cartilaginous ring was formed surrounding the anterior end of the nasal tube.) The posterior terminal processes of the connecting bar arise opposite the ends of the third and fourth arches. They are short, extending downward, inward and forward, ending in a free tip. The posterior two nasal arches are either fused together in the median dorsal line, or connected by a short process. Their ends are not connected. The last nasal arch connects with the cartilages

of the olfactory capsule by five processes, one median, two
lateral, and two ventral, as already described.   The penulti-
mate and antepenultimate arches always appear concave for-
wards, when viewed from above.

The extraordinary degree of variation in the external form
of Bdellostoma, and especially in the number of gills and teeth,
is a fact which has already been emphasized by Dr. Ayers.
It is even more remarkable to find a similar variation in the
more deeply lying structures, including the skeletal parts.
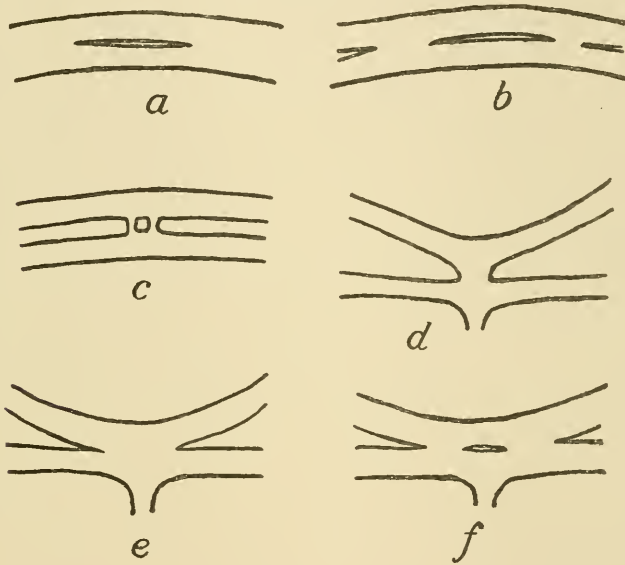
Some of the more important variations have already been
referred to, but in order to give a more adequate idea of
the actual amount of variation, we shall give as an example
the main points of difference found in the nasal arches of
a dozen specimens taken at random.

The nasal arches are found to vary to a considerable extent
in number, form, and size, both relative and absolute.   In
number, as has been stated, the nasal arches vary from eight
to ten, but they are usually nine in number.   In this count
the two transverse bars of the olfactory capsule, though seri-
ally homologous with the nasal arches, are not included.   The

number of arches seems, in general, to vary according to the
size of the specimen, but this is by no means always the case.
In one instance a very small specimen (about fifteen inches
long) possessed ten nasal arches. In an 8-arched form the
fifth arch was unusually wide, and was divided in the dorsal
portion by a small slit (Fig. *a*), indicating the fusion of two
arches. In a 10-arched form a rudimentary eleventh arch was
represented by a lateral piece between the sixth and seventh
arches on the right side, which extended dorsally nearly to the
median line. Below, it fused with the seventh arch near the
end. On the left side in this same (seventh) arch the lower
end was bifurcated. In the same specimen there was a short
process projecting backward from the sixth arch, a little to
the right of the median line. In a 9-arched form the fifth
and sixth arches were connected by a median longitudinal bar.
In another 9-arched form the fifth and sixth arches were
partly fused together dorsally, a small slit separating them in
the median line, as shown in Fig. *b*. In a 10-arched form the
sixth and seventh arches were somewhat similarly joined on
each side of the median dorsal line, as in Fig. *c*. The last
two arches were always found joined in the median dorsal line.
Usually this was accomplished by a short longitudinal bar, as
in Fig. *d*; but in several cases the two arches were partly
fused, as in Fig. *e*, and once as in Fig. *f*. The anterior arch
is wide and thin, usually perforated by from two to four circu-
lar foramina on the dorsal side. In one case the arch was
found unperforated. It was usually smooth on the anterior
margin, but in one case it was found marked by several irregu-
lar notches. The lateral plates of the olfactory capsules were
found in a few instances perforated by two foramina, but
usually unperforated. They vary considerably in shape and
extent, as previously stated. Having completed the descrip-
tion of that part of the skull which constitutes the smaller
dorsal tube, we shall now consider those structures which
surround and support the larger ventral tube. In the antero-
lateral region, just below the level of the floor of the nasal tube,
are two bars, the *cornual cartilages* (Pl. XXII, Figs. 5, 6;
Pl. XXIII, Fig. 7, *cc*). They are a pair of slender cartilaginous

rods, lying in the same horizontal plane, but curved so as to be convex outward. They are closest together posteriorly, where they are attached on each side of the palatine commissure (*cm*). Anteriorly they diverge laterally and curve around so that the most anterior portions run parallel and end in a slender tip on each side of the nasal tube, a short distance behind its anterior end. The anterior ends of the cornual cartilages are connected by ligaments with the lateral labial cartilages at the base of the first tentacular cartilage.

In the median line between the cornual cartilages, and directly under the floor of the anterior portion of the nasal tube, is the unpaired *subnasal cartilage* (Pl. XXII, Fig. 6; Pl. XXIII, Fig. 7, *N*). This is a rather strong, fusiform rod, thickest in the middle portion, somewhat nearer to the anterior end, and tapering anteriorly and posteriorly. Near the posterior end it becomes flattened and at the extremity expands laterally into two small processes. The posterior end is attached to the dorsal side of the palatine commissure, just in front of its posterior margin. Anteriorly it attaches in the median line to the transverse labial cartilage.

The *labial cartilages* (Pl. XXII, Figs. 5, 6; Pl. XXIII, Fig. 7) support the oral margin dorsally and laterally, and give attachment to the tentacular cartilages. The *transverse labial cartilage* is an unpaired transverse bar lying in the dorsal portion of the oral margin. The lateral halves meet at an angle in the median line, so that the bar may be described as an arch, concave forward. Laterally the ends of the bar are directly continued into the second pair of tentacular cartilages (t_2). These extend outward and forward, tapering gradually to a point. In the median line, posteriorly, the transverse labial cartilage is attached to the anterior end of the subnasal cartilage, as already mentioned.

The *lateral labial cartilages* (Pl. XXII, Figs. 5, 6; Pl. XXIII, Fig. 7, *Lc*) are a pair of somewhat slender cartilaginous rods lying in the lateral margin of the mouth, surrounded by muscles, and supporting the first and third pairs of tentacular cartilages. Posteriorly they are attached to the antero-external angles of the basal plate. From this point they curve forward,

upward, and inward. A little below the level of the floor of
the nasal tube, and almost directly below the anterior ends of
the cornual cartilages, they give off, on each side, a process
which continues outward and forward as the third tentacular
cartilage (t_3). Like the second pair, these cartilages are coni-
cal in shape, tapering gradually to a point. A little further
upward and inward, the lateral labial cartilage gives off a short
process (mp) which extends inward toward the median line, and
abuts against the side of the nasal tube, just above the longi-
tudinal connecting bar. This process is attached to the nasal
tube by a membranous ligament, but is not joined directly to any
cartilages of the nasal tube. The lateral labial cartilage now
curves outward, and then turns sharply upward, forward, and
inward to terminate in the long, slender, conical cartilage of
the first tentacle (t_1). It is at the base of this tentacle that the
ligament connecting with the anterior end of the cornual carti-
lage is attached. The fourth tentacular cartilages (Pl. XXII,
Fig. 6; Pl. XXIII, Fig. 7, t_4) are remarkable for their shape
and position. They are irregular, somewhat elliptical, thin
plates of cartilage, nearly vertical in position, and curved later-
ally so as to be slightly convex anteriorly. From the upper
end a short process extends upward, forward, and outward
toward the base of the third tentacle, with which it is connected
by a ligament. The cartilaginous plates of the fourth tenta-
cles are not fused with any other cartilages, but are supported
by the surrounding muscles and connective tissue. It is ques-
tionable whether they are true tentacles in the sense that the
other three pairs are. In life they appear as slight folds in
the skin on the infero-lateral margin of the oral opening. The
floor of the skull is formed by a large, strong, trough-shaped
cartilaginous apparatus, the *basal plate* (Pl. XXII, Figs. 5, 6;
Pl. XXIII, Figs. 7–9, B, B', B''). The basal plate may be divided
into three segments, an anterior (B), a middle (B'), and a pos-
terior (B''). The anterior segment (Pl. XXIII, Figs. 8, 9, B)
is made up of two pairs of flattened longitudinal bars, an inner
and an outer pair. The inner pair (Ai) is the smaller. In the
anterior two-thirds they are fused together in the median line,
no trace of the fusion showing on the ventral side, and only a

faint indication on the dorsal side. This anterior fused portion
is divided by a transverse suture which is concave forward, and
is well marked on the ventral side, but less distinctly on the
dorsal side. In the posterior third, the inner pair of bars
diverge slightly, enclosing in the angle between them the ante-
rior half of a diamond-shaped space. The posterior ends are
attached to the inner thirds of the anterior ends of the median
cartilages (*Mc*). At the anterior end, the inner bars, just in
front of the fused portion, are extended outward, forward, and
upward as a pair of short, flattened processes. The flattened
inner bars are not placed in the horizontal plane, but are in-
clined toward each other so that a dorsal groove is formed by
their meeting in the median line. This groove is shallow pos-
teriorly but deeper anteriorly. In it the median portion of the
overlying dental plate glides. The anterior continuation of
this groove is formed by the anterior processes, whose faces
look forward, as well as upward and inward. An anterior
notch is thus formed, through which the tendon of the pro-
tractor muscles of the dental plate glides. The outer pair of
bars belonging to the anterior segment is larger, thicker, and
stronger than the inner (Pl. XXIII, Figs. 8, 9, *Ac*). They are
somewhat flattened dorso-ventrally, and are wider at the ante-
rior end. Their posterior ends are attached to the outer two-
thirds of the anterior ends of the median cartilages, besides the
posterior ends of the inner pair. Anteriorly, the outer bars at
first diverge laterally and then curve inward slightly toward the
anterior ends, which are truncated and lie a short distance in
front of the anterior ends of the inner bars. The external
angles of the anterior ends give attachment to the lateral labial
cartilages (*Lc*). The outer bars are separated from the inner
pair by a narrow longitudinal slit. The ventral surfaces of the
outer bars are convex, the dorsal surfaces nearly plane. The
latter are inclined so as to be nearly in the same plane as the
corresponding inner bar, so that they aid in forming the grooved
dorsal surface on which the dental plate glides. The outer
bars are nowhere directly connected with the inner, the two
being bound together by strong ligamentous bands. The mid-
dle segment, *B'*, of the basal plate lies immediately behind the

anterior segment, with the cartilages of which it is connected
by a slightly movable joint.   The middle segment is made up
of a pair of flattened plates, large, thick, and strong, which are
closely united in the median line, excepting at the anterior
end (Pl. XXIII, Figs. 8, 9, *Me*).   In the anterior fifth, the
plates diverge laterally and enclose the posterior half of the
diamond-shaped space which extends forward between the pos-
terior ends of the inner bars of the anterior segment.   At the
postero-external angle on each side a short cylindrical process
(Pl. XXIII, Figs. 7, 8, *sb*) is given off which extends upward,
outward, and backward to fuse with the lower ends of the first
two branchial arches (br_1 and br_2).   The plates of the median
segment are nearly rectangular in outline, thicker externally
than internally, and curved so as to be convex ventrally and
concave dorsally.   They are about as long as the cartilages of
the anterior segment and as wide as both anterior pairs com-
bined.   They are inclined so as to form a continuation of the
trough described in the anterior segment, the chief difference
being that the trough is narrower and deeper in the middle seg-
ment.   Moreover, each of the plates of the middle segment
has two longitudinal grooves on its dorsal surface.   The first
is along the internal margin and unites with that of the oppo-
site plate to form a median groove which transmits the main
division of the *M. retractor mandibuli* tendon.   The second
groove is along the median dorsal surface, and serves to trans-
mit, on each side, the lateral division of the same tendon (see
Pl. XXIII, Figs. 10, 11, *t*).[1]   Anteriorly, as already described,
the middle segment articulates with the cartilages of the ante-
rior segment.   Posteriorly it is immovably attached to the
third segment, *B''*.

The third segment, *B''*, forms the posterior division of the
basal plate, and is considerably longer than both the first and
second segments combined.   It is a heavy, unpaired structure,
corresponding in width to the middle segment anteriorly,

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[1] It is worthy of mention that on the ventral surface the cartilages of the
median segments show distinct longitudinal markings on each side, which may be
obsolescent sutures indicating that the pair of plates is composed of *two* fused
pairs, corresponding to the two pairs of the anterior segment.

but tapering to a point posteriorly. It has a deep dorsal groove corresponding to that formed by the anterior segments, and transmitting the tendon of the *M. retractor mandibuli.* Consequently it is *U*-shaped in cross-section. Posteriorly this groove flattens out, and the third segment of the basal plate becomes continuous dorsally and laterally with the fibers of the *constrictor musculi mandibuli* muscle. It is quite evident that the third segment is not a true cartilage, but is formed by a chondroidal modification of the tendon of the "constrictor" muscle. It is white in color when preserved in formalin, contrasting strongly with the reddish-colored anterior cartilaginous segments. A histological examination shows it to be made up of a very peculiar tissue, more nearly related to notochordal tissue than to the cartilage found in the other skeletal parts.

The *dental plate* (Pl. XXII, Fig. 6, *D.* ; Pl. XXIII, Figs. 10, 11) is a cartilaginous structure resting upon the anterior segment of the basal plate and supporting the four rows of horny teeth. It is a flattened framework, consisting of two arches, an anterior and a posterior. The anterior arch is made up of a median piece (Pl. XXIII, Fig. 11, *Md*), two lateral plates (*aa*), and two pairs of connecting processes (*r, v*).

The median piece is a thin elliptical plate, with its long axis in the median line. It is curved from side to side, the downward convexity fitting into the dorsal groove of the anterior segment of the basal plate. The anterior end is somewhat depressed where it passes into the protractor tendon.

The anterior half of the median piece extends farther forward than the anterior margins of the lateral plates. Connecting the median piece with the lateral plates are two pairs of lateral processes (Fig. 11, *r, v*). The anterior processes (*r*) are short and are given off from the middle of the median piece on each side, and extend backward, outward, and slightly upward to the antero-internal angles of the corresponding lateral plates. The posterior processes (*v*) are longer. They extend from the posterior angles of the median piece on each side, backward, outward, and upward to join the lateral plates about the middle of their internal margin. The lateral plates

(*aa*) are large, thin, oval sheets of cartilage, broad anteriorly
and narrower posteriorly.   They are curved slightly so as to
be somewhat convex upward and concave downward.   Their
ventral surfaces rest directly upon the external bars of the
anterior segment of the basal plate.   The upper surfaces of
the two plates are, therefore, correspondingly slightly inclined
toward each other in the normal position.   The external
margins of the plates are smooth and rounded.   The internal
margins are nearly straight, and approach each other in the
median line anteriorly (being separated from each other only
by the median piece and anterior processes) but diverge pos-
teriorly, being widely separated at the posterior ends.   The
internal margins are connected with the median piece, as
already described.   The posterior processes are separated from
the anterior, and from the lateral plate, on each side, by a
long, narrow slit, parallel to the inner margin of the lateral
plate.   On account of this slit, the posterior processes, which
are long and slender, have the appearance of a third arch
closely united with the anterior arch.   At the posterier ex-
tremity, each lateral plate has two processes, an external (*e*)
and an internal (*i*).   The external process curves around up-
ward and inward, and ends freely in a fold of the mucous
membrane just behind the inner row of teeth.   The internal
process curves backward, upward, and inward.   It fuses with
the end of the lateral bar of the posterior arch.   On their
dorsal surfaces the lateral plates bear the matrices in which
are imbedded the four rows of teeth.   The posterior arch
(Pl. XXIII, Figs. 10, 11, *pa*) is composed of two curved flat-
tened bars.   These bars are rather wide anteriorly, but narrow
abruptly as they approach, to fuse with each other in the
median line by a short commissure.   The anterior half of the
arch curves downward to form a convex projection correspond-
ing to that formed by the median piece of the anterior arch,
and fitting in the median dorsal groove of the basal plate below.
The posterior halves of the lateral bars diverge posteriorly and
become narrower.   Near the posterior end of each there is a
slight enlargement, at which the bar turns sharply outward to
fuse with the internal process (*i*) from the lateral plates of the

anterior arch. The posterior arch does not support any teeth. The tendons of the retractor, as well as the protractor, muscles are attached, not to the cartilages directly, but to the strong fibrous membranes which surround them.

The foregoing description has included the skeletal parts making up the dorsal and ventral portions of the skull and the cartilages of the mouth region. The lateral walls of the skull, especially in the posterior region, are supported by a framework of cartilages (" Schlundkorb " of Johannes Müller). A comparison of Figs. 5, 6, and 7 will show that this framework is, for the most part, composed of a number of vertical arches, connected on each side by two longitudinal bars, a dorsal and a ventral. The cartilages of this framework, as we shall see, are really metamorphosed visceral arches. Beginning anteriorly, we find the lateral walls of the skull, in front of the auditory capsules, and external to the trabeculae, supported by the heavy irregular *palato-pterygo-quadrate* bars (Plate XXII, Figs. 5, 6 ; Plate XXIII, Fig. 7, *PQ, Pl.*). The *palato-pterygo-quadrate* on each side has two main divisions — an anterior, the palatine bar, and a posterior, the *pterygo-quadrate* cartilage, *PQ*. The *palatine bars* (Figs. 5, 6, 7, *Pl*) are a pair of large, strong, cartilaginous rods, extending along either side of the nasal capsule and the posterior fourth of the nasal tube, at about the level of the floor of the latter. The olfactory capsule and nasal tube rest upon the flattened surfaces of the palatines, which look upward and inward, but there is no cartilaginous connection between them. Anteriorly the palatine bars converge slightly toward the median line. Finally they turn abruptly toward each other and are connected at the anterior end by a short, broad, flattened transverse commissure (Fig. 7, *cm*) which faces upward and slightly forward. To the inner margins of the palatines, a short distance behind the commissure, are attached the anterior processes of the hypophysial plate. On the upper surface of the commissure, in the median line near the posterior margin, is attached the posterior end of the subnasal cartilage. At the anterior external angles are attached the posterior ends of the cornual cartilages, as previously mentioned. At the posterior

extremity the palatine bar is connected by a short process with the corresponding trabecula. The main bar of the palatine passes directly backward and downward into the anterior process of the pterygo-quadrate.

The posterior division of the palato-pterygo-quadrate carti- lage is the *pterygo-quadrate* (Figs. 5, 6, 7, *PQ*). This cartilage is triradiate, being composed of three large flattened processes, the anterior, the superior, and the inferior. The anterior process runs forward, upward, and inward, to fuse with the posterior end of the palatine bar. It is flattened and slightly curved, its outer surface looking outward, upward, and slightly forward. Posteriorly it fuses into the superior and inferior processes. The superior process is a flattened bar passing upward, back- ward, and inward. On reaching the level of the cranial floor it passes inward and slightly forward and fuses with the tra- becula just in front of the auditory capsule. The anterior margin of the superior process, the upper margin of the anterior process, and the external margin of the trabeculae together enclose a large oval fenestra (1). The anterior and superior processes together constitute the subocular arch. In its upper portion the superior process gives off posteriorly a large flattened connecting piece which runs directly backward to fuse with the hyoid arch, a short distance from the auditory capsule. The posterior margin of the superior process and the superior margin of the connecting piece are separated from the external wall of the auditory capsule by a narrow, curved fenestra (2). The inferior process runs outward, downward, and backward, enlarging at the lower extremity, where it fuses with the hyoid arch. The inferior process is flattened laterally. Its upper margin, together with the posterior margin of the superior process, the inferior margin of the connecting piece, and the anterior margin of the hyoid arch, encloses a large fenestra (3) nearly circular in general outline.

The hyoid arch (Pl. XXII, Figs. 5, 6 ; Pl. XXIII, Fig. 7, *Hy*) is a flattened, irregular, vertical bar placed behind the pterygo- quadrate. The upper end fuses with the posterior half of the external wall of the auditory capsule. From the point of fusion the hyoid arch extends outward and downward, almost

immediately expanding into a broad plate which fuses anteriorly with the connecting piece from the pterygo-quadrate, as previously described, and posteriorly, at the same level, with the anterior end of the superior lateral bar (b). The hyoid arch then narrows somewhat and continues outward and downward, its anterior margin forming the posterior boundary of the large circular fenestra (3). From the anterior margin of the hyoid a short flattened oval process extends forward, nearly to the center of this space. Inferiorly the hyoid arch again broadens out, connecting anteriorly with the inferior process of the pterygo-quadrate, and posteriorly with the inferior lateral bar (b'). In the angle between the hyoid and the inferior lateral bar a short process curves upward, backward, and then slightly downward, ending freely in the large fenestra (4). This fenestra is bounded anteriorly by the posterior margin of the hyoid, above by the superior lateral bar, below by the inferior lateral bar, and posteriorly by the upper end of the second branchial bar (br_2). The first branchial arch (Pl. XXII, Figs. 5, 6; Pl. XXIII, Fig. 7, br_1) is a slender cartilaginous bar arising from the superior lateral bar at some distance behind the hyoid. It curves at first outward, backward, and downward, over the middle part of the fenestra (4), previously described; then it curves forward and inward to fuse with the posterior process of the second basal segment (sb), together with the lower end of the second branchial arch (br_2).

The superior lateral cartilage (Figs. 5, 6, 7, b) arising anteriorly from the upper portion of the hyoid arch, and continuing directly backward, gives attachment externally to the first branchial arch, as just noted. Then it continues horizontally backward and finally curves slightly outward and downward, broadening out into a flattened, somewhat triangular plate, which terminates posteriorly in a sharp apex. The lower angle of the triangle is continued into the upper division of the second branchial arch (Figs. 5, 6, 7, br_2). The upper division extends downward into a more or less prominent process, then turns sharply backward and fuses with the inferior lateral bar (b). This bar, as previously noted, arises anteriorly from the inferior portion of the hyoid, being at first a rather strong

bar, which becomes more slender posteriorly.   After fusing
with the upper division of the second branchial it passes back-
ward and slightly upward, ending in a sharp apex just below the
posterior end of the superior lateral bar.   Near the posterior
end the lower division of the second branchial arch begins
from the inferior margin and extends forward, downward, and
inward, to fuse with the posterior process (*sb*) of the second
segment of the nasal plate, as previously described.

Lying in the median dorsal pharyngeal wall, between the
posterior portions of the superior lateral bars, is a flattened
plate, the suprapharyngeal cartilage (Pl. XXII, Figs. 5, 6, *S*).
It is composed of a body and two lateral horns.   The body
is a flattened, nearly circular plate, somewhat wider than the
notochord.   The plate is usually perforated by two openings
lying one behind the other in the median line, but there is
considerable variation in different specimens.   On each side
of the plate is given off a slender lateral horn which extends
outward and slightly downward, with a sharp apex ending free
near the tip of the superior lateral bar.   The lateral horns are
continued anteriorly with the anterior connecting processes
(*a*) from the velar cartilages.   From the body of the supra-
pharyngeal cartilage there is also given off anteriorly, in the
median line, a short process that fuses with the vertical median
connecting process (*m*), which passes directly downward to the
underlying velar cartilages.

The velum, or pharyngeal valve, is formed by an evagination
of the mucous membrane from the dorsal and lateral walls of
the pharynx.   The velum projects backward in the pharyngeal
cavity and is supported by a framework of velar cartilages.
This includes the external and internal lateral bars, connect-
ing bars, and connecting processes.   The *external lateral velar
bars* (Pl. XXII, Fig. 6; Pl. XXIII, Fig. 7, *V*) are the largest
and support the lateral margins of the velum.   Anteriorly the
bar is large, thick, strong, and flattened laterally.   Posteriorly
it tapers very gradually to a point.   The rod is slightly curved,
the posterior end being nearer the median line.   The anterior
end of the bar is continued into a large, strong, articular process,
extending outward and slightly downward.   This process

is firmly attached by ligaments to the pharyngeal wall, just anterior and internal to the short anterior process from the hyoid arch. About the middle of the external lateral velar bars, in the region of the first branchial arch, the anterior ends of the internal lateral velar bars are attached. From this point the external velar bar on each side passes backward and slightly inward, tapering to a point in the posterior margin of the velum a short distance behind and below the posterior end of the corresponding superior lateral velar bar. The *internal lateral velar bars* (Figs. 6, 7, *V'*) are smaller than the externals. Beginning at their attachment to the middle point of the internal margin of the external velar bars, they pass backward and inward until they are directly over the lateral margins of the basal plate. Here they are connected by a transverse velar bar (*ab*). The internal lateral velar bars then pass directly backward parallel to each other. A second velar transverse bar (*pb*) connects them with the lateral velar bars and the anterior connecting velar bar, and encloses a fenestra almost square. The internal lateral velar bars then continue backward and slightly outward, each tapering to a point in the posterior margin of the velum near the posterior end of the corresponding external lateral velar bar. The posterior transverse velar bar (*pb*) has a process extending directly backward in the median line which ends in the margin of the velum. The posterior end of this process is usually bifid. The anterior transverse velar bar (*ab*) has three connecting processes. The median process (Fig. 7, *m*) passes at first backward, then turns directly upward and fuses with the anterior median process of the suprapharyngeal cartilage. The anterior connecting processes (*a*) arise one on each side of the median line and curve forward, upward, and backward, to unite with the anterior surfaces of the lateral horns of the suprapharyngeal plate (Figs. 5, 7, *a*). These connecting processes form an elastic apparatus by means of which the velum, under ordinary conditions, is suspended above the floor of the pharynx.

In the posterior part of the fascia of the club-shaped muscle body are found two whitish elongated bars lying in the median line, one above the other. These are the *superior* and *inferior*

chondroidal bars (Pl. XXII, Fig. 6, *cs*, *ci*). These bars are somewhat flattened dorso-ventrally and serve for the attachment of muscles and fascia. The superior bar is somewhat larger and placed slightly anterior to the inferior bar. These bars are not true cartilage but are composed of a chondroidal tissue similar to that of the posterior segment of the basal plate. They are not, therefore, to be regarded as skeletal derivatives of the visceral or branchial arches, but simply as chondroidal modifications (*i.e.*, condensations of connective tissue) in the muscular fascia.

The gill cartilages (Pl. XXII, Fig. 6, *gb*) are a remarkable series of small cartilaginous bars which are so bent as to encircle the walls of the external gill passages lying near the external gill openings. The broad faces of the bands, when straightened out, present a somewhat semilunar shape (see Fig. 12), being composed of a wide central portion and slender extremities. The central portion is often perforated, as shown in Fig. 12. Of the lateral pieces, the outer is longer than the inner. When in position the wide central portion of the band is applied to the posterior surface of the tube, the band surrounding it so that the ends approach each other on the anterior surface of the gill passage. The ends are at a slightly lower level than the central portion of the gill cartilage, and consequently nearer the mouth of the passage. In many cases the ends of these gill cartilages fuse together, forming a complete ring around the tube. There is much variation in different specimens in this respect. It is evident that these gill cartilages serve to keep the mouths of the gill passages open under ordinary circumstances.

Since the myotomes and gills of the opposite sides are not opposite but alternate, the gill bars, of course, have the same arrangement.

The position of the gills and gill bars, however, does not seem to have any fixed relation to the position of the myotomes, for the openings of the external passages correspond sometimes to myotomes, sometimes to intermuscular septa. They are, therefore, neither regularly opposite to, nor alternate with, the myotomes.

Moreover, the relations which exist on the opposite sides, even of the same animal, are quite different in all the specimens examined. The heterogeneity of segmental arrangements in myxinoids, of which this is an example, is one of the most remarkable characteristics of their anatomy.

The last external gill passage on the left side always, so far as our observations yet go, opens into the oesophago-cutaneous duct, instead of having an independent opening on the surface of the body. In the wall of the oesophago-cutaneous duct is a cartilage of peculiar shape (Pl. XXII, Fig. 6, *oes. c.* ; Pl. XXIII, Fig. 13). This is the only part of the gill-cartilage system heretofore observed in Bdellostoma.

It is irregular in shape and varies considerably in different specimens. Apparently it has been derived by the fusion of two cartilages such as we find in the other gill tubes ; one corresponding to the last left gill tube, and the other to the oesophago-cutaneous duct. It is an important fact that one process from this cartilage (Pl. XXIII, Fig. 13, *h*) often passes up the duct wall *and into the wall of the oesophagus, i.e., pharynx.*

The number of gill cartilages (including that of the oesophago-cutaneous duct), of course, corresponds to the number of gills. This number, as Dr. Ayers has pointed out, varies to a remarkable degree. In 354 specimens of Bdellostoma dombeyi collected by him from Monterey Bay in the spring of 1892, the following variations occurred :

101	individuals had 11	gills on both sides.
26	"	" 11	"	" one side,
		and 12	"	" the other side.
208	"	had 12	"	" both sides.
11	"	" 12	"	" one side,
		and 13	"	" the other side.
8	"	had 13	"	" both sides.

354

Of the eleven to twelve variation, where the position of the gills was noted, four had eleven gills on the right side and twelve on the left ; while four were just the reverse, with twelve gills on the right side and eleven on the left. The

same alternate variation was true of the twelve to thirteen variety.

In 162 specimens collected by Mr. Jackson in Monterey Bay in July, 1897, the following variations were found:

Number of Individuals.	Number of Gills on Left Side.	Number of Gills on Right Side.
1	10	10
50	11	11
17	12	11
88	12	12
1	12	13
2	13	12
3	13	13
162		

The variation in the number of gills and hence of gill cartilages is, therefore, in no way dependent upon the formation of the oesophago-cutaneous duct, which is always upon the left side.

Neither does the variation bear any constant relation to the size or sex of the specimen. The average length of the 162 specimens was 18.3 inches (varying from 14 to 23 inches).

The 10-gilled form measured 19 inches, while one of the 13-gilled forms measured only 17 inches.

Behind the gill region there is a considerable portion of the body devoid of any skeleton, excepting those structures already mentioned in connection with the notochord. In the posterior region of the body, however, we find the large caudal fin supported by an extensive set of cartilages (Pl. XXIII, Fig. 14). The caudal fin arises about the middle of the body in the median dorsal line as a slight ridge of the integument, which gradually increases in height and is well developed posteriorly. It passes around the posterior end of the body and terminates on the ventral side in the median line just behind the cloaca.

For convenience of description, the caudal fin may be divided into a dorsal fin and a ventral fin (Pl. XXIII, Fig. 14, *DF*, *VF*). Each of these is supported by a number of slender, cartilaginous fin-rays, the most of which are fused at their proximal ends with a pair of longitudinal bars, dorsal and ventral.

The fin-rays are imperfectly segmented, and are surrounded by a sheath of connective tissue, which is also constricted in places, but not always in agreement with the cartilage segments. The bodies of the fin-rays are conical, their bases being the proximal ends. They are often bifurcated at their distal ends, sometimes twice, so that each ray may have three or four terminal twigs.

In the anterior portions of the fins one can recognize a general segmental arrangement of the fin-rays, though the agreement between myotomes and fin-rays as well as gills, slime glands, etc., is by no means perfect. Near the posterior end of the body the fin-rays are much more numerous than the myotomes.

The *dorsal fin* (Fig. 14, *DF*) extends through the body region occupied by about the fiftieth to the ninety-fifth (last) myotome. Anteriorly the first three or four fin-rays are very small and scattered, being imbedded in the median dorsal septum just outside the muscles. A little farther back the rays are better developed. Their bases lie in the roof of the neural tube (skeletogenous layer), and they extend obliquely backward and upward through the median dorsal septum to the margin of the fin. The proximal ends of the fin-rays are therefore imbedded between the muscles of the right and left halves of the body, while the distal ends lie between the layers of skin forming the fin fold. The bases of the most anterior rays are free and independent. A little farther back a slender cartilaginous process extends forward in the median line from the base of each fin-ray. Each ray with its corresponding process is at first independent; but posteriorly the processes become larger and longer, and finally (about seventy-fifth to eightieth myotome) they fuse together with the bases of the fin-rays to form the longitudinal *median dorsal bar* (Fig. 14, *MD*). This bar is slender anteriorly and triangular in cross-section, but posteriorly it passes around the end of the tail into the vertical plate (see Pl. XXIII, Fig. 15, *MD*). The median dorsal bar extends along the roof of the neural canal. To it are fused twenty-five to forty fin-rays. (Total number of fin-rays in dorsal fin, fifty to sixty.) In some cases small extra fin-rays are found in the

fin between the outer ends of the ordinary fin-rays, but not connected with them. The dorsal fin is tallest a short distance from its posterior end. Posteriorly it is directly continuous around the posterior end of the body with the ventral fin. The median dorsal bar is continuous with the median ventral bar.

The *ventral fin* (Fig. 14, *VF*) begins in the median line just behind the cloaca, and extends from the seventy-fifth to the ninety-fifth myotome (posterior end of the body). Its fin-rays are from thirty to thirty-five in number. They are all well developed and branched at the outer ends, especially in the posterior region, where the branches are in some cases longer than the main stalk. The rays of the ventral fin form two divisions. Those of the anterior division (eleven to fifteen) are larger, and approximately segmental in arrangement. Their bases end freely above, and are not directly connected with the notochord or the median ventral bar. From their bases they extend downward and slightly backward in the median line. The first ray is the largest and longest, extending back over the median dorsal roof of the cloaca.

Beginning about the eighty-fifth myotome, the fin-rays of the posterior division are smaller and more closely crowded together, being about twenty, corresponding to the last ten myotomes. Their upper or proximal ends are fused with a large vertical plate, the *median ventral bar* (Fig. 14, *MV*). This cartilage arises anteriorly about the cloacal region as a *pair* of slender bars running along the infero-lateral angles of the notochord in the skeletogenous layer. Anteriorly each of these lateral bars breaks up in some cases into a short chain of small cartilage patches, *segmentally arranged*, corresponding to the neighboring myotomes. Posteriorly the lateral bars become wider, and finally fuse across the median line below the notochord, to form the median ventral bar. This portion of the bar lies above the anterior division of the ventral fin-rays, but is not fused with them. About the region of the eighty-fifth myotome the median ventral bar suddenly enlarges into a vertical plate, with which the remaining fin-rays are fused (see Fig. 14). The anterior inferior angle of this plate extends forward as a process (Fig. 14, *c*) which forms a *T*-shaped plate,

flattened dorso-ventrally.   This plate forms the ventral wall of a pocket in which the posterior end of the large dorsal abdominal vein lies, its main trunk terminating here.   A similar structure has been described by Cleland for Myxine.   In the anterior region the dorsal surface of the median ventral bar is merely in contact with the ventral surface of the notochord.   Posteriorly, however, the ventral bar sends up lateral extensions around the sides of the notochord, so that the latter lies in a groove on the dorsal surface of the bar.   Near the posterior end these lateral plates extend still farther up around the sides of the neural tube, so that the median ventral bar is *Y*-shaped in cross-section.   The appearance near the end of the notochord is shown in Pl. XXIII, Fig. 15.   Just behind the plane of the section shown in this figure a cartilaginous band rises on each side from the median ventral bar, and fuses with the median dorsal bar, *forming a complete neural arch around the neural tube* (Pl. XXIII, Fig. 14, *r*).   This arch lies just anterior to the last two myotomes.   The posterior end of the neural tube is slightly dilated and surrounded by cartilage, excepting the upper lateral portions.   The spinal cord itself *is not dilated* at the posterior end.

A very thin irregular sheet of cartilage is found in the wall of the cloaca, especially in the anal region (Fig. 14, *av*).   It extends across the cloaca in the postanal septum.   The development of this cloacal cartilage varies a great deal in different specimens.   In a few cases it is found well developed, in others only to a slight extent.   It is possible that this cartilage serves to expand the anal opening in anal respiration.

Exoskeleton.

The only skeletal parts which belong to the integument are the horny teeth.   The *dorsal tooth* (Pl. XXII, Fig. 6, *dt*) is a single corneous conical structure in the roof of the mouth.   Its base is imbedded in a firm, disc-shaped matrix which lies in the median line immediately below the palatine commissure (*cm*).   The tooth is light brown in color, thick at the base, but slender toward the end, which curves downward and backward,

ending in a very sharp point. This tooth prevents the forward slipping of any object which is being subjected to the rasping action of the ventral teeth.

The *ventral teeth* are arranged in four rows, two upon each side of the dental plate (Pl. XXII, Fig. 6; Pl. XXIII, Fig. 10). The larger rows are anterior and external. Each tooth is some-what conical, with a large hollow base, and a smaller apical portion which is somewhat flattened laterally. The free distal portion of each tooth curves upward, backward, and inward, tapering to a sharp point. The teeth of each row are united firmly to their bases, the corneous material of the teeth being continuous through an entire tooth row. The separate rows are not united in any way. Occasionally, however, the ante-rior and the posterior teeth of the row are not fused with the remainder. The teeth in each row decrease in size from before backward. They are yellowish-brown in color, and the number of teeth varies to a considerable extent.

In the following tables the formula used to indicate the number of teeth in the several rows is given in the subjoined diagram :

Left outer row.	Right outer row.
Left inner row.	Right inner row.

The limits of the variability in the teeth of Bdellostoma, so far as known, are as follows :

Bdellostoma from the Cape of Good Hope (Müller), 6 to 7 gills, $\frac{8}{7} \mid \frac{8}{8}$, $\frac{11}{11} \mid \frac{11}{11}$, $\frac{12}{11} \mid \frac{12}{12}$.

Bdellostoma from the coast of Chili, 10 gills, $\frac{11}{7} \mid \frac{11}{7}$ (Lacépède), $\frac{12}{11} \mid \frac{12}{11}$, $\frac{13}{12} \mid \frac{13}{12}$.

Bdellostoma from the coast of California, 11 to 13 gills, $\frac{9}{9} \mid \frac{8}{9}$, $\frac{12}{11} \mid \frac{12}{11}$.

In the California series examined by Dr. Ayers the dental formulae were as follows : In 22 individuals with 11 gills,

1 $\frac{10}{9} \mid \frac{9}{9}$, 1 $\frac{10}{10} \mid \frac{9}{10}$, 4 $\frac{10}{9} \mid \frac{10}{9}$, 1 $\frac{10}{9} \mid \frac{10}{10}$, 1 $\frac{10}{10} \mid \frac{10}{10}$, 1 $\frac{10}{10} \mid \frac{10}{9}$, 1 $\frac{10}{9} \mid \frac{11}{9}$, 1 $\frac{10}{10} \mid \frac{11}{10}$, 3 $\frac{11}{10} \mid \frac{10}{10}$, 1 $\frac{11}{11} \mid \frac{10}{10}$, 5 $\frac{11}{10} \mid \frac{11}{10}$, 1 $\frac{11}{11} \mid \frac{11}{10}$, 1 $\frac{12}{9} \mid \frac{11}{9}$, 2 $\frac{12}{11} \mid \frac{12}{11}$.

In 62 individuals with 12 gills the following dental formulae occurred: $1\ \frac{8}{9}\,|\,\frac{10}{10}$, $1\ \frac{9}{9}\,|\,\frac{8}{9}$, $8\ \frac{9}{9}\,|\,\frac{10}{9}$, $1\ \frac{9}{10}\,|\,\frac{10}{9}$, $1\ \frac{9}{10}\,|\,\frac{10}{10}$, $2\ \frac{10}{9}\,|\,\frac{9}{9}$, $1\ \frac{10}{10}\,|\,\frac{9}{9}$, $1\ \frac{10}{10}\,|\,\frac{9}{10}$, $10\ \frac{10}{9}\,|\,\frac{10}{9}$, $4\ \frac{10}{10}\,|\,\frac{10}{9}$, $6\ \frac{10}{10}\,|\,\frac{10}{10}$, $7\ \frac{10}{9}\,|\,\frac{10}{10}$, $1\ \frac{10}{9}\,|\,\frac{11}{9}$, $1\ \frac{10}{10}\,|\,\frac{11}{10}$, $1\ \frac{11}{9}\,|\,\frac{10}{9}$, $1\ \frac{11}{10}\,|\,\frac{10}{9}$, $10\ \frac{11}{10}\,|\,\frac{10}{10}$, $3\ \frac{11}{10}\,|\,\frac{11}{10}$, $1\ \frac{11}{10}\,|\,\frac{12}{10}$, $1\ \frac{12}{11}\,|\,\frac{12}{11}$.

On combining the dental formulae of the 11-gilled and 12-gilled variations, the following numbers were found to obtain in the 86 individuals whose dental formula was carefully examined on both sides of the dental plate.

NUMBER OF INDI-VIDUALS.	DENTAL FORMULA.	NUMBER OF INDI-VIDUALS.	DENTAL FORMULA.
1	$\frac{9}{9}\,|\,\frac{8}{9}$	2	$\frac{10}{9}\,|\,\frac{11}{10}$
8	$\frac{9}{9}\,|\,\frac{10}{9}$	1	$\frac{10}{10}\,|\,\frac{11}{9}$
1	$\frac{9}{10}\,|\,\frac{10}{9}$	2	$\frac{10}{10}\,|\,\frac{11}{10}$
1	$\frac{9}{10}\,|\,\frac{10}{10}$	1	$\frac{11}{9}\,|\,\frac{10}{9}$
3	$\frac{10}{9}\,|\,\frac{9}{9}$	1	$\frac{11}{10}\,|\,\frac{10}{9}$
1	$\frac{10}{10}\,|\,\frac{9}{9}$	13	$\frac{11}{10}\,|\,\frac{10}{10}$
2	$\frac{10}{10}\,|\,\frac{9}{10}$	1	$\frac{11}{11}\,|\,\frac{10}{10}$
14	$\frac{10}{9}\,|\,\frac{10}{9}$	8	$\frac{11}{10}\,|\,\frac{11}{10}$
8	$\frac{10}{9}\,|\,\frac{10}{10}$	1	$\frac{11}{11}\,|\,\frac{11}{10}$
4	$\frac{10}{10}\,|\,\frac{10}{9}$	1	$\frac{11}{10}\,|\,\frac{12}{10}$
7	$\frac{10}{10}\,|\,\frac{10}{10}$	1	$\frac{12}{9}\,|\,\frac{11}{9}$
1	$\frac{10}{9}\,|\,\frac{11}{9}$	3	$\frac{12}{11}\,|\,\frac{12}{11}$
	Total number	86	

In the series of 162 specimens from Monterey Bay examined by Mr. Jackson, the following dental formulae were observed: Branchial formula, 10–10 : — $1\ \frac{9}{9}\,|\,\frac{9}{9}$.

Branchial formula, 11–11 : — $1\ \frac{10}{9}\,|\,\frac{10}{8}$, $11\ \frac{10}{9}\,|\,\frac{10}{9}$, $4\ \frac{10}{10}\,|\,\frac{10}{10}$, $1\ \frac{11}{9}\,|\,\frac{11}{9}$, $3\ \frac{11}{10}\,|\,\frac{10}{9}$, $4\ \frac{10}{10}\,|\,\frac{11}{10}$, $2\ \frac{10}{10}\,|\,\frac{10}{9}$, $1\ \frac{10}{9}\,|\,\frac{11}{10}$, $1\ \frac{11}{9}\,|\,\frac{11}{8}$, $2\ \frac{11}{9}\,|\,\frac{10}{10}$, $3\ \frac{11}{10}\,|\,\frac{10}{10}$, $7\ \frac{11}{10}\,|\,\frac{10}{10}$, $1\ \frac{11}{10}\,|\,\frac{12}{9}$, $1\ \frac{11}{10}\,|\,\frac{12}{10}$, $1\ \frac{11}{11}\,|\,\frac{12}{10}$. Total, 50.

Branchial formula, 12–11 : — 3 $\frac{10}{9}$ | $\frac{10}{9}$, 3 $\frac{10}{9}$ | $\frac{10}{10}$, 3 $\frac{10}{10}$ | $\frac{10}{10}$, 1 $\frac{10}{10}$ | $\frac{11}{9}$, 2 $\frac{10}{10}$ | $\frac{10}{9}$, 2 $\frac{11}{10}$ | $\frac{10}{10}$, 3 $\frac{11}{10}$ | $\frac{11}{10}$. Total, 17.

Branchial formula, 12–12 : — 1 $\frac{9}{9}$ | $\frac{9}{9}$, 1 $\frac{9}{9}$ | $\frac{9}{10}$, 2 $\frac{9}{9}$ | $\frac{10}{9}$, 16 $\frac{10}{9}$ | $\frac{10}{9}$, 4 $\frac{10}{9}$ | $\frac{10}{10}$, 1 $\frac{10}{9}$ | $\frac{11}{10}$, 1 $\frac{10}{10}$ | $\frac{10}{9}$, 3 $\frac{10}{9}$ | $\frac{11}{9}$, 3 $\frac{11}{9}$ | $\frac{10}{9}$, 23 $\frac{10}{10}$ | $\frac{10}{10}$, 3 $\frac{10}{10}$ | $\frac{11}{10}$, 4 $\frac{11}{10}$ | $\frac{10}{10}$, 2 $\frac{11}{10}$ | $\frac{10}{9}$, 1 $\frac{10}{10}$ | $\frac{11}{10}$, 1 $\frac{10}{10}$ | $\frac{11}{9}$, 2 $\frac{11}{9}$ | $\frac{10}{10}$, 2 $\frac{11}{10}$ | $\frac{10}{9}$, 13 $\frac{11}{10}$ | $\frac{11}{10}$, 2 $\frac{11}{10}$ | $\frac{11}{9}$, 1 $\frac{11}{10}$ | $\frac{10}{10}$, 1 $\frac{11}{10}$ | $\frac{11}{11}$, 1 $\frac{12}{9}$ | $\frac{12}{9}$. Total, 88.

Branchial formula, 12–13 : — 1 $\frac{11}{9}$ | $\frac{10}{10}$.

Branchial formula, 13–12 : — 1 $\frac{11}{9}$ | $\frac{10}{10}$, 1 $\frac{11}{10}$ | $\frac{10}{10}$.

Branchial formula, 13–13 : — 1 $\frac{10}{9}$ | $\frac{10}{9}$, 1 $\frac{10}{9}$ | $\frac{10}{10}$, 1 $\frac{10}{10}$ | $\frac{10}{10}$. Total, 3.

The following table is a summary of all the variations found in the 162 specimens observed.

NUMBER OF INDIVIDUALS.	DENTAL FORMULA.	NUMBER OF INDIVIDUALS.	DENTAL FORMULA.
2	$\frac{9}{9}$ | $\frac{9}{9}$	6	$\frac{11}{10}$ | $\frac{10}{9}$
1	$\frac{9}{9}$ | $\frac{10}{10}$	2	$\frac{11}{10}$ | $\frac{11}{9}$
2	$\frac{9}{9}$ | $\frac{10}{9}$	2	$\frac{10}{10}$ | $\frac{11}{9}$
1	$\frac{10}{9}$ | $\frac{10}{8}$	1	$\frac{10}{9}$ | $\frac{11}{10}$
31	$\frac{10}{9}$ | $\frac{10}{9}$	24	$\frac{11}{10}$ | $\frac{11}{10}$
10	$\frac{10}{9}$ | $\frac{10}{10}$	9	$\frac{11}{10}$ | $\frac{10}{10}$
4	$\frac{10}{10}$ | $\frac{10}{9}$	9	$\frac{10}{10}$ | $\frac{11}{10}$
2	$\frac{10}{10}$ | $\frac{9}{10}$	1	$\frac{10}{10}$ | $\frac{10}{11}$
5	$\frac{10}{9}$ | $\frac{11}{9}$	1	$\frac{11}{10}$ | $\frac{11}{11}$
4	$\frac{11}{9}$ | $\frac{10}{9}$	1	$\frac{11}{10}$ | $\frac{12}{9}$
1	$\frac{11}{9}$ | $\frac{11}{8}$	1	$\frac{11}{10}$ | $\frac{12}{10}$
1	$\frac{11}{9}$ | $\frac{11}{9}$	1	$\frac{11}{11}$ | $\frac{10}{10}$
1	$\frac{10}{9}$ | $\frac{11}{10}$	1	$\frac{11}{11}$ | $\frac{12}{10}$
6	$\frac{11}{9}$ | $\frac{10}{10}$	1	$\frac{12}{9}$ | $\frac{12}{9}$
31	$\frac{10}{10}$ | $\frac{10}{10}$		
	Total number	162	

From a comparison of the above dental formulae, including in all 248 specimens, we conclude :

(1) That there is an exceedingly great variation in the number of the teeth, even more striking than in the case of the gills. Thus these two characters (the number of teeth and gills), the only two "constant" characters which Johannes Müller could find upon which to base his classification, are both proven to be *extremely variable*.

(2) In a large number of cases the two sides of the dental plate are not symmetrical with regard to the number of teeth. It is to be feared that the dental formulae given in systematic accounts of Bdellostoma are, in many cases, based upon counts of *one side only* of the dental plate.

(3) There is no constant relation between the number of teeth and the number of gills. If there is any difference at all worthy of note, the individuals with the larger number of gills have a smaller proportion of teeth than might be expected.

(4) There is no constant relation between the number of teeth and the size or sex of the individual. The size and sex, though not given in the above tables, were noted in every case. While we should naturally expect that the larger individuals would have a larger number of teeth, this is usually not the case. In a 23-inch specimen, for example, which is considerably above the average size, the dental formula was $\frac{9}{9} \mid \frac{10}{9}$.

(5) The outer rows of teeth have in a majority of cases a greater number of teeth than the inner. In 312 cases the teeth of the outer row were more numerous than those of the corresponding inner row. In 178 cases they were equal. In only 6 cases had the inner rows a greater number of teeth.

(6) The dental formulae occurring oftenest are : $\frac{10}{9} \mid \frac{10}{9}$ (45), $\frac{10}{10} \mid \frac{10}{10}$ (38), $\frac{11}{10} \mid \frac{11}{10}$ (32), $\frac{11}{10} \mid \frac{10}{10}$ (22). It is evident that we cannot speak of any one "typical" or predominant formula. More than half the rows of teeth number 10 however, and in nearly half the cases the corresponding outer and inner row each contains 10 teeth. The number 9 is given next in frequency, but occurs less than half as often as 10. More than 95% of the rows include either 9, 10, or 11 teeth.

The Chilian specimens seem to average a larger number of

teeth, for Girard counted in his type specimens with 14 gills $\frac{12}{12} \mid \frac{12}{12}$, while Putnam found $\frac{13}{12} \mid \frac{13}{12}$ or $\frac{12}{11} \mid \frac{12}{11}$ in the material he studied from Talcahuano Bay (Hassler expedition), reported as having 10 gills.

Lacépède's example from Chili had the very unusual dental formula of $\frac{11}{7} \mid \frac{11}{7}$.

A critical histological study of the dental structures of Bdellostoma is reserved for a separate paper. But it may be mentioned that the hollow bases of the corneous teeth rest upon soft dental papillae, which are fused together below into a bar extending the entire length of the row of teeth. These papillae are epidermal in origin, and the teeth are simply cornified sheaths of the epidermal elevations upon the dental plate. They are easily sectioned when imbedded in celloidin, and show no traces of dentine, enamel, bone, or calcareous matter of any kind.

March, 1898.

SYNONYMOUS TERMS FOR SKELETAL PARTS.

Ayers and Jackson.	Johannes Müller.
Notochord	Gallertsäule.
Cellular sheath of same	Innere Schicht.
Fibrous core	Faser-Faden.
Notochordal sheath	Innere Scheide der Gallertsäule.
Skeletogenous layer, elastica externa	Aeussere Scheide der Gallertsäule.
Neural tube	Rückenmarksrohr.
Auditory capsule	Gehörkapsel.
Parachordals	Knöcherne Basis cranii.
Trabeculae	Flügelfortsätze derselben.
Hypophysial plate	Gaumenplatte.
Subnasal cartilage	Knöcherne Stütze der Schnautze.
Transverse labial cartilage	Innerknorpel.
Lateral labial cartilage	Knorpel-Fortsatz am vordern Ende des Zungenbeins.
Nasal tube	Nasenrohr.
Olfactory capsule	Nasenkapsel.
Cranium	Gehirnkapsel.
Tentacular cartilages	Mundknorpel.
Dental plate	Zunge.
Teeth	Zähne.
Anterior segment of basal plate	Vordere Reihe der Zungenbein-Knochenstücke.
Middle segment of basal plate	Hintere Reihe der Zungenbein-Knochenstücke.
Posterior segment of basal plate	Knorpeliger Kiel des Zungenbeins.
Palatine bars	Gaumenleisten.
Cornual cartilages	Knorpel-Fortsatz am vorderen Ende der Gaumenleiste.
Pterygo-quadrate	Unterer Fortsatz der Gaumenleiste.
Hyoid arch	Verbindung der Fortsätze mit der Gehörkapsel.
Superior lateral cartilage	Oberer Fortsatz des Schlundkorbes.
Inferior lateral cartilage	Unterer Fortsatz des Schlundkorbes.
First branchial arch	Grosses Horn des Zungenbeins.
Second branchial bar	Kleines Horn des Zungenbeins.
External lateral bar	Hauptstück des Schlundsegels.
Internal lateral bar	Mittel-Riemen des Schlundsegels.
Suprapharyngeal plate	(In part-) Aufsteigende Fortsätze des Mittelriemens.

EXPLANATION OF PLATE XXII.

FIG. 1. A cross-section of the notochord, spinal cord, and sheaths of Bdellostoma a short distance behind the cranial region (× 10, camera lucida outlines).

FIG. 2. A cross-section of the notochord, spinal cord, and sheaths of Bdellostoma in the posterior gill region (× 10, camera lucida outlines).

FIG. 3. Section of the notochord and its envelope.

FIG. 4. A cross-section of the cranium of Bdellostoma through the region of the auditory capsules (× 20, camera lucida outlines).

FIG. 5. Dorsal view of the skull of Bdellostoma (× 2).

FIG. 6. The skull and the skeleton of the pharyngeal region of Bdellostoma, lateral view. The roof of the spinal canal, the oesophagus, the gills and gill passages, and the retractor mandibuli muscle are outlined with dotted lines (× 2).

A.	= auditory capsule.	*m.p.*	= internal process of lateral labial cartilage.
a.	= anterior connecting process.		
B.	= anterior segment of the basal plate.	*N.*	= notochord.
		n.	= nucleus.
B.'	= middle segment of the basal plate.	*n. sh.*	= notochordal sheath.
		N.	= subnasal bar.
B.''	= posterior segment of the basal plate.	*N. T.*	= nasal tube.
		Oes.	= oesophagus.
b.	= superior lateral cartilage.	*oes. c.*	= oesophago-cutaneous duct.
b.'	= inferior lateral cartilage.	*O.C.*	= olfactory capsule.
br.1	= 1st "branchial" arch.	*P.Q.*	= pterygo-quadrate.
br.2	= 2d "branchial" arch.	*Pl.*	= palatine bar.
Br.	= brain.	*p.c.*	= parachordal cartilage.
Cr.	= cranium.	*S.*	= supra-pharyngeal plate.
c.c.	= cornual cartilages.	*sc.*	= neural tube.
c.s.	= superior chondroidal bar.	*s.b.*	= basal process.
c.i.	= inferior chondroidal bar.	*sk.*	= skeletogenous layer.
D.	= dental plate.	*Sh.1*	= internal layer of notochordal sheath.
d.t.	= median dorsal tooth.		
ex.	= external cellular layer.	*Sh.2*	= middle layer of notochordal sheath.
F.	= "fatty" tissue of the neural tube.		
		Sh.3	= external layer of notochordal sheath.
F.'	= similar layer within the cranium.		
		Sp.	= spinal cord.
F.c.	= fibrous core.	*t.1, t.2. t.3, t.4*	= 1st, 2d, 3d, 4th tentacular cartilages.
F.s.i.	= fascia superficialis interna.		
g.b.	= gill bar.	*Tr.*	= main bar of the trabecula.
Hy.	= hyoid arch.	*tr.*	= anterior horn of the same.
H.p.	= hypophysial plate.	*t.*	= tendon of retractor mandibuli muscle.
i.m.s.	= intermuscular septum.		
L.c.	= lateral labial cartilage.	*vc.*	= vacuole.
L.p.	= lateral (ethmoidal) plate.	*V.*	= external lateral velar bar.
m.l.e.	= membrana limitans externa.	*V.'*	= internal lateral velar bar.
m.d.s.	= median dorsal septum.	1, 2, 3, 4	= fenestrae of skull.
m.	= median connecting process.		

Pl. XXII

EXPLANATION OF PLATE XXIII.

Fig. 7. A dorsal view of the skull of Bdellostoma, cranium, olfactory capsule and nasal tube removed ($\times$ 2½).

Fig. 8. The basal plate of Bdellostoma, ventral view ($\times$ 1).

Fig. 9. A dorsal view of the basal plate of Bdellostoma ($\times$ 1).

Fig. 10. Dorsal view of the teeth and the dental plate of Bdellostoma ($\times$ 2).

Fig. 11. The dental plate of Bdellostoma, ventral view ($\times$ 2).

Fig. 12. A gill bar of Bdellostoma, stretched out ($\times$ 8).

Fig. 13. The cartilage of the oesophago-cutaneous duct of Bdellostoma ($\times$ 4).

Fig. 14. The skeleton of the posterior region of Bdellostoma ($\times$ 1).

Fig. 15. A cross-section of the tail of Bdellostoma, taken just in front of *r*, Fig. 14. Dorsal and ventral portion of section not shown ($\times$ 10).

A.i. = internal bars of anterior segment.

A.c. = external bars of anterior segment.

a.a. = lateral dental plate.

a.v. = cloacal cartilage.

a.b. = anterior transverse velar bar.

b.v. = blood vessel.

c.m. = palatine commissure.

c. = process of median ventral cartilage.

cn. = connective tissue.

D.m. = dermal muscle layer.

D.F. = dorsal fin.

e. = posterior external process of lateral plate.

ex.g.p. = external gill passage.

f. = auditory foramen.

f.r. = fin-ray.

g. = dorsal tendon groove.

h. = superior process of oesophago-cutaneous cartilage.

i. = posterior internal process of lateral plate.

K. = anterior process of hypophysial plate.

M.D. = median dorsal bar.

M.V. = median ventral bar.

Me. = median cartilage bar.

M.d. = median piece of dental plate.

My. = myotome.

oes.b. = the oesophago-cutaneous bar.

p.b. = posterior transverse velar bar.

p.a. = posterior arch of dental plate.

r. = anterior connecting process of lateral plate.

s. = sheath of fin-ray.

S.I. = epidermis.

T.I. = transverse labial cartilage.

tr. = anterior process of trabeculae.

V.F. = ventral fin.

v. = posterior connecting process of lateral plate.

Pl. XXIII.

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THE ORGANIZATION OF THE EGG OF UNIO, BASED ON A STUDY OF ITS MATURATION, FERTILIZATION, AND CLEAVAGE.

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INTRODUCTION.

THIS piece of work was originally undertaken in 1896 as a study of the behavior of the centrosomes in the fertilization of the egg of *Unio*, but I was soon led into a *general* study of the centrosomes in this egg. The results which I obtained seemed to be irreconcilable with the views then current of the functions of the sperm-centrosomes in fertilization, and with

the general theory of the permanence of the centrosome as the special organ of cell division, or the center of organic radii. Brief abstracts of the results were published in the form of two short papers, read before the American Society of Morphologists in the winter meetings of 1897 and 1898, summaries of which appeared in *Science* in March, 1897 and 1898. The second paper was also published in full in the *Zoölogical Bulletin*, Vol. I. Part of the subject was described for the first time in my paper on "Adaptation in Cleavage," in the *Biological Lectures* for 1898 (Lillie, '97–'99).

Since this work was begun the entire centrosome question has entered on a new phase of development, and the subject now appears to me to be in such a condition that the great need is for careful observations rather than for any attempt to found new theories or defend old ones; any such attempt in the present state of our knowledge would be premature. It is in this spirit that I publish the following detailed account of my observations. The work, moreover, has had for me a greater embryological than purely cytological interest ; hence I lay the greatest weight on the evidence as to the organization of the egg. The conclusions concerning this subject link themselves directly to my earlier observations ('95) on the cell-lineage of *Unio*, and are a further elaboration of the principles laid down in that paper.

The egg of *Unio* is most definitely oriented. The orientation is not due to the arrangement of the deutoplasmic materials, for the yolk-granules have no definite abiding-place in this egg, but are driven here and there by cytoplasmic movements. Yet of all the many hundreds and even thousands of eggs examined, not one was found in which the polar globules were not formed opposite to the micropyle, which thus occupies the center of the vegetative pole. It is here, of course, that the spermatozoön enters.

The eggs are fertilized in the suprabranchial chamber by spermatozoa that enter with the inhalent current of water. Apparently, contact with the water may act as stimulus for the first breaking of the germinal vesicle ; because, so far as my observations go, the spindle is not formed in the ovary, and I

have found unfertilized eggs with the first maturation spindle fully formed. Moreover, I have never found an egg, into which the spermatozoön had entered, in which, at the same time, the first maturation spindle was not forming.

It seems probable, from some observations I have made on the breeding habits of *Unio*, that the presence of spermatozoa in the water may act as a stimulus to the female, causing the extrusion of eggs.

The general course of events after the entrance of the spermatozoön is this : a sperm-amphiaster is formed very soon after the penetration of the sperm-head, which moves up towards a definite zone above the equator of the egg and there comes to rest. The sperm-amphiaster gradually disappears, and the sperm-nucleus, which has enlarged considerably during its penetration, contracts in volume nearly to its original bulk. By the time that this is accomplished the first maturation spindle is entering on its metaphase. The sperm-nucleus undergoes no further changes until after the completion of the second maturation division, at which time it grows in size synchronously with the egg-nucleus and begins to describe its copulation path. The copulation path of the germ-nuclei is by no means simple, but they ultimately come into contact at or near the center of the egg. The first cleavage spindle is then formed, and moves axially to one end of the egg ; thus the first cleavage is very unequal.

I. The Behavior of the Spermatozoön in the Egg up
to the Time of Extrusion of the Second
Polar Globule.

1. *The Sperm-Nucleus.*

The spermatozoön enters through the micropyle shortly after the breaking down of the germinal vesicle. In mounts of entire eggs at this stage one can see a number of spermatozoa clustered around the region of the micropyle, and may even see one within it. But it very rarely happens that more than one enters and fuses with the egg, for I have not seen

more than two or three cases of polyspermy in the thousands of eggs examined. The head of the spermatozoön is shaped like a blunt cone, and so should furnish evidence of rotation, if this really occurs. But as it rounds off almost immediately on entering, and as the sperm-aster does not form at once, the evidence is inconclusive.

The usual path of the spermatozoön is shown in Pl. XXIV, Figs. 1 and 2. It is always towards one side of the egg and in the direction of the animal pole. The path is frequently quite direct, as in Pl. XXIV, Figs. 1 and 2, but is often curved more or less. When the sperm-nucleus has passed above the equator a little way, its movement stops and it remains quiescent until after the formation of the second polar body.

During its penetration the sperm-nucleus swells up somewhat by the accumulation of caryolymph within it, forming a nucleus with a thick chromatic wall and a reticulum of a few strands only. At this time it possesses almost invariably a sharp apex directed forwards and a broad base, thus corresponding very nearly in form to the head of the spermatozoön before its entrance. But as it has power, after entering, to change its form very considerably, it does not of necessity follow that the apices correspond in the two cases.

Arrived at a point of equilibrium in the egg-substance, its place of rest, the sperm-nucleus rounds off and contracts in volume. As it does so, the chromatic reticulum becomes very regular (Pl. XXIV, Figs. 7, 8, 9 *a* and 9 *b*). By careful study it is possible to determine that there are about sixteen regular hexagonal interchromatic areas on the surface; and as this agrees with the number of the chromosomes that ultimately develop from it, it seems probable that each of these areas corresponds to a chromatin-vesicle or chromosome. The contraction proceeds during the anaphase of the first maturation division, until the cavity of each vesicle becomes indistinguishable, and the sperm-nucleus is then a small mulberry-like body, staining intensely black. Sobotta ('97, p. 46) has described in *Amphioxus* a similar enlargement and subsequent contraction of the sperm-nucleus.

There is nothing specially worthy of note in the further
behavior of the sperm-nucleus during its resting period ; appar-
ently it undergoes no further change, and certainly makes no
other movements until after the formation of the second polar
body.   Then it begins to enlarge again by the accumulation of
caryolymph in its interior, keeping pace with the growth of
the egg-nucleus ; and both nuclei begin to move inward at
the same time.   The details of the growth and movements
of the germ-nuclei are better considered together, and will be
described thus after the section on the maturation of the egg.

2. *The Sperm-Aster and Amphiaster :   Origin and Disappearance.*

a. *Observations.* — I have not been able to determine the
exact origin of the sperm-centrosome in the egg of *Unio*,
and have nothing to contribute to this subject.   The aster
is comet-like in form during the penetration, and possesses
a small apical centrosome which soon divides ; separation of
the halves produces a typical amphiaster.   The amphiaster
frequently precedes the sperm-nucleus, with its axis in the line
of movement and its long rays trailing behind (Pl. XXIV,
Figs. 1 and 2) and losing themselves in the cytoplasmic retic-
ulum.   But the centrosomes may separate in a direction at any
angle to the penetration path up to a right angle (Pl. XXIV,
Fig. 4).   It seems probable that the latter is the case, when
the division of the centrosome is delayed until the penetra-
tion path is nearly completed.   Frequently the rays of the
amphiaster engage with those of the inner aster of the matura-
tion spindle, *and may become continuous with them.*   In one
case I found an apparently perfect spindle, one pole of which
was formed by the inner centrosome of the maturation spindle,
and the other by one of the sperm-centrosomes.   This would
seem to indicate development of the fibers from a common
reticulum or foam-structure.

The comet-like form of the aster in the egg of *Unio* seems
to be quite unique.   That it is not due to any peculiarity
in the structure of the protoplasm of this egg is shown by

the fact that the same aster becomes radial after the penetration path is complete. Therefore, it is due in some way to the conditions of the penetration ; these probably affect the form of the aster in two ways : 1. It is noticeable that rays form more readily in protoplasm free from yolk-granules ; there is a yolk-free path behind the amphiaster, and the rays would naturally be longer within this area. 2. The mere forward movement of the centrosomes would tend to draw out the rays extending behind and to shorten those in front.

Shortly before the metaphase of the first maturation spindle the sperm-asters undergo retrogressive metamorphosis, and, as they disappear, the yolk-granules flow in and gradually obliterate all traces of the clear area. Figs. 7 *a*, 7 *b*, 8, and 9 *a*, Pl. XXIV, illustrate progressive phases of this process. Figs. 7 *a* and 7 *b* are two sections of the same amphiaster two sections apart. The centrosome nearer the sperm-nucleus (7 *a*) is united to the tip of the latter by a delicate fiber, and the radiations originally surrounding it have completely disappeared. The more distant centrosome is still surrounded by a well-defined aster, which, however, in the irregularity of the rays and the number and size of the microsomes on them, shows plain signs of degeneration. In Pl. XXIV, Fig. 8, we have a more advanced stage of degeneration ; the nearer centrosome can now no longer be distinguished among the microsomes, and the more distant one is barely discernible, the rays having nearly disappeared ; the yolk-granules have encroached on the clear area. In Pl. XXIV, Fig. 9 *a*, neither centrosome can be distinguished, and the clear area is nearly obliterated. In the last stages of degeneration of the aster the centrosome is distinguishable from a cytomicrosome only by its position. When the aster is entirely gone, one sees in its place simply ordinary vesicular protoplasm with nodal microsomes. There can be no doubt of the resolution of the radiations into the cytoplasm ; and if the centrosomes do not simply become microsomes, they certainly become indistinguishable from them by any visible morphological character. The view that they persist as definite entities of specific structure and function is not inherently impossible, but there is nothing in the observations to indicate this, and

the subsequent history of centrosomes in this egg gives no support to such an hypothesis.

b. *Literature.* — There are so many observations of the undoubted origin of the sperm-aster around the middle-piece of the spermatozoön that there is a strong presumption in favor of assuming such an origin even when it cannot be directly observed (Wilson and Mathews, '95; Fick, '93; Foot, '98; Van Der Stricht, '98, and others). But the time at which this aster appears varies greatly in different cases. Foot says ('98, p. 51): " In studying the literature I have not been able to find any satisfactory evidence of the appearance of a sperm attraction sphere earlier than the anaphase of the first maturation spindle." It certainly seems a general rule that the radiations do not appear until about this time, however early the spermatozoön may enter. The case of *Unio* is a striking exception to this rule, for here the sperm-aster is formed, and divides and vanishes before this time. In *Arenicola* (Child, '98) the sperm-aster also appears early, just after the first maturation spindle has taken a radial position, but division follows much later.

Disappearance of the sperm-asters and centrosomes has been observed in a number of cases, though the process is not usually described in detail. On account of the importance of this phenomenon I will give rather full citations :

Van Name ('99) states that in *Planocera* he has not been able to learn anything of the subsequent fate of the sperm-centrosomes, "nor to demonstrate any connection between them and the cleavage centrosomes." From this I conclude that they disappear before the first cleavage spindle is formed.

In *Arenicola* (Child, '97) the sperm-amphiaster is well developed during the last phases of formation of the second polar body. After this is formed "the two centrosomes have disappeared, the polar regions of the spindle being occupied by a fine network. The rays are evidently disintegrating, and the spindle is barely indicated in the cytoplasm. These structures present this appearance with regularity at this stage. It is certainly not due to imperfect fixation or errors in technique, for on the same slide are eggs in somewhat earlier stages, with

distinct 'male' centrosomes, spindle, and radiations. The following stages afford a further confirmation of the view that this disintegration is a part of the normal history of these structures, for a little later there is no trace of asters or spindles in this egg." Concerning the cleavage centers, Child says : "I am inclined to regard the cleavage centrosomes as new formations and as not related to the 'male' centrosomes."

Wheeler ('97) finds no sperm-aster in *Myzostoma*, and though Kostanecki ('98) has since observed its appearance exceptionally in this egg, he has not been able to trace it into the first cleavage spindle. Wheeler finds that the cleavage centers are furnished by the egg.

In *Pleurophyllidia* (MacFarland, '97) the sperm-asters disappear entirely during the formation of the second polar body. The centrosomes can no longer be distinguished.

In *Allolobophora foetida* "the sperm attraction sphere is present until the head of the spermatozoön begins to develop into the male pronucleus, when it also totally disappears. Both spheres, *i.e.*, sperm and egg 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." (Foot, '97, p. 811).

In *Prostheceraeus* (Klinckowström, '97) both egg and sperm asters disappear completely before the union of the germnuclei.

In *Cerebratulus* (Coe, '99), "after the germ-nuclei are nearly in contact, the sperm-asters have served the purpose for which they were intended, and completely disappear in a manner quite similar to that of the aster remaining in the egg after the formation of the second polar body. Even the centrosomes are lost from sight in most cases." Exactly the same thing is true of *Physa* (Kostanecki and Wierzejsky, '96).

In Echinoderms the sperm-asters become much less distinct at the time of meeting of the germ-nuclei. Coe states ('99, p. 455): "Observations which I have made on the eggs of *Echinus*, of *Sphaerechinus*, and of *Strongylocentrotus*, lead me to

believe that this diminution of the asters just before the union of the germ-nuclei is much greater than has been described."

II. The Maturation of the Egg.

I have not observed the breaking down of the germinal vesicle; but from the study of ovarian eggs I have found that before extrusion it lies excentrically near the animal pole. In the earliest stage of the first maturation spindle observed (not figured) the two centrosomes lie near the periphery in the neighborhood of the animal pole, and at the same distance from it; the chromosomes lie between them, but much nearer the center of the egg, so that the spindle is bent in the middle. The asters are barely indicated in this stage. The axis of the spindle straightens by the inward migration of the centrosomes to the level of the chromosomes, so that the whole spindle lies very little above the center of the egg. Then follow the rotation, elongation, and peripheral migration of the entire spindle (Pl. XXIV, Figs. 1, 2, 5, 6, and 9).

1. *The Chromosomes in Maturation.*

I have paid relatively little attention to this subject, as I was chiefly concerned with the study of cytoplasmic phenomena. However, some of the observations are of interest. Sixteen chromosomes are formed in the germinal vesicle. In the earliest stage seen they are apparently typical tetrads, which, however, elongate before they become arranged in the equatorial plate. Each chromosome then takes on the form of a longitudinally split rod with two constrictions. The first division is at right angles to the axis of the rod, and passes midway between the two constrictions. The chromosomes take on the very characteristic forms represented in Pl. XXIV, Figs. 1, 2, 5, and 9; material accumulates at the two ends of each chromosome, and, as in a heterotypic division, also in the middle. The chromosome finally parts in the center of this median accumulation. As the chromosomes diverge towards the poles, they undergo typical changes in form, until, when

they reach the poles, each has reassumed the 6-partite form,
i.e., a double rod with two transverse constrictions (Pl. XXIV,
Figs. 11, 12). The second division of the chromosomes for
the formation of the second polar globule is longitudinal, as
shown in Pl. XXV, Figs. 19–21.

The forms of the chromosomes in the maturation spindles
of the egg of *Unio* resemble closely those in corresponding
stages of the eggs of *Prostheceraeus* (Klinckowström, '97), of
Thalassema (Griffin, '99), and of *Zirphaea*, another lamelli-
branch (Griffin, '99). The first division is certainly at right
angles to the long axis of the chromosomes, as these lie in the
equatorial plate, and the second division is with equal certainty
longitudinal. But as the history of the tetrads in *Unio* is
unknown, it is quite possible that the first division is mor-
phologically a longitudinal division and the second transverse,
that is, a reducing division in the usual sense. Klinckowström
comes to the conclusion that this is probably the case in the
egg of *Prostheceraeus*, though the evidence he offers is not
conclusive. Griffin ('99) has made a very detailed study of this
form of chromosome, and has shown in an ingenious manner
that the first division may be interpreted as following the
longitudinal division of the original spireme, and the second
as transverse to this, that is, a reducing division in the ordi-
nary sense. If his interpretation should turn out to be just,
it would have a wide application, seeing that this form of
chromosome is found in flatworms and Gephyrea as well as
in mollusks.

2. *Achromatic Structures.*

As already stated, the earliest cytoplasmic phases of matura-
tion have not been observed; thus the question of the origin
of the egg-centrosomes will not be considered. We may
begin, therefore, with a description of the aster of the first
maturation spindle at the time of the metaphase. At this
time the aster at either end of the spindle has the following
structure (Pl. XXIV, Figs. 9 and 10); in the exact center is a
minute centrosome, which has begun to elongate, subsequent
to division. This is imbedded in a substance, homogeneous

in appearance, staining uniformly in Bordeaux red, which is traversed by fine radiations centering in the centrosome. A short distance from the centrosome, and at the periphery of the homogeneous substance surrounding it, each ray possesses a distinct microsome; these microsomes together bound a definite body, which I have called *the inner sphere,* and the next row of microsomes on the rays bounds a fairly definite *outer sphere.* Beyond this the radiations pass out into the general cytoplasm for a considerable distance. From the cen-trosome to the outer sphere each ray is a thread or fiber, but beyond the spheres the rays can plainly be seen to be united by more or less regular anastomoses; and farther out the branch-ing of the rays and the increase in number of the anastomoses cause the rays, as such, to disappear in the general cytoplasmic network. The whole appearance of the aster is such as to give the impression that it has arisen by a central strain in the cytoplasmic network; and the history of the origin of the aster in this and other places in this egg is such as to make this conclusion a practical certainty. I am inclined to believe that the cytoplasmic groundwork in this egg is primarily an alveolar structure, which may become reticular or filar (as in the center of the aster) by the breaking down of alveolar walls. In this opinion I agree with Wilson's conclusions in his recent work (1900) on the structure of protoplasm.

The existence of double rays is a characteristic appearance in this stage of karyokinesis (Pl. XXIV, Fig. 10, and others). However, I do not interpret these as due to " splitting " of rays (Kostanecki, '97), but to approximation of independent rays. One would naturally expect this to occur if the aster arises in the manner indicated.

During the anaphase the centrosome divides into two at each end of the spindle (Pl. XXIV, Fig. 11); and, as the proto-plasm begins to protrude for the formation of the first polar body, each one of these four centrosomes becomes double or quadruple (Pl. XXIV, Fig. 12). Another process, which has been going on during the anaphase, is the peripheral accumu-lation of the ground-substance of the inner sphere and the disappearance in this substance of the bounding row of micro-

somes so that the inner sphere becomes a vesicle with deeply
staining wall and clear contents (Pl. XXIV, Figs. 9, 11, and
12). The rays which were originally attached to the centro-
somes are now attached to the bounding membrane of the
inner sphere. Within the sphere are fibers attaching the cen-
trosomes to the wall, but independent of the radiations of the
aster.

When the chromosomes are drawn up close to the inner
spheres (Pl. XXIV, Figs. 11 and 12), the functions of the cen-
tral spindle and asters are for the time at least completed, and
both the latter become thickly studded with large microsomes.

Then follows the outpushing of the first polar body
(Pl. XXIV, Figs. 12, 13, and 14); as it is being formed, the
egg-sphere and chromosomes move up near to the periphery
of the egg, at the point of formation of the polar globule.
New rays are seen at this time extending from the central
sphere to the periphery of the egg; it is probably through the
activity of these that the sphere and chromosomes approach
the surface (Pl. XXIV, Fig. 13), for the rays disappear as soon
as the chromosomes have reached their most peripheral position
(Pl. XXIV, Fig. 14). This peripheral transfer of the sphere
and chromosomes results in the drawing out of the remains
of the original aster into fibers, as shown in Pl. XXIV, Figs.
13 and 14.

During the last phases of the formation of the first polar
body there is a very considerable diminution in size of the
sphere. This has the effect of bringing the centrosomes much
nearer together (Figs. 13 and 14). Sometimes, apparently, they
fuse; or the process may go farther, and both centrosomes and
sphere may disappear.

The mode of formation of the spindle of the second polar
body varies, according to whether the centrosomes and sphere
left over from the first division disappear or not. Let us con-
sider the latter case first.

In this case there is within the egg at the close of the first
maturation division the sixteen bivalent chromosomes, and a
sphere containing two compound centrosomes. This aggrega-
tion lies near the periphery of the egg. The aster that origi-

nally surrounded the sphere is still in process of degeneration, and the yolk-granules are beginning to intrude on it (Pl. XXIV, Fig. 14).   The fibers that reached from the sphere to the periphery of the egg have completely disappeared.   This is a period of inactivity.

Figs. 15–20, Pl. XXV, illustrate the development of the second maturation spindle ; they are drawn from eggs in which the spindle formed in its definitive radial position.   But in other eggs of the same set the spindle was found forming at various angles with the axis of the egg, later swinging into a radial position.   It will be noticed that there is no inward migration of the chromosomes and sphere prior to the spindle formation, as in the eggs of some other animals, and as in those eggs of *Unio* in which the " division-apparatus " of the first maturation division disappears.

The first step in the formation of the new spindle is the enlargement of the sphere ; new radiations appear around it (Pl. XXV, Fig. 15) ; the centrosomes diverge, frequently in a radial direction, as has been already mentioned, and a very minute central spindle is seen between them ; the boundary of the sphere still persists. *As will be shown later, the entire inner· sphere is the product of a single centrosome ; hence in a certain sense " Centrosoma und Centralspindel bilden ein Ganzes "* (Heidenhain).

By elongation of the central spindle the centrosomes are brought in contact with opposite sides of the wall of the sphere, and, pressing against the latter, cause it to elongate (Pl. XXV, Fig. 16).   The central spindle, filling the entire interior of the sphere, stretches between the centrosomes, which have become larger and more subdivided, and stain intensely black in iron-haematoxylin.   The fibers of the central spindle have been formed out of the ground-substance of the sphere, either by direct conversion of its substance or by growth of the few original fibers at the expense of the ground-substance.   The radiations which have arisen anew (Fig. 16) are related to the sphere as a whole, and not to the centrosomes as independent centers.

As the spindle elongates still more, the wall of the sphere

becomes stretched out to form the periphery of the central spindle (Pl. XXV, Fig. 17), and the radiations entirely disappear, leaving the spindle in the center of a mass of vesicular cytoplasm. The outer end of the spindle pushes up through the group of chromosomes nearly to the periphery of the egg. In Fig. 17 the first faint mantle-fibers can also be seen stretching from each chromosome to the inner centrosome.

Fig. 18, Pl. XXV, illustrates a later important phase in the elongation of the spindle. One of the most interesting features of this stage is the change which the inner centrosome has already undergone, to be described in detail immediately below. The elongation of the spindle, after the outer end has become fixed at the periphery of the egg, has resulted in the passive drawing out of the protoplasmic vesicles in straight rows extending to the surface. The appearance of crossing of rays seen in this and other figures is due, I am convinced, to a double strain on the same mass of protoplasm. The chromosomes have now been drawn, apparently by their attached fibers, around the equator of the spindle. The asters are beginning to develop at both ends by continuous arrangement of adjacent alveolar walls. This is very plainly shown by comparison of Figs. 17 and 18, Pl. XXV.

Figs. 19, 20, and 21, Pl. XXV, illustrate successive later stages of the same processes and need not be described in detail here. They carry us to the metaphase of the second maturation spindle. The structure of the centrosome, spheres, and aster in Fig. 20, Pl. XXV, is precisely the same as in the stage with which we started (Pl. XXIV, Figs. 9 and 10). Indeed, at the metaphase of any spindle we have the same structures. *It is always at the metaphase that the centrosome is smallest; at this stage the inner and outer spheres are also invariably marked by concentric rows of microsomes.*

Now, how are these structures related to the stage of Fig. 15, Pl. XXV, with which we ceased our description of centrosome structure? In this stage the large centrosome has simply increased in size and at the same time become more subdivided. Figs. 24–27, Pl. XXV, illustrate the changes which the centrosome undergoes in assuming the form charac-

teristic of the metaphase.   These figures represent tangential sections of the outer aster and centrosome in stages corresponding to those of the figures immediately above them, *i.e.*, to Figs. 17–20.   To quote from my earlier paper on " Centrosome and Sphere in the Egg of Unio " ('98) :   " These figures show two processes taking place : (1) the subdivision of the relatively large centrosome granules and their distribution in the form of a sphere ; and (2) the increase of the red-staining substance in which the granules are imbedded.   The peripherally distributed granules become the stratum of microsomes bounding the inner sphere.   One of the granules remains behind as the ' centrosome ' of the newer sphere ; which one is, apparently, determined entirely by position.   The outer sphere has developed during this process.

" The black granules in the inner sphere of Fig. 27 are plainly much less in bulk than those of Fig. 24.   There is no doubt that a large part of the centrosome granules has been changed into the red-staining substance of the sphere, which is identical in all noticeable respects with the substance from which the central spindle was formed.   In a later stage the fibers of the latter are dotted with large, deeply staining microsomes."   Here is evidence of the lack of persistence of the *microsomes* as definite morphological elements.

" From this description it would seem to follow that the centrosome of one cell generation becomes the inner sphere of the next ; and this is undoubtedly true at times.   But I do not believe that the inner sphere has *necessarily* any such definite morphological value as this would seem to imply. For it may disappear between the first and second maturation divisions, and is then re-formed as the first step in the prophase of the second maturation spindle from the cytoplasm. The same method of formation may also be observed in other places (*e.g.*, formation of the first cleavage spindle).

" Both Van Beneden's and Boveri's conceptions of this structure appear as phases in the history of the mitosis, though Boveri's ' centrosome ' is really inner sphere, and his ' Centralkorn ' or ' centriole ' really the centrosome."

I am now, however, inclined to modify the qualification that

the inner sphere has not necessarily a definite morphological value, in the sense that it is always, probably, a product of growth of the centrosome. But it seems to me probable that the latter is of purely functional significance.

"To the criticism that the centrosome phases shown in Figs. 24–27, Pl. XXV, may be pathological, *i.e.*, due to imperfect extraction of the haematoxylin or other action of the reagents, it may be replied : first, that they are found with different killing fluids ; second, that the changes are perfectly uniform in all cases, so that, knowing the stage of development of the spindle, one can be certain that a definite stage of the centrosome will be found ; third, that inasmuch as the inner aster develops much more rapidly than the outer, the inner centrosome passes through these phases more rapidly. Thus one often finds a spindle, *e.g.*, Figs. 18 and 19, Pl. XXV, in which the inner centrosome has already passed through the entire metamorphosis, while the outer is still in the stage of Fig. 24 or 25, Pl. XXV." Thus one has a typical centrosome at one end and the supposedly pathological centrosome at the other end of the same spindle !

In regard to the formation of the entire central spindle of the second maturation division from the inner sphere ("centrosome," MacF.) my results are in complete accord with those of MacFarland on *Diaullula* ('97). But concerning the origin of the inner sphere ("centrosome," MacF.) of the second maturation spindle I believe my results to be more trustworthy than those of MacFarland. This author describes the inner sphere as arising around the minute "Centralkorn" by a condensation of the substance of the central spindle at either end. I have sometimes found appearances similar to Mac-Farland's figures in sublimate-acetic preparations which give a poor fixation of the eggs of *Unio* ; but when the fixation is good I have always found the centrosomes standing out with the greatest clearness, and their differentiation into the inner sphere with the contained centrosome could be plainly traced. The lack of detail in MacFarland's figures of the cytoplasm gives the impression that the fixation of his materials was not of the best, which might explain our differences in regard to

this point ; for it seems to me unlikely that where the agreements are so many such important differences should also exist.

I have already said that the sphere and centrosomes remaining within the egg after the first maturation division may disappear prior to the formation of the second polar spindle. When this happens, the process of formation of the second maturation spindle is (naturally) somewhat different from that already described. There next follows a stage with a single centrosome, whether one of the old ones or not I cannot say ; this lies just within and partly surrounded by the chromosomes, and is first recognizable as the center of a small aster. The regular arrangement of the microsomes on the rays of the latter gives the appearance of a regular sphere, analogous, I believe, rather to the outer than to the inner sphere already described. The centrosome divides in two, and the whole combination of chromosomes and aster moves in towards the center of the egg. A minute central spindle can be seen between the two centrosomes, which develops as the latter move apart. The developing spindle takes up a horizontal position central to the chromosomes and a little above the center of the egg (Pl. XXV, Fig. 30). The sphere has dilated and continues to include the entire spindle, although it forms no part of the latter, as in the former method ; and the rays of the aster are related to the sphere and do not penetrate within it.

The centrosomes at either end of the spindle now divide repeatedly, forming a group of centrosome granules at either end ; and the chromosomes are gradually drawn around the periphery of the spindle at its center. Asters begin to develop at either end, and the whole structure revolves into a radial position (Pl. XXV, Fig. 31), moving towards the surface as it does so. The centrosomes at both ends then simultaneously go through exactly the same process of development as in the former method.

This mode of formation of the second maturation spindle was found in all of the eggs, which were preserved at this stage, of one female ; the modification was undoubtedly due

to some slight difference in the constitution of the cytoplasm in the two cases. That such different courses of events can yet lead to the same typical result indicates a most remarkable power of self-regulation of the entire ovum, and at the same time demonstrates that the mechanical processes of spindle and aster formation are not the primary factors in determining the position of the maturation spindles.

The later history of the sphere and centrosomes of the second maturation spindle is shown in Figs. 22, 23, Pl. XXV, and in Fig. 32, Pl. XXVI. During the anaphase the inner sphere becomes hollowed out in the interior, just as in the corresponding phase of the first maturation spindle; and, as there, so here, the wall of the sphere becomes the central area of attachment of the rays, the centrosome itself being united to the wall of the sphere by a few fibers, which are independent of the aster proper. In the stage of Fig. 23, Pl. XXV, the centrosome is invariably placed excentrically within the sphere. What the meaning of this may be I have no idea. The centrosome does not divide during the anaphase of the second maturation division. But in the stage of Pl. XXVI, Fig. 32, the sphere has enlarged considerably, and its interior is occupied by a reticulum, at the nodes of which are a number of microsome-like bodies.

The accessory aster. — Shortly after the metaphase of the second maturation spindle an accessory aster is formed in the egg. It usually arises quite near the center of the egg and bears no fixed relation either to the maturation spindle or the sperm-nucleus ; the center of the aster is occupied by an exceedingly minute centrosome. The latter divides and a small amphiaster is formed (Pl. XXV, Fig. 28) which entirely disappears before the stage of Pl. XXV, Fig. 23.

What is the significance of the accessory aster ? Is it simply due to a renewal of activity of the sperm-centrosomes which disappeared a cell generation before, or is it an entirely new formation ? I do not believe that it has anything to do with the original sperm-asters for several reasons : in the first place, there are two sperm-centrosomes, and the accessory aster is at first single and later divides ; secondly, the usual place of

origin of the accessory aster does not correspond with the usual place of disappearance of the sperm-amphiaster ; and, finally, in the egg of *Crepidula* (Conklin, '98a) there are two or three accessory asters formed at about this time, while the sperm-aster is perfectly distinct in connection with the sperm-head. Carnoy ('86) has also observed accessory asters of three orders in the egg of *Ascaris megalocephala* during maturation.

This negative result is the only definite conclusion at which I have been able to arrive. It would seem that in this stage the protoplasm of the egg is readily incited to aster formation (*Unio, Crepidula, Ascaris*), and if one cannot refuse the name of centrosomes to the central granule of such asters, it would certainly seem to follow that there are present in the egg other bodies than the sperm- or egg-centrosomes capable of similar function.

III. Growth, Migration, and Union of the Germ–Nuclei ; Behavior of the Sphere Substance ; Origin of the Cleavage Centrosomes.

1. *Observations.*

Immediately after the formation of the second polar globule the egg-sphere undergoes a most extraordinary enlargement, which takes place so rapidly that it has been almost impossible to find stages intermediate between Figs. 32 and 33, Pl. XXVI. The interior of the sphere is filled with a very wide-meshed reticulum or alveolar structure, in the walls and at the nodes of which are deeply staining granules. The boundary wall of the sphere can be seen during the greater part of the time of expansion ; but ultimately (Pl. XXVI, Fig. 34) it becomes stretched beyond the limits of visibility, and the sphere becomes directly continuous with the general cytoplasm. There is thus between the egg-nucleus and the center of the egg at this time (Fig. 34) a mass of vesicular cytoplasm, derived from the inner sphere, entirely devoid of yolk-granules. As this substance preserves its individuality for a considerable time

and acts in a specific manner, it is best to designate it by a special name. So I shall speak of it as the *sphere substance*, following Conklin.

During the growth of the sphere substance the two germ-nuclei also have been enlarging. The individual chromosomes do not form separate vesicles, as in so many eggs, but are intimately united, apparently by processes sent out from the chromosomes. The two germ-nuclei begin their growth at the same time and keep pace with each other throughout the process; so that it would be impossible to distinguish them apart were it not for their positions and the relation of the egg-nucleus to the sphere substance.

The migration of the two germ-nuclei begins simultaneously some time before they have attained their full size. *The egg-nucleus is preceded in its first movements by the sphere substance, which stretches out towards the side of the egg opposite to the sperm-nucleus* (Pl. XXVI, Figs. 35 and 36); *at the same time the sphere substance undergoes a significant change of form, elongating in an equatorial plane at right angles to the line uniting the two germ-nuclei* (Pl. XXVI, Figs. 37 and 37 *a*).

The egg-nucleus thus moves at first away from the sperm-nucleus, which takes up its march in the direction of the former (Figs. 35 and 36). So far as my observations go, this is invariably the case. The two nuclei may meet a little to one side of the center of the egg, in which case they soon move to the center, or they may first come in contact at the center. *At the time of their meeting there is no indication of an aster in any part of the egg;* and I have only once found any such structure in the sphere substance; in this case the aster was exceedingly minute and no doubt sporadic in its appearance. In yet another egg I found an amphiaster in connection with the egg (?) nucleus some time before the meeting of the two nuclei. But this also was extremely unusual.

To return to the sphere substance: I have already spoken of its elongation in a horizontal plane at right angles to the line uniting the two germ-nuclei (Pl. XXVI, Fig. 37). *This elongation continues until there is a relatively narrow band of sphere substance stretching almost entirely across the egg* (Fig. 37 *a*), *at*

a time when the two germ-nuclei are still separated by a quarter of the diameter of the egg. The line of elongation of the sphere substance marks the long axis of the first cleavage spindle. Thus the first cleavage must pass very nearly through the point at which the sperm-nucleus has been resting.

The invariable migration of the sphere substance towards the side of the egg opposite to that in which the sperm-nucleus lies indicates one of two things : either that the sperm-nucleus has, so to speak, driven it away, or else that it is moving along lines of orientation of the egg-substance. The first alternative appears to me manifestly absurd, and the corollary of the second is that the sperm-nucleus has occupied throughout its entire resting period a definite position in the egg ; and this can be explained only on the assumption of a definite orientation of the egg-substance, not only polar but also corresponding to the chief axes of the embryo.

It might very readily be assumed, on the other hand, that the plane of the first cleavage is determined by the copulation path of the germ-nuclei, as is stated to be the case in the ova of some other animals, frog (Roux, **87**) and *Toxopneustes* (Wilson, '**95**). But whoever should take this position for *Unio* would have to explain how it happens that the sphere substance elongates in the plane of the future first cleavage spindle before the germ-nuclei come together. It would be necessary, I believe, to assume that the distant sperm-nucleus exercises an influence on the direction of elongation of the sphere substance in the first cleavage, although in the next division the sphere substance acts independently. And this assumption is absurd on the face of it. (For discussion of this, see p. 262.)

In the movements of the germ-nuclei there are two factors concerned : first, certain dynamic relations between the egg-substance and germ-nuclei, under which we include the influence exerted by the orientation of the egg-protoplasm ; and, second, the mutual attraction of the germ-nuclei. The latter controls the course of the copulation path of the germ-nuclei to a great extent, but not altogether. It is generally, however, so strong a force that the influence of the former factor is not very apparent during the copulation path, but, after the germ-nuclei

have met, it is again in evidence in determining subsequent
movements to the place of origin of the cleavage spindle, and
later in determining the ultimate position of the latter.   I am
convinced, from its subsequent behavior, that the penetration
path of the sperm-head is conditioned by dynamic relations with
the egg-substance dependent on the orientation of the latter.
" The paths of the germ-nuclei are determined by at least two
different factors, one of which is an attraction or other dynami-
cal relation between the nuclei and the cytoplasm, the other
an attraction between the nuclei.   The former determines the
entrance path of the sperm-nucleus, while both factors probably
operate in the determination of the copulation path, along which
it travels to meet the egg-nucleus.   The real nature of neither
factor is known " (Wilson, '96, pp. 151 and 152).

Origin of the Cleavage Centrosomes.

When the two germ-nuclei come together there is no indica-
tion of an aster in any part of the egg.   But, after they have
been in contact for a short time, two centrosomes arise quite
near together, one in contact with the egg-nucleus and the
other with the sperm-nucleus, but both close to the plane of
contact of the two (Pl. XXVI, Fig. 38.   See description of
this figure).   I feel quite sure that the two centrosomes do not
arise from the division of one immediately preceding, but are
formed independently ; for there is no trace of a central spin-
dle between them, and at this stage not more than two rows of
cytoplasm alveoli are involved in the rudimentary asters.   All
of my efforts to find in earlier stages any indication of origin from
a single centrosome, either in closer approximation of the two,
or in union by a central spindle, have been in vain, in spite of
a superabundance of good material covering the critical period.

I was under the impression at the time of my first paper on
this subject (*Science*, March, 1897) that the cleavage centro-
somes arose in the sphere substance, and thought it possible,
therefore, that they might be traced back to the egg-centro-
some of the maturation divisions.   But, although the centro-
somes do arise very close to or on the margin of the sphere

substance (see Pl. XXVI, Fig. 38), I no longer believe that they are descendants of the egg-centrosome. Still less can they be derived from the sperm-amphiaster. *I believe that they are egg-products of new origin formed under the influence of the two germ-nuclei. It seems to me probable that each nucleus has the power in a certain condition of maturity to enter into a reaction with the cytoplasm, which results in the formation of an aster with its centrosome, and thus initiates the process of karyokinesis.* For instance, it sometimes happens in the egg of *Unio* that after the first cleavage the chromosomal vesicles unite so as to form two separate nuclei in each cell, instead of a single one. After these two nuclei have moved apart an amphiaster is formed in connection with each, and two karyokinetic spindles arise.

However, I have found no evidence in favor of the view of Carnoy et Le Brun ('97) as to *Ascaris*, that the cleavage centrosomes are directly derived from the nucleoli of the germ-nuclei. This view would be reduced to absurdity in the case of the egg of *Unio*, where in cleavage stages each chromosomal vesicle apparently forms a nucleolus.

The further formation of the first cleavage spindle is illustrated in Pl. XXVI, Figs. 39–43. The two centrosomes move to opposite ends of the plane of contact of the germ-nuclei. *Each subdivides several times to form a group of granules, which represents the rudiment of the inner sphere.* The outer sphere is well marked by the absence of transverse anastomoses of the rays. One of the centrosome granules then forms the center of the inner sphere, and the remainder arrange themselves around the periphery (Figs. 39–42). The rays of the aster form by continuity of neighboring alveolar walls, as is shown in the series of figures, and the central spindle is formed from the small amount of cytoplasm between the germ-nuclei on the one hand and the two centrosomes on the other.

Behavior of the Chromatin in the Germ-Nuclei.

During the growth of the germ-nuclei there is an enormous increase in the quantity of the chromatin (Pl. XXVI, Figs. 34–40), but a small proportion of which is used in the

formation of the chromosomes of the first cleavage spindle (Pl. XXVI, Figs. 41–43). What becomes of the chromatin which does not pass into the chromosomes ? If the basichromatin granules, which stain so intensely in haematoxylin, were thrown out of the nucleus as such, it would be an easy matter to follow them, but apparently they are first transformed into oxychromatin, which does not stain in haematoxylin, and in this condition they are found on the mantle-fibers of the spindle, which differentiate out of the intranuclear network (Pl. XXVI, Fig. 42). However, some of the unaltered basichromatin is found near the spheres (Pl. XXVI, Figs. 42 and 43). A great part of the chromatin, then, is probably transformed into spindle fibers, as Wilson has maintained in the case of the sea-urchin ; and a smaller part probably enters into close relations with the sphere substance, which it may influence through transfusion or other mode of transference.

The germ-nuclei never " fuse " to form a " segmentation nucleus," but from each arises a group of chromosomes. The chromosomes are at first long and narrow, and longitudinally split from the time of their first appearance (Pl. XXVI, Figs. 42 and 43). Later they shorten and condense, staining more intensely, and become short, bent rods (Pl. XXVI, Fig. 44, and Pl. XXVII, Fig. 45).

2. *Literature and Theory of Fertilization.*

It is well known that, in the majority of animals in which the process of fertilization has been studied with sufficient care, the cleavage centers arise from the sperm-amphiaster. (See Boveri, '87, '95; Crampton ;[1] v. Erlanger, '97, '98; Fick, '93; Griffin, '96, '99; Hill, '95; Korschelt, '96; Kostanecki, '96; Mead, '95, '98; Sobotta, '97; Van Der Stricht, '97; Wilson and Mathews, '95.) When I began the study of the egg of *Unio* I had no reason to anticipate any other result than this, and had

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[1] Crampton's work on the fertilization of the egg of *Molgula* (an Ascidian) is not yet published, but, as it has been ready for some time, the author kindly permits me to refer to it. This egg offers especially clear evidence of the persistence of the sperm-centrosomes in the cleavage, because there are no asters associated with the maturation spindles, and the sperm-asters are perfectly distinct, and persistent from the time of entrance of the spermatozoön.

certainly no prejudice against Boveri's theory. The reasons why it is impossible to take this view of the origin of the cleavage centers in *Unio* are : 1. The sperm-amphiaster disappears utterly before the metaphase of the first maturation spindle, and the sperm-centrosomes become indistinguishable from cytomicrosomes. 2. There is no evidence of the persistence of the sperm-centrosomes during the maturation as anything more than cytomicrosomes, unless we identify with them the centrosomes of the accessory aster, which, however, contains at first only a single centrosome. 3. The cleavage centers *always* arise in a definite position, *viz.*, on each side of the plane separating the two germ-nuclei, one in contact with each nucleus.

If the cleavage centrosomes really are the persistent sperm-centers, it becomes necessary to assume : 1. That the sperm-centrosomes, which are no larger than cytomicrosomes, maintain a persistent identity among these, though the chief distinguishing mark of a centrosome is its position in the center of an aster or centroplasm. 2. That one of the sperm-centrosomes becomes active during the second maturation division (accessory amphiaster) and divides again, the resulting amphiaster then vanishing. 3. That two of the three sperm-centrosomes migrate *to the perfectly definite position*, in which the cleavage centers arise, and there again resume activity. If any theory of fertilization, *otherwise tenable*, depended on such assumptions, I should feel justified in making them. But it seems to me that Boveri's justly celebrated theory must be given up, in large part at least, for other reasons to be spoken of below.

As already mentioned, disappearance of the sperm-centrosomes has been observed by a number of authors (pp. 233, 234). Of these authors, *viz.*, Child, Van Name, Foot, MacFarland, and Klinckowström, only MacFarland positively maintains that the cleavage centers arise from persistent sperm-centers, and his argument appears to me most inadequate. The only reason that he gives for his positive identification is, that the cleavage centers may arise in almost any position relative to one another and the germ-nuclei, though always well above the vegetative pole ; and the sperm-centrosomes exhibit a similar relationship,

or lack of relationship to anything definite, before their disappearance. The case of *Arenicola* (Child, '98) gives especially clear evidence of independent origin of the cleavage centers, for in this case the sperm-centrosomes do not disappear until after the formation of the second polar body, and the cleavage centrosomes arise soon after in such a position that it seems simply impossible that they should represent persistent sperm-centrosomes. Child himself is " inclined to regard the cleavage centrosomes as new formations and as not related to the 'male' centrosome." In the other cases there is certainly no objective evidence of identity of sperm-centrosomes and cleavage centrosomes, indeed much that speaks against it.

I agree, therefore, with Wheeler and with the views expressed by Korschelt (95b, p. 655) and Brauer ('93) that the centrosomes are of no special significance in fertilization. The cleavage centers may arise from the sperm-centers, or from the egg-center (doubtfully, *Myzostoma*,[1] Wheeler, '97), or in

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[1] I cannot regard Kostanecki's observations on *Myzostoma* ('98) as *proving* the origin of the cleavage centers from the sperm-centrosomes in this case. According to both his observations and Wheeler's ('97) no sperm-aster is visible (until at least after the formation of the second polar body, Kostanecki), although the spermatozoön enters the egg before the breaking down of the germinal vesicle. The sperm-nucleus always lies toward the vegetative pole. The germ-nuclei enlarge simultaneously, and begin to approach, the sperm-nucleus making a more extensive migration than the egg-nucleus, so that their place of meeting is always nearer the animal pole. When the nuclei have approached within a short distance of each other, there appears between them a usually double aster (source of cleavage centrosomes). Sobotta's observations on the mouse are entirely similar. What is the origin of these centrosomes ? The author (Kostanecki) sees only two possibilities : either they are the egg-centrosomes reappearing, or the sperm-centrosomes.

" Ich habe aber überdies *vereinzelte* Bilder bei Myzostoma gesehen, die direct für die Herkunft der Centrosomen von Spermatozoon sprechen : wenn die beiden Geschlechtskerne die Gestalt von grösseren Bläschen angenommen haben, konnte ich an der Eimitte zugekehrten Seite des Spermakerns *bisweilen die Andeutung* einer Strahlung sehen, die jedoch mehr durch eine radiäre Anordnung der kleinen Dotterkörnchen zum Ausdruck kam. Die radiär angeordneten Körnchen umgaben ein helleres Feld, in dessen Mitte ein oder zwei kleine Punkte zu sehen waren. . . . Ich glaube in allen diesen Fällen (Pl. XXIV, Fig. 14; Pl. XXV, Figs. 15, 16) das Spermacentrosoma oder die Spermacentrosomen vor Augen gehabt zu haben, wenn auch in Anbetracht des Umstandes, dass das Hauptkriterium für die Existenz von Centrosomen, nämlich eine *deutliche* Strahlung, hier fehlte, eventuelle Zweifel nicht mit absoluter Sicherheit zurückgewiesen werden können."

But these " doubtful " sperm-centrosomes were never traced to the cleavage

such a way that there is no discernible relation to either (*Unio, Arenicola, Allolobophora, Pleurophyllidia, Planocera, My-zostoma, Mus*). (For the last case see Sobotta, '95.)

It must certainly be admitted that the most recent observations on the fertilization and early cleavage of the ovum demonstrate the inadequacy of Boveri's theory of fertilization, *viz.* : "The ripe egg possesses all of the organs and qualities necessary for division excepting the centrosome, by which division is initiated. The spermatozoön, on the other hand, is provided with a centrosome, but lacks the substance in which this organ of division may exert its activity. Through the union of the two cells in fertilization all the essential organs necessary for division are brought together ; the egg now contains a centrosome, which by its own division leads the way in the embryonic development." (Quoted from Wilson, '96.) Certain observations, such as those of Wilson, Mead, Griffin, and others, showing that the sperm-centrosomes become the cleavage centers, support it. But in other cases it is not possible to trace any connection between the centers of the first cleavage and the sperm-centrosomes. And it has been shown by Morgan ('99) that certain chemical substances may initiate cleavage of the unfertilized egg by stimulating the egg-cytoplasm. Loeb ('99) has recently shown by an experiment, whose conception and brilliant success must win our warmest admiration, that in the egg of *Arbacia* not only may division be initiated by adding certain chemical substances to the sea water, but that the entire typical development may take place under these circumstances without fertilization. Neither the sperm-centrosome nor nucleus is required for the typical development of the sea-urchin egg, which has long served, on account of the clear way in which the sperm-centers may be shown to become the cleavage centers in normal development, as the paradigm of Boveri's theory. Certainly in this case the egg is not without the mechanism of cell division, though this mechanism may usually be inhibited by the more active development of the sperm-centers. If not here,

centrosomes. These observations can thus be convincing evidence of the spermatic origin of the latter only for those whose minds are made up in advance.

then nowhere, can the theory hold. Moreover, it seems to me that the theory loses all its significance if the view that the centrosome is a unique and persistent organ of the cell should be given up, as now seems necessary, since it has been shown that centrosomes may arise at any place within the cell, by the observations of Mead ('97), Morgan ('96 and '99), Reinke ('94), and Watasé ('94).

Fertilization usually accomplishes two purposes : 1. It sets in motion the series of developmental phenomena, the entire mechanism for which is in the egg, awaiting only the proper occasion or stimulus. This is not effected primarily by the sperm-centrosomes furnishing cleavage centers, as shown above, but probably by some stimulus, chemical or mechanical, emanating from the part of the spermatozoön that penetrates.[1] 2. Fertilization restores the typical number of the chromosomes. In this we must recognize its chief function and so return to earlier theories of fertilization. The intercalation of a sexual generation in the series of asexual or parthenogenetic generations, wherever these may occur alike in plants and animals, demonstrates, as has been so often pointed out, the fundamental significance of amphimixis. We may regard the first function of fertilization, that of setting in motion the series of ontogenetic changes, as an accessory one adapted to secure the fertilization of as many eggs as possible and to prevent recurrent parthenogenesis. This is of course but a modification of Boveri's theory, that the *postulated degeneration* of the egg-centrosome is an adaptation against parthenogenesis. Perhaps Mead's suggestion, "The inhibition of division would seem to depend upon the metabolic activity peculiar to the cell by virtue of its internal structure," may partly explain the mechanism of this adaptation of the egg-cell (Mead, '98b, p. 218). Or Loeb's view : "The ions and not the nucleus in the spermatozoön are essential to the process of fertilization" ('99, p. 137).

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[1] The universal, or almost universal, occurrence of sperm-asters might thus be interpreted simply as the first response of the egg-cytoplasm to this stimulus.

IV. Movements of the First Cleavage Spindle; First and Second Cleavages.

The first cleavage spindle is invariably formed in the exact center of the egg, as already described, and parallel to the plane of elongation of the sphere substance, which stretches completely across the egg. The spindle remains in the center of the egg until just before the metaphase, and then, without undergoing any considerable change in form or size, it moves end first towards one side of the egg, until one sphere comes almost in contact with the cortical alveoli (Pl. XXVII, Figs. 45 and 46). This movement is a necessary preparation for the first cleavage, which is very unequal. But it is difficult to understand why the spindle should first form in the center of the egg and secondarily change its position. The metaphase and almost the entire anaphase (Pl. XXVII, Fig. 47) of the spindle are spent in this position, and then the spindle shifts back a little (Pl. XXVII, Figs. 48 and 49) to the definitive position of the cleavage plane. (See descriptions of these figures.) It is possible that there may be other lesser oscillations of the spindle before the point of equilibrium is found.

It is perfectly plain from this description that the position of the spindle does not primarily determine the place or direction of the first cleavage plane, but that, on the contrary, the position of the spindle is controlled through the cytoplasm, as a needle in a magnetic field oscillates until equilibrium is attained. Now there are no visible differences in structure in different parts of the egg, which might explain the movements of the spindle, but it seems to me that the movements themselves are an indication of a definite bilateral orientation ; for I cannot conceive it as being possible that in an isotropic substance movements of such definiteness and prospective value in the cleavage should take place. Thus, although there is no other evidence that the movement of the spindle in determining the inequality of the first cleavage is in one direction rather than the other, I am convinced that it is always in the same direction under the influence of some predelineated organization of the cytoplasm.

The behavior of the spheres in the first cleavage is entirely similar to that of the egg-sphere during the copulation phases of the germ-nuclei. The enlargement of the inner spheres begins during the anaphase (Pl. XXVII, Fig. 47) and goes on rapidly during the telophase, until a large amount of sphere substance is produced (Pl. XXVII, Figs. 48 and 49). The sphere substance then elongates in the direction which the second cleavage spindles will assume much later (Pl. XXVII, Fig. 50, horizontal section, and Figs. 51 and 52, vertical section, of the larger cell *CD*). *At the same time each cell takes on the form of a dividing cell* (seen especially well in the larger cell, Pl. XXVII, Figs. 50–52), *although the nuclei are not yet reconstructed since the preceding division.* I regard this as a premature expression of the forces within these cells that later cause an unequal division in the region of the constrictions, after the spindles have been formed. Later both cells round off, then become closely appressed, and, when the spindles form, they move into the positions previously indicated by the elongation and constriction of the cells and sphere substance.

The sphere substance is thus divided in both the first and second cleavages; from its position and form it seems likely that it is distributed between the cells very nearly in proportion to their size. It is, however, impossible to be certain of this, because one cannot follow it in any division up to the time of the definitive placing of the spindle; before this time the intrusion of yolk-granules on it has hidden it from view.

V. Concerning the Sphere Substance.

From the preceding account it will be plain that I regard the entire sphere substance as the product of growth or inflation of a single centrosome. There can be no doubt that it is derived from the inner sphere, so that everything depends on the interpretation of this structure. On pp. 240–242 I have given in detail my reasons for believing that each of the inner spheres of the second maturation spindle is derived from a single centrosome. I can add nothing to the description there given, except the statement that I have worked over this series of

phenomena repeatedly with the utmost care, and have demonstrated the sections to several colleagues with perfect satisfaction. The inner sphere at the central end of the spindle becomes the sphere substance of the fertilized egg. The evidence that the sphere substance formed at the poles of the first cleavage spindle has a similar centrosomic origin is equally conclusive, as the figures show. It seems very probable, although I cannot speak with assurance on this point, that part of the material for the growth of the sphere substance is derived from the nucleus. If this be so, the later wide dispersal of this substance through the cell may give a useful clue to the manner in which the nucleus exercises its undoubted formative influence on the cell.

A similar growth of the spheres towards the end of karyokinesis is now known to be of very wide occurrence (Agassiz and Whitman, '89; Boveri, '95; Erlanger, '98; Herfort, '99; Conklin, '99; Vejdovský, '88). Opinions as to the morphological significance of these structures vary. Erlanger ('98) declares: " Ferner entsprechen die Centroplasmen oder ' Sphären ' durchaus nicht riesig angeschwollenen Centralkörpern wie Boveri angiebt." My own observations would support Boveri's interpretation. Erlanger also suggests an interaction between nucleus and sphere substance: " Den hier entwickelten Anschauungen gemäss dürfte in den mittleren Phasen der Mitose ein enges Verhältniss zwischen den Centroplasmen, inclusive Centralkörpern, und dem Kern, beziehungsweise Kernspindel, herrschen." Herfort's observations ('99) lend themselves to a similar interpretation.

Conklin ('98) was the first to recognize the possible importance of the sphere substance in differentiation. He summarizes his results thus : In the egg of Crepidula, "after the first two cleavages, the sphere substance is differently distributed to the different cells, the entire sphere substance of one generation always going into those cells of the next generation which lie nearest the animal pole. This differential distribution of the spheres has been followed through every cleavage up to the 24-cell stage. As the form of cleavage is perfectly constant, it follows that the sphere substance of any generation

goes into certain definite cells which have a perfectly constant origin and destiny.

"This differential distribution of the spheres is not caused by their specific weight, since their movements are the same in whatever position the egg may be placed. It seems to be the result of a form of polarity, which, like that of the egg itself, is not the result of gravity.

"The centrosomes do not, apparently, arise from the sphere substance of the previous division, but some distance from it, and the sphere substance itself never divides, but each sphere ultimately grows ragged at its periphery, and gradually fades out into the general cytoplasm.

"The differential distribution of the spheres and their subsequent conversion into cytoplasm suggests that they may be important factors in the differentiation of cleavage cells, and if further investigation should establish the fact that they are in part composed of the oxychromatin of the nucleus, it would furnish a basis in fact for certain well-known speculations of de Vries, Weismann, and Roux."

VI. Two Recent Theories of Unequal Cleavage.

Before proceeding to a general consideration of the organization of the egg of *Unio* let us take up two recent theories of unequal cell division. Those views that seek a simple mechanical explanation either in the arrangement of yolk or other inclusions, or in extrinsic forces, have been already sufficiently criticised by Jennings ('96), Conklin ('97), zur Strassen ('98), myself ('95 and '99), and others.

1. *Ziegler's Theory of the Unequal Power of the Centers.*

Ziegler has suggested the theory of centers of unequal power for the explanation of certain unequal cleavages in the ontogeny of nematodes and the ctenophores ('96 and '98). He styles an unequal cleavage in which the centers are of unequal power "heterodynamic." Ziegler does not claim, himself, to have observed any difference in the centers in such cases, but he

believes that in many cases of unequal cleavage there is no other explanation possible. The theory certainly is an advance over the blind faith in invisible yolk that plays so prominent a part in Hertwig's theorizing ('92 and '98) in such matters, inasmuch as it squarely meets the conditions of the problem — unequal cleavage in a mass of protoplasm, in which yolk may be absent or uniformly distributed. But it appears to me to be without satisfactory foundation in fact. If the postulated difference really exists, it should be evident from the start in unequal mastery of the cytoplasm by the two centers ; but if we take the case that Ziegler himself instances as the most marked example of "heterodynamic" cleavage, the formation of the polar globules, we find that in the first maturation spindle, when first formed and lying horizontally, there is no discoverable difference in the size or appearance of the centrosomes or asters at the ends of the spindle. It is only after the spindle has rotated, and one end has moved out to the surface, that a difference is to be noted ; and this is a secondary difference, due not to unequal force but to unequal opportunity. The same is true of every unequal cleavage of the entire ontogeny. As Conklin has pointed out, the centrosomes accommodate themselves to the size of the mass of cytoplasm *in which they come to lie*, being at first equal. Inequality of centers and asters is an effect, not a cause, of unequal cleavage.

But one has a right to expect of a theory meant to be purely formal, that it should be at least far-reaching ; here, however, we are told simply, the cause lies not in the protoplasm but in the centrosomes. How the centrosomes become of unequal power, and how this is adapted to embryo formation, of this not the slightest clue.

It goes without saying, then, that I cannot find in this theory any explanation of the unequal cleavage under discussion. I have looked in vain, though with great care, for any difference in the two centers of the first cleavage, while the spindle still occupies the center of the egg. A difference becomes noticeable only after the spindle has become excentric, and this is plainly due to the fact that the centers are now operating in areas of unequal extent.

Child ('97) finds a difference in the size of the cleavage asters, and suggests that "this anticipates and indicates the difference in size of the first two cells of cleavage." The segmentation nucleus is shown in an excentric position in the figure illustrating this, and I would suggest that the difference in the asters may be due to this.

2. *Conklin* ('99) *ascribes unequal cleavage to movements of the cytoplasm:* "When the cell movements carry the mitotic spindle out of the middle of the cell, unequal cleavage results." The nature and cause of the movements are thus described: "With the escape of nuclear sap into the cell body at the beginning of mitosis, vortical movements are set up in the cytoplasm, the poles of the spindles being the centers of such vortices." The movements are thus "due to the appearance of unlike substances in different parts of the cell." The extremely delicate and beautiful observations on which Conklin's conclusions rest form a model of morphological methods. I fully believe that we have here a factor that must be reckoned with in our theories of early differentiation; it may even have all the importance that Conklin claims for it, while it certainly fails, like all its predecessors, to give any clue to the solution of the adaptive aspect of these phenomena. It may well be that the shiftings of the first cleavage spindle in *Unio* are due to currents in the cytoplasm; the changes in form of the sphere substance and the elongation of the cells after cleavage furnish possible evidence of such currents. But why these currents should relate themselves to subsequent events by placing the spindle in its definite position we cannot say. It would seem also to be necessary to distinguish carefully between random cytoplasmic streamings and those that are definitely adapted to purposes of differentiation. For instance, Ziegler ('96) describes how the germ-nuclei in the egg of *Diplogaster*, lying near opposite ends of the egg, are carried around at first in an aimless fashion by streaming movements of the cytoplasm, which do not, however, prevent the union of the germ-nuclei near the end of the egg, destined to become the posterior end of the embryo. The spindle is then formed and rotates so as to lie in the long axis of the egg, frequently oscillating for a

time back and forth across the line of equilibrium.   Even after
this the spindle may be carried around more or less by stream-
ing of the protoplasm, and yet it comes to rest for the division
in a definite place ; but it *may* be that the random streaming
has become converted into a slow, directive movement that
determines the position of the spindle.   This *must* be the case,
if the theory is to apply here.   The theory would seem to
imply that protoplasmic currents may be adapted to purposes
of differentiation, not that all streaming is definitely directed ;
just as cell division may be adapted to purposes of differ-
entiation, though it may equally be without definite refer-
ence to it.

VII. Organization of the Egg.

We have been assured frequently of late by Driesch (*e.g.*, '99,
p. 717) that descriptive investigations can at most yield nega-
tive results, that only experimental results justify general
theorizing.   While I would personally agree with Driesch that
such theorizing as he sometimes indulges in cries aloud for
justification, I do not find anything in his remarks to cause
descriptive writers the slightest qualms in drawing conclusions
from their observations.   " Negative " and " positive " are
purely relative terms, and as the scientific method at present
universally followed consists in testing working hypotheses by
all means available, I cannot see why a fact of pure observation
should be " negative " in a different sense from one based on
experiment ; or less instructive, especially in biology, where
most of the conditions are unknown, and the experimenter is
generally unaware of much that he has modified.   I should be
the last one to deny the value of the results reached in the
field of experimental embryology, in which Driesch has done
such brilliant work ; but surely it is time to object when
this writer claims monopoly of the faculty of judgment for
those who have shaken eggs.   Rather in our speculation
should we check our observations with experiment and our
experiments with further observation, for only one thing is
more deadly than a careless observation, and that is a doubtful
experiment, and either may lead astray.   Surely one who has

so often been led astray, on his own admission, by doubt-
ful experiments should realize that everything lies in the
interpretation.

One would suppose that even Driesch would admit that the
problem of the development of an entire egg is no more com-
plicated than that of a part ; and that the fact that a part may
develop into a normal embryo gives no explanation of how a
normal embryo is formed, either in "absolut-normal" or in
modified cases.

The evidence on which I base my argument for a bilateral,
and more than bilateral, organization of the cytoplasm of the
egg of *Unio* may be summarized thus : 1. The polar differentia-
tion is clear and definite, but without reference to the distribu-
tion of yolk. 2. Evidence of a *bilateral* orientation of the
protoplasm is given by the behavior of the germ-nuclei and of
the sphere substance ; the line uniting the two germ-nuclei just
before they come together is at right angles to the long axis of
the future first cleavage spindle, and the plane of elongation of
the sphere substance is parallel to the future axis of this
spindle. We have, then, two independent phenomena that
have definite reference to the future axes of the embryo, for
the first cleavage spindle has such a definite relation. In the
absence of definite elongation of the sphere substance it would
be possible to interpret the determination of the axes of the
embryo as due to the plane of meeting or of copulation of the
germ-nuclei. The coincidence of two such independent phe-
nomena can only be interpreted as due to a third factor, *viz.:*
bilateral orientation of the egg-cytoplasm. 3. Evidence of dif-
ferentiation of anterior-posterior proportions is found in the
shifting of the first cleavage spindle. We have seen that this
shifting can be due neither to unequal distribution of the yolk
nor to unequal power of the cleavage centers. Moreover, the
final position of the spindle has a differential value in the
subsequent ontogeny, *i.e.*, it is adapted to the formation of a
large shell gland and mesodermal structures, as I showed in
my paper on "Adaptation in Cleavage."

In this paper I pointed out that the only possible basis for
a rational interpretation of the rate, direction, and place of

cleavage in the cells of the egg of *Unio*, and of other animals possessing strictly determinate cleavage, is a prospective one. " In the cleavage of the egg of *Unio* there are marked variations in the size of the cells and in the rate and direction of their cleavages; in every case these possess prospective significance, and by means of them the organism is able, so to speak, to realize, in the most direct manner possible, on its available capital, the substance of the egg. *To this principle I have given the name of adaptation in cleavage*" (*l.c.*, p. 53). I must refer to this paper and to an earlier one, " Embryology of the *Unionidae*" ('95), for the details of proof that there is no relation between the form of the cleavage and the distribution of the yolk or any extrinsic forces.

The larger of the first two cells is destined to form the posterior part of the embryo, including the mesoblast and the immense shell gland. The smaller cell will form the relatively small anterior portion of the embryo. The proportions of these parts are, then, marked out by the first cleavage. The place of the first cleavage is due to the position ultimately occupied by the first cleavage spindle. The manner in which this reaches its excentric position has already been described in detail. It is certainly due to inherent forces of the undivided protoplasm. Before the egg of *Unio* has divided for the first time it thus foreshadows not only the polarity (position of ectoderm and endoderm), but also the position and *proportions* of anterior and posterior parts of the embryo. *That is to say, the forces within the unsegmented egg of Unio are those not only of a bilaterally symmetrical animal, but of such an animal of definite proportions.* This much at least is evident ; how far other features of the embryo are actually existent in the unsegmented egg, and how far they arise in response to subsequent conditions, internal and external, I am unable to determine. I simply repeat that, independently of cleavage or other of the phenomena usually styled developmental, the bilaterality and the proportions of the embryo of *Unio* are dependent on a certain distribution of protoplasmic (as opposed to nuclear) forces. Nor are these forces in any way complicated or modified by subsequent events ; rather is

the course of subsequent events guided by their unbroken continuance.

These forces are responsible in part for the paths of the germ-nuclei, the elongation of the sphere substance, the definite position and shifting of the spindle, and later, to a great extent, for the equality or inequality, the rate, and the direction of the early cleavages.

The observations and experiments that force on us the conclusion that the cytoplasm of the egg is definitely organized are now so numerous that a book would be required for their adequate consideration. I will therefore refer to only a few typical examples. Watasé ('90) shows that the unsegmented blastoderm of the squid (*Loligo*) is bilateral in structure, the nucleus being situated excentrically nearer the end of the blastoderm that is to become the posterior end of the embryo. The blastoderm is at the pointed end of the nearly oval egg, which is more convex on the surface corresponding to the anterior end of the blastoderm. Watasé does not hesitate to attribute the bilaterality of the blastoderm to the distribution of different protoplasmic substances. He cites the following interesting extract from van Beneden and Neyt on *Ascaris* (1887) : " Les premiers blastomères ont, comme l'œuf fécondé, non seulement une symétrie monoaxone, mais une structure bilatérale. Il est probable que c'est là un caractère commun à toute cellule et l'on doit concevoir un organisme cellulaire, non comme formé de couches concentriques, mais comme présentant essentiellement un axe à extrémités différents et un plan unique de symétrie. Cette symétrie bilatérale de la cellule est probablement la cause de la symétrie bilatérale des organismes plus complexes, des animaux en particulier."

McMurrich's observations ('95a, '95b) furnish interesting evidence of a high grade of organization of the ova of Isopoda. In *Jacra* the segmented egg is a syncitium, the cells being really local nucleated accumulations of a continuous cytoplasm in which the yolk is imbedded. The egg is oval, so that it is possible to recognize an anterior (broader) and posterior (narrower) end. In the 8-celled stage the arrangement of the cells is as follows : four cells at about equal distances apart surrounding

the anterior end of the ovum, near the posterior pole a second circle of three, "while the eighth cell *D* occupies an almost polar position." In the undivided egg there is a central nucleated mass of protoplasm connected by strands with a peripheral layer. Divisions begin in this central mass and do not affect the yolk, so the cells are united by protoplasm, although they move apart. This lack of contact of the cells characterizes the early cleavage throughout, so that, as McMurrich points out, none of the laws of alternating cleavages or mutual pressure can influence the form of cleavage. Yet the cleavage does follow an invariable law of its own, and the arrangement described for the 8-cell stage is brought about partly by the orientation of the spindles and partly by extensive migrations of cells. Now it is to be observed that this arrangement is an adaptive one in the sense that it predelineates definite systems of organs. The four anterior cells give rise to the ectoderm, the three next to entoderm and some mesoderm, while the posterior cell forms the vitillophags, "still later giving rise to certain mesodermal structures."

"In the later stages of *Jaera* there is a concentration of cells towards one surface of the ovum, which will eventually become the ventral surface of the embryo ; concomitantly with this concentration the outlines of the naupliar region of the embryo being formed." But in *Porcellio*, "at the period at which the concentration of the peripheral protoplasm occurs," to form the ventral region of the embryo, "the nuclei are separated from it by equal and considerable distances, being united with it, however, by the protoplasmic network, and it is difficult to conceive how any of them could be able to influence the peripheral cytoplasm in such a way as to produce the concentration. It seems rather that we have to do with an independent action of the cytoplasm, which precociously prepares for the formation of the blastoderm."

Have we not here a beautiful picture of the way in which the egg acts as an undivided organism, controlling some events by orientation of cleavage planes or shifting of cells, and others by protoplasmic movements clearly independent of cell boundaries ? Is it not evident that the orientation of the cytoplasm

is the primary factor in these cases? and that this organization
must include at least bilateral symmetry and possibly definite
proportions?

In his "Inadequacy of the Cell Theory of Development,"
Whitman has given a picture of the way in which crystallization
of the embryo sets in in the undifferentiated mass of cells com-
posing the blastodisc of the pelagic fish-egg. "It is well known
that the transformation of the blastodisc just before the appear-
ance of the germ-ring is quite rapid, at least in the pelagic
fish-egg, and also *quite independent of cell formation.* The
discoidal germ-mass suddenly thins out, but not uniformly in
all parts. The half of the disc in which the embryo is to be
formed remains thick, anticipating, as it were, the axial concen-
tration which is to follow, while the half lying in front of this
is rapidly reduced to a thin epithelial membrane. This *regional*
differentiation of the outer layer and the concomitant formation
of the germ-ring, including the forward movement of the embry-
onic plate ('head process'), which advances in an axial direction
to the very center of the disc, are indubitably accomplished,
not by the aid of cell formation, but by formative processes
of an unknown nature, but, nevertheless, real and all-control-
ling. Cell formation, to be sure, goes on, but it seems to
me certain that it has no *directive* influence on the formative
process. The cleavage runs on from beginning to end, regularly
or irregularly, without modifying in any essential way the form
of the blastodisc. All at once, when this segmentation has
been carried to a certain point, the transformation sets in and
goes rapidly on, without interrupting cell formation, but to all
appearance quite independently of it."

The view that the cytoplasm of the egg possesses at least a
definite bilateral orientation is not in the least weakened by
cases of non-determinate cleavage. In such cases all one can
say is, that cell formation is not directly adapted to purposes
of differentiation. But is this view disproved by such observa-
tions as those of Wilson ('95) and Ziegler ('96) that the orienta-
tion of the embryo may bear no definite relation to the polarity
of the egg? We must inquire here what is meant by this
expression, *polarity of the egg.* Two things, clearly, are meant

by this, and it is necessary to sharply separate these in our thinking, if not in our vocabulary. In the first place is meant the distinction that is marked by the appearance of the " polar " globule at a point (*i.e.*, " pole ") on the surface ; in the second place is meant the distinction that is based on the existence of ectodermal and entodermal portions of the blastula or gastrula. Now formation of polar globules is historically a very different thing from embryo formation, and there is no other reason for assuming that the axes marked out by these distinct processes *must* correspond, than that apparently they almost always do. Therefore I fail to see why such observations as those of Wilson and Ziegler furnish evidence against such an organization of the egg-cytoplasm as we have maintained. If there be an ectental orientation of the egg-cytoplasm prior to maturation, it would be natural to expect a control of the place of polar globule formation by this, of such a sort that the polar globules would tend to arise at the ectodermal (animal) pole, as is usually the case, or entodermal (vegetative) pole, as happens apparently in the Ascidian egg (Castle). But one can readily imagine that other forces might operate more powerfully and determine a different location of the polar globules.

Just as little do I consider that observations of the development of the egg under pressure or of the development of parts into whole embryos militate against the view here upheld. The eggs, like the adults of many animals, possess great power of reorganization. In such cases the reorganization of the egg-cytoplasm would be a necessary preliminary to typical development, however we may think of it as taking place.

Finally, even if it should be proved that the axis of the embryo may be determined either by the point of entry or by the copulation path of the spermatozoön, we should have here no evidence of lack of previous organization in the cytoplasm, but simply another striking confirmation of the wonderful power of reorganization of the egg-cytoplasm, for it has been shown that parts of fertilized eggs in which this process is held to occur (frog, sea-urchin) are capable of typical development, in which case the path of the spermatozoön can exercise no possible directive influence.

If the basis of these adaptive cleavage forms is cytoplasmic, as I believe, the complexity of the cytoplasmic organization must be great. The preceding examples illustrate this. But no phenomena of this sort give so overwhelming a sense of the inadequacy of our theories of development as the fact that cleavage may actually exhibit ancestral reminiscence (Wilson, '98). Admitting that reminiscence of ancestral conditions is ever shown in ontogeny, no one who is conversant with the literature of cell lineage can doubt that Dr. Wilson has placed the phenomena which he describes in their natural category ; they belong to the same order of events as the development of tooth germs in a whale embryo. No one who has become convinced that true homologies are found in cleavage, and that the cleavage may exhibit adaptive modification, can be surprised by this last discovery. But these phenomena bring us so near to the unsegmented egg, that the conclusion is unavoidable that homology, adaptation, and ancestral reminiscence have a protoplasmic basis, and are not merely dependent on the recurrence of similar conditions.

Another conclusion that follows directly from this is that the egg transmits far more than the spermatozoön, *at least in these early stages*. All those features of the early development that are dependent on the organization of the cytoplasm must be transmitted by the egg alone. Driesch ('98) has gone to work in the right way with his experiments on hybridization, to determine what features of the early development are purely maternal in their origin. Before his experiments were published I had begun some similar experiments on fish-eggs to determine the influence of hybridization on the cleavage ; but reached no certain results, owing to difficulties offered by the material. Experiments of this sort on eggs exhibiting determinate cleavage are much to be desired. The annelids would offer, perhaps, the best opportunities for this kind of work.

Coördinated and definitely directed forces imply a mechanism of definite configuration ; what idea can we form of the configuration of this mechanism? In the first place, it should be observed that, inasmuch as the capacity for typical development is not dependent on the maintenance of the integrity of

the entire egg, but is, as has been shown for echinoderms especially, the property of any nucleated part of sufficient size, the mechanism cannot be *primarily* due to structurally differentiated germ-areas. Even if such areas can be shown to exist in any unsegmented egg, it would be necessary to regard this as a secondary effect of the primary mechanism upon the unsegmented egg.

It has not been shown for the egg of *Unio* that separated nucleated parts of definite size are capable of typical development; but even if it should be shown that they are not, that typical development is dependent on the integrity of the original egg, I could not consider that as evidence in favor of localized germ-areas; for it has been clearly demonstrated, in the case of the frog at least, that partial development of isolated blastomeres may be due to certain physical properties of the protoplasm. Whether we are studying the typical development of a part of an egg or of the entire egg, we are confronted with the same problem; and all that the facts of typical development of a part teach us is, that neither the nuclei nor yet the protoplasm of early cleavage stages are unchangeably specified. Are these then the " positive results " that we are led by Driesch to expect? So far as positive results are concerned the entire problem still faces us, and the negative conclusions were clearly seen by some naturalists as the result of interpretation of facts of observation (" Nature's Experiments "), long before the experiments were performed.

But we may be told that complete and typical development of a part, even from the moment of its separation, is no argument against the original localization of germinal areas, for the typical regeneration of a fragment of *Hydra* does not alter the fact that the parent animal had differentiated parts. But in the absence of ocular demonstration of areas in the whole egg, or of experimental proof of their occurrence, and in the face of the fact that currents of rotation and translocation in the protoplasm of the egg in no wise affect the power of typical development (Ziegler, '96), the theoretical necessities must be hard that will drive us to such an hypothesis. Moreover, if certain areas be removed from the egg, for instance, A and B

from an egg *A B C D*, then, according to the hypothesis, *C* and *D* must reconstitute *A* and *B* before the typical development can begin. But it can be shown that they are not reconstituted by growth; and *if complete reorganization of the areas take place in the material of the part, their typical distribution and proportions must be due to some primary mechanism of the entire protoplasm independent of regional differentiation.* Finally, in the regeneration of *Hydra*, the fact that equal parts from different regions form similar individuals shows that the power of regeneration does not depend upon the visible structural features, but on some underlying principle of organization common to all parts of sufficient size. To argue, therefore, for germinal localization from the facts of regeneration is to miss the most characteristic feature in such regeneration as that of *Hydra*, for the visible localization of parts has no reference to the formative forces.

Driesch has also been concerned with the problem of the configuration of the protoplasmic mechanism in the egg. In his paper "Lokalisation morphogenetischer Vorgänge" he formally announces his discovery that the location of morphogenic phenomena is a problem *sui generis*. After devoting a considerable part of the introduction to the demonstration of this, he begins Part I thus: "Having shown that the localization of ontogenetic processes is a possible problem, we begin by instancing a further example of this problem by recording the results obtained on blastomeres of the echinoderm egg." Does Driesch really believe that all the rest of the world has remained in ignorance of the existence of such a problem? Is he not aware that the theory of localized germ-areas is shaped largely to the requirements of this problem? What does he suppose that Loeb has in mind when he propounds the question, "What are the circumstances which determine that only one kind of organs originate at *certain places* in the body?" ("On Some Facts and Principles of Physiological Morphology," *Biological Lectures*, 1893, p. 38). It has long been generally recognized that the morphological problems of any ontogenetic process involve at least three factors: (1) its localization; (2) the time of its origin; (3) its specific nature: (place, time, structure).

I dwell on this because I am especially concerned here with a problem of localization, *viz.*: What determines the position of the first cleavage plane? and this, as I have attempted to show, is equivalent to asking, What determines the general proportions of the embryo of *Unio*? With Driesch I recognize that the conditions of this problem are found in a mass of protoplasm, the parts of which cannot be shown to be different.

Now how does Driesch conceive of this mechanism? In the first place, he attempts to explain the polarity of the egg by assuming polarity of the protoplasmic parts or elements, without considering further the nature of these; and as he cannot find the causes of the bilateral structure of the embryo in external conditions, he endows the parts with bilaterality also, so that each is doubly heteropolar. The problem of the polarity and bilaterality of the egg is thus transferred to its parts, so that the organization of the whole might be pictured as due to a "magnetization of the parts." But these postulates are still inadequate to explain the localization of ontogenetic processes; why the stomodaeum, for instance, arises in a certain part, or why the intestine of the pluteus becomes divided into three parts of definite proportions. How is this condition satisfied in Driesch's system?

"Let us first examine the simpler case of differentiation in a system with but one heteropolar axis, for instance, the intestine of the echinid gastrula, or the stem of *Tubularia*. Here we can represent the process in 'causal' form to ourselves by assuming that one of the end points of the axis is the seat of incitive (auslösender) 'Fernkräfte'; this spot, which is distinguished as something different from other points of the system by the very fact that it is the end of the axis, produces, therefore, an effect (*Wirkt* also); that on which the effect is produced must be able to answer to the cause, as in all cases where effects are produced; and in this manner a kind of causal harmony is postulated by this conception of the matter.

"In what way, now, does this particular designated place of the axis affect the system? Let us explain this first in the

simple case of the segmentation of the intestine of the echinid gastrula.

"Here at first only two effects are produced; the formation of two constrictions takes place. That the effect happens to be the formation of constrictions does not concern us here, for it is founded on the potency of the system, and is only called forth by the exciting cause; but the cause is decisive for the place of origin of the constrictions. As already said, we *must* (italics mine) conceive of this cause as 'Fernkräfte,' more particularly as 'Fernkräfte' acting at a particular distance. But the distance of the effect (which is inherent) is not absolute, because in gastrulae of any size, produced by operations, the intestine segments equally well into the proper proportions; thus the effective distance of the 'Fernkräfte' acting from one end point of the system is given not as an absolute but as a relative amount, being dependent on the length of the axis of the differentiating system.

"The establishment of heterogeneity affords new places for new kinds of 'Fernkräfte,'" *und so weiter.* Driesch passes here from saying in one paragraph we *can* represent the process by *assuming* "Fernkräfte" acting from a fixed point, to the assertion in the next that we *must* conceive of the cause as "Fernkräfte." Hereafter, then, he regards his postulate as a logical necessity, no longer a mere intellectual convenience, like Maxwell's demon, although it is an hypothesis of a precisely similar nature.

It is pertinent to ask whether in any extremity the assumption of "Fernkräfte" is permissible; but, passing this by, the postulated force must either be a product of the system or something independent of the system. If a product of the system, when all parts are alike, it must be postulated of each part; each element then is not only bilaterally symmetrical, but has a "Fernkraft" "seated" at one of its poles; as soon as the heterogeneity demanded by this is established, the elements in each section become endowed with new kinds of "Fernkräfte." Unless these arise absolutely *de novo* (!), they must have existed in a latent form previously, and there would appear to be no escape from the conclusion that each element of

the protoplasm must be endowed originally with all the " Fern-kräfte " of every localization of the entire ontogeny. But are the " Fernkräfte " independent of the system ? What sort of science would this be? Yet Driesch appears, without actually discussing these alternatives, at times to lean to this view. For instance, p. 95 : " The actual adaptiveness of the process *appears* thus in this case to be contained *both* in the causing spot (verursachenden Ort) and the forces proceeding from it, *as well as* in the system concerned." He explains this "appears," on the next page, by saying that in reality the conception of adaptiveness belongs only to the system, because this controls by its absolute size the effective radius of the " Fernkräfte."

Does the conception of " Fernkräfte " reduce itself to ab-surdity or does it not ? One must admit that it is often difficult to extract the meaning from the welter of words, and so I may have misinterpreted Driesch, though I have honestly tried to understand and represent his ideas fairly. Driesch believes his theory of " Fernkräfte " to be rational vitalism ; I should prefer to style it irrational mysticism.

On the other hand, the theory of the bilaterality of the pro-toplasmic elements appears to me a useful hypothesis, even though it may be difficult to see that their endowment with bilaterality alone is a logical halting ground. In the case of the egg of *Unio*, at least, the theory would seem to be of greater use if we were also to postulate of the elements certain antero-posterior proportions.

That the entire organism in every stage of its development exercises a formative influence on all its parts appears to me an absolutely necessary hypothesis. Only thus can we explain the reorganization of portions of the egg or of the adult into complete individuals, or form a satisfactory picture of the maintenance of individuality. The theory of division of labor needs reinforcing by a companion principle of unity of organ-ization. The comparison of the metazoan body to a com-munity or state is inadequate ; it is this inadequate conception that has led to the extreme cell theory of development, as upheld by Hertwig and the majority of zoölogists. The mis-conception may be traced back to Haeckel's comparison of the

egg to a protozoan, and the cleavage stages to colonies of protozoa. We are even beginning to doubt that *phylogenetically* the protozoan-colony theory is a necessary view of the origin of the metazoan body; ontogenetically this view has become impossible.

But though the hypothesis I have just stated above appears necessary to many at the present time, I think there are very few who will follow Driesch in his rapid flight from one extreme, the dominant cell theory which he upholds in his earlier papers, to the opposite : that only incomprehensible and intangible vital forces, such as " Fernkräfte," are adequate to explain the unity which, as we are beginning to see, pervades all the metamorphoses of any species. I think that most naturalists will resolutely suppress the feeling, almost of panic, that accompanies the revelation of the real complexity of the problems of development, and will stand firm in the unassailable position that, until it is shown that there enter into the composition of protoplasm elementary substances not known elsewhere, vital properties must be explained as in some way due to the qualities of that matter which physicists and chemists investigate. On the other hand, better understanding of the marvelous properties of protoplasm furnish problems to the physicist and must inevitably lead to a reconsideration of the properties and theories of matter.

Zur Strassen ('98) has been led by his studies on nematode development to reject mechanical views of cleavage. He is impressed by the adaptive nature of the earliest cleavages and exclaims : " It is as though the cleavage cells possessed a perfect guiding *instinct*." " Ich zögere wirklich kaum, diesen Begriff allen Ernstes für das Verhalten der Blastomeren in Anspruch zu nehmen." He gives a vivid description of the manner in which the *T*-shaped early 4-cell stage is converted into a rhomboid by active amoeboid migrations of the cells, sometimes carried out in the face of considerable difficulties, as in the giant eggs, where the cells have to force past a constriction of the egg-membrane. " Ich sah zwei Organismen ein Ziel, ihre Vereinigung, erstreben und dieses Ziel erreichen. . . . Die Zellen sind nicht Bausteine der Entwickelung, die durch

fremde Kräfte geformt und an ihren Ort geschleppt worden, sondern Steine und Baumeister zugleich."

The use of the term "instinct" for such phenomena of irritability and response as the parts of an embryo exhibit is questionable, and is not in any case very illuminating. While I heartily agree with Zur Strassen that we must look within the egg for the chief causes of embryo formation, it appears to me that he attributes too much to "social instinct" of the cells. In this he resembles Hertwig, indeed, sometimes using similar expressions, as where he speaks of single cells of the 4-cell stage as "organisms." The four cells form but one organism; "it is neither functional *economy* nor social instinct that binds the two halves of the egg together, but the constitutional bond of *individual organization*" (Whitman, '93, p. 115).

The view that I have attempted to demonstrate is: 1. That the cytoplasm of the egg of *Unio* possesses a definite organization involving bilateral symmetry and certain antero-posterior proportions; 2. That this is not primarily dependent on localization of specific substances, but in some way results from the interaction of the idiosomes.

I believe: 1. That this particular kind of organization is continued throughout the entire life cycle, and is one of the main factors in axial regeneration where this occurs; 2. That in every stage of the organism it is an undivided force. It therefore implies protoplasmic continuity throughout.

I regard this as one of the most important factors in ontogeny, and think it important to clearly distinguish it from the other factors, such as reactions of nucleus and cytoplasm, tropisms and taxic phenomena, and all relations of parts. It differs from these in being the property of the cytoplasm as a whole.

It appears to me that the problem we are considering recurs in fundamentally the same form in the reorganization of a fragment of a stentor or a planarian into a complete individual. The materials in which the formative forces [1] are at work are different in these cases, and so there are different modes through which they reach their expression. But whether these forces are working through adaptive cleavage, or in a

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[1] I use this term in a purely descriptive sense.

mass of undifferentiated cells, such as the chick blastoderm in
an early stage, or in a mass of material composed of specific
tissues, as in a portion of a planarian, it appears to me that
they are in principle everywhere the same; I would say that
the same cytoplasmic organization is concerned alike in the
development of the planarian from the egg and from the frag-
ment of a mature individual.   Where are we to place it except
in those protoplasmic elements of whose nature we are so
ignorant?

"If the formative processes cannot be referred to cell divi-
sion, to what can they be referred?   To cellular interaction?
That would only be offering a misleading name for what we
cannot explain; and such an answer is not simply worthless,
but positively mischievous, if it puts us on the wrong track.
Loeb's experiments on heterogenesis furnish a refutation of
the interaction theory.   The answer to our question may be
difficult to find, but we may be quite certain that when found
it will recognize the regenerative and formative power as one
and the same thing throughout the organic world.   It will
find, as Wiesner has so well insisted, a common basis for every
grade of organization, and it will abolish those fictitious dis-
tinctions we are accustomed to make between the formative
processes of the unicellular and multicellular organisms.   It
will find the secret of organization, growth, development, not
in cell formation, but in those ultimate elements of living mat-
ter, for which *idiosomes* seems to me an appropriate name."
(Whitman, "The Inadequacy of the Cell Theory of Develop-
ment.")

I think that we need not ascribe to the egg a more complex
organization than it may be ultimately possible to discover in
it by observation and experiment, and should attempt to explain
the subsequent development, phenomenally at least, in a purely
epigenetic manner.   I am very far from asserting that in the
case of the egg of *Unio* we have fully described the organiza-
tion in the cytoplasm, to say nothing of the nuclear material.
But what I do mean to assert is that we should rid ourselves of
all mysticism in dealing in a purely scientific manner with the
problem of the organization of the egg, or the nature of the

primary formative forces.   These should be matters of pure description of facts observed, or inferences of a descriptive nature directly drawn from these facts ; theories of *potency* should be left to the metaphysicians.   No amount of observation or experiment will enable us to add anything to metaphysical theories of potency ; if we are to devote ourselves to these, we might as well shut up our laboratories.

It does not appear to me that in the above sense there is any insoluble problem in the whole field of heredity.   We may hope, by sufficiently long, patient, and searching investigation, some day to be able to trace the organism through all phases of its metamorphoses from the egg of one generation to that of the next, to describe the exact nature of the organization that is continued throughout, to estimate at its proper value the influence of external conditions at each step, and so solve the old riddle of evolution and epigenesis, and that of the influence of the soma on the germ.   That there is continuity of organization we know, but what the organization that is continued may be, we do not know.   This is the question that presses most at the present time for solution.

The solution of the problem of differentiation would be given by an *exact* description of *every* step in the process.   If this ideal should ever be realized in any one case, there would be no further need of *scientific theories* of development.   But it seems extremely improbable that it can ever be actually realized, though I believe that it will be possible by sufficiently detailed observation so closely to approximate what we call stages of development to each other that but a single rational theory will exist capable of filling up the gaps.   Therefore, observations such as those of Boveri ('99), Conklin ('99), and those described here seem to me the surest means of approaching this theory.   That experimental methods will be of immense service no one can doubt.   The study of living material will also be an indispensable method.

In conclusion, then, I would state as my opinion that the organization that is continued from one generation to another consists of nuclear material in a mass of cytoplasm possessing a definite orientation, the extent and nature of which must be

left to future investigation; I have described here only what seemed to me necessarily to follow from the phenomena accompanying the maturation, fertilization, and cleavage in *Unio*. It is this orientation in the cytoplasm that gives a differential value to mere position (Driesch), for "position" in an unorganized mass of cytoplasm is unthinkable. We have sufficiently considered the evidence for believing that this organization must be a property of the idiosomes; and it seems to me at present that Driesch's conception of bilaterality of these elements is likely to prove a fruitful one.

Vassar College,
May, 1900.

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DESCRIPTION OF PLATES.

All figures are from camera drawings of sections of the eggs of *Unio compla-nata*. Most of the figures are from single sections, but when two or more sections are combined in a single drawing this is always stated in the description. The lenses used for the drawings were Zeiss Apochromat 2 mm. Homog. Immers. and compensating ocular No. 6, except where otherwise stated. The figures are all drawn from eggs killed in Boveri's picro-acetic mixture, and stained on the slide with Heidenhain's iron-haematoxylin, followed or preceded by Bordeaux red in aqueous solution. Needless to say other methods were used, but the drawings are not published. The sections were 5 or 6 2/3 μ in thickness.

EXPLANATION OF PLATE XXIV.

FIG. 1. Radial section through the egg. Early stage of the first maturation spindle. Sperm-head and comet-like sperm-amphiaster.

FIG. 2. Perfect axial section. Part of the egg-membrane with the micropyle is shown. Somewhat later stage of the first maturation spindle than in Fig. 1. The entire path of the spermatozoön is shown. Peripheral distribution of yolk-granules. The section was injured before the completion of the drawing, so the maturation spindle is represented somewhat diagrammatically.

FIG. 3. Sperm-nucleus in the same position as in Figs. 1 and 2; but the sperm-centrosome is just dividing.

FIG. 4. Sperm-head and amphiaster from a stage of maturation similar to Fig. 2. The plane of separation of the sperm-centrosomes is nearly at right angles to the penetration path. Many similar eggs found. The distal sperm-centrosome is divided in three parts.

FIG. 5. Turning of the first maturation spindle; stage intermediate between Figs. 1 and 2. Combination of two successive sections.

FIG. 6. Section of unfertilized egg with first maturation spindle. In the other eggs of the same lot, which were fertilized, the first polar globule was already fully formed, and the second in process of formation.

FIGS. 7 *a* and 7 *b*. Sperm-nucleus and sperm-aster from different sections of the same egg, in a stage of maturation intermediate between Figs. 2 and 5. The centrosome in 7 *a* is attached by a fiber to the tip of the sperm-nucleus, and the aster is gone. The aster around the centrosome in 7 *b* is already breaking up.

FIG. 8. Slightly later stage of degeneration of the sperm-asters. But one centrosome could be found; the one that originally lay nearer to the sperm-nucleus is entirely indistinguishable.

FIG. 9. Radial section of the first maturation spindle at the time of the metaphase; one of the chromosomes has already divided transversely. Part of the inner aster was drawn from another egg, where it showed more plainly.

FIG. 9 *a*. Sperm-head in the same egg from which Fig. 9 was drawn. The sperm-centrosomes are now no longer distinguishable; and the clear area has

become much smaller. The sperm-nucleus is contracting, and may be seen to be composed of about sixteen chromatic vesicles. Only those of one surface shown.

FIG. 9*b*. Sperm-nucleus of another egg in the same stage of maturation, to show the vesicular structure.

FIG. 10. Tangential section through the outer aster in the stage of Fig. 9, to show the concentric spheres, inner and outer, and the rays penetrating to the dumb-bell-shaped centrosome.

FIG. 11. First maturation spindle just before the beginning of the protrusion of the first polar globule. The centrosomes at each end are double, and the chromosomes are assuming the form characteristic of the second maturation division, thus repeating their form in the first maturation spindle. The microsomes are beginning to grow large in the interzonal region where the stress has ceased. *Cf.* Fig. 8.

FIG. 12. First maturation spindle. In this stage the two centrosomes of each inner sphere are distinctly compound, double or quadruple. The enormous size of the microsomes in this stage is especially noticeable. The chromosomes have completely assumed the form characteristic of the second maturation division.

FIG. 13. Constriction of first polar globule. The inner sphere and group of chromosomes have moved bodily near to the surface, to which they are attached by a new set of radiations. The sphere remaining in the egg is smaller than in the preceding stage (Fig. 12), and the centrosomes within it are closer together.

FIG. 14. The first polar globule is fully formed, but is still attached by means of "Zwischenkörper" fibers to the surface of the egg. (These were found in the next section, which is not figured.) The centrosomes of the egg-sphere are now in contact, and the radiations which in Fig. 9 extend from the sphere to the surface have now been resolved into vesicular cytoplasm.

EXPLANATION OF PLATE XXV.

FIG. 15. Earliest stage in the formation of the second maturation spindle. The central spindle arises within the inner sphere. Each of the centrosomes is at least quadruple. Radiations surround the entire sphere, and are not specially related to the centrosomes of the new central spindle.· The fibers of the aster of the first maturation spindle form a reticulum with large, deeply staining microsomes.

FIG. 16. Slightly later stage; the elongation of the central spindle has stretched the sphere into an elliptical form. The boundary of the sphere forms the periphery of the new central spindle, from all parts of which radiations proceed.

FIG. 17. Further elongation of the central spindle ; the radiations of the preceding stages (Figs. 15 and 16) have become resolved into vesicular cytoplasm, and new asters are forming in this from each of the compound centrosomes ; the aster at the inner end of the spindle is much farther developed than that at the outer end. Mantle-fibers are attached to the chromosomes, which are being drawn towards the equator of the spindle. The centrosomes are larger and more subdivided than in the preceding stages, but as they are somewhat flattened radially this is not very evident in this figure. See, however, Fig. 24.

FIG. 18. Later stage. The chromosomes are now nearly at the equator of the spindle. The inner centrosome has nearly undergone the metamorphosis more fully pictured in Figs. 24–27. The outer end of the spindle is in contact with the surface, apparently as a result of the elongation of the spindle ; fibers pass from the inner centrosome to the surface.

FIG. 19. In this stage the outer centrosome still consists of a number of deeply staining granules, each of which under ocular 18 seems to be compound. The outer sphere is now established by the arrangement of the first row of microsomes.

FIG. 20. The outer centrosome has now completed its metamorphosis into a sphere (inner sphere) with central granule (centrosome). The chromosomes are splitting longitudinally. For the sake of clearness only about one-third of the chromosomes are drawn in.

FIGS. 21–23. Metaphase and anaphase of the second maturation spindle. Note the peripheral distribution of the yolk in Fig. 23.

FIGS. 24–27. These are tangential sections through the outer centrosome of the second maturation spindle in the stages of the spindles figured immediately above them (Figs. 17–20). They are camera drawings with comp. oc. No. 8 and hom. imm. apo. 2 mm. of Zeiss.

FIG. 28. *Accessory amphiaster.* The inner end of the second maturation spindle (in the anaphase), cut obliquely, is to the right. The sperm-nucleus in this egg was found in another section near the side of the egg opposite to the accessory amphiaster.

FIG. 29. Figs. 29–31 illustrate the second mode of formation of the second maturation spindle. In Fig. 29 the aster and centrosome of the first maturation spindle have almost entirely disappeared.

FIG. 30. The chromosomes have moved in towards the center of the egg, and the central spindle is forming in a horizontal plane. Each end of the spindle is occupied by a group of centrosome granules.

FIG. 31. The spindle is completely formed and has swung into its definitive radial position. At each end of the spindle is a large group of centrosome granules, which later undergo the metamorphosis shown in Figs. 24–27. There is no such difference in the time of the metamorphosis of the centrosomes in this mode of formation of the spindle as in the other mode.

EXPLANATION OF PLATE XXVI.

Fig. 32. Telophase of the second maturation spindle. The inner sphere has enlarged considerably, and in place of the single centrosome there are a number of deeply staining bodies united by a delicate reticulum within the sphere. The entire sphere stains more darkly than before. The aster shows the usual appearance of degeneration, being thickly studded with large microsomes.

Fig. 33. Beginning of reconstruction of the egg-nucleus. The inner sphere has swollen up to a relatively enormous size, and is occupied by a loose-meshed reticulum with deeply staining microsomes on the fibers.

Fig. 34. The egg-nucleus has enlarged considerably, and the sphere substance is continuous with the general cytoplasm.

Fig. 35. Beginning of the migration of the germ-nuclei. The egg-nucleus, accompanied by the sphere substance, is moving towards the side of the egg away from the sperm-nucleus, which has taken up its march in the general direction of the egg-nucleus. Zeiss 4/2 mm. Combination of four sections.

Fig. 36. Similar section in a slightly later stage of the migration. In this stage the sphere substance has elongated in a plane at right angles to a line uniting the germ-nuclei and to the axis of the egg. Zeiss 4/2 mm.

Fig. 37. Section in the plane indicated on Fig. 36 to show the elongation of the sphere substance.

Fig. 37a. Outline of sphere substance in a later stage stretching completely across the egg.

Fig. 38. Meeting of the germ-nuclei. This is the earliest stage in which the cleavage centrosomes can be distinguished. The figure is a combination of two successive sections; hence the appearance of overlapping of the nuclei. *The centrosomes are therefore simply seen through the nuclei. They do not lie in them.* For the sake of clearness the chromatin has been omitted, except in the right-hand border of the egg-nucleus. The latter can be distinguished from the sperm-nucleus by its relation to the sphere substance, which can still be distinguished.

Figs. 39–43. Illustrate origin of asters, spheres, and chromosomes of the first cleavage spindle. The position of every chromatin granule and spindle fiber in Figs. 39–43 is drawn in as accurately as possible.

Fig. 39. Combination of two successive sections; before the formation of the central spindle. The centrosomes are broken into a number of parts imbedded in a red-staining mass. Outer sphere well marked; mag. Zeiss 8/2 mm.

Fig. 40. Slight advance in aster and spindle formation. First rudiments of chromosomes may be distinguished; mag. Zeiss 8/2 mm.

Fig. 41. Origin of central spindle; chromosomes and mantle-fibers forming. Notice chromatin masses near the centrosomes. Slight diminution in amount of basichromatin. Zeiss 8/2 mm.

Fig. 42. Combination of two successive sections. Great diminution in amount of basichromatin; long chromosomes appearing. Other chromatin granules in spindle fibers, and yet others in the neighborhood of the centers. Remainder in the form of oxychromatin (?). Zeiss 8/2 mm.

Fig. 43. Early stage of the first cleavage spindle; the two groups of chromosomes readily distinguished; each chromosome is very long and delicate but distinctly double.

Fig. 44. Later stage of the first cleavage spindle before its shifting. Vesicular "centrosome" (inner sphere).

Pl. XXVI.
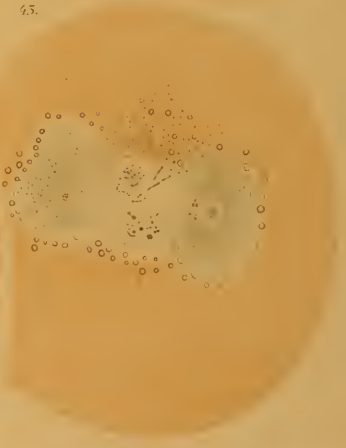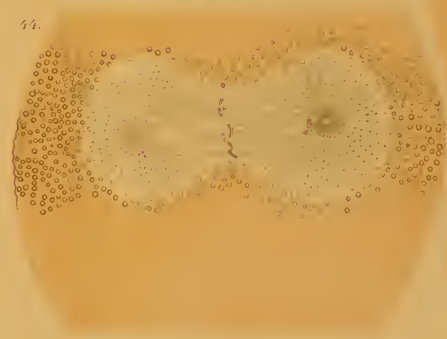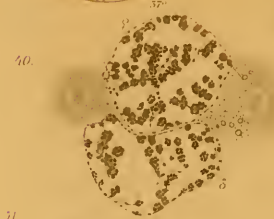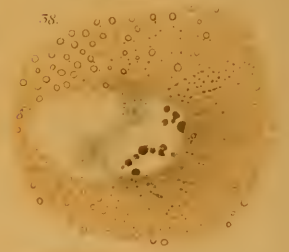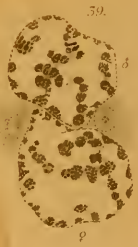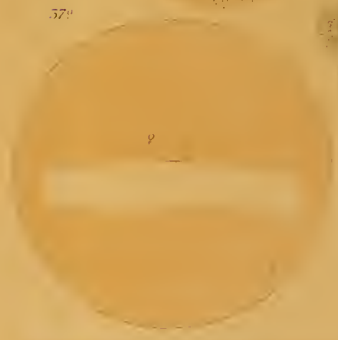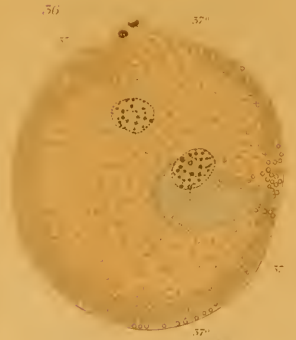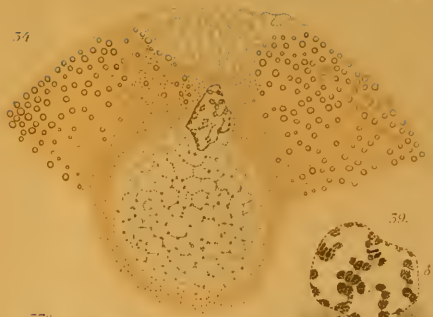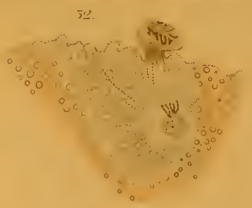
EXPLANATION OF PLATE XXVII.

FIG. 45. Shifting of first cleavage spindle to one end of the egg. Observe that the spindle does not elongate as it moves. Stage immediately preceding the metaphase.

FIG. 46. Metaphase of the first cleavage spindle; for the sake of clearness only about one-third of the chromosomes in the section were drawn. In this stage there is a single centrosome within a hollow inner sphere. The egg is perfectly round.

FIG. 47. Anaphase. Egg slightly elongated in the plane of the spindle. The inner sphere is enlarged and occupied by a delicate reticulum.

FIG. 48. Beginning of telophase; the spindle has shifted to its definitive position; the line of shifting is plainly marked. The outer sphere has disappeared; the inner spheres have enlarged yet further, and are occupied by a reticulum with microsomes at the nodes.

FIG. 49. Combination of two sections. Constriction in first cleavage plane begun. The inner sphere has grown much more and altered its form; its reticulum is wider-meshed. The remains of the aster is a close-meshed reticulum (or foam). The migration of the sphere substance in the larger cell is already indicated.

FIG. 50. Combination of two sections. The sphere substance has lost its sharp boundaries, and in the larger cell is migrating towards one side of the cell which is elongating in that direction.

FIGS. 51 and 52. Two sections of the larger cell parallel to the first cleavage plane in a slightly later stage. The nucleus is yet in process of reconstruction, and the sphere substance is stretching across the cell. The furrow is on the vegetative pole surface of the cell.

Pl. XXVII.
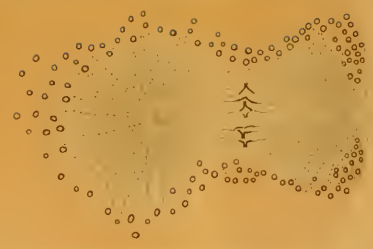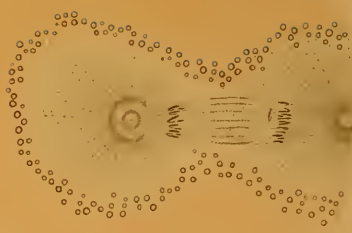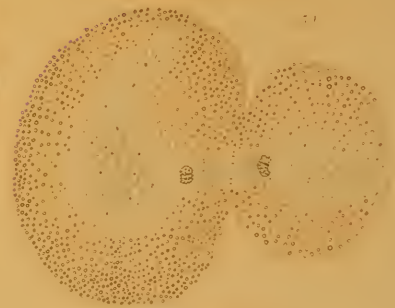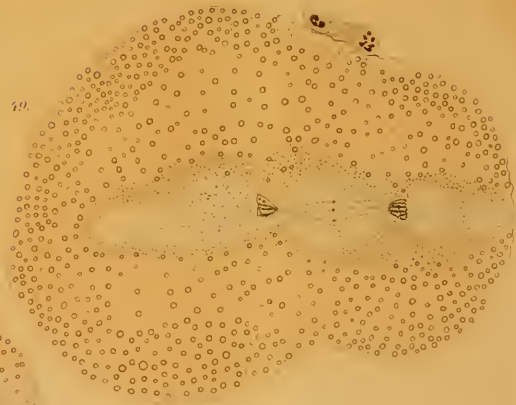
THE MATURATION AND FERTILIZATION OF THE EGG OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

CONTENTS.

I. INTRODUCTION.

SINCE the publication, in 1883, of van Beneden's classic paper, "Recherches sur la maturation de l'œuf, la fécondation, et la division cellulaire," an extensive literature has appeared

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dealing with the various problems of spermatogenesis and oögenesis. The invertebrates have furnished such favorable material for investigation along these lines that as yet but comparatively little work has been done on the higher forms.

Schultze ('87) was the first to give an accurate account of the ripening of the amphibian egg, and his results have been most ably supplemented by those of Born ('92), Fick ('93), and Jordan ('93). None of these investigators have, however, given the details of the breaking down of the germinal vesicle, of the formation of the first polar spindle and the subsequent polar divisions, nor of the origin and division of the segmentation spindle. It was with the hope of throwing some light on these questions that the present work was undertaken at Bryn Mawr College in the spring of 1896. To Prof. T. H. Morgan I wish to express my thanks for many valuable suggestions and for a careful supervision and criticism of my work.

II. Material and Method.

In the vicinity of Bryn Mawr the common toad, Bufo lentiginosus, usually comes from its winter hibernation during the first warm days of April, and there is then a period of about three weeks in which the long strings of freshly laid eggs can be easily procured.

As eggs laid in the laboratory had been found to develop into perfectly normal tadpoles, copulating animals were placed in large glass aquaria containing a small amount of water, and left undisturbed until the eggs were deposited and fertilized. Then, by preserving a number of eggs every five minutes during a period of an hour and a half, a complete series of the fertilization stages was obtained.

Material for the study of the maturation processes was acquired in the following manner: females were captured as soon as possible after they had emerged from their hibernation and killed immediately. If the eggs were still in the ovaries, they were transferred to jars of fresh water and a few of them fixed at frequent intervals for several hours, as in three cases maturation was found to continue under these conditions.

Generally the eggs were found in the body cavity and oviducts
of the female, and such eggs were fixed at once.   For the pur-
pose of comparison with those normally fertilized, eggs taken
from the lower part of the oviducts were artificially fertilized
by being gently shaken in a vessel of water containing small
pieces of testes.   Young ovarian eggs were obtained in the
latter part of September, just before the beginning of the
hibernation period.

The difficulty which many investigators have found in sec-
tioning and staining the amphibian egg in a perfectly satisfac-
tory manner will, perhaps, warrant a somewhat detailed account
of the method by which I was enabled to obtain unbroken
serial sections from 4 μ to 6 μ thick, and also to differentiate
sharply various parts of the egg.

Killing reagents which have given excellent results on other
eggs — Hermann's fluid, Flemming's solution (strong), picro-
sulphuric, picro-acetic, and alcoholic-acetic — cannot be used
on the egg of Bufo.   The first two cause the eggs to disinte-
grate in a short time, while the others render the eggs so
brittle that they cannot afterwards be sectioned successfully.
By far the best killing reagent for the toad's egg is corrosive-
acetic.   Eggs left from five to ten minutes in a saturated solu-
tion of corrosive sublimate containing 5 per cent glacial acetic
acid, washed in 50 per cent alcohol and preserved in 80 per
cent alcohol, give particularly good results in the maturation
stages.   If the amount of acetic acid is increased to 10 per
cent or decreased to 2 per cent, there is no marked effect on
the egg.   It is necessary to remove the jelly-like coating of the
eggs within a few days after fixation, as the eggs soon disinte-
grate if it is allowed to remain.

For the fertilization stages the following solution (a slight
modification of that used by Schultze) is recommended :

        1 per cent chromic acid . . . . 25 parts
        Glacial acetic acid . . . . 10 "
        Distilled water . . . . 65 "

Eggs are left in this solution from eighteen to twenty-four
hours, then washed thoroughly with distilled water and carried

through the various ascending grades of alcohol (remaining in each about ten hours) and preserved in 80 per cent alcohol.

A third method of fixation which gives good results with the ovarian egg, but which I have not tried with the fertilization stages, is that used successfully by Jordan on the egg of the newt, namely, immersion for a few seconds only (from thirty to forty) in distilled water at a temperature of 80° C. The eggs are then transferred to 35 per cent alcohol and passed *slowly* through the various ascending grades of alcohol to 80 per cent alcohol, in which they are preserved.

If Flemming's solution (strong) be diluted one-half with distilled water, it gives fairly good preparations; but owing to the difficulty of staining after its use, this method was seldom employed.

The preparation for sectioning is the same for all methods of fixation. From 80 per cent alcohol the eggs are put into 90 per cent alcohol for half an hour; 95 per cent alcohol for one hour; absolute alcohol for two hours; turpentine from two to three hours; fifteen minutes each in paraffin with a melting point of 48° C., of 52° C., and of 56° C. (a longer time makes the eggs brittle); and imbedded in paraffin with a melting point of from 56° to 58° C.

Owing to the large amount of pigment in the upper hemisphere, the two poles of the egg are readily distinguished and orientation in any desired plane is easy. The eggs were cut either parallel to an axis passing through the light and dark poles or at right angles to this axis.

Heidenhain's iron-haematoxylin, which has given such brilliant results with many kinds of eggs, cannot be used to advantage on the egg of Bufo, as the yolk colors as deeply and retains the stain almost as tenaciously as does the chromatin. Staining the entire egg with borax-carmine, the method recommended by Schultze, Fick, and Jordan, gives good preparations; but far more satisfactory results can be obtained by staining the sections fastened to the slide by Meyer's albumen fixative, with the following solution:

Saturated solution of Bleu de Lyon in 95 per cent alcohol . . . . 1 part
Borax-carmine (Grübler's) . . . . 2 parts
90 per cent alcohol . . . . 1 part

Eggs fixed with hot water or corrosive-acetic stain in from one to three hours ; those fixed with chromic-acetic stain much more slowly and must remain in the solution from eighteen to twenty-four hours ; if the material is not fresh, two or three days may be required to give satisfactory results. When removed from the solution the sections appear to have taken only the borax-carmine, but a good differentiation can be obtained by a careful use of 90 per cent alcohol which has been strongly acidulated. When rightly differentiated, the sections should appear a reddish-purple to the naked eye.

At various stages in their development the eggs react somewhat differently toward this stain. In general, the chromatin, cytoplasm, and nuclear sap of the ovarian egg take the carmine, the yolk granules, nucleoli, and egg-membrane staining blue. In the fertilization stages the chromatin as well as the yolk granules and egg-membrane take the Bleu de Lyon, the cytoplasm and amphiaster showing a stronger affinity for the borax-carmine.

III. Maturation of the Ovum.

1. *The Unripe Ovarian Egg.*

Normally segmenting eggs of the same female have been found to vary in size from 0.6 mm. to 1.5 mm. in diameter, and comparative measurements of a large number of eggs seem to show that the average diameter of the fertilized egg and of the ovarian egg just before the beginning of the hibernation period is 1.1 mm. From these facts it seems probable that the eggs attain their maximum growth before the animal goes into its winter sleep, and that little, if any, significance can be attached to the fact that eggs taken from a female late in the fall usually show a considerable variation in size.

At the time of hibernation the ovarian egg is nearly spherical and is surrounded by three distinct membranes (Pl. XXVIII, Fig. 2). The two outer ones (Fig. 2, *A, C*) are thin and homogeneous ; follicle cells and blood corpuscles are frequently found between and under them. These membranes do not properly

belong to the egg, as they are a part of the inner and peritoneal epithelium of the ovary and are never found around an egg taken from the body cavity.

The third egg-membrane, the "Chorion" of van Bambeke ('80), the "Dotterhaut" of Schultze, and the "Zona pellucida" of Fick, arises apparently from the follicle epithelium. It has a thickness of about 2 μ and is seemingly structureless (Fig. 2, *ZP*). Various writers, Joly ('72), Robin ('74), Stieda ('75), and Schultze, have asserted that the Chorion is the only true membrane belonging to the amphibian egg. Schultze, however, finds between this membrane and the egg a narrow zone which shows fine striations. He suggests for this zone the name "Zona radiata," and considers it to be the peripheral yolk-layer of the egg, and not a membrane in any sense of the word. Fick, on the other hand, has stated that in eggs taken from the body cavity there is a distinct inner yolk-membrane beneath the Zona pellucida, which is not found in younger eggs.

The ovarian egg of Bufo has a distinct Zona radiata which, with the combination stain I have used, colors a pale blue with darker striations, and stands out in sharp contrast to the deep blue Zona pellucida above it and the brown pigment layer below (Fig. 2, *ZR*). Although at this stage the Zona radiata can hardly be considered a real membrane, and is, apparently, but the "äusserste körnchenfreie Dotterschicht," as Schultze believes, I am inclined to consider it the beginning of the inner yolk-membrane described by Fick. When the first polar spindle is formed, the pigment layer comes to the extreme edge of the egg, and there is no sign of a striated zone above it. In favorable sections, however, there is distinctly visible a thin, faintly staining, apparently homogeneous membrane (Pl. XXX, Figs. 35, 36), which is sometimes separated from the surface of the egg and closely attached to the Zona pellucida above it.

In proportion to its size, the egg of Bufo is probably as deeply pigmented as are any of the amphibian eggs. Individual eggs vary greatly in the amount of pigment they contain ; but, in all cases, there is a thick layer of compact pigment

granules around the upper pole which gradually becomes thinner towards the equator (Pl. XXVIII, Fig. 1). Beyond this point the egg usually appears unpigmented, although sections through the egg often show a narrow zone of light brown pigment around the periphery of the lower hemisphere. Scattered pigment granules are found throughout the egg, being much more numerous above than below the equator.

The yolk, as in other amphibian eggs, is composed of yellowish round or oval granules, which vary greatly in size, some being three or four times as large as others. In general, as Fick has stated, the size of the yolk spherules increases from the dark to the light pole; but large granules are always found in the vicinity of the germinal vesicle, and are often massed around the first polar spindle after it has reached the periphery of the egg, in the latter case furnishing a means for determining the position of the spindle under a very low magnification. Between the yolk and pigment granules is a loose network of finely granular cytoplasm, which is most abundant in the upper part of the egg.

At various points, particularly in the upper hemisphere, the yolk-membrane is separated some distance from the surface of the egg, and in the spaces thus formed there is often found a slightly granular substance, the so-called "perivitellin." Sometimes this same substance is found in the pigment layer just beneath the surface of the egg (Fig. 2, *P*). Fick seems to be the only investigator thus far who has discovered perivitellin in the unripe ovarian egg, although von Baer ('34), Hertwig ('76), Schultze, Jordan, and van Bambeke find it in the amphibian egg, and van Beneden ('75) in the rabbit egg, after maturation is completed. All these investigators consider this substance to be a portion of the germinal vesicle which has been forced out of the egg, perhaps by contractions of the yolk, as suggested by Hertwig.

As far as can be judged from its reactions to various staining fluids, the perivitellin of the ovarian egg of Bufo has exactly the same structure as the substance which is found around the lower part of the germinal vesicle at this period (Pl. XXVIII, Figs. 1, 3, *CY*). The presence of perivitellin at this stage of

the development precludes its arising from distintegrating material of the vesicle, and, therefore, I believe it to be but a portion of the cytoplasm of the egg, forced up to or through the surface by contractions in the egg-substance. After the formation of the first polar spindle, I have found no marked increase in the amount of perivitellin at the upper surface of the egg, as one would naturally expect to find if a portion of the substance of the germinal vesicle had been extruded from the egg during the later maturation processes.

Fig. 3 shows the germinal vesicle of the unripe ovarian egg. It is oval in outline, with a somewhat jagged border, and its size is enormous when compared with that of the egg, as it measures 0.24 mm. by 0.34 mm. in an egg with a diameter of 1.1 mm. At this stage the vesicle has left the center of the egg, and lies a little eccentrically towards the black pole, with its longitudinal axis oblique to an axis passing through the light and dark poles of the egg (Pl. XXVIII, Fig. 1, GV). Corresponding to Figs. 20, 21, of Schultze, the greater mass of the nucleoli form a ring at the center of the vesicle; the few nucleoli that have not migrated to the center are at the periphery, very close to the nuclear membrane.

While most of the nucleoli are round or oval, occasionally one is found which is triangular or decidedly oblong (Pl. XXVIII, Fig. 4). There is a great difference in the size of these bodies : some are exceedingly small and can be seen only under an immersion lens ; others are distinctly visible with the lowest power of the microscope. The larger nucleoli generally contain one or two vacuoles, but the smaller ones always appear perfectly homogeneous and take the same stain as the chromatin.

At this period all the chromatin threads are collected in the center of the vesicle. They lie in the finely granular nuclear sap, surrounded by the circle of nucleoli, and are composed of very short, rod-shaped microsomes, which are arranged with their longitudinal axes at right angles to the main axis of the chromosome (Pl. XXVIII, Fig. 5). The chromosomes are of various lengths and usually much bent and twisted. A number of them may be closely intertwined, and two may cross (Fig. 5,

A and *B*); but at this period there is never found a paired arrangement of all of the chromosomes which is so characteristic of a later stage in the egg of Bufo, as well as in the egg of Triton, Axolotl, and the selachian.

In some sections it is easy to follow the entire course of one or two individual chromosomes, so that there can be no doubt that the chromatin is in the form of distinct rods and not in a continuous skein ; but as the chromatin stains very faintly at this stage, and as most of the chromosomes are so long and closely interwined that it is impossible to distinguish between them, I have been unable to count their number satisfactorily. In some cases there appear to be fourteen or sixteen of these chromatin threads ; in others, at least twenty. I have not been able to make out definitely a higher number, yet presumably there are twenty-four chromosomes present, as that is the number which is found at the end of the hibernation period.

The chromosomes occasionally appear to end in small round homogeneous balls greatly resembling the smaller nucleoli (Fig. 5, *A*, *B*, and *C*). Whether these bodies are nucleoli with which the chromatin threads have come in such close contact that the two appear to be united, or whether they are rounded masses of fused chromatin microsomes, I have been unable to determine.

The membrane of the germinal vesicle never has a perfectly smooth contour, but is decidedly wavy, showing a number of small projections and indentations. This form of outline is considered by many investigators to indicate that the vesicle leaves the center of the egg and migrates towards the upper pole by amoeboid movements. While in the Axolotl, frog and newt the germinal vesicle reaches the periphery of the egg before disintegrating, in Bombinator igneus, according to Goette ('75), and in Bufo lentiginosus, dissolution begins when the vesicle is but little more than halfway between the center and the periphery. As there is subsequently a flowing of the nuclear substance to the upper pole of the egg (Pl. XXIX, Figs. 17, 19), which obviously cannot be due to amoeboid movements, I am inclined to believe that the early eccentric movement of the vesicle, as well as the later migration of its

disintegrating substance to the periphery, is brought about by some rearrangement in the distribution of the yolk and cytoplasm which shows itself only in its results.

With all methods of fixation, there is found at the side of the germinal vesicle nearest the center of the egg an accumulation of a granular substance, which, as before stated, has exactly the same appearance as the perivitellin. Under a low power (Pl. XXVIII, Figs. 1, 3) this substance contrasts sharply with the rest of the contents of the egg; but under an immersion lens it is found to be connected with the reticular cytoplasm extending throughout the egg. Born ('94), in describing the ovarian egg of Triton with a diameter of not more than 90 μ, says: "Im Eiprotoplasma tritt dicht um den Kern eine besondere körnigfädlige Schicht auf, so dass man mitunter Mühe hat, die feine Kernmembran zu erkennen." As this substance is found in the eggs of Triton and Bufo before the germinal vesicle has attained its full size, I cannot agree with Goette and Schultze that it is a part of the granular nuclear sap forced through the membrane by shrinkage of the vesicle during its dissolution; nor do I believe with Jordan that it is due to osmotic action during the processes of killing and hardening. Whatever its nature, this substance seems to play an important rôle in the changes accompanying the dissolution of the germinal vesicle, and it is certainly a normal structure in the toad's egg, if not in other amphibian eggs.

There seems to be some question as to whether the large space often found around the germinal vesicle in preserved amphibian eggs is normal or not. According to Fick: "Die scharfe Begrenzung, sowie zum Theil auch die rundliche Form und endlich die ziemlich konstante Grösse der Höhle, sprechen entschieden dagegen, dass es sich hier nur um eine Kunstprodukt handelt." On the other hand, Schultze, Hertwig, van Bambeke, and Jordan believe this space to be an artificial product of the reagents used in killing and hardening.

To determine this point definitely for the toad's egg, a number of ovarian eggs from the same female were intentionally left in the different killing and hardening fluids varying lengths of time and the effects noted. In eggs badly preserved the

germinal vesicle always appears somewhat shrunken and shows long pseudopodium-like processes, as seen in Schultze's Fig. 5. In all such cases there is a large round space surrounding the vesicle, as shown in Fick's Fig. 1. *The outline of the germinal vesicle becomes more regular and the space around it decreases in size the better the egg is preserved.* From this it follows that the small space often found at the upper side of the germinal vesicle in eggs apparently well preserved (Pl. XXVIII, Fig. 7) is artificially produced by reagents, and that, in the living egg, the vesicle is in close contact with the yolk and cytoplasm.

2. *Dissolution of the Germinal Vesicle and Formation of the First Polar Spindle.*

I am aware that the rather remarkable metamorphosis which I have found the germinal vesicle undergoing in preparation for the first polar division may be considered as due entirely to pathological changes produced in the eggs by removing them from the body of the female and allowing them to continue their maturation in water, thus subjecting them to abnormal conditions. Against any such criticism I can only urge the following facts :

1. That when the females were killed before the later maturation processes had already begun, the nuclear membrane remained intact and no changes were noticed in the germinal vesicle. In such cases the eggs usually disintegrated in the course of four or five hours after they had been placed in water. In order that maturation shall continue, the female must be killed but a few hours before the eggs would normally have left the ovaries.

2. That many developing eggs which had been in water for some twenty hours exhibited no apparent signs of disintegration, and that sections through a large number of such eggs show that the first polar body had been given off in the normal position and presumably in a normal manner.

3. That the progress of maturation was uniformly along the same lines in all the eggs that continued their development.

4. That eggs fixed as soon as possible after the females had been killed were not all in the same stage of maturation ; in many cases normal changes were taking place exactly similar to those found in a number of eggs that had been developing in water for at least half an hour.

The processes which are about to be described appear, therefore, to be nearly if not entirely normal, and they may serve to throw some light on a stage in the maturation of the amphibian egg which has not yet been worked out.

In the youngest eggs obtained in the spring the germinal vesicle usually lies with its longitudinal axis parallel to the upper surface of the egg (Pl. XXVIII, Fig. 6). It is generally found to be slightly larger than before the hibernation period, measuring 0.31 mm. by 0.39 mm. in an egg with a diameter of 1.1 mm. Its upper surface is never nearer than 0.08 mm., nor farther than 0.12 mm. from the periphery of the egg, and in general contour it greatly resembles the germinal vesicle of an earlier stage (Pl. XXVIII, Fig. 1).

During the hibernation period, marked changes have taken place in the nucleoli and in the structure of the chromatin threads. The latter are no longer composed of a series of short rod-shaped microsomes, as in Pl. XXVIII, Fig. 5, but have nearly the filamentous structure seen by Rückert ('92) in the selachian egg, and by Born ('92) in the egg of Triton. The axial part of each chromosome is composed of a number of fine fibers, which are thicker at the center of the chromosome where they stain quite intensely, and much finer at the periphery where they stain very faintly and appear to run into the fine reticulum of the nucleoplasm (Pl. XXVIII, Fig. 16). The fibers are placed radially to the longitudinal axis of the chromosome, so that in cross-section the chromosome presents "das Bild eines Sternes mit dunkler Mitte," as described by Flemming ('82) for chromosomes of like structure in the egg of Siredon.

Scattered among the axial fibers are a number of small, round, deeply staining chromatin granules which apparently bear no constant relation to the axial portion of the chromosome either in size, number, or position. In some chromosomes

there are very few of these chromatin granules present ; in others there are a large number which lie, for the most part, in the center of the chromosome.   In the latter case there are fewer of the axial fibers and they appear much finer.   Rückert has stated for the selachian egg that the microsomes of which the chromosomes are composed at an earlier period change into fine fibers, so that at one time the whole chromosome appears as a filamentous structure.   Such a stage, if it occurs in the egg of Bufo, will be found during the hibernation period.   The presence of a varying number of chromatin microsomes in the midst of the axial fibers of the chromosomes in the youngest eggs obtained in the spring shows that the filamentous structure of the chromosomes is being resolved into a microsome structure.   At this period the chromosomes still occupy the center of the germinal vesicle, although they are no longer surrounded by a ring of nucleoli, as most of the latter have migrated to the side of the germinal vesicle nearest the periphery of the egg (Pl. XXVIII, Fig. 6).   Owing to the different lengths of the chromosomes, to their close intertwining, and to the fact that they stain very faintly at best, it has been impossible to count their number accurately.   In many cases the chromosomes appear to be separated off in pairs ; but this arrangement is by no means general until the next stage, when the axial fibers have entirely disappeared and the chromosomes are composed of a single series of perfectly round microsomes which take a very intense carmine stain (Pl. XXVIII, Fig. 8). Instead of forming a compact mass at the center of the germinal vesicle, as in the previous stages, the chromosomes now become scattered over a considerable area, and all are distinctly paired as described by Rückert in the later maturation stages of the selachian egg, by Fick in Axolotl, and by Born in Triton.   There is still considerable variation in the size of the chromosomes, as well as in their arrangement.   Some are long and slender, others short and thick ; but the two chromosomes forming a pair are always of the same length.   Two of the chromosomes may be united in the form of an X or Y, a single or double figure eight, or they may lie parallel for part of their length and the ends intertwine in various ways (Fig. 8).

Although usually turned towards each other, the ends of the chromosomes are generally distinct (Fig. 8, *C* and *E*), but it is not uncommon to find a fusion of two chromosomes at one end only (Fig. 8, *B*). Very rarely at this stage both ends of a pair of chromosomes are united so that a closed ring is formed (Fig. 8, *F*).

The number of chromosomes characteristic of the somatic cell of Bufo lentiginosus is twenty-four. *There are twelve pairs of chromosomes in the germinal vesicle at this stage.* If, as believed by Rückert, the paired arrangement of the chromosomes is due to a longitudinal splitting of single chromatin threads, which takes place at a very early period, possibly during the transition from the oögonium to the oöcyte, then there must have been a reduction to one-half the number of chromosomes normal for the species before the longitudinal splitting occurred. On the other hand, if there has been no longitudinal division and but a pairing off of the separate chromatin threads, then the normal number of chromosomes is still present at this stage in the maturation, and the reduction to one-half the normal number must come in the second polar division.

The nucleoli are now beginning to disintegrate; but the changes which take place in them are not simultaneous. The same section of an egg will show a large number of nucleoli that stain an intense blue and contain but few vacuoles (Pl. XXVIII, Fig. 11); others with a yellowish tinge that take only a faint blue stain and are completely filled with vacuoles; and still others that no longer show any affinity for stains and appear as yellowish-green, highly refractive bodies, having apparently a thick outer membrane, yet retaining the original outline of the nucleolus (Pl. XXVIII, Fig. 12, *A*). The very smallest nucleoli still appear homogeneous and take the deep carmine stain characteristic of the chromatin. In addition to these nucleoli, there are now found in each germinal vesicle, usually near the periphery, from two to six bodies which are distinguished from the nucleoli by their enormous size (*cf.* Figs. 9 and 10 with Fig. 11). The youngest stage of these bodies which I have thus far been able to find is seen in

Fig. 9. Here the interior of the structure contains several large and many smaller vacuoles; the latter have arisen presumably from the breaking down of the larger ones. In an older stage (Fig. 10) the vacuoles are very numerous and they are more nearly uniform in size. These nuclear bodies decrease considerably in volume during the formation of the first polar spindle and stain less intensely, always, however, retaining their vacuolated appearance. They are frequently found near the upper pole of the egg during the extrusion of the polar bodies, but they are soon after absorbed and no trace of them can be found in the later fertilization stages. As these bodies first appear during the hibernation period, it is impossible to say whether they arise from the fusion of two or more small nucleoli, from the rapid growth of a single nucleolus, or whether they are only a late secretion product of the germinal vesicle. The last assumption seems the most probable, as these structures apparently undergo an entirely different process of absorption from that of the other nuclear bodies.

Another egg from the same series as the one just described shows the next steps in the process of maturation. The substance around the germinal vesicle has differentiated into two parts (Pl. XXVIII, Fig. 7): the one nearer the vesicle is a dense band of uniform thickness (Fig. 7, LR), composed of very fine, closely packed fibers, the greater number of which run parallel to the nuclear membrane; the other part consists of coarse homogeneous granules imbedded in a fine reticulum which is continuous with the denser portion above and also with the cytoplasm around it (Fig. 7, A).

The lower pole of the germinal vesicle is apparently the place where the disintegration processes originate. In this region the nuclear membrane first becomes very irregular and then gradually disappears; here, too, the finely granular nuclear sap first begins to be formed into large granules, as described by Born in the germinal vesicle of the egg of Triton.

As dissolution continues, the germinal vesicle becomes filled with round, deeply staining granules of nucleoplasm. In the center and upper portion of the vesicle the granules, which are very small, form a loose reticulum in which the chromatin

threads and nucleoli lie (Pl. XXVIII, Fig. 14, *R*). In the lower part of the vesicle the granules are extremely coarse and somewhat irregular in outline (Fig. 14, *N*); they do not form a reticulum, but are closely crowded around the periphery of the vesicle. When the nuclear membrane disappears at the lower pole of the vesicle (Pl. XXVIII, Fig. 13), fibers from the dense substance below extend up between the granules, and, except that the nuclear granules are somewhat smaller, there is no difference in appearance between the structure of the nucleoplasm and that of the granular substance below the dense dividing line. After the use of the combination stain, all the granules appear deep carmine, and the dense fibrous band stains an intense blue.

Many eggs removed from the female and left in water for half an hour show phenomena similar to those which have just been described in eggs known to be normal. Others, however, show more advanced changes. The fibrous band around the lower part of the germinal vesicle shortens gradually, and from extending nearly halfway around the vesicle, as in Pl. XXVIII, Fig. 1, it comes to occupy only a relatively small space at what was the lower pole of the vesicle. In further description I shall call this band a "line of radiation," although I am aware that the term is not entirely appropriate. The nuclear membrane has now completely disappeared, yet the contents of the vesicle lie in a space sharply marked off from the cell contents. I have found the line of radiation nearly straight (Fig. 14, *LR*), or curved in either direction (Pl. XXVIII, Fig. 13; Pl. XXIX, Fig. 17), when the adjacent parts of the egg were in relatively the same condition, so I do not think that the form of the line of radiation is at all constant, or that it can have much significance. As it becomes shorter the line of radiation broadens considerably; its fibers are withdrawn from the substance beneath, so that the lower part of this structure becomes a narrow, compact, fibrous band, sharply defined below, and gradually extending into a loose upper meshwork from which a larger number of fibers extend up between the coarse granules of nucleoplasm (Pl. XXVIII, Fig. 14). These fibers are much longer than in the preceding stage;

they end apparently in the fine granular network which fills the greater part of the space once occupied by the germinal vesicle, and are beginning to have a radiate appearance. In the succeeding stages, in proportion as the radiation becomes more marked the rays become longer, thicker, more numerous, and from being apparently homogeneous as when first formed, they are seen to consist of a series of minute round microsomes. The granules of nucleoplasm, on the contrary, constantly decrease in size and number and stain less intensely (Pl. XXVIII, Fig. 15). When the radiation has reached its greatest extent (Pl. XXIX, Fig. 18), every trace of the nuclear granules has disappeared. The change is so marked that it admits of but the one interpretation, that the rays grow at the expense of the granular substance once a part of the germinal vesicle.

During this period a series of changes has also taken place below the line of radiation. After the withdrawal of the fibers from above, the fine reticulum extending between the coarse granules in this region disappears and the granules themselves break up into smaller ones (Pl. XXVIII, Fig. 14, *A*). At the next stage (Pl. XXVIII, Fig. 15), this substance is a uniformly finely granular mass, which stains a light pink and contains a few scattered pigment granules. Meanwhile there has been a gradual movement of the line of radiation, and all the substance formerly contained in the germinal vesicle, towards the upper pole of the egg (Pl. XXIX, Fig. 17). This movement must be brought about by some rearrangement in the yolk and cytoplasm of the egg, as it is inconceivable that these structures can have the power of independent movement.

Hertwig has figured the germinal vesicle of the frog's egg just before its disintegration as a flattened oval structure with a distinct membrane, lying close to the upper surface of the egg. As the phenomena which take place during the maturation of the egg of the frog and toad appear so closely similar in most respects, it seems probable that in the former as well as in the latter the germinal vesicle breaks down some distance below the surface of the egg. Presumably, therefore, Hertwig's Fig. 7, which shows the germinal vesicle, still surrounded by its membrane, lying close to the upper surface of

the egg, is comparable to my Fig. 19 (Pl. XXIX), which shows
a portion of the nuclear substance that has moved up under the
black pole after the breaking down of the nuclear membrane.
After the stage of Fig. 19, the nuclear débris begins to be
uniformly distributed throughout the upper part of the egg,
and by the time fertilization takes place nearly every trace of
it has disappeared. I have seen no indication that this sub-
stance is ever extruded from the egg to form perivitellin.

When the radiation has reached its greatest extent, the line
of radiation occupies a position in the egg as seen in Pl. XXIX,
Fig. 17. Its fibers no longer form a meshwork, but they lie
nearly parallel and at right angles to the longitudinal axis of
the line of radiation, running directly into the rays which form
the radiation above (Pl. XXIX, Fig. 18). At the extreme lower
border (very rarely at the upper border) the fibers are closely
packed together, forming a well-defined boundary line which
separates the line of radiation from the granular substance
beneath. From this time on, the line of radiation and the
radiation above it undergo a rapid transformation. The rays
become shorter, finer, and again appear homogeneous. Where
they converge towards the line of radiation there are found a
number of small round granules which take the carmine stain
(Pl. XXIX, Fig. 20). These granules quickly increase in size
and number and later become fused into irregular masses which
lie, for the most part, in the finely granular substance below
the line of radiation (Pl. XXIX, Figs. 21, 22). After the
hibernation period, the chromatin threads never terminate
in the round, nucleolar-like bodies found in an earlier stage
(Pl. XXVIII, Fig. 5, *A*, *B*). It is probable that these bodies
separate from the chromosomes during the hibernation period
and come to lie among the nucleoli, being indistinguishable
from the smallest of these bodies, which always stain exactly
like chromatin. Although definite proof is lacking, I am inclined
to believe that all the minute homogeneous bodies in the ger-
minal vesicle are composed of chromatin which did not go
into the formation of the twelve pairs of chromosomes. Their
history cannot be followed after the granular reticulum is
formed in the germinal vesicle, yet I feel confident that they

are broken up into still smaller granules, which later fuse to
form the irregular masses found near the line of radiation.
This assumption is certainly correct if reactions towards stains
form any criterion on which to base a judgment of the nature
of a substance ; for when the nucleoplasm can again be distin-
guished as such by its light pink color, these bodies take the
deep carmine stain which is so characteristic of chromatin.   I
can offer no explanation as to why the chromatin nucleoli
should be broken up into minute particles, and these particles
subsequently reunited into large masses.

The processes which Osterhaut ('97) describes as leading to
the formation of the karyokinetic spindle in the spore-mother
cells of Equisetum bear a striking resemblance to the changes
occurring in the breaking down of the germinal vesicle in the
toad's egg.   In Equisetum, when the chromatin skein is be-
ginning to break up into chromosomes, a layer of cytoplasm
lying close around the outside of the nucleus, the " Kinoplasm "
of Strasburger ('92), stains differently from the rest of the
trophoplasm and broadens apparently at its expense.   The
kinoplasm first forms a fine meshwork, but soon changes to a
coarse, irregular reticulum, which contains a number of small
granules and forms a thick layer close to the nuclear wall.
The threads of this layer thicken, stain more deeply, and
arrange themselves at right angles to the nuclear membrane in
the form of a pronounced radiation which later extends up into
the nucleus and becomes the spindle.   Osterhaut believes
that the kinoplasm is probably derived primarily from the
trophoplasm ; but as the kinoplasm is not apparent in the
resting cell, he cannot tell whether or not it is a specific sub-
stance in the sense of Boveri's "archoplasm."

At the stage of Pl. XXIX, Fig. 17, with the exception of the
peculiar large nuclear bodies which appear during the hiberna-
tion period, and the small ones presumably composed of chro-
matin, all the nucleoli have changed into yellowish-green
refractive bodies and are going through the final processes of
disintegration.   From the apparently normal form (Pl. XXVIII,
Fig. 12, *A*) several small round portions are first separated off
(Fig. 12, *B*) ; the central part then breaks up (Fig. 12, *C*), and

finally there is formed a mass of small round bodies (Fig. 12, *D*) which, for a time, lie scattered in the substance of the vesicle, but which have completely disappeared by the time the segmentation spindle is formed.

The twelve pairs of chromosomes can be traced throughout all the changes that have been described. They shorten gradually and become proportionally thicker, still, however, showing a distinct microsome structure. When the nuclear membrane disappears, the pairs of chromatin threads are scattered over a considerable area, and the ends of each pair have fused completely, so that twelve closed chromatin rings are formed (Pl. XXVIII, Fig. 8, *F*). These rings apparently have no connection with the line of radiation or with the radiation itself. When the line of radiation is beginning to decrease in length there suddenly appears near each chromatin ring a small, well-defined aster (Pl. XXIX, Fig. 24) in which the delicate rays converge towards a central part seemingly composed of only the ends of the astral rays united into a closed meshwork. Iron-haematoxylin with all methods of fixation fails to disclose a centriole or any definite body in the center of these asters that might be considered a centrosome according to Boveri's ('88) definition of the word. There may be several asters scattered throughout the nuclear substance which are not connected with chromatin rings, but after the asters have once appeared, no chromatin ring is ever found without one.

Unfortunately there is a break in my series of preparations at this point, so that I am not able to follow the next changes that occur in the egg.[1] About one hour after the stage of Pl. XXIX, Figs. 21 and 22, the nuclear substance is nearly uniformly distributed throughout the upper hemisphere, and the fully formed spindle, undoubtedly derived from the line of radiation, is found at some distance below the surface of the egg. The spindle lies in the midst of yolk granules and is not surrounded by any marked accumulation of cytoplasm. Its

[1] During the spring of 1899, material was obtained giving a complete history of the formation of the first polar spindle. A detailed account of these changes will be published later.

fibers are well defined and extend unbroken from pole to pole, where they converge sharply and run into two distinct astrospheres (Pl. XXIX, Fig. 26). These astrospheres are considerably larger than the asters which accompanied the chromatin rings at an earlier period, and the rays are much longer and thicker. Their structure, however, appears to be similar to that of the asters in that the center of each astrosphere seems to be composed of only the ends of the rays closely massed together, and all methods of fixation and staining fail to disclose the presence of a distinct central body.

The asters accompanying the chromatin rings at the stage of Fig. 24 have entirely disappeared by the time the spindle is formed, and the rings are apparently being drawn towards the spindle by some sort of an attractive force, the nature of which cannot be determined. The chromatin rings that have reached the spindle (Figs. 25, 26) are found to be much larger than those some distance away (Pl. XXIX, Fig. 27), and instead of being composed of a single series of large microsomes, as in the previous stage, they are now distinctly seen to consist of a double series of smaller microsomes. *The rings must, therefore, undergo a longitudinal splitting at this stage.* Each of the sister rings has two knob-like thickenings, undoubtedly representing the place of union of the two chromosomes that fused to form the ring (Fig. 26). Chromatin rings with such thickenings have been found by Flemming ('87) in the heterotypic type of division in the spermatocytes of the salamander testes. After the double chromatin rings have arranged themselves on the spindle each breaks into four parts at its knob-like thickenings (Pl. XXIX, Fig. 28), and thus the chromosomes which fused to form the rings are separated and split longitudinally.

During its migration towards the black pole, the spindle shortens and becomes much more slender, — a phenomenon seen by Korschelt ('95) during the migration towards the periphery of the first polar spindle of Ophryotrocha, by Bütschli ('76) in Cucullames, and by Erlanger ('95) in Macrobiotus macronyx. The astrospheres fade away and have completely disappeared by the time the spindle has reached the periphery, so

that the spindle poles in eggs taken from the body cavity show no traces of the radiation found by Sobotta ('95), Wheeler ('97), Kostanecki ('98), and Korschelt at the poles of the first polar spindle when it is preparing for the cutting off of the first polar body. There may be a small amount of cytoplasm containing a few pigment granules at the spindle poles, but there is no marked accumulation of either protoplasm or pigment as has been described by Born ('92) and Michaelis ('97).

The spindle migrates towards the upper surface of the egg with its poles at right angles to the direction of the movement (Fig. 28). There is a quick turning when the periphery is reached, so that the spindle lies either radially or slightly oblique to the surface when it is ready to give off the first polar body.

3. *The First Polar Division.*

At the upper pole of eggs taken from the body cavity there is usually seen a small, round, well-defined area which appears white in contrast to the dark pigment around it. To this area Prévost and Dumas ('24) gave the name " Cicatricula," and von Baer ('34), " Keimpunkt." After the polar divisions are completed, Fick divides this " light-spot" into two portions ; a small depression at or near the center, which he believes to be caused by the pressure of the first polar body, is called " Richtungsdelle," and the term " Fovea Germinativa," as used by Max Schultze ('63), is considered to apply only to this portion and not to the whole area, as several writers have stated. To the remainder of the structure Fick gives the name " Richtungsfleck."

Newport ('51) considered the Cicatricula to be the outlet of a canal passing from the exterior to the germinal vesicle, caused by an imperfect closing of the " yelk-cells " around the vesicle. Schultze, with whom Fick agrees, has advanced a very simple explanation that the Cicatricula is at first due to the presence of the germinal vesicle at the periphery of the egg, the pigment being still kept out of this region after the breaking down of the vesicle by the diffused portions of the disintegrating nuclear substance. I cannot but agree with Jordan that this explanation

hardly covers the facts. It is perfectly apparent that the Cicatricula is caused by a lack of pigment granules in a definite region of the upper surface of the egg, so that the light-colored substance beneath becomes visible at the surface ; but the Cicatricula is often sharply defined when the débris from the germinal vesicle is nearly absorbed and the yolk granules are again uniformly distributed over this region. If the yolk granules can again invade this region, why cannot the pigment granules ? Jordan suggests that an unknown repellent force is exerted by the substance which forms the polar spindle and later the female pronucleus, for it is only after the pronucleus has moved away from the periphery of the egg that the Cicatricula disappears and the pigment is again uniformly distributed over the surface of the upper hemisphere.

Sections through an egg from the body cavity (Pl. XXX, Figs. 29–31) invariably show the first polar spindle lying close against the periphery, directly under the Cicatricula, and usually at the base of the small depression in the surface of the egg. The spindle axis is generally approximately radial, but it is often very oblique even during the anaphase (Pl. XXX, Fig. 32). In a longitudinal section of the spindle the chromosomes are sometimes found to be arranged in a row at the equator (Fig. 29) ; more often, however, this stage has not yet been reached and the chromosomes are scattered in groups along the spindle (Figs. 30, 31), each group seemingly composed of four rounded chromatin granules lying close together and thus showing a striking resemblance to the tetrad groups found by Boveri ('87) in the first polar spindle of the egg of Ascaris. A transverse section of the spindle at the stage of the equatorial plate, or a longitudinal section of the spindle during the anaphase (Fig. 32), will show conclusively that there are no tetrad groups in the first polar spindle of the egg of Bufo. The chromosomes in Figs. 30 and 31 are in reality dumb-bell-shaped loops arranged in pairs, with the angles of the loops turned towards the center of the spindle, the knob-like ends of a pair of chromosomes giving the appearance of a typical tetrad group when the spindle is cut longitudinally.

There are but twelve groups of chromosomes to be found in

eggs taken from the body cavity, and these chromosomes must
be derived from the twelve chromatin rings which were found
in connection with the first polar spindle before its migra-
tion to the periphery (Pl. XXIX, Figs. 25–27). One of the
twelve groups of chromosomes found in eggs from the body
cavity must, therefore, be equivalent to one of the chromatin
rings of the earlier stage. Unfortunately I have not a com-
plete series of preparations showing all of the transitional
stages from Figs. 25, 26, 27 to Fig. 28 ; yet it may be of inter-
est to trace as closely as possible the changes occurring during
this period. After the twelve chromatin rings have reached
the spindle, they split longitudinally (Figs. 26, 27), so that
there are twenty-four chromosomes in the form of closed rings.
Each ring then separates into two parts at its thickened por-
tion, and thus forty-eight half-rings are formed, each ending in
knob-like thickenings. This second separation of each ring
into two parts is not a transverse division, but merely a break-
ing apart of the chromatin threads which originally fused to
form the ring. During the migration of the spindle towards
the periphery, a concentration of the chromatin material must
take place, as the chromosomes found on the spindle after the
periphery is reached are very much smaller than those at the
stage of Fig. 28. Besides this concentration, there is also an
apparent decrease in the number of chromosomes, as there are
but twelve groups or twenty-four chromosomes to be counted
in the equatorial plate of the spindle in eggs taken from the
body cavity (Pl. XXX, Figs. 30, 31). Each of these chromo-
somes must, therefore, be equivalent to one-half of a chromatin
ring before it underwent longitudinal division at the stage of
Pl. XXIX, Fig. 26; that is, it must represent one of the two
original chromosomes from the germinal vesicle which fused
to form the ring. The apparent lessening of the number of
chromosomes from forty-eight to twenty-four might be brought
about in two ways : either the two sister portions of one-half a
chromatin ring which were completely separated in an earlier
stage (Fig. 26) have fused again, or they lie so close together
that it is impossible to distinguish between them. These
changes must take place very soon after the stage of Fig. 26,

as the apparent union of the two sister portions of each half ring has already occurred and the double segments have come to lie parallel, when the spindle is still some distance below the surface of the egg (Pl. XXIX, Fig. 28). There are certainly not more than twenty-four distinct chromosomes present at the stage of Fig. 28, although I have not been able to make out the exact number satisfactorily. On account of the small size of the chromosomes, it is extremely difficult to follow the changes they undergo, and only a more extended research with an abundance of very favorable material can explain these stages satisfactorily. Cases in which the chromosomes have split longitudinally and then apparently re-fused are not unknown. In Ophryotrocha, Korschelt has found that there is a distinct longitudinal splitting of the four chromosomes during the formation of the first polar spindle, and a subsequent apparent disappearance of the division, so that, in the equatorial plate of the spindle after it has reached the periphery, there again appear but four chromosomes, seemingly forming a tetrad group. The splitting reappears after the chromosomes have separated and are migrating towards the poles of the spindle.

While the eggs of Bufo are in the upper part of the oviducts, the chromosomes shift their position on the spindle, and in the anaphase (Pl. XXX, Fig. 32) the angles of the chromatin loops are turned towards the spindle poles so that their true shape can readily be seen. In favorable cases some of the chromosomes show their double structure; but the reappearance of the splitting is best seen in the equatorial plate of the second polar spindle (Pl. XXX, Fig. 37).

After metakinesis, the chromosomes separate in such a manner that twelve double chromosomes, one from each group, migrate to each pole. *The first polar division, therefore, separates the two chromosomes which originally fused to form a chromatin ring.*

After the separation of the chromosomes, the distal end of the spindle extends up into a small projection of egg-substance (Pl. XXX, Fig. 33) which is then pinched off to form the first polar body. During this process the spindle becomes

somewhat distorted and its fibers appear granular and very indistinct.

The first polar body (Pl. XXX, Figs. 35–37) is usually oval, rarely round. It is surrounded by a thin delicate membrane, and contains chromatin, cytoplasm, pigment granules, and occasionally yolk spherules. The chromosomes are soon collected in an irregular-shaped mass in the center of the polar body and every trace of their individuality is lost. I have seen no indication of a later division of the first polar body as has been observed by Fick in Axolotl and by Korschelt in Ophryotrocha.

The first polar division occurs while the egg is in the oviduct, and here too the egg receives its outer coat of thick, jelly-like substance, which swells up as soon as the egg is laid, and serves to protect the egg during the cleavage and early embryonic stages of development.

4. *The Second Polar Division.*

All the eggs taken from the lower part of the oviduct show the fully formed second polar spindle lying at the periphery of the egg, just below the first polar body. It can be readily distinguished from the first polar spindle by its more slender shape and more delicate fibers. The fibers of this spindle also converge to distinct points, but there is absolutely no sign of a centrosome, a radiation, or an accumulation of either protoplasm or pigment at the spindle poles. The six groups of chromatin loops are arranged at the equator with the angles of the loops turned in, as they were in the first polar spindle (Pl. XXX, Fig. 34). The sister chromosomes, which have apparently been fused, soon separate, so that, in a longitudinal section of the spindle, there appear twelve pairs of dumb-bell-shaped chromosomes which again give the impression of being tetrad groups (Pl. XXX, Figs. 35, 36). In this case, however, each chromosome no longer has a double value, as it represents but one-fourth of a chromatin ring. The chromosomes separate in such a manner that one from each pair, *i.e.*, twelve chromosomes, goes into the second polar body. *The second polar division*

separates sister-halves formed by a longitudinal splitting of the chromatin rings, and there is an "equal division" in Weismann's ('92) sense.

The second polar body (Pl. XXX, Fig. 39) is extruded about ten minutes after the spermatozoön has penetrated into the egg. It is somewhat smaller than the first polar body, and consists of a rounded mass of naked protoplasm enclosing the twelve chromosomes and a few pigment granules, but no yolk spherules. The chromosomes retain their individuality much longer in the second polar body than they do in the first, but eventually they fuse into a central mass which, as far as I have been able to determine, never makes any preparations for a further division.

5. *The Female Pronucleus.*

After the extrusion of the second polar body, the egg contains but twelve chromosomes, one-half the number normal for the somatic cells of the species. These lie in a small accumulation of granular substance arising presumably from the breaking up of the spindle fibers which had already become granular and decidedly irregular before the second polar body was cut off. The chromosomes soon fuse into a rounded mass (Pl. XXX, Fig. 40), and from them the pronucleus is formed as a small round body staining so faintly that its structure cannot be determined. At a later stage, when its migration from the periphery has begun (Pl. XXXI, Fig. 49), the pronucleus has increased greatly in size, and stains much more intensely. It is either round or slightly oval, with a perfectly smooth membrane which never shows any irregularities in well-preserved specimens. The interior of the pronucleus is filled with a clear, colorless nuclear sap, and a reticulum composed of irregular pieces of chromatin bound together by delicate linin fibers. The reticulum becomes more regular as the pronucleus increases in size (Pl. XXXI, Fig. 50), and is then apparently composed entirely of linin, the chromatin being collected in a number of rounded masses which, for the most part, lie close to the nuclear membrane or at the points of intersection of the linin fibers. It is impossible to say whether all the deeply

staining rounded masses in the pronucleus are composed of chromatin or whether some may not be newly formed nucleoli. The enormous volume of these bodies as compared with that of the chromosomes which went into the formation of the pronucleus, would seem to favor the second alternative; but the question cannot be settled definitely, because these structures all stain alike, and it is impossible to differentiate them at any stage in the history of the pronucleus.

6. *Nature of the Nucleoli.*

The germinal vesicle of all amphibian eggs contains a large number of nucleoli whose function is not known with any degree of certainty. After describing the disintegration of the nucleoli into small granules, Schultze adds: "Man überzeugt sich, dass die Körnchen, die ich jetzt wohl Mikrosomen nennen darf, allmählich zur Erzeugung eines Fadenknäuels zusammentreten, der also nicht aus einem präformierten Kerngerüst entsteht, sondern sich direkt aus den winzigen Keimkörperchen herausbildet." This view was completely overthrown by Born's discovery that the chromatin threads in Triton are distinctly traceable throughout the whole history of the germinal vesicle, existing before, as well as during, the migration of the nucleoli towards the center of the vesicle.

The general opinion of the nature of the nucleoli in all eggs seems to be that these bodies either represent some kind of a reserve substance as maintained by Pfitzner ('83), Rhumbler ('93), and Korschelt, or that they are a secretion product of the nucleus. The latter view was first advanced by Leuckart ('53), and has since been advocated by Flemming ('80), Häcker ('93, '95), and Wheeler ('97).

There are at least three different kinds of nucleoli in the germinal vesicle of the toad's egg: the very large vacuolated bodies appearing during the hibernation period, which are slowly absorbed without any apparent change in their structure; the large nucleoli which break up into small yellowish refractive granules before disappearing; and the minute homogeneous nuclear bodies which are apparently unchanged when

the nucleoplasm breaks up into granules and stains like them, thus making it impossible to follow their further history. Only a much more extended study of the nucleoli in the egg of Bufo can determine which of the various explanations that have been advanced to account for the presence of these bodies is substantiated by facts.

7. *Chromatin Reduction.*

Several prominent investigators have recently emphasized the fact that the question of reduction involves more than a separation of the chromosomes during the two polar divisions, and they have asserted that the key to the solution of the problem is to be sought in the origin of the so-called tetrad groups.

In the egg of Bufo the question is a most perplexing one, owing to the peculiar changes occurring during the formation of the first polar spindle. As the substance which forms the irregular masses near the first polar spindle is undoubtedly chromatin, *fully one-half of the chromatin substance of the germinal vesicle does not go into the chromosomes of the first polar spindle.* On Hertwig's ('90) assumption that reduction is " eine Einrichtung um zu verhindern dass durch die Befruchtung eine Summirung der Kernmasse und der chromatischen Elemente herbeigeführt werde," it is not conceivable why the mechanism of karyokinesis should be called upon to lessen again the amount of chromatin. If we disregard these masses and consider only the chromatin material which forms the twelve chromatin rings of the first polar spindle, then the problem is somewhat simplified. Judging from the results which have been obtained by a study of other eggs, it seems highly probable that the paired arrangement of the chromosomes in the early maturation stages of the toad's egg is brought about by a longitudinal division of chromatin threads existing in the germinal vesicle from the time of the formation of the oöcyte. Rückert ('92), who has met with the same paired arrangement of chromosomes in the ovarian egg of the selachian, states that " Die Verdoppelung geschieht beim Uebergang des Ureies zur Eimutterzelle und zwar, wie sich mit Wahrscheinlichkeit darthun lässt, durch eine eigentümliche

Längspaltung der Chromosomen im Dyaster der letzten Teilung des Ureies."

After the formation of the first polar spindle in the egg of Bufo, the subsequent longitudinal splitting of the chromatin rings can be definitely proved (Pl. XXIX, Fig. 25). Hence, assuming the rings to be originally composed of two sister-halves of a split chromatin thread, both polar divisions are "equal divisions" and there is no qualitative division of the "ids" as Weismann's theory demands.

In the results obtained by Boveri ('87) on Ascaris, ('90) on Petrotrachea and other mollusks; by Brauer ('89) on Branchipus, ('93) on Ascaris; by Hertwig ('90) on Ascaris; by Moore ('96) on elasmobranchs; and by Meves ('97) on Salamander, there is manifestly a disagreement with the reduction process as described by Weismann ('85, '92), in that the tetrad groups arise from a double longitudinal division of primary chromatin rods, and not from one longitudinal and one transverse division.

Guignard ('91) and Strasburger ('94) have stated that there is no evidence of a transverse division of the chromosomes in flowering plants. They find only a reduction in the number of chromosomes, which is brought about by a segmentation of the spireme thread into one-half the usual number of chromosomes. These chromosomes do not form tetrads, but undergo simple longitudinal splitting at each succeeding division.

Equally strong evidence in favor of the Weismann hypothesis has been given by vom Rath ('92, '93, '95) from an exhaustive study of the tetrad groups in Salamander, Gryllotalpa, and various copepods; by Henking ('91) from his work on insects; by Häcker ('95) and Rückert ('93, '96) from investigations on various copepods; and by Calkins ('97) from a study of the tetrad formation in Pterodophytes. According to vom Rath, there is first a longitudinal splitting of a primary chromatin rod, the ends then unite and open out to form a ring, which subsequently breaks into four parts by a separation of the two halves corresponding to the original longitudinal splitting, followed by a second transverse division of each half. The latter is the "reduction division," by which there is a qualitative division of the chromatin substance.

If the fundamental cause of reduction is alike for all forms, the conflicting results which have been obtained from a study of oögenesis and spermatogenesis in both plants and animals obviously cannot be explained by the theory advanced by Weismann. Strasburger ('94) has recently asserted that: "The morphological cause of reduction in the number of chromosomes, and of their equality in number in the sex cells, is phylogenetic. There is a return to the original generation from which, after it had obtained sexual differentiation, an offspring was developed having a double number of chromosomes. It is not the outcome of the gradually evolved process of reduction, but the reappearance of the primary number of chromosomes as it existed in the nuclei of the generation in which sexual differentiation first took place." To this hypothesis, which was expressed in substance by Whitman in 1876, Wilson ('96) has raised objections, and a satisfactory solution of the problem seems as remote now as when, in 1891, Boveri stated : "Dass uns aber eine wirkliche Einsicht in diesen Vorgang bis jetzt fehlt. Es bleibt weiterer Forschung vorbehalten, dieses Dunkel aufzuhellen."

IV. The Spermatozoön.

The spermatozoön of Bufo lentiginosus greatly resembles that of Bufo cinereus as described by La Valette St. George ('96), and is similar in all essential respects to that of Alytes obstetricans (Ballowitz, '90). The head (Pl. XXX, Fig. 41, H), which is usually slightly sickle-shaped, is a cylindrical structure 0.11 mm. long and 0.08 mm. thick. It stains an intense black with iron-haematoxylin and a deep red with the combination stain used, and appears perfectly homogeneous in all its parts. The anterior end of the head is slightly rounded and is perfectly distinct from the apex, which is an awl-shaped structure 0.025 mm. long, ending in a fine point (Fig. 41, A) and showing no affinity for either iron-haematoxylin or the combination stain. As the anterior end of the head or the base of the apex apparently contains the centrosome, if one is present in the spermatozoön, I have very carefully examined a large

number of spermatozoa in the hope of confirming Field's ('96) results on the echinoderm spermatozoön, that a slight depression exists in the anterior end of the head in which the centrosome lies. All attempts to discover such a depression in the spermatozoön of Bufo have failed to show other than a sharp *outward* curving line between the head and the apex, and if a centrosome is present in this part of the spermatozoön it is imbedded in the substance of the head and is too minute to be detected.

At the posterior end of the head is the so-called " middle-piece " (Fig. 41, *M*), a cylindrical rod 0.012 mm. long and 0.006 mm. wide. *After the use of iron-haematoxylin, this portion of the spermatozoön appears uniformly homogeneous and perfectly colorless, showing no sign of any structure that might be considered a centrosome.* In all cases the middle-piece appears to be sharply differentiated from the head, and if a projection from it extends into the head as found by Ballowitz in the spermatozoön of Triton, and by Fick in that of Axolotl, its presence cannot be detected in the spermatozoön of Bufo lentiginosus, owing to the deep staining of the substance of which the head is composed.

Fick has conclusively demonstrated for Axolotl that the " attraction spheres " in the fertilized egg arise from the middle-piece of the spermatozoön. He has stated that it is the *whole middle-piece* which stains an intense black with iron-haematoxylin, the apex and head remaining *absolutely colorless.* This result is in striking contrast to that obtained on the spermatozoön of Bufo lentiginosus by the use of iron-haematoxylin. In this case *the head alone stains black, the apex, middle-piece, and tail remain uncolored.* If, as maintained by Heidenhain ('92) and others, iron-haematoxylin is a specific centrosome stain, then it follows that *the middle-piece of the spermatozoön of Bufo lentiginosus does not contain a centrosome, and that, if one is present, it must be imbedded in some part of the head.*

The tail of the spermatozoön of Bufo varies from 0.28 mm. to 0.37 mm. in length. It consists of two thread-like fibers which come out from the center of the lower surface of the middle-piece. One of these fibers is shorter, straighter, and a

little finer than the other. The two fibers are united by a thin
transparent membrane which narrows at the posterior end
where the two fibers run together. The longer fiber extends
some distance beyond the place of union and forms a pointed
termination of the tail. The delicate membrane between the
fibers may be easily destroyed by reagents, leaving the fibers
completely separated, so that, as stated by La Valette St.
George ('76), the spermatozoön appears to have two thread-
like tails which are in no way connected after they emerge
from the middle-piece.

No portion of the tail colors intensely with either iron-haema-
toxylin or the combination stain, although with either stain the
fibers take a faint bluish tint, while the membrane remains
colorless.

V. Fertilization of the Ovum.

1. *The Penetration of the Spermatozoön into the Egg and the Formation of the Astrosphere.*

The pointed head of the spermatozoön so quickly bores its
way through the thick membrane surrounding the egg that fer-
tilization is usually effected within three or four minutes after
the egg is laid. The spermatozoön can penetrate at any point
of the upper hemisphere of the egg, but I have not been able
to confirm the result of Fick, Jordan, and Roux that it can also
enter any portion of the lower hemisphere.

Kupffer ('82) has observed the entrance of the spermatozoön
into the living egg of Bufo variabilis and describes it thus:
When the head of the spermatozoön touches the egg-mem-
brane, the protoplasm of the egg draws back slightly at the
point of contact but quickly returns again to its first position.
The period of penetration of the spermatozoön from the
moment of contact of the sperm-head until the spermatozoön
disappears into the egg lasts in some cases from one to one
and a half minutes, in other cases but three-fourths of a
minute.

In preserved specimens of newly fertilized eggs of Bufo len-
tiginosus there is usually a slight depression in the surface of

the egg where the spermatozoön has entered. I have frequently found an extrusion of egg-plasm at this point to form an "entrance cone" (Pl. XXX, Fig. 42), as described by Fick and Michaelis, although such a structure is not invariably present. The spermatozoön enters the egg obliquely, and as soon as its pointed apex has penetrated below the surface, and *before the middle-piece is even in contact with the egg,* there is found at its anterior end a small round area containing a finely granular substance which always stains a uniform light pink after the use of the combination stain, and remains uncolored after the use of iron-haematoxylin (Pl. XXX, Fig. 43). This area, which is the beginning of the astrosphere, is free from yolk granules and is surrounded by a thick layer of pigment.

The most careful examination of a large number of eggs in this and the succeeding fertilization stages, preserved and stained in various ways, utterly fails to show any central granule in the astrosphere which might be considered a centriole; nor is there at any stage a collection of granules or a thickening of the substance of the astrosphere that could be called a centrosome.

That the astrosphere is called into existence by the presence of the spermatozoön in the egg, there can be no doubt ; but I have not been able to determine whether it is formed from a substance brought in by the head of the spermatozoön, whether it is an accumulation of a specific substance of the egg, the "archoplasm" of Boveri, or whether it is a condensed portion of the general cytoplasm as believed by Eismond ('94), Erlanger ('97), and vom Rath. It certainly cannot come from the swelling up of a substance in the middle-piece of the spermatozoön, as found by Fick to be the case in Axolotl. The appearance of the astrosphere in connection with the spermatozoön before the formation of the male pronucleus, and its early division into two distinct parts while the female pronucleus is still near the periphery of the egg, invalidate for this egg the explanation of the origin of the astrosphere advanced by Carnoy and Lebrun ('97), namely, that each pronucleus contains a centrosome which migrates into the cytoplasm when the two pronuclei come together, and so influences it that two

radial cytoplasmic systems are formed which become the poles of the segmentation spindle.   Whatever its origin, and I am inclined to agree with those who consider the astrosphere to be purely cytoplasmic, the astrosphere is present through all stages of fertilization, and apparently plays an important rôle in the various processes leading to the division of the cell.

The entire spermatozoön enters the egg.   The tail (Pl. XXX, Fig. 45) is found for a short time and is then absorbed, as in Axolotl.   I have been unable to follow the history of the middle-piece after it enters the eggs, as it stains very faintly ; but I believe it is absorbed with the tail and takes no part in the fertilization processes.

Hill ('96), Kostanecki and Wierzejski ('96), Wilson ('95), and others have described the rotation of the sperm-head through an angle of 180° after it enters the egg.   This is done, apparently, in order that the middle-piece from which the astrosphere arises, according to these investigators, may precede the male pronucleus in its migration towards the female pronucleus. I have found no indication of a rotation of the sperm-head in the egg of Bufo.

The path traced by the entering spermatozoön is similar to that described by Roux for the frog's egg.   The sperm moves at first centripetally, describing the " entrance path," and later curves more or less abruptly towards the female pronucleus, thus describing the " copulation path " (Pl. XXX, Fig. 46).

In all pigmented amphibian eggs the sperm-path is marked from its beginning by a well-defined trail of pigment granules. Many investigators agree with Hertwig : " Dass von der pigmentirten Rindenschicht ein vom Kern ausgezogener Theil sich abschnürt und mit nach dem Centrum wandert; hierbei lösen sich Pigmentkörperchen von Stelle zu Stelle ab und lassen so noch später die Strasse erkennen, auf der die Einwanderung des Spermakerns erfolgt." Fick's objections to this explanation of the origin of the pigment trail seem well founded.   If the sperm-head can penetrate the thick egg-membrane, it is inconceivable that it cannot also bore its way through the pigment layer below.   Moreover, the sperm-path is so deeply pigmented that it does not seem possible that so

much pigment could be torn from the peripheral layer without
leaving the place where the sperm entered nearly devoid of
pigment granules, and yet this region does not appear to have
lost any of its pigment.   Again, if Hertwig's view is correct,
there should be a large amount of pigment around the enter-
ing sperm, which should steadily decrease as the sperm pene-
trates deeper into the egg ; but according to Fick's Fig. 27,
the sperm-head, very soon after entering the egg, has almost no
pigment around it.   Not only is the same thing true of the
entering sperm in the egg of Bufo, but the male pronucleus,
when it is about to fuse with the female pronucleus, often
has more pigment around it than when it was first formed
(Pl. XXXI, Fig. 50).

Fick believes that there are three factors at work in the
formation of the pigment trail.

1. The entrance of the spermatozoön.   This apparently
takes place so quickly that a small amount of pigment is pushed
in before the head of the spermatozoön.   It is this pigment
from the peripheral layer which forms the funnel-shaped base
of the pigment trail (Pl. XXX, Fig. 46, *A*).

2. The spermatozoön exerts an attraction on the surrounding
pigment.[1]

3. The spermatozoön stimulates the protoplasm of the egg
to produce new pigment.

The fact that when the spermatozoön penetrates into the
lower part of the egg there is no indication of a pigment trail,
does not invalidate this last assumption ; because the proto-
plasm of this portion of the egg never forms much pigment,
and in some eggs none at all.

2. *The Male Pronucleus.*

The sperm-head enters the egg as an apparently homogene-
ous structure.   It soon becomes shorter and thicker, and breaks
up into a number of closely packed rounded granules (Pl. XXX,

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[1] Jordan considers that this attractive force diminishes as the spermatozoön
penetrates deeper into the egg, in consequence of which pigment grànules are
discarded along the way and mark the path taken by the spermatozoön.

Fig. 44), from which a small pronucleus develops in about fifteen minutes after the egg is fertilized.

Pl. XXXI, Fig. 48, shows a very young male pronucleus. It is round in outline, measuring 0.008 mm. in diameter, and lies in a small accumulation of cytoplasm directly behind the astrosphere which is preparing to divide. The male pronucleus has at first an irregular reticulum surrounded by a colorless nuclear sap. In its later growth it goes through the same processes as described for the female pronucleus, so that when the two pronuclei come together it is no longer possible to distinguish between them.

3. *Growth and Division of the Astrosphere.*

By the time the entire spermatozoön has entered the egg, the astrosphere has become an oblong structure composed of a central uniformly granular portion, the centrosphere, from which a constantly increasing system of rays extends out in all directions (Fig. 44). Pigment granules which previously formed a thick layer around the astrosphere now mark the course of the rays, the yolk spherules occupying an inter-radial position. At this stage the astrosphere greatly resembles the oblong "attraction sphere" found by Zimmermann ('93) in the pigment cells of Sargus annularis.

The next changes in the astrosphere are shown in Pl. XXX, Fig. 47. The greater mass of the substance of the centrosphere has collected in two knob-like ends which are connected by a narrow bridge of the same substance, the whole structure appearing dumb-bell shaped. It contains numerous fine threads which extend, for the most part, parallel to the longitudinal axis of the centrosphere. The male pronucleus has formed by this time and lies just back of the connection between the two centers into which the astrosphere is dividing. The radial system has become much more pronounced, the rays being longer and more numerous and ending apparently in the protoplasmic reticulum of the egg.

As the centers separate, they increase in size and the fine threads of the centrosphere begin to arrange themselves in an

irregular reticulum (Pl. XXXI, Fig. 50) which shows no signs
of the thickenings found by Eismond ('94) in the astrospheres
of Triton. The radial systems, having reached their greatest
extent at the previous stage, are now less pronounced, although
they are still distinctly visible. A layer of pigment granules
forms around the centrospheres, and also around the male pro-
nucleus, which, now that the connection between the centers is
broken, occupies a position between them. In the next stage
(Pl. XXXI, Fig. 51), when the two pronuclei are in contact,
every trace of the radial systems has disappeared and the astro-
spheres are round masses containing a distinct granular reticu-
lum and surrounded by a very thick layer of pigment which is
evidently formed of the pigment granules which previously
marked the course of the astral rays.

These later changes are comparable to those described for
Axolotl, where, at the time the two pronuclei are about to
unite, the radiation around the two centers has entirely disap-
peared and the centers themselves appear as compact, round
structures showing no sign of a centrosome.

4. *Fusion of the Pronuclei and Formation of the First Segmentation Spindle.*

The male pronucleus grows very rapidly and soon outstrips
the female pronucleus, measuring 0.022 mm. in diameter when
the latter has a diameter of but 0.018 mm. (Pl. XXXI,
Fig. 50). In structure both pronuclei appear the same, each
being nearly spherical and containing a reticulum seemingly
composed of fine linin fibers. A number of rounded nuclear
masses, presumably chromatin, lie either at the intersections
of the fibers of the reticulum or in the nucleoplasm close to
the nuclear membrane.

The two pronuclei can be readily distinguished until they
come in contact and are about to fuse (Pl. XXXI, Fig. 51).
From the moment of its formation the female pronucleus lies
naked in the protoplasm in close contact with the yolk granules.
It is never accompanied by an astrosphere or any radiation ; nor
is it ever surrounded by a layer of pigment or an accumulation

of cytoplasm.   The male pronucleus, on the other hand, is always in close contact with the astrosphere, lying between the two centers after their separation and being surrounded by the same pigment layer enclosing them at the stage of Fig. 50.

Fick considers the migration of the two pronuclei to be due to amoeboid movements; but the pseudopodium-like processes which he finds in the pronuclei of Axolotl are not present in the egg of Bufo.   It seems more probable, as Wilson ('96) says, that: "The paths of the germ-nuclei are determined by at least two different factors, one of which is the attraction or other dynamical relation between the nuclei and the cytoplasm, the other an attraction between the nuclei.   The former determines the entrance path of the sperm-nucleus, while both factors probably operate in the determination of the copulation path along which it travels to meet the egg-nucleus."

The place of union of the two pronuclei is never the geometric center of the egg, but is much nearer the upper pole, apparently in the neighborhood of the position occupied by the germinal vesicle just before its dissolution.   It is impossible to distinguish between the two pronuclei just before they have fused.   They are both about 0.024 mm. in diameter, and in each the reticulum has become very fine and stains but faintly, while the rounded nuclear masses are much more numerous and stain more deeply.   About three-quarters of an hour after the egg is fertilized, the two pronuclei fuse completely.   The segmentation nucleus is at first decidedly oblong (Pl. XXXI, Fig. 56) and shows a pronounced reticulum, in the meshes of which are the rounded nuclear masses.   When the segmentation nucleus rounds up (Pl. XXXI, Fig. 53), the reticulum begins to disappear and all the nuclear bodies become collected at the nuclear membrane.

Meanwhile the astrospheres have undergone a considerable transformation.   From an irregular meshwork (Pl. XXXI, Fig. 51), the fibers of each astrosphere become arranged radially, as in Fig. 56.   The central parts of these radiations show no centrosome, and, as in the case of the astrospheres at the poles of the first polar spindle, they appear to be formed only

of the massed ends of the radiating fibers. The rays increase quickly in number, and they extend out in every direction, pushing the pigment layer before them (Fig. 53). Their course is no longer marked by pigment granules, as in the earlier stages.

At the sides towards the segmentation nucleus, certain rays from each astrosphere grow more rapidly than the others (Fig. 53). These rays meet, forming the central spindle, in which the segmentation nucleus lies with its membrane still intact. The spindle rays seem to fuse completely in the equatorial region, so that the fibers of the fully formed spindle appear to extend unbroken from pole to pole where they, too, run into the central mass of the astrosphere.

A similar origin of the segmentation spindle is described by MacFarland ('97) in the egg of Pleurophyllidia californica. Here, after the division of the sperm-centers, the two daughter spheres with their radiating systems separate, showing no visible connection between them. The rays from both centers then completely disappear for a time, but they appear again later, and a central spindle is formed by a fusion of definite rays from each center.

Opposed to the general acceptance of the cytoplasmic nature of the spindle are the views of Flemming ('80), Moore ('96), and Erlanger ('96), which ascribe to the spindle a dual origin : its superficial portion and its extreme ends originating in the cytoplasm, while its greater internal and equatorial mass arises from the substance of the segmentation nucleus.

In the egg of Bufo it is not improbable that a part of the substance from the segmentation nucleus goes into the equatorial mass of the spindle, as the nuclear membrane breaks down before the spindle is completely formed. However, the spindle is primarily of cytoplasmic origin, as clearly demonstrated by Hermann ('91) in the spermatocytes of Salamander, and its partial formation before the nuclear membrane has disappeared precludes its arising entirely from the substance of the germ-nuclei, as maintained by Wilson ('95) for the segmentation spindle in the sea-urchin's egg, and by Carnoy and Lebrun ('97) for the egg of Ascaris.

5. *Division of the Segmentation Spindle and Formation of the Daughter Nuclei.*

After the disappearance of the nuclear membrane the chromatin of the segmentation nucleus becomes arranged at the equator of the spindle in the form of very short rods (Pl. XXXI, Fig. 54) which divide in the usual way and migrate to each pole (Pl. XXXI, Fig. 55). Here they apparently lose their identity, as they become fused into a rounded mass from which a daughter nucleus develops. At first each daughter nucleus is somewhat irregular in outline and contains a coarse reticulum and several masses of chromatin (Pl. XXXI, Fig. 52). Later the nucleus rounds up, and the chromatin breaks up into small chromosomes in preparation for the second division.

During the breaking down of the segmentation nucleus and the arrangement of the chromosomes at the equator of the spindle, the astrospheres increase enormously in volume. Each centrosphere again consists of an irregular reticulum, the fibers of which are directly continuous with the numerous fine rays extending out in every direction. After its complete formation the spindle is barrel-shaped and the ends of its fibers do not converge to any definite points as in the polar spindles, but they run nearly parallel into the reticulum composing the centrospheres. In the anaphase the astrospheres decrease in size, their central reticulum becomes decidedly granular, and the few rays that have not disappeared are again marked by pigment granules (Pl. XXXI, Fig. 55). When cell division takes place all the rays have disappeared and the astrospheres appear as rounded granular bodies surrounded by a pigment layer. In preparation for the second division, the astrospheres again show a reticulum composed of fine threads (Pl. XXXI, Fig. 52). They divide into two parts and the segmentation spindles are formed as before.

There is no period when the astrospheres entirely disappear, so they become, in one sense, permanent organs of the cell. Yet it is quite conceivable, as suggested by Kostanecki and Siedlecki ('97), that the substance of which the astrospheres are composed would become equally distributed throughout the

cell if the cell divisions did not follow each other quite so rapidly.

After the breaking down of the segmentation nucleus, there are no nucleolar-like bodies found near the spindle, as one would expect to find if some of the rounded masses in the pronuclei were true nucleoli. The entire substance of the segmentation nucleus is either absorbed at once or else it goes into the formation of the spindle. The changes occurring at this period take place very quickly, and I have been unable to follow them in all their details.

Although a definite central body has been found in the attraction spheres of various annelids, mollusks, echinoderms, and vertebrates, as yet it has not been discovered in any unsegmented amphibian egg, even with methods of preservation and staining which have clearly demonstrated its presence in other eggs. Eismond finds condensed portions in the attraction spheres of Triton and Axolotl, which he thinks may be comparable to the centrosome found in other forms. These bodies are not solid, are in no way constant in size, number, or position, and are undoubtedly, as Eismond himself suggests, the result of certain mechanical conditions. Braus ('95) has described typical centrosomes in the many-layered blastula cells of Triton; but he states that these centrosomes are very difficult to find in the two-layered blastula cells and makes no mention of their existence in the unsegmented egg.

At no period in the fertilization of the egg of Bufo is there the slightest trace of a definite central point in the astrospheres from which the rays diverge. The nearest approach to it is in the stage of Pl. XXXI, Fig. 56, where the rays seemingly converge towards a center; but this center is never a single granule or a mass of granules; it always appears to be composed of the massed ends of the radiating fibers. In other stages the reticulum of the astrospheres is perfectly uniform. Therefore, in the egg of Bufo, and presumably in other amphibian eggs, either the reagents used in killing and hardening do not penetrate the egg sufficiently well to preserve the delicate structure of the centrosome, or the mechanism of cell division can be carried on independently of a centrosome, and this structure

does not have, in all cases, the morphological value that has been ascribed to it.

VI. POLYSPERMY.

It cannot be doubted that polyspermy occurs in many eggs which subsequently undergo a perfectly normal development. Foot ('94) has found it in the egg of Allolobophora, Oppel ('92) in the reptilian egg, Rückert in the selachian egg, and van der Strict ('95) in the egg of Amphioxus. According to Jordan, Fick, Kupffer, Braus, and Michaelis, polyspermy is also normal in the amphibian egg. Born, differing from the last two observers, has maintained that but one spermatozoön normally enters the egg of Triton, and Roux has made the same statement regarding the frog's egg. Although Kupffer observed several spermatozoa entering the living egg of Bufo variabilis, my own results are not in accord with these. In the examination of a large number of both normally and artificially fertilized eggs, I have never found a single egg containing more than one spermatozoön. I am inclined to believe, therefore, that the eggs observed by Kupffer were undergoing pathological changes permitting the entrance of several spermatozoa, and that polyspermy is not a normal occurrence in the egg of Bufo.

SUMMARY.

1. The unripe ovarian egg of Bufo lentiginosus is surrounded by four membranes. The first and second are a part of the epithelium of the ovary; the other two, the chorion and the inner yolk-membrane, belong to the egg; beneath them perivitellin is found.

2. The three kinds of nucleoli in the ovarian egg disappear at the end of maturation.

3. The ovarian egg contains twenty-four separate chromosomes. These are at first composed of rod-shaped microsomes, later they have a filamentous structure, and at the end of hibernation each appears to be composed of a single series of rounded microsomes.

4. Before the disintegration of the germinal vesicle begins, a portion of the egg cytoplasm near the lower pole of the nucleus forms a fibrous band — the beginning of the line of radiation.

5. The nucleoplasm apparently forms the rays that run into the line of radiation after the breaking down of the nuclear membrane.

6. The line of radiation shortens gradually and a number of small granules appear in the midst of the rays. These granules soon fuse into irregular clumps staining like chromatin.

7. The débris from the germinal vesicle is carried to the black pole and forms the cicatricula.

8. At the beginning of nuclear disintegration the chromosomes become arranged in twelve pairs. The ends of each pair fuse, forming a closed ring near which a small aster suddenly appears.

9. The first polar spindle is formed at some distance below the surface of the egg, probably from the line of radiation. The large polar astrospheres have no centrioles.

10. The asters accompanying the rings have disappeared when the chromosomes are arranged on the spindle. The twelve chromatin rings split longitudinally, forming twenty-four closed rings which later divide at the points of union of the two original chromosomes, forming forty-eight half rings.

11. On its way to the periphery, the spindle loses its astrospheres. The chromatin material becomes greatly concentrated, halves of sister chromosomes apparently re-fuse, and the spindle seems to have but twenty-four dumb-bell-shaped chromosomes.

12. The first polar division separates the two chromosomes which originally fused to form a chromatin ring.

13. The second polar spindle has no astrospheres and is smaller and more slender than the first polar spindle. In the second polar division sister-chromosomes separate and there is an equal division in Weismann's sense. Twelve chromosomes, one-half the normal number, are left in the egg after the second polar body is given off.

14. There is no evidence that a centrosome is contained in any part of the spermatozoön of Bufo lentiginosus.

15. The spermatozoön can penetrate any point of the upper hemisphere. An astrosphere is formed at its anterior end before the middle-piece has entered the egg. The entire spermatozoön enters the egg, the tail and middle-piece soon disappearing.

16. The male and female pronuclei are apparently alike in structure ; but they can be distinguished until nearly the time of fusion, as the former is always accompanied by the astrosphere ; the latter lies in close contact with the egg substance and has no sign of a radiation around it.

17. The astrosphere is at first round and uniformly granular. It soon becomes oblong, many rays appear around it, and it then divides into two separate astrospheres.

18. The two pronuclei fuse in the upper hemisphere. The segmentation nucleus lies between the two astrospheres, which appear as rounded structures composed of an irregular meshwork, the rays having entirely disappeared.

19. The barrel-shaped segmentation spindle is formed primarily by the meeting of some of the rays which are again sent out by the astrospheres.

20. When cell division occurs, the rays of the astrospheres again disappear. The astrospheres become much smaller, but do not disappear entirely at any stage.

21. The astrospheres of the chromatin rings, of the first polar spindle and of the segmentation spindle do not contain a centriole at any period of their history.

22. Polyspermy is not normal in the egg of Bufo lentiginosus.

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EXPLANATION OF PLATE XXVIII.

All figures were drawn with the aid of the camera lucida. Plate XXXI is reduced one-third.

FIG. 1. Vertical section through the black pole of a young ovarian egg, showing the distribution of the yolk (Y) and pigment (PL) and the position of the germinal vesicle (GV) just before the beginning of the hibernation period. Diameter of the egg, 1.1 mm. Size of the germinal vesicle, 0.24 mm. × 0.36 mm. Zeiss AA., Oc. 4.

FIG. 2. A portion of the upper surface of a young ovarian egg, showing the egg membranes. A and C, membranes belonging to the wall of the ovary; ZR, Zona radiata; P, perivitellin; B, blood corpuscles. Zeiss apoc. 2 mm., Oc. 8.

FIG. 3. Transverse section of the germinal vesicle in the same stage as that of Fig. 1, showing the position of the chromatin threads and the nucleoli. C, chromatin threads; Cy, fluid substance surrounding the lower part of the germinal vesicle; N, nucleoli. Zeiss D., Oc. 2.

FIG. 4. Nucleoli from the germinal vesicle of an ovarian egg at the stage of Fig. 1. Zeiss apoc. 2 mm., Oc. 4.

FIG. 5. Chromatin threads from the germinal vesicle of an ovarian egg at the stage of Fig. 1. Zeiss apoc. 2 mm., Oc. 8.

FIG. 6. Vertical section through the black pole of an ovarian egg, showing the position of the germinal vesicle just before the beginning of its dissolution. Diameter of the egg, 1.1 mm. Size of the germinal vesicle, 0.31 mm. × 0.39 mm. Zeiss AA., Oc. 2.

FIG. 7. The germinal vesicle of an ovarian egg at the beginning of its dissolution. LR, line of radiation; A, granular substance below the line of radiation. Zeiss D., Oc. 4.

FIG. 8. Paired arrangement of the chromatin threads at the stage of Fig. 7. Zeiss apoc. 2 mm., Oc. 8.

FIG. 9. Youngest stage of the large nucleolar-like bodies found in the germinal vesicle at the end of the hibernation period. Zeiss apoc. 2 mm., Oc. 4.

FIG. 10. Later stage of the same. Zeiss apoc. 2 mm., Oc. 4.

FIG. 11. Ordinary nucleolus found in the germinal vesicle at the end of the hibernation period. Zeiss apoc. 2 mm., Oc. 4.

FIG. 12. Stages in the disintegration of the nucleoli during the dissolution of the germinal vesicle. Zeiss apoc. 2 mm., Oc. 4.

FIG. 13. The lower pole of the germinal vesicle after the disappearance of the nuclear membrane. LR, line of radiation from which fibers extend both above and below; N, nucleoplasm breaking up into coarse granules; A, granular substance below the line of radiation. Zeiss apoc. 2 mm., Oc. 4.

FIG. 14. The lower pole of the germinal vesicle at a somewhat later stage than that of Fig. 13. The line of radiation is composed of a dense lower part sharply defined below, and an upper looser meshwork from which fibers extend up between the granules of nucleoplasm. Zeiss apoc. 2 mm., Oc. 4.

FIG. 15. Beginning of a pronounced radiation extending from the line of radiation. Zeiss apoc. 2 mm., Oc. 4.

FIG. 16. Filamentous chromosomes found in the germinal vesicle of the youngest ovarian eggs obtained at the end of the hibernation period. Zeiss apoc. 2 mm., Oc. 8.

Pl. XXVIII
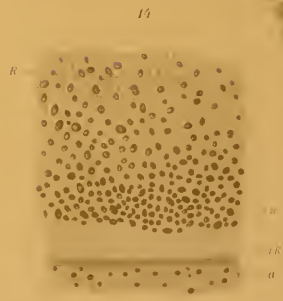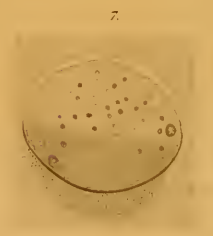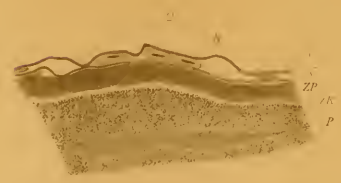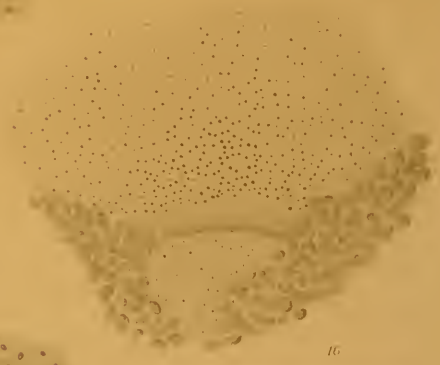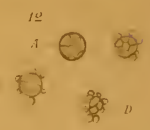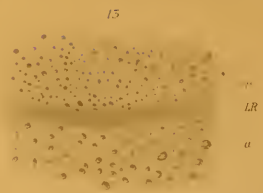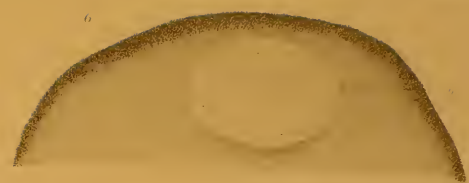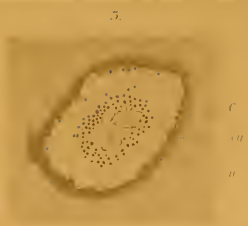
EXPLANATION OF PLATE XXIX.

FIG. 17. Vertical section through the black pole of an ovarian egg, showing the migration of the nuclear débris towards the upper pole after the breaking down of the nuclear membrane. Zeiss D., Oc. 4.

FIG. 18. A high magnification of the region of the line of radiation at the stage of Fig. 17. The radiation has reached its greatest extent, and the coarse granules found both above and below the line of radiation at an earlier period have entirely disappeared. Zeiss apoc. 2 mm., Oc. 4.

FIG. 19. Vertical section through an egg at a later stage than that of Fig. 17. The nuclear débris has taken a position directly under the black pole. Zeiss D., Oc. 4.

FIG. 20. A somewhat oblique section through an egg in the stage succeeding that of Fig. 18. The radiation is decreasing and the line of radiation has shortened considerably. First appearance in the radiation of rounded granules staining like chromatin. Zeiss apoc. 2 mm., Oc. 4.

FIG. 21. A later stage than that of Fig. 20. The granular masses seen in Fig. 20 have increased in size and number and take a much deeper stain. Zeiss apoc. 2 mm., Oc. 4.

FIG. 22. Next section in the same egg as Fig. 21, showing the rest of the granular substance in the radiation. Zeiss apoc. 2mm., Oc. 4.

FIG. 23. Masses of granular substance near the first polar spindle. From the same egg as Figs. 25–27. Zeiss apoc. 2 mm., Oc. 4.

FIG. 24. The twelve chromatin rings with accompanying asters, found in the nuclear substance at the stage of Fig. 19. Zeiss apoc. 2 mm., Oc. 8.

FIG. 25. A somewhat oblique section of the first polar spindle before its migration to the periphery of the egg, showing the longitudinal division of the chromatin rings and a distinct aster at one pole. Zeiss apoc. 2 mm., Oc. 4.

FIG. 26. Next section in the same egg as the preceding. Zeiss apoc. 2 mm., Oc. 4.

FIG. 27. Next section to the preceding. Zeiss apoc. 2 mm., Oc. 4.

FIG. 28. The first polar spindle migrating towards the periphery, with its longitudinal axis parallel to the upper surface of the egg. The asters at the spindle poles have entirely disappeared, and the double chromatin rings have separated into four parts. Zeiss apoc. 2 mm., Oc. 4.
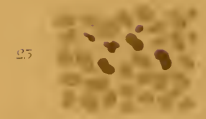
EXPLANATION OF PLATE XXX.

FIG. 29. Vertical section through an egg from the body cavity. The first polar spindle occupies a radial position at the surface of the egg directly under the black pole. The chromosomes are arranged at the equator of the spindle. Zeiss apoc. 2 mm., Oc. 4.

FIG. 30. Vertical section through an egg from the body cavity at a stage preceding that of Fig. 29. *ZP*, Zona pellucida; *YM*, inner yolk-membrane. Zeiss apoc. 2 mm., Oc. 4.

FIG. 31. Next section in the same egg as Fig. 30. Zeiss apoc. 2 mm., Oc. 4.

FIG. 32. Vertical section through an egg from the upper part of the oviduct. Anaphase of the first polar spindle. Zeiss apoc. 2 mm., Oc. 4.

FIG. 33. Section through an egg from the middle part of the oviduct. Cutting off of the first polar body. Zeiss apoc. 2 mm., Oc. 4.

FIG. 34. Vertical section through an egg from the lower part of the oviduct. The second polar spindle lies at the periphery with its chromosomes arranged at the equatorial plate. Zeiss. apoc. 2 mm., Oc. 4.

FIG. 35. Vertical section through an egg from the lower part of the oviduct. Separation of the sister chromosomes which were apparently fused in the first polar spindle. *PB*, first polar body. Zeiss apoc. 2 mm., Oc. 4.

FIG. 36. Next section to the preceding. Zeiss apoc. 2 mm., Oc. 4.

FIG. 37. Horizontal section through an egg from the lower part of the oviduct, showing the first polar body and the equatorial plate of the second polar spindle. Zeiss apoc. 2 mm., Oc. 4.

FIG. 38. Vertical section through a newly fertilized egg, showing the late anaphase of the second polar spindle. Zeiss apoc. 2 mm., Oc. 4.

FIG. 39. Section of a newly fertilized egg, showing the second polar body. Zeiss apoc. 2 mm., Oc. 4.

FIG. 40. The chromosomes remaining in the egg after the cutting off of the second polar body preparing to form the female pronucleus. Next section in the same egg as Fig. 39. Zeiss apoc. 2 mm., Oc. 4.

FIG. 41. The spermatozoön of Bufo lentiginosus. *A*, apex ; *H*, head stained black with iron-haematoxylin; *M*, middle-piece; *T*, tail. Zeiss apoc. 2 mm., Oc. 8.

FIG. 42. Depression in the surface of an egg caused by the entrance of the spermatozoön. There has been an extrusion of egg-plasm at this point to form an "entrance cone." Zeiss apoc. 2 mm., Oc. 4.

FIG. 43. Penetration of the spermatozoön into the egg and the formation of the astrosphere at its anterior end. Zeiss apoc. 2 mm., Oc. 4.

FIG. 44. The sperm-head after it has entered the egg breaking up into rounded chromatin granules preparatory to the formation of the male pronucleus. The astrosphere has become decidedly oblong and a radiation marked by pigment granules is forming around it. Zeiss apoc. 2 mm., Oc. 4.

FIG. 45. The middle-piece and tail of the spermatozoön after their entrance into the egg. Zeiss apoc. 2 mm., Oc. 4.

FIG. 46. Vertical section through an egg about one-half an hour after fertilization. A pigment trail marks the path taken by the spermatozoön in the egg. Zeiss AA., Oc. 2.

FIG. 47. Division of the astrosphere into two parts, each surrounded by a pronounced radiation marked by rows of pigment granules. Zeiss apoc. 2 mm., Oc. 4.

Pl. XXX
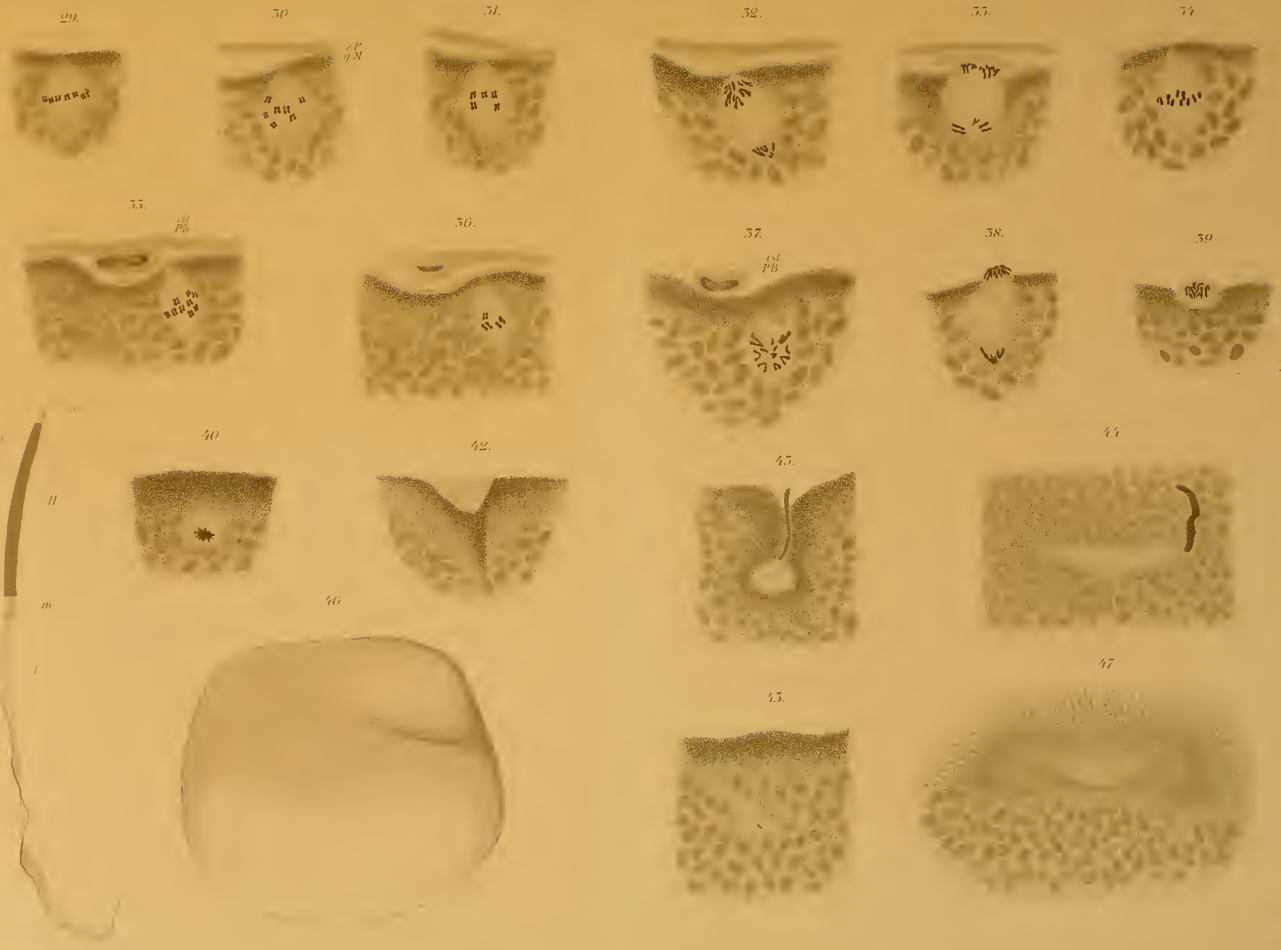
EXPLANATION OF PLATE XXXI.

FIG. 48. The male pronucleus about fifteen minutes after the egg has been fertilized. The radiation around the pronucleus centers in the astrosphere which is in the next section of the egg. Zeiss apoc. 2 mm., Oc. 8.

FIG. 49. The female pronucleus beginning its migration from the surface. About fifteen minutes after fertilization. Zeiss apoc. 2 mm., Oc. 8.

FIG. 50. The two pronuclei approaching each other about one-half hour after fertilization. The male pronucleus lies between the two centers into which the astrosphere has divided. Zeiss apoc. 2 mm., Oc. 8.

FIG. 51. Apposition of the two pronuclei about three-quarters of an hour after fertilization. Zeiss apoc. 2 mm., Oc. 8.

FIG. 52. A somewhat oblique section showing the resting daughter nucleus with its astrosphere preparing to divide into two parts in preparation for the second cleavage. Zeiss apoc. 2 mm., Oc. 8.

FIG. 53. Formation of the segmentation spindle by the union of rays from the two astrospheres. The segmentation nucleus has rounded up and occupies a position at the equator of the forming spindle. Zeiss apoc. 2 mm., Oc. 8.

FIG. 54. The segmentation spindle after the breaking down of the segmentation nucleus. The chromosomes lie at the equatorial plate of the spindle; the astrospheres have reached their greatest extent. (A combination of two sections.) Zeiss apoc. 2 mm., Oc. 8.

FIG. 55. Metaphase of the segmentation spindle. Decrease in size of the astrospheres and disappearance of many of the rays. (A combination of two sections.) Zeiss apoc. 2 mm., Oc. 8.

FIG. 56. The segmentation nucleus. The section is cut somewhat obliquely, so that only one astrosphere is shown. Zeiss apoc. 2 mm., Oc. 8.

Pl. XXXI.

JOURNAL

OF

MORPHOLOGY.

ON LOXOSOMA DAVENPORTI SP. NOV.

AN ENDOPROCT FROM THE NEW ENGLAND COAST.

W. S. NICKERSON.

CONTENTS.

INTRODUCTION.

ALL representatives of the genus Loxosoma heretofore studied have been European. One species has been mentioned

(Hincks, '**77**) as occurring in the Gulf of St. Lawrence, but no study of it has been made, nor has a satisfactory description been published.   Verrill ('**79**) has also mentioned Loxosoma as occurring on the New England coast, but without identifying the species or giving any description from which it could be recognized.   With these exceptions no representatives of the genus have been known to occur outside of European waters. During the summer of 1897 I found a species of Loxosoma living in abundance upon the New England coast.   To it, the first member of this genus to be described from the western hemisphere, I have given[1] the name " Loxosoma Davenporti," in honor of the foremost American student of the Bryozoa, who was also the first to recognize it as a new species, from a mounted specimen sent him.   A study of this form has revealed several structural features not found in European species, and incomplete studies of its development give promise of novel embryological data.   The present paper will be confined to a description of the structural characters, leaving its embryology to be described subsequently after the opportunity for more thorough investigation.

Orientation of Loxosoma. — The terms "dorsal," "ventral," "transverse," "longitudinal," etc., will be used in accordance with the interpretation of the relations of the body given by Harmer, '**86** (not '**85**).   According to this view, the antero-posterior axis of the animal coincides with its longest diameter, and a section perpendicular to this axis is a transverse section. The ventral surface includes the whole inner face of the lophophore, the side of the body and stalk toward which this is turned, and the foot or surface for attachment.   Ventral is, therefore, essentially synonymous with anterior.   The dorsal (= posterior) surface is that between the distal extremity of the lophophore and the posterior margin of the foot.

Brief Statement of External Characteristics. — Loxosoma Davenporti is a robust little animal between .75 mm. and 2.4 mm. in length when fully expanded, the stalk making up about one-half of the total length.   The average individual is about

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[1] See Reports of Meetings of American Morphological Society, in *Science* (N.S.), vol. vii, 1898, p. 220, and vol. ix, 1899, p. 366.

1 mm. long.   The stalk is terminated at its basal end by an orbicular foot which has neither foot-gland nor lateral expansions of any kind.   Muscles having a spiral course are present on the anterior side of the stalk connecting with the margins of the foot.   The number of tentacles most commonly observed is between twenty-two and twenty-six.   Buds are usually present, varying in number from two to twelve.   A single row of large, prominent cells is present in the mid-dorsal line of the body-wall.   A pair of organs not mentioned as occurring in any other species, and which will be described as flask organs, are frequently present attached to the anterior wall of the body, nearly opposite the oesophageal end of the stomach.   A modification of the floor of the atrium between epistome and rectum will be described as a mammary organ.   To this the developing embryos attach themselves, and from it they derive their nourishment.   The anus opens in the center of the lophophore upon a small papilla or anal cone, the posterior wall of the rectum being attached throughout its length to the lophophore.   Dorsal sense organs do not occur in this species.

The Body-Wall and its Modifications. — The body-wall is formed of a single layer of flattened epithelial cells, outside of which is a thin, structureless cuticula 1–2 μ thick.   The cells forming the inner wall of the lophophore are somewhat more attenuated than those upon its outer side.   The tentacles which arise from the margin of the lophophore have the epithelium on their inner faces covered by long cilia, such as also line the atrial groove at their bases.   The only other portion of the superficial epithelium which bears cilia is the oral face of the epistome (Pl. XXXII, Figs. 1, 7, 9, 10).   The tentacles vary in number from about sixteen or eighteen, in the young individual just separated from the parent, to twenty-six or twenty-seven in the fully developed adult.   The numbers most commonly observed are from twenty-two to twenty-six.   No species has been described heretofore as having more than eighteen tentacles.   New tentacles arise alternately upon either side of the median plane at the distal portion of the lophophore, as shown by the frequent occurrence of one or two immature tentacles in that position (Fig. 1).   When two are

present, one is always larger than the other. Cross-sections of
tentacles are represented in Figs. 9 and 10. Each consists of a
single layer of epithelial cells surrounding a central core of the
gelatinous parenchyma, the cells on the inner or atrial side
being ciliated. Kowalevsky ('65) has described the tentacles
of L. Neapolitanum as having their anterior faces composed of
three rows of cells, definitely arranged, the middle row being
depressed to form a groove of which the two lateral rows
form the edges and bear cilia. No such definite arrangement
of cells is present in L. Davenporti, and the distribution of
cilia is uniform over the whole inner surface. Muscle fibers
have been described as extending into the tentacles, by Kowa-
levsky in L. Neapolitanum, and by Davenport ('93) in Urna-
tella. I have preparations of L. Davenporti stained by the
methylen-blue *intra vitam* method, which show two fibers, one
on each inner lateral face, extending into each tentacle and
there dividing into fine branches. These fibers I have not
observed either in sections or in entire preparations stained by
other methods. I have been unable positively to determine
whether the fibers in question are muscular or nervous in func-
tion, though I incline strongly to the latter view. The two
kinds of fibers are so intimately associated in the marginal por-
tion of the lophophore, and react so nearly alike to the methy-
len-blue stain, as to make it often extremely difficult to decide
in which class a particular fiber belongs.

A thin fold of the epithelium, the velum, extends outward
from the margin of the lophophore, partially closing the atrial
aperture when the lophophore is contracted. It is attached to
the outer side of each tentacle, near its base, at which point a
little protuberance is formed (Pl. XXXII, Fig. 3) which con-
tains a single peculiar large cell. In Pl. XXXII, Fig. 10, is
shown the appearance of one of these cells, as seen in a cross-
section of the tentacle. The large, nearly spherical nucleus is
situated in the basal portion, while through the cytoplasm a
number of delicate lines extend perpendicularly toward the
surface. The cuticula over the cell is thickened to form a
flattened or sucker-shaped protuberance (Figs. 3 and 10). The
explanation of the function of these cells is furnished by a

study of the living animal. If a number of specimens in clear sea water in a smooth glass vessel be observed attentively, individuals may often be seen lying on the dorsal surface with the lophophore fully expanded. If a current of water from a pipette be directed against an animal in this position, it becomes evident at once that the creature is attached quite firmly by the lophophore margin ; and though the foot end may be lifted up by the motion of the water, the hold of the animal is loosened only by a very strong current. This observation makes it evident that the cells in question serve as a means of attachment. They are to be regarded as unicellular suckers, which are of use to the animal by enabling it to keep a hold upon its host while changing its foot attachment. The projecting saucer-shaped portion of the cuticula probably serves as a disc for attachment, and the lines traversing the cytoplasm as differentiations enabling the cell to contract in one direction, perpendicular to the surface. Harmer ('85) described certain large cells in the margin of the lophophore of L. tethyae, and considered them glandular in function. That they are homologous with those which I have found in L. Davenporti seems extremely probable, and renewed investigation may be expected to show a similarity in function.

When the body is in a contracted condition, the lateral margins protrude on either side as thin outfoldings of the bodywall or alae, which are represented in transverse section in Pl. XXXII, Fig. 2. In Pl. XXXII, Fig. 1, the alae are represented as they appear from the ventral side in a partially contracted individual. In the fully expanded condition (Pl. XXXII, Fig. 11) no trace of them is to be seen. Similar alate expansions of the body have been described by Schmidt ('76) for L. raja (= L. Neapolitanum Kow.) and by Prouho ('91) for L. annelidicola. In the latter species they appear to be much more prominent structures than in L. Davenporti.

In the median line of the posterior side of the body is a peculiar row of prominent cells shown in Pl. XXXII, Figs. 2, 7, and 8. These cells are very noticeable, both in the living animal and in preserved specimens. They are sharply set off from those upon either side, and are arranged with perfect

regularity in a single row, extending from a short distance above the margin of the foot to a region behind the intestine, where the peculiarities gradually become less marked and the cells become indistinguishable from those about them. The character of the cells is shown in Pl. XXXII, Figs. 7 and 8. The nuclei are large, rounded, and near the basal ends. A narrow marginal zone of the cytoplasm is clear and free from granules, and stains very faintly. The remaining portion is filled with fine granules which stain so deeply with iron-haematoxylin as to make the body of the cell appear almost black. The cuticula covering these cells seems to be in no way different from that of adjoining regions. Concerning the function of the cells I have little to offer. The character of the contents would seem to indicate a secretory or glandular function, though no conditions have been observed which point to a discharge of the contents ; neither does there appear to be any avenue provided for the discharge of the secretion. A row of similar cells was described and figured by Salensky ('77) for L. crassicauda and L. tethyae ; he considered them gland cells. Harmer ('85) failed to find them in the specimens which he studied.

The structure known as foot-gland in several other species is lacking in L. Davenporti. Neither in the adult nor in the developing bud is any trace of it to be seen. Its place is probably taken functionally by certain unicellular glands, from twenty-five to thirty in number, distributed chiefly around the margin of the foot (Pl. XXXII, Fig. 15). They open by a sort of bordered pit (Pl. XXXIII, Fig. 34), which resembles a minute sucker, with which Kowalevsky compared the corresponding structures in L. Neapolitanum. They resemble very closely the cells which he figured, the chief difference being that in the American species a greater number is present. Around the margin of the foot these cells are often grouped in clusters of two or three, which may in some cases open by a common pore. Those farther from the margin are more scattered and open singly. These gland cells arise in the bud by the elongation and differentiation of certain cells of the body-wall in the region which will become the margin of the foot when the

separation between bud and parent shall have taken place (Pl. XXXIII, Fig. 32).

The epistome is present in L. Davenporti as a portion of the atrial epithelium, which forms a projecting lip at the posterior margin of the mouth (Pl. XXXII, Figs. 5, 7, and 11). Its oral face is ciliated.

I shall describe under the name of flask organs certain structures of unknown function not mentioned for other species of Loxosoma, nor for other Bryozoa. They are illustrated in Pl. XXXII, Fig. 11, and Pl. XXXIII, Figs. 16–19. The flask organs are attached by their rounded bases to the wall of the body, near the basal end of the stomach, and project laterally outward and obliquely forward. The length of the organ is from one-half to three-quarters the diameter of the stalk of the animal to which it is attached. Many individuals have two of these organs, one on either side ; others have one, while in some specimens they are entirely absent. The peripheral portion of the organ (Pl. XXXIII, Fig. 16) is composed of a single layer of epithelial cells similar in character to those covering the body of the animal. The interior is occupied by gland cells, about a half dozen in number, which have rounded bases and long, tapering peripheral ends extending out into the pointed extremity of the flask. At the extreme end of the organ there exists a minute opening. Several stages in the life history of these structures are shown in the figures. The first indication of their formation is given by a thickening of the body-wall, as represented in Pl. XXXIII, Fig. 17. The ectodermal cells at this point have become greatly elongated, forming a conical elevation, of which the central cells have already begun to be unlike the peripheral. A little later stage (Pl. XXXIII, Fig. 18) shows that these central cells have become clearly differentiated from the rest ; their nuclei have become larger and have taken a position near the basal end, and an abundance of fine granules have made their appearance in the cytoplasm. The surrounding cells have become shorter, and at the base of the elevation the beginning of a constriction has appeared. In the fully developed condition shown in Pl. XXXIII, Fig. 16, the whole body of the glandular cells is packed full of fine

granules which stain both with carmine and iron-haematoxylin, though not intensely. The nuclei are small and lie at the extreme basal ends of the cells. A condition which evidently follows that just described is shown in Pl. XXXIII, Fig. 19. The form of the flask is retained, but the secretion has been discharged, and the interior contains only the shriveled remains of the gland cells. Several facts lead me to believe that the whole organ soon afterward disappears. First, the observation that in some individuals the organs are entirely absent; and these individuals are frequently large, mature, actively budding animals. Second, the presence or absence of flask organs is not correlated with the sexual condition of the individual. Third, animals having no flask organs have been observed bearing buds on which flask organs were present. Fourth, animals having immature flask organs have been observed bearing buds on which flask organs, which appeared to be fully developed, were present (*cf.* Pl. XXXII, Fig. 11). Fifth, mature individuals have been found with the flask organs present on one side and wanting on the other, but showing no indication that the missing organ had been lost through mechanical injury. The only reasonable interpretation of these facts seems to be that the organs arise from certain ectodermal cells in the manner described, form and discharge a secretion, and afterwards disappear. The fact that buds sometimes have flask organs larger than those of the parent probably indicates that the organs arise more than once in the life of the individual. The observed facts give no basis for determining more definitely the function of these organs.

It seems rather remarkable that no organs have been described from other members of the genus Loxosoma which appear capable of being homologized with these structures. The only organs known to me which have been described for other Endoprocta, and which appear in any respect comparable with the flask organs of L. Davenporti, are certain structures described by Ehlers ('90), p. 33, as occurring at intervals in the stalk of Ascopodaria. Slight elevations of the cuticula, pierced at the summit by a minute pore, cover little conical groups of cells which differ in size and appearance from the surrounding

ectodermal cells. These cells, usually three in number, have deeply staining protoplasm and basal nuclei, and with their distal ends surround a minute cavity with which the pore communicates.

Musculature. — The most prominent part of the musculature consists of fibers extending longitudinally just beneath the epithelium (Pl. XXXII, Fig. 15). They are attached about the margin of the foot and extend upward to the lophophore, at the margin of which those upon the anterior side become inserted. Those in the lateral and dorsal portions pass obliquely outward into the marginal portion of the lophophore and help to make up the atrial sphincter. At about the height of the stomach the dorsal longitudinal fibers bend laterally to enter the marginal portion of the lophophore, leaving the central part of the organ immediately about the rectum free from muscle fibers. It is obvious that the contraction of the longitudinal muscle fibers causes a shortening of the body of the animal, and in conjunction with the atrial sphincter, the closing of the lophophore.

Oblique muscle fibers are present in the lower portion of the stalk, just beneath the longitudinal muscles, and chiefly upon the anterior side. They are attached all around the margin of the foot and extend obliquely upward and toward the anterior side. Their obliquity is greatest near the foot, where those from opposite sides cross in the median part of the ventral side. Higher up the obliquity becomes less, and toward the stomach the oblique fibers become nearly parallel with the longitudinal. On the dorsal side of the stalk oblique fibers are present only at the extreme basal portion where they attach at the margin of the foot. These fibers may be assumed to be of service in aiding the animal to turn and bend its body in various ways, though I have seen no indication of their use in the manner described by Prouho ('91) for corresponding muscles in L. annelidicola. The latter species, which lives like L. Davenporti, attached to a tubicolous annelid, can quickly rotate itself 180° upon its base of attachment by the contraction of its oblique muscles, and so assume the position in which it is least liable to injury by the movement of the worm up or down in its tube.

When the American species is fixed by its foot to the bottom of a glass vessel and bending its body in various directions, if it be touched very gently upon one side by a needle, instead of rotating out of harm's way, as L. annelidicola is described as doing, it responds by drawing in its tentacles and contracting its body.

A band of circular fibers, the atrial sphincter, extends around the margin of the lophophore in the velum, just over the bases of the tentacles (Pl. XXXII, Fig. 3). By its contraction the infolding of the tentacles and the closure of the atrium are accomplished. These fibers are shown as seen in section in Pl. XXXII, Figs. 5 and 7.

Delicate fibers extend around the upper portion of the oesophagus. By their contraction they reduce the lumen of the organ (Pl. XXXII, Fig. 2).

Similar fine fibers surround the intestine and rectum, serving as constrictors for these portions of the tube.

A number of muscle fibers extend from either side of the oesophagus backward and slightly upward over the stomach to the vicinity of the body-wall on either side of the intestine. Whether they attach to the sides of the oesophagus and intestine respectively or to the body-wall near them, I have not determined, but in either case they form virtually a band enclosing the two structures and serving by their contraction to draw the intestine forward and so to aid in the closure of the atrium.

Muscle fibers also pass from the vicinity of the stomach obliquely outward to the region on either side of the oesophagus where the buds are formed, and in some cases may be seen extending into the developing buds, to which they furnish a mechanical support. On either side of the oviduct a few fibers are found extending from the vicinity of the intestine upward and forward into the mammary organ. A certain amount of variability in the number and arrangement of the muscle fibers traversing the part of the body between the stomach and the floor of the atrium may reasonably be expected to occur, dependent upon the presence of buds and the state of development of the mammary organ. I have, however, no data for determining the amount of such variability.

The structure of the muscle cells has been made out only in the case of the longitudinal and oblique muscles of the stalk. It is best shown by preparations stained by methylen-blue. The nucleus is situated at one side of the fiber, and usually at a short distance from it, surrounded by a small amount of cytoplasm, which serves also as a stalk to attach it to the muscle fiber. The fibers are usually branched at their ends and so have several points of attachment.

Parenchyma.— As in other Endoprocta, a definite body cavity is lacking. The space between body-wall and intestinal tract is occupied by the parenchyma, a gelatinous material containing branched anastomosing cells. Through this extend the muscle fibers and within it lie the brain, sexual and excretory organs. A number of rounded or ovoidal highly refractive bodies are found irregularly distributed through the parenchyma. Some of these are represented in Pl. XXXIII, Fig. 25. They are found in every part of the animal in which the parenchyma is present. They remain nearly uncolored when treated with carmine stains, and show within a variable number of minute spherical particles which resemble oil globules in optical qualities, but do not blacken when treated with osmic solutions. In sections stained by iron-haematoxylin these bodies take an intensely black color, the larger spherical particles showing frequently as lighter spots within. These bodies are often surrounded by a clear space, when seen in sections (Pl. XXXII, Fig. 16), suggesting the presence of a system of spaces or canals in the parenchyma. In preparations of entire animals, the bodies in question appear arranged in rows or lines forming an anastomosing network, most clearly seen in the expanded lophophore. I believe that it is true that the spaces in the parenchyma form an irregular system of channels through which a body fluid circulates, propelled by the contractions of the animal, and that the rounded bodies described are physiologically comparable with blood discs or corpuscles of some higher forms.

These bodies arise in developing buds chiefly in the axial portion of the stalk. This is composed mainly of elongated cells attached at the upper end to the stomach wall and extending

longitudinally downward. The interior of these cells becomes hollowed out to form elongated tubules and the cytoplasm metamorphosed into the bodies described, the process agreeing closely with that described by Davenport ('93), p. 7, in the formation of the yolk granules of Urnatella.

In fully mature animals the interior of the stalk appears to be filled in some cases by a gelatinous mass almost destitute of structure, and the rounded cytoplasmic bodies are scattered through every part of the parenchyma.

Digestive System. — The digestive system in L. Davenporti is similar to that described for other members of the genus (Pl. XXXII, Fig. 7). It consists of a *U*-shaped tube having its oral end just inside the anterior margin of the lophophore and opening at the anal end upon a small elevation or papilla rising from the center of the lophophore into the cavity of the atrium. Its wall is composed of a single layer of epithelial cells which bear long, thickly set cilia, except those forming the glandular portions of the wall of the stomach. The descending limb of the *U* is formed by the oesophagus or gullet, which is overhung at its oral end by the epistome, a fold of the vestibular epithelium ciliated upon its oral face (Pl. XXXII, Figs. 5 and 7). At its lower end it is slightly narrowed and bends sharply inward to open into the basal portion of the stomach.

The stomach, which is formed by a considerable enlargement of the digestive tube, has its long diameter nearly parallel with the long axis of the body. The cilia are here shorter than in other regions, and are entirely absent from the portions which are of a distinctly glandular nature. These regions are three in number, two of which occur as a pair of thickened ridges near the basal end of the organ, one on either side of the opening of the oesophagus (Pl. XXXII, Fig. 4 ; Pl. XXXIII, Fig. 24). In the living animal they appear whitish and opaque. The cells forming these thickenings are long, narrow, and filled with fine granules which stain deeply with iron-haematoxylin, giving to the cells of this region a dark, slaty-blue color. The nuclei are basal in position. A few small vacuoles are sometimes present, while at the free ends of the cells, occupying a narrow strip next to the lumen, there is often a zone of small spherical

granules which take an intense stain. The third region is what has been described in other species as the liver. This comprises nearly the whole anterior wall of the stomach above the opening of the oesophagus, and is made up of elongated cells containing clusters of coarse granules which in the living condition give a color to the organ, varying in different individuals from yellow to reddish brown. These cells, with the exception of the nuclear chromatin, are but faintly colored by iron-haematoxylin, the coarse granules of the cytoplasm remaining unstained. Many cells show conspicuous granular yellow masses in the center of large clear vacuoles, while in the cavity of the stomach similar masses are found which appear to have been discharged from corresponding vacuoles in the liver cells.

The intestine leads away from the stomach in a course nearly parallel with the oesophagus, when the animal is expanded, and is lined with cilia similar to those of the oesophagus. A slight constriction of the tube marks the passage from the intestine to the rectum. The cilia lining the rectum are the longest present in any portion of the alimentary tract.

Nervous System. — Further investigation with special nerve methods will be necessary in order to get a knowledge of the details of this system. The brain lies just in front of the intestine and above the stomach, between it and the floor of the atrium, and shows essentially the characters described for several other species of Loxosoma (Pl. XXXII, Fig. 7 ; Pl. XXXIII, Fig. 27). It is elongated transversely, the two rounded ends being composed of a peripheral layer of cells and an inner fibrous portion continuous with the delicate fibers composing the commissure (Pl. XXXIII, Figs. 21, 22, 29, and 30). From each end of the brain two bundles of fibers are given off, but their distribution has not been traced in detail. One on each side passes into the lophophore and extends around parallel with its edge to the median distal region, giving off on the way frequent branches which pass out into the lophophore margin. Sensory bristles are visible upon the tentacles of the living animal as described by Harmer ('85) and others, but their relation to the nerve fibers has not been determined. Fibers, probably nervous,

entering the tentacles have already been mentioned (p. 354).
Dorsal sense organs, as described for many other species, are
absent in this one.

A connection between the brain and the outer ectoderm of
the body is represented in Pl. XXXIII, Figs. 30 and 31. Each
cell of the pair of large bipolar cells is apparently in connection
with the brain at one end, and at the other is continued into
a long process which extends outward to the body-wall on the
ventral side near the oesophagus. This condition was observed
in a young animal not yet separated from the parent. From
their close relation to the brain, I regard these cells as nervous
in function, although their later history is not known.

Reproductive System. — The rare condition of proterogynic
hermaphroditism is illustrated in the reproductive system of
L. Davenporti. In the single pair of gonads both male and
female genital products are developed, the ova preceding the
sperm. By far the greater number of individuals examined
possessed ova in various stages of development, but no trace of
male sexual cells. In other individuals, developing spermatozoa
were found and no trace of ova. But that both male and
female elements are developed in the same gland is demon-
strated in Pl. XXXII, Fig. 6. As shown here, the sexual gland
of one side of the body is an ovary ; that of the other side con-
tains, besides an evidently degenerating ovum, a mass of male
sexual cells in different stages of development up to the fully
grown spermatozoa with tails. The evidence of degeneration on
the part of the ovum is found in the shriveled condition of the
germinative vesicle and the absence of chromatic material.
While in most cases both sides of the body showed well-
developed ovaries in an apparently functional condition, in
others the ova of both glands were evidently suffering degen-
eration, and certain of the follicle cells undergoing division
exhibited what seemed to be early stages in the development of
the spermatozoa. Why it is that the female condition is so much
more frequently found I have not determined. The relative
scarcity of individuals in the state of male functional activity
is in agreement with the observations of other students upon
the genus Loxosoma, although no observations have been

reported up to this time which demonstrate that both sexual conditions occur in the same animal at different times.

The gonads are two nearly spherical masses of cells lying one on either side of the median plane of the body in front of the intestine and above the stomach (Pl. XXXII, Fig. 4). Their position is subject to modification by the contractions of the body accompanying the closing of the lophophore. Pl. XXXII, Fig. 2, represents a section across a very strongly contracted animal and shows the displacement of the gonads thus produced. Here they are seen to lie one on either side of the stomach and at some distance from the intestine. Each gonad, when functional as an ovary (Pl. XXXII, Figs. 4 and 13), consists of a layer of smaller peripheral cells which surround a central space occupied by one or occasionally two or three larger cells or ova. The size of the ovary varies considerably, according to the degree of maturity exhibited by the contained ova. Enclosing the whole organ is a delicate structureless membrane. Pl. XXXII, Fig. 4, represents a section through an ovary containing a nearly mature ovum. The cytoplasm is finely and uniformly granular throughout, and appears to contain but a small amount of deutoplasm evenly distributed. The germinative vesicle is central, spherical, and has all of the chromatic substance gathered into a single mass or nucleolus. This is centrally placed and stains deeply with iron-haematoxylin.

In the phase of activity when the gonads are functional as spermaries, the whole mass is made up of many small, deeply staining cells, as shown in Pl. XXXII, Fig. 2. The cells of the peripheral layer are smaller and less evident, and in sections can be distinguished often around only a portion of the margin. From the upper side of each gonad a narrow duct passes upward and inward, just beneath the surface of the brain (Pl. XXXIII, Fig. 20), to the median plane, where it meets its fellow from the opposite side. At the point of meeting a swelling of the duct occurs, forming a little chamber, or sack, which lies immediately below the brain commissure. From it a short duct leads upward, to open in front of the commissure into the cavity of the atrium, just anterior to the intestine (Pl. XXXII, Fig. 4 ; Pl. XXXIII, Figs. 22 and 23). In the

male phase the chamber functions as a seminal vesicle and the efferent duct as a ductus ejaculatorius. Lying below and in front of the brain commissure, and partly surrounding the median sexual chamber and its ducts, is a mass of large granular cells (Pl. XXXII, Fig. 4 ; Pl. XXXIII, Figs. 20–24), which undoubtedly function as glands. The facts observed indicate that during times of female functional activity these cells secrete a substance which forms a covering for the embryos in early stages of their development and attaches them to the parent. Pl. XXXIII, Fig. 23, shows the unpaired portion of the oviduct, and at its mouth an early blastula surrounded by a delicate membrane which, on the side toward the parent, is continued out into a stalk or pedicel. This stalk occupies the lumen of the terminal portion of the duct. There seems to be no reason for doubt that this structure owes its origin to the large gland cells described, and that these cells pour out their secretion around the egg as it passes them on its way from the ovary to the exterior. Prouho ('91) has described for L. annelidicola under the name of shell gland (*glande de la coque*) a group of similar cells, to which, as the name implies, he attributed the formation of a protective membrane around the developing egg. The facts observed give no suggestion as to what the function of these glands may be when the animal is functionally male.

The existence of hermaphroditism has long been suspected in the genus Loxosoma, but heretofore no one has proved this supposition correct. Harmer ('85) expressed a belief that both male and female sexual products arise in the same gonad at different times, the male being formed before the female. My observations establish the fact of hermaphroditism, but show that, contrary to Harmer's view, in L. Davenporti the female sexual products arise before the male. It is possible, however, that several periods of sexual activity, alternately male and female, may occur in the same animal, in which case there would be no conflict between Harmer's view and my observations. The fact that we worked upon different species may also afford a possible explanation of the differences in our conclusions. Prouho ('91) observed that in L. annelidicola the

sexes are separate, and that the males are larger than the
females, although he considers the number of his observations
insufficient to establish the law. Since, according to my ob-
servations on L. Davenporti, the male condition indicates a
greater age than the female, we might reasonably expect that
the average male individual would exceed the female in size.
Nevertheless, I do not find evidence that such is the case.
All of the males which I have studied (about ten) were of
nearly average size. All of the exceptionally large individuals
which I have observed, specimens 2 mm. or more in length,
have without exception been females. In view of these facts,
Prouho's generalization cannot be accepted as applying to the
American species. Neither do I consider that my observations
upon so small a number of males justify the opposite conclusion,
namely, that the average size of the females exceeds that of the
males. So far as size may be considered to have any bearing
upon the matter, the facts observed would seem to lend support
to my suggestion that several periods of sexual activity, alter-
nately male and female, occur in the life of a single individual.

The gland cells, which have been described hitherto as occur-
ring in the vicinity of the brain, have been those of the shell
gland. Pl. XXXIII, Fig. 27, represents a cell which evidently
plays a different rôle. Although lying closely associated with
the cells of the shell gland, it sends out a long process extending
to the epithelium of the upper part of the oesophagus. From
the coarsely granular character of the cytoplasm and the appear-
ance of the process (Pl. XXXIII, Fig. 28), I consider it a gland
which pours its secretion into the upper part of the oesophagus,
probably to aid in the entanglement and deglutition of food
particles. This condition has been observed only in the case
figured, which is probably to be accounted for by the fact that
only in an exceptionally favorable section (longitudinal and ob-
lique to the sagittal plane) could both the cell and its process
be made out completely. A fact which is of interest in this
connection is that in sagittal sections the cilia of the upper
part of the oesophagus just beneath the epistome have been
noticed in some cases to stain differently with iron-haema-
toxylin and orange from those of other regions. While the

cilia in other places were colored only by orange, those of this region took a decided haematoxylin color. It is possible that the difference in staining qualities resulted from the presence in that region of a secretion of cells such as I have described. Oesophageal glands have not been described for other species of Endoprocts.

Mammary Organ. — I shall describe as a mammary organ a peculiar modification of the epithelium of the floor of the atrium. This organ is found only in females with embryos. The tissue between the epistome and the opening of the oviduct becomes swollen and rises up as a projection into the atrium (Pl. XXXII, Fig. 7; Pl. XXXIII, Fig. 27), often extending as high as the anus. The epithelium covering this elevation is very much thickened, and the cytoplasm of the cells shows the presence of granules which do not stain with iron-haematoxylin but are faintly colored by orange. The nuclei of the cells are generally near their basal ends, and the granules are most numerous in the same region and fewer toward the free ends of the cells. This condition is represented in Pl. XXXII, Fig. 7, which is drawn from a median sagittal section through an individual in which the mammary organ is in a condition often observed both in living animals and in series of section.

A condition of the organ which has been observed in only one case is shown in Pl. XXXII, Fig. 5, which represents a portion of a section deviating slightly from the median sagittal plane. As there shown, the distal extremity of the mammary organ has become secondarily invaginated, giving rise to an oval sack-shaped cavity open at the end. This cavity is occupied by a mass of nearly spherical cells filled with granules similar in character to those already mentioned, but more completely filling the cytoplasm. Many of the cells appear to lack a nucleus, and where present it is small and inconspicuous. In the atrium outside of the mouth of this sack are many loose cells similar to those within the cavity. Although no conditions intermediate between the two here figured have been observed, it seems likely that the cells shown in the latter case have arisen by a proliferation of the epidermal cells covering the organ. The cells probably contain an elaborated food

material or yolk, deposited in them for the purpose of supplying
nutriment to the developing embryo.

The embryos fix themselves to this organ, and it is notice-
able that the cells immediately under the attached embryo are
always greatly reduced in height and have lost the characteris-
tics of the mammary epithelium (Pl. XXXII, Fig. 7, *emb.*). It is
probable that the contents of such cells have been absorbed
by the embryo. Modifications of the atrium, for holding the
developing embryos and providing them with nourishment, occur
in several of the endoprocts. That most closely resembling the
condition in L. Davenporti has been described by Ehlers ('90)
for Ascopodaria. The epithelium lining portions of the atrium
on either side of the rectum become modified to form a layer
of high columnar secreting cells ; it also becomes folded to
form pouches (*Bruttaschen*) in which the developing embryos
are retained and nourished by the secretion of the glandular
lining. Just in front of the sexual aperture the modified
epithelium is raised up to form an irregular projection, the
Embryoträger, to which the youngest embryos are found
attached. This structure corresponds with a partially de-
veloped mammary organ in L. Davenporti. In the latter
species, however, *Bruttaschen* are not developed. A provision
for the nourishment of the embryo by means of the atrial epi-
thelium has also been mentioned by Harmer ('85) as occurring
in one of the species which he studied, but no structure resem-
bling the mammary organ of L. Davenporti has been described
as occurring in any species of Loxosoma.

Budding. — Asexual reproduction in L. Davenporti takes
place by means of buds which arise from the anterior surface
of the body. They are found upon either side of the median
plane in the region of the ventral wall of the stomach. As
many as six buds upon each side may be present, representing
different stages of development, although the numbers more
commonly observed are but two, three, or four upon a side.
With the exception of L. Kefersteinii, no species has been
described as having so large a number of buds.

New buds arise alternately upon opposite sides, as is shown
by the fact that no two buds borne by the same parent are of

exactly the same size and stage of development, and those most nearly alike are on opposite sides. The younger buds are near the median plane and the older ones farther from it (Pl. XXXII, Figs. 1 and 2). The oral side of the bud corresponds in position with that of the parent. This was observed by Schmidt ('76), and is in agreement with the statement of Davenport ('93) for Endoprocta in general, that the oral side of the bud is always turned toward the region of proliferation. The formation of buds is not dependent upon the sexual condition of the parent, for they are as numerous upon the functionally male individuals as upon the females.

The details of the process of bud formation have not been followed, but the observations which I have made appear to be in accordance with the account given by Seeliger ('90).

With the ordinary buds, which resemble the parent in all essential characteristics, others are occasionally present which I shall describe as abnormal buds. They arise in the same way as other buds and in corresponding positions. They appear quite unlike the normal buds, having small rounded bodies supported upon slender elongated stalks (Pl. XXXII, Fig. 14). They lack tentacles, digestive system, sexual organs, and very possibly a brain. Within the body may be distinguished in some cases a rounded mass of cells which, from its position, probably represents the rudiment of a stomach. On either side of this mass is a smaller group of cells which perhaps represents a gonad. The lophophore has a blunt projection on what corresponds with the oral side ; through the action of the highly developed sphincter this is capable of being opposed to the distal margin, thus closing the atrial aperture. The epithelium lining the atrium is shown by sections to be composed of large glandular cells whose cytoplasm is filled by granules which stain deeply with acid dyes (orange).

The relation of these buds to the parent, which is in no way different from that of normal buds, renders it evident that their attachment can endure for but a limited period, while their lack of organs for obtaining and digesting food shows quite as conclusively that they are incapable of leading an independent existence. More than fifty such buds have been observed, a

number much too great to be explained as a chance variation. My observations seem to show that they are more abundant during the breeding season (July and August) than earlier in the summer. That they are not the result of insufficient nutrition, due to the development at the same time of sexual products, is shown by the fact that the same parent frequently bears a number of other buds all perfect and normal.

The most reasonable explanation of these abnormal buds, in view of all the facts observed, is suggested by comparing these with specialized individuals of a colony, the avicularia or vibracula of certain Ectoprocta. If Loxosoma be assumed to be derived from stock-building ancestors, the imperfect buds may be readily explained as manifestations of the tendency — not yet eliminated — to produce similarly modified individuals. This I regard as probably the correct view, though it is contrary to the one generally accepted, according to which Loxosoma is regarded as a primitive form which branched off from the main stem of the Endoprocta before the colonial habit of life was acquired. It seems to me more probable that Loxosoma has secondarily abandoned the colonial habit in adaptation to the commensal or parasitical mode of life.

The number and size of the buds found attached to a parent suggest that this species comes nearer perhaps to the formation of stocks than any of the species of Loxosoma which have been described heretofore.

Excretory Organs. — Two forms of excretory organs have been reported for the genus Loxosoma. That described by Harmer ('85) was essentially an intracellular tubule terminated by a flame cell and resembling in all important characteristics the excretory organs of flatworms, annelid larvae, rotifers, and several other genera of Endoprocta (Pedicellina, Urnatella, etc). The other form of excretory organ is that described by Prouho ('91) for L. annelidicola. In this case the ciliated canal to the exterior could be seen in the living animal, but its internal ending could not be made out clearly. However, the author could get no evidence that the tube ended blindly (*en cul-de-sac*). Near the inner end of the tubule was a group of two or three very large cells filled with yellowish granules. The cells were

flattened against one another, forming a rounded mass, but were not perforated by a tubule, as Harmer believed to be the case in L. crassicauda, and as Foettinger ('87) described in Pedicellina. Furthermore, Prouho found two clusters of excretory cells on each side of the body, although he was unable to make out a connection between them.

My own observations accord much more nearly with those of Prouho than with those of Harmer, Foettinger, and Davenport ('93) on Urnatella. In L. Davenporti there is a pair of ciliated (?) tubules opening into the atrium. Near the inner end of each of these tubules is a rounded cluster of large vacuolated cells appressed one against another so as to produce flattened surfaces of contact. Seen in the living animal, these cells show an orange or reddish color, evidently due to the contained granules, and the same color is present in the tubule to the exterior, although a direct continuity of the color from the cells to the tubule is not apparent. In the living animal I have not been able to make out ciliary action in any part of the organ. A second cluster of the large orange-colored cells is also present at some distance from the excretory tubule (Pl. XXXII, Fig. 4, *ex., ex'.*), but no connection between the two clusters has been observed. Pl. XXXIII, Fig. 33, shows the appearance of the organ as seen in a section of an animal killed by corrosive-sublimate-formaldehyde mixture. The large excretory cells are very highly vacuolated, the larger vacuoles containing a granular coagulum, and the small nuclei appearing as though forced to one side by the vacuoles. In this case each cell is bordered on one side at least by a distinct space which partially surrounds the cluster of cells. The duct to the exterior shows in this section an irregular lumen, within which lies a cord of material which is probably a coagulum precipitated by the reagents from the fluid contained in the tubule. The walls of the tubule give faint indications of being lined by fine cilia, though there is room for reasonable doubt as to the correctness of this interpretation. The lumen of the duct appears to communicate with the space around the excretory cells. Though no connection has been made out between the secondary cluster of excretory cells and those here figured, it is nevertheless extremely probable that the

two groups have a common function from the fact that they have the same histological characteristics and in life the same color. The later study of sections of animals fixed by other methods (Hermann's and Flemming's fluids) has led me to doubt whether the space as shown in Pl. XXXIII, Fig. 33, around the cluster of excretory cells, is normally present. The failure to find it in such sections has led me to suspect that it may be an artifact produced by the shrinking of the cells by reagents.

Several questions must be left for the time undetermined. I am convinced, however, that the excretory organ in this species is composed chiefly of clustered vacuolated cells which are not arranged in linear order and which are not pierced by an intracellular canal. It will be impossible to determine the structure more exactly until many more sections made from animals fixed by a variety of reagents shall have been studied.

Relation of L. Davenporti to Other Species. — That L. Davenporti is a distinct species is shown by the possession of flask organs and mammary organ, structures heretofore unknown for the genus, by the greater number of tentacles, and by the condition of the foot. In these respects it differs from all other species known. It resembles most closely L. crassicauda, with which it corresponds in the general proportions of the parts of the body, in the possession of the row of large dorsal cells, in the absence of a foot-gland, and in the rapidity of asexual reproduction and the persistence of the buds.

Material and Methods. — L. Davenporti occurs in Cotuit Harbor, a tributary of Vineyard Sound, associated with the annelid Clymene producta. This worm inhabits a long tube extending vertically downward into the sand for more than a foot and made up of sand grains cemented together. Though the nearly related worm, Axiothea (" Clymenella "), lives under almost exactly the same conditions and in the same sand flat, I have never succeeded in finding a specimen of Loxosoma associated with it. The flat in which the worms burrow is composed of almost pure sand and is bathed constantly by a strong current of clear sea water.

No means have been discovered for stupefying the Loxosomae in an expanded condition, although a number were tried. The

little creatures are extremely sensitive to the condition of the water, and the addition to it of any foreign substance, even in the most minute quantity, is invariably followed by a contraction of the body, which is never succeeded by full expansion. They may readily be killed in an expanded condition, however, by the sudden application of hot fixing reagents. Much of the material I have used for study has been killed in this way by corrosive-sublimate solution or by a corrosive-sublimate-formaldehyde mixture. The best preservation of details of cytological structure is given, however, by the osmic fluids (Hermann's and Flemming's). Only ordinary stains have been used, iron-haematoxylin giving the best results and being the one most employed.

Summary.

1. L. Davenporti is a new species; the first of the genus to be described from the North American coast. It is characterized by 18 to 29 tentacles; 2 to 6 buds on each side; foot orbicular, and destitute of foot-gland; stalk short (about equal in length to body), having a row of large columnar cells along dorsal side and spiral arrangement of muscles to the foot; possession, in many cases, of a pair of flask organs; presence of mammary organ in females with embryos.

2. A single large cell, which acts as a sucker, is present on the outer side of the base of each tentacle.

3. Lateral expansions (alae) are formed by the contraction of the body.

4. A single row of large glandular (?) cells occupies the mid-dorsal line of stalk.

5. Unicellular glands occur in the foot chiefly about its margin.

6. Flask organs, which are secretory structures, arise by differentiation of ectoderm cells of a certain region, discharge their contents, and drop off. They are capable of being regenerated. No similar organs have been described for Endoprocta.

7. The musculature of the stalk shows a spiral arrangement of fibers in the region of the foot.

8. Rounded or elliptical bodies containing refractive spherules are present in all parts of the parenchyma. These probably

circulate in a body fluid through irregular spaces in the paren-
chyma and perhaps act physiologically somewhat as blood
corpuscles.

9. In the stomach three glandular regions are distinguish-
able : (*a*) paired lateral regions near the lower end of the
stomach, composed of elongated, finely granular, non-vacuo-
lated cells, which stain deeply; (*b*) the " liver " in the anterior
wall, composed of elongated cells containing coarse yellow
granules in large vacuoles.

10. The brain, which is dumb-bell-shaped, is situated in
front of the intestine and gives off a pair of nerves from each
end. Sensory hairs occur on the surface of the tentacles.
Dorsal sense organs are absent.

11. Proterogynic hermaphroditism occurs in L. Davenporti,
ova and later spermatozoa developing in the same gonad.

12. Gland cells below the commissure of the brain secrete
an envelope which surrounds and attaches the developing egg.

13. A pair of large gland cells below the brain pour their
secretion into the upper part of the oesophagus.

14. The mammary organ, a structure for the nourishment of
the embryo, is formed by a modification of the floor of the
atrium between the epistome and the opening of the sexual
duct. The developing embryos attach themselves to it and
obtain from it their nutriment.

15. Buds, up to twelve in number, are found attached to the
parent.

16. Imperfect buds are occasionally developed, lacking tenta-
cles and digestive organs. They may correspond to vibracula
or avicularia of Ectoprocta, and probably indicate the derivation
of Loxosoma from a stock-building ancestry.

17. Excretory organs are clusters of large vacuolated cells with
ducts to the exterior. They are not provided with flame cells.

NOTE. — This paper was accepted by the editor of the *Journal* in June, 1898,
and the drawings for the plates sent to the lithographer at that time. I have
recently revised the text, however, making such minor changes as later obser-
vations made necessary in so far as this could be done without new figures.

UNIVERSITY OF MINNESOTA, MINNEAPOLIS,
January, 1900.

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EXPLANATION OF PLATES.

All figures were made with the aid of an Abbé camera lucida from preparations of Loxosoma Davenporti.   In finishing details of all drawings having a magnification of 495 diameters or more the $\frac{1}{12}$ immersion objective (Leitz) was used.

ABBREVIATIONS.

a.	Anus.	*l.*	Liver.
al.	Ala, alae.	*lph.*	Lophophore.
atr.	Atrium.	*m.*	Mouth.
atr. g.	Atrial groove.	*mam. org.*	Mammary organ.
atr. sp.	Atrial sphincter.	*o.*	Ovum.
b.	Bud.	*oes.*	Oesophagus.
br.	Brain.	*ov.*	Ovary.
br. com.	Brain commissure.	*ovd.*	Oviduct.
d. c.	Cells of dorsal row.	*ped.*	Stalk (pedicel).
emb.	Embryo.	*r.*	Rectum.
ep.	Epistome.	*sem. ves.*	Seminal vesicle.
ex.	Excretory organ.	*s. gl.*	Shell gland.
ex'.	Secondary group of excretory cells.	*st.*	Stomach.
		ten.	Tentacle.
ex. d.	Excretory duct.	*ten. c.*	Cell at base of tentacle.
fl. org.	Flask organs.		
in.	Intestine.	*v. d.*	Vas deferens.

EXPLANATION OF PLATE XXXII.

FIG. 1.   Ventral view of large adult represented as an opaque object ; the lophophore is partially retracted.   × 59.

FIG. 2.   Transverse section of a strongly contracted male specimen.   × 114.

FIG. 3.   A portion of the margin of the lophophore, outer surface, showing bases of five tentacles, two of which are extended and the other three folded inward beneath the margin of the lophophore.   × 160.

FIG. 4.   Portion of a total preparation, dorsal aspect, showing relations of the brain, the ovaries and their ducts, the shell gland, the excretory organs, and the digestive tract.   × 205.

FIG. 5.   Sagittal section through upper portion of body showing mammary organ in condition of highest development.   × 205.

FIG. 6.   Portion of transverse section through intestine and sexual organs ; in the gonad on the left side of the figure is shown a degenerating ovum surrounded by a mass of cells showing various stages in the formation of spermatozoa. × 255.

FIG. 7.   Sagittal section of a fully expanded individual.   × 165.   A portion of an embryo attached to the mammary organ is shown in this section.

FIG. 8.   A few cells of the dorsal band showing appearance when more highly magnified.   × 675.

FIG. 9.   Transverse section of a tentacle.   × 675.

FIG. 10.   Transverse section through base of a tentacle showing unicellular sucker.   × 675.

FIG. 11.   View of individual with fully expanded lophophore and four buds, the parent and the two larger buds having flask organs.   × 57.

FIG. 12.   The digestive tract from a total preparation, dorsal aspect.   × 57.

FIG. 13.   Section of ovary containing a nearly mature ovum.   × 480.

FIG. 14.   An abnormal bud drawn as an opaque object.   × 250.

FIG. 15.   Portion of stalk and foot as seen from ventral side ; showing glands in foot and oblique muscle fibers on anterior side of stalk.

Pl. XXXII.

EXPLANATION OF PLATE XXXIII.

Fig. 16.   Longitudinal section of a mature flask organ.   × 495.

Figs. 17, 18.   Sections showing two stages in the formation of flask organs. × 675.

Fig. 19.   A flask organ in which the gland cells have discharged their contents. × 495.

Figs. 20–22.   Three consecutive transverse sections showing relative positions of the gonads and their ducts, the median unpaired chamber (*sem. ves.*), the shell gland, the brain, and the duct from the excretory organ.   × 250.

Fig. 23.   Portion of sagittal section showing manner in which young embryos are attached to parent.   × 250.

Fig. 24.   An optical frontal section of stomach showing lateral glandular tracts near basal end.   × 114.

Fig. 25.   Rounded corpuscles from parenchyma.   × 675.

Fig. 26.   Two mature spermatozoa from seminal vesicle.   × 1280.

Fig. 27.   Portion of nearly sagittal section through upper portion of body to show relations of oesophageal gland cell.   × 250.

Fig. 28.   Oesophageal gland cell shown in Fig. 27, more highly magnified. × 675.

Figs. 29–31.   Three consecutive sections through brain and adjacent organs of bud still attached to parent ; showing a pair of bipolar cells connecting brain and ventral wall of body.   × 675.

Fig. 32.   Section through stalk of bud showing attachment to parent.   × 495.

Fig. 33.   Section through excretory organ and duct.   × 495.

Fig. 34.   Portion of margin of foot showing sucker-like openings of gland cells.   × 250.

Pl. XXXIII.

SENSORY AND GLANDULAR EPIDERMAL ORGANS IN PHASCOLOSOMA GOULDII.

MARGARET LEWIS NICKERSON.

DURING the summer of 1897, while enjoying the advantages of the Marine Biological Laboratory at Woods Holl, Mass., I had an opportunity to apply the methylene-blue *intra vitam* nerve stain to a study of the epidermal organs of the Gephyrean worm Phascolosoma Gouldii. This form lends itself well to this method of study, as the tough body wall is not easily torn in the process of injection, and the copious body fluid surging back and forth quickly carries the injected stain to all parts of the peritoneal cavity. The use of methylene blue gave as results some facts regarding the peripheral nervous system which could probably not be obtained in any other way. These were supplemented and confirmed, as far as possible, by maceration preparations and by sections prepared by the ordinary histological methods, as well as by the picro-osmic-acetic-platinic chloride method of Vom Rath ('95). This latter method was found to be especially useful in the study of glandular structures.

A brief description of the structure of the body wall will be given, not with the idea of presenting anything new, for the general anatomy of the worm is well known (Andrews, '90), but for the purpose of making clearer my account of the epidermal organs.

The body wall consists of three parts : the cuticula, the epidermis, and the muscular layers. In the regions of the proboscis and of the tail the cuticula is thrown into elevations or papillae, but in the middle region of the body it forms a comparatively smooth covering. If the cuticula from this middle region is studied in sections, it will be seen to show numerous large excavations upon its inner surface. The epidermis, which consists of a single layer of columnar epithelial cells resting

upon a basement membrane,[1] rises at frequent intervals above its general level to follow the inner surface of the cuticula. On the proboscis and tail it lines the cuticula covering the papillae, while in the body region it follows the contour of the excavations mentioned. Between the epidermis and the circular muscle is a quantity of loose connective tissue. Scattered abundantly over the proboscis and body of the worm are found the well-known epidermal organs which form the subject of the present paper. On the introvert and tail these organs occupy the papillae already described, while in the body region they are partially included in the large excavations in the under portion of the cuticula. They are ovoidal in shape; the smaller end is directed outward, while the base rests upon the circular muscle.

The Epidermal Organs.

The epidermal organs may be divided into two classes: one containing gland cells, the other not. Each class may be subdivided into two types. The two types belonging to the first or non-glandular class are distinguished by the presence or absence of a bulb over the organ; the two types belonging to the second or glandular class by the presence or absence of intracellular sacks in the gland cells. Each of the four types contains sensory cells. Except in these organs I obtained no indication of nerve cells or nerve terminations in the epithelium covering the animal. Nerve endings are probably present in the tentacles surrounding the mouth, but no observations upon these structures are included in the present paper. Each epidermal organ is flask-shaped and is surrounded by a delicate membrane, probably an invagination of the basement membrane, and in each type considerable variation is shown in distribution and appearance, corresponding to the different regions of the body in which they occur. For convenience, the two types of non-glandular organs will be first considered, and afterwards the two types of glandular organs. This

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[1] Andrews's statement, that no basement membrane is present, is certainly a mistake, as a basement membrane can always be demonstrated in material which has been well preserved and stained.

classification has no reference to the distribution of these
bodies; for in the integument of the worm glandular and non-
glandular organs are found intermingled with one another, with
no definite arrangement.

Organs of the First or Non-Glandular Class (First Type).

The organs of this type are most abundant on the anterior
end of the introvert, in which region, indeed, none of the other
types are found. Above each organ the cuticula is much re-
duced in thickness, the reduction being made from the inner
surface. Each organ has quite a large number of sensory cells,
as many as six or seven of these often responding to the blue
stain. Moreover, in nearly all cases there is evidence that
there are sensory cells which have not taken the stain or which
have taken it only in a slight degree. A drawing of one of
these organs (Pl. XXXIV, Figs. 3 and 10) shows that the group
of sensory cells is situated near the middle of the flask, and that
the long axis of the individual cells is perpendicular to the ring
muscles. The sensory cells are spindle-shaped and are all
bipolar. The peripheral process is stouter than the central
process, and at a short distance below the cuticula, is somewhat
broadened and thickened, making a club-shaped end. From
this expanded end a slender sensory hair ascends to the cutic-
ula and passes through it to the exterior. It is only in exceed-
ingly fortunate preparations that the sensory hairs have retained
the stain, but it is probable that in all cases such hairs are
present. The central processes from the sensory cells emerge
from the flask in one bundle, pass inward across the band
of circular muscles, and enter one of the main nerves coming
from the ventral cord. So far as these central processes were
followed they were never seen to branch.

Organs of the First or Non-Glandular Class (Second Type).

These organs, like those of the first type, are strictly non-
glandular and evidently have some marked sensory function.
Like those of the first type, again, they are each flask-shaped.
They are to be distinguished, however, even under a low power

of the microscope, by the presence of a bulb-like structure projecting above the general surface of the cuticula. It is this bulb which constitutes the distinction between the first and second types of epidermal bodies. In other respects there is much similarity, and the two types are perhaps to be regarded as fundamentally the same. A comparison of the two types might suggest that the bulb is a temporary structure produced by the effect of reagents or by a violent muscular contraction; but examination of a large number of sections from specimens preserved in different ways showed that no matter what the method of killing, organs exhibiting these bulb-like protuberances were always present, even when the cuticula had been removed before placing the tissue in the fixing fluid. Again, pieces of the body wall cut from living worms and examined under the microscope showed the presence of these bulbs.

The organs of this type first appear on the introvert somewhat anterior to the nephridial openings. Here they are very rare. They increase in number, however, backward, until in the region of the nephridia they are so numerous that several are found in one transverse section. In the tail region they are even more abundant. Occasionally there may be found in a section two successive organs belonging to the same type, but far more often they are separated by a considerable distance. They are found at intervals in all parts of the body of the worm except the most anterior portion of the introvert. In sections they are more conspicuous in the middle region of the body than upon the introvert, hence the projecting bulb appears more prominent.

These organs gave a very successful reaction to the blue stain, and frequently single sections were obtained which showed all the details here presented regarding the individual cells and their connection with the main nerves coming from the ventral cord. The number of cells stained by the blue showed considerable variation, but the best effects were obtained when not more than six cells had taken the blue color. Organs in which only one or two sensory cells were stained were best for studying the peripheral endings (Pl. XXXIV, Figs. 4 and 6). The enlarged inner portion of the flask contains a group of bipolar nerve cells very similar in shape to the sensory cells of the

organs of the first type. The body of each of these bipolar sense cells is of an elongated spindle shape, and it possesses a large nucleus which is often differentiated by the stain (Pl. XXXIV, Figs. 6 and 7). The group of cells lies in the middle or just above the middle of the flask, and the long axes of the cells are perpendicular to the ring muscles. The peripheral processes of the cells are longer than those of the sensory cells belonging to the first type of organ, as they must traverse both the upper portion of the flask and the entire length of the bulb before reaching the exterior. In passing through the neck of the flask the peripheral processes of these sensory cells sometimes show spiral twists and turns very difficult to reproduce (Pl. XXXIV, Fig. 6).

The fibers passing inward from the group of sensory cells lie close together and occupy the central axis of the organ. Emerging in a bundle from the base of the flask, they run for a longer or shorter distance in a horizontal direction under the epidermis and, crossing the circular muscle, enter one of the main nerves coming from the central nervous system (Pl. XXXIV, Figs. 4 and 6).

The peripheral process of each sensory cell passes up through the neck of the bulb and at a little distance from the surface becomes broadened and thickened in the manner described for the sensory cells of the first type of organ. From this peripheral expansion a delicate process or hair extends through the cuticula covering the bulb (Pl. XXXIV, Figs. 6 and 11). In some instances this sensory hair could not be traced quite through the cuticula to the exterior, but such appearances were probably due to defective staining, as in a large number of instances the hair could be seen distinctly projecting beyond the surface. Immediately over the center of the bulb in the region through which the sensory hairs pass, the cuticula shows a marked concavity of the outer surface, in some cases being so reduced in thickness that only a line was visible (Pl. XXXIV, Figs. 6 and 7).

The organs of both types possess cells which are evidently not sensory but supporting epidermal cells. No particular attention has been paid to such cells and no description of them appears necessary.

Organs of the Second or Glandular Class (First Type).

The structures belonging to this class are rather small, glandular bodies, containing a few — probably never more than eight or ten — large gland cells, each of which is rudely pyramidal in shape. Fig. 20 (Pl. XXXV) represents a section through one of these organs from a preparation made by the Vom Rath method. Over the middle of the organ is a tubular excavation in the outer surface of the cuticula, which probably represents the common duct of the gland cells. Besides the large gland cells, two nuclei are shown situated near the base of the organ, and another lying close to the periphery of the upper portion of the flask. These nuclei are best interpreted by comparison with Fig. 5 (Pl. XXXIV), which represents a section through one of these same organs from a worm injected with methylene blue. It is here demonstrated that sensory as well as glandular cells are present, and that the nuclei near the base of the first figure, in all probability, correspond to the sensory cells which are colored blue in the second. These cells are evidently bipolar with a small cell body. They are situated near the base of the organ, and in its central axis, or else close to the limiting membrane of the organ, outside the gland cells. The peripheral as well as the central process is evidently very slender, and the small cell body is almost entirely occupied by the nucleus. The exact method of termination of the peripheral process was not determined by this method, and indeed the results of the methylene-blue staining were in all respects far less satisfactory for this type of organ than for any one of the other three types. This was probably due, in some cases, to the fact that the glandular secretion was stained somewhat diffusely with the blue and so tended to obscure the sensory cells. In sections stained by the Vom Rath method the nuclei belonging to the large gland cells were not visible, but in sections from material treated with methylene blue and afterwards stained by alum carmine a small round nucleus was demonstrated near the base of each gland cell.

Fig. 22 (Pl. XXXV) shows one of these organs containing about the maximum number of cells and presenting a condition

in which the different cells show varying phases of activity. While in most of the cells the contents appear finely granular, in one the secretion is formed into spherules, and these spherules I believe to represent a very late stage in the activity of the cell.

Organs of the Second or Glandular Class (Second Type).

This type, by far the most interesting of the four classes, includes certain large glandular organs characterized by the presence of some remarkable intracellular canals within the gland cells. These organs are found abundantly in all parts of the body of the worm, with the exception of the anterior portion of the proboscis. In the region of the nephridial openings and the anus they are the most common of the four types, as many as ten different organs often being shown in a single section. Fig. 16 (Pl. XXXV) represents a section through one of these bodies, and shows that, as in case of the other three classes, a thin membranous sack encloses the whole organ, which contains at least two types of cells, *viz.*, gland cells and sense cells. By ordinary methods of preparation the sense cells would probably escape notice entirely, but by the use of the blue stain they are rendered conspicuous, the gland cells as a rule remaining entirely unstained. Figs. 9 and 12 (Pl. XXXIV) are drawings from methylene-blue preparations. As figured here, the sensory cells are bipolar and resemble in some respects those of the first type of glandular organ, the peripheral and central processes being both very slender. The peripheral endings are similar to those found in the non-glandular organs, for at a little distance below the cuticula the external process of the cell shows an expansion, and from this expanded end a sense hair ascends to the cuticula and passes through it to the exterior. The long axes of the cell bodies are, as a rule, nearly vertical to the cuticula, and the central processes either take a course perpendicular to the ring muscle, through the middle of the organ, or else lie close to the membranous covering of the organ, the processes seeming, as it were, to creep over the gland cells. In sections of material prepared

by the Vom Rath mixture, the cell body with its characteristic nucleus can be distinguished readily as that of a nerve cell, although neither process of the cell can be followed for any distance.

Besides these sensory cells, each organ contains a large number, often as many as twenty or thirty large gland cells, each of which is broadened at the base and narrowed somewhat toward the outer end. The nucleus lies near the base of the cell, and above the nucleus is an intracellular ampulla opening into a canal which unites with similar canals coming from the other gland cells of the organ. By the union of all these canals a duct is formed which communicates with the exterior by means of a pore in the cuticula situated over the summit of the organ (Pl. XXXV, Fig. 15). As the sacks are nearly always plump, it is inferred that they are filled with a fluid secreted from the gland cells, although the sack contents were unaffected by any of the stains used.

So much may be said in the way of a general description of these organs ; but for a correct understanding of the individual cells and intracellular structures, the various conditions presented by the different phases of activity must be noted. At what is probably a comparatively early stage in the activity of one of these gland cells, there are present outside each of these membranous sacks a very large number of delicate radiating threads which form a rather broad radial zone or vesicle between the sacks and the general cytoplasm of the cell. These threads are attached by one end to the sack wall, and are limited at the other end by the general cytoplasmic reticulum, with which they are probably continuous. The width of this radial zone may be equal to that of the sack (Pl. XXXV, Fig. 16).

A condition considerably later than the one described is shown in Pl. XXXV, Fig. 17. As figured here, there appear to be large vacuoles in the protoplasm, which in some cases occupy nearly the whole of the cell. The sack within now almost or quite fills the vacuole, and the delicate threads shown in the first figure are not visible in case of the enlarged sacks. The ducts from the different sacks unite as before and finally form a single tube. In the case of two of the cells the condition shown in the first figure is retained. Other organs show

a condition in which the radial zone is enlarged, and extends down into the protoplasm of the cell, while the sack within remains small and surrounded by an extensive zone of delicate threads.   At what appears to be a very late phase the walls of some of the sacks are seen to be broken down, the sacks are confluent, and in all probability entire cells disintegrate.

After a comparative study of a large number of these organs representing many conditions, the following explanations of the different appearances seemed justified.   The transparent zone traversed by radial fibers as shown in Pl. XXXV, Fig. 16, enlarges and occupies more of the cell.   The sacks within enlarge in turn, and finally, as the periphery of the cell is approached, the sack comes to occupy the whole space.   The enlargement of the sack within the radial zone is the result of the secretory activity of the cell — the sack being filled with a clear secretion which is conducted to the outside by means of the common duct arising from the union of the several smaller canals.   The delicate radiating threads surrounding the sack probably represent the protoplasmic reticulum filling the cell, the threads of the reticulum in the vicinity of the sack being perhaps stretched by the accumulation of the secreted material in this region and thus caused to assume the radiating character shown.   That the space between the sack and the wall of the vacuole, as figured in Pl. XXXV, Figs. 15, 16, and 19, is not an artifact due to the action of reagents, is proved, I think, by the examination of fresh tissue under the microscope, in which case the sack appears as a highly refractive body, surrounded by a second clear zone which evidently represents the region traversed by the delicate radiating threads.

Comparison with other accounts of the epidermal organs of Gephyreans. — Of the various accounts which have been given of the epidermal organs of the Gephyreans, the only ones which will be considered here are those of Andrews ('90), Ward ('91), and Jourdan ('91), these being the only papers, so far as I know, which offer observations upon the epidermal organs, interesting for comparison with the results here presented.

Andrews, who deals with the same worm as the one treated in the present paper, divides the epidermal organs into three

classes — two glandular and one sensory and non-glandular. The two glandular classes which he mentions are probably to be identified with the glandular organs described in this paper, although he makes no mention of intracellular sacks and ducts. In regard to his third class, the sensory organs, I am not able to infer such a correspondence, as he limits these organs to the anterior and posterior extremities of the body, while in the present account sensory organs have been described for the mid-body region as well as the extremities of the worm. The histological details of the epidermal organs, as given by Andrews, possess but little in common with the observations here presented. The sense cells of both types of glandular organs are not mentioned by him, and the large sensory organs with protruding bulb seem also to have escaped his notice entirely, as he neither describes nor figures anything of the sort. His statement (p. 393) that the delicate processes of the vacuolated gland cells of the multicellular organs are to be regarded as nerve processes are not supported by the observations here presented, and in view of the modern conception of nerve fibers and their relationships, such a condition as continuity of gland cell and nerve process is highly improbable. The structure of the cells in the second type of glandular organ has been very incompletely described by Andrews ; the intracellular sacks and ducts and the relation of these sacks to one another being unnoted, as well as the extreme variety of conditions presented by these structures.

Ward ('91), in his paper on the anatomy and histology of Sipunculus nudus, describes a condition of the bicellular glands of this Gephyrean which resembles to a slight extent the condition found in the second type of glandular organs described in this paper. He says (p. 153) : " Whether resting or active, a clear zone of plasma forms the periphery of the cell on all sides, and is, therefore, adjacent to the vacuole as well as to the external surface of the cell. This zone is traversed radially by delicate fibrils, the beginnings of the plasma reticulum which fills the cell, but which is ordinarily seen only in this clear zone." These delicate fibrils are probably to be compared with the filaments surrounding the intracellular sack in the gland cell, as figured in this paper. Ward states that the gland cells

of the multicellular glands are in direct connection with nerve fibers. This view, which harmonizes with that of Andrews, seems to me untenable, and to be due to the lack of special nerve methods.

Jourdan ('91), who investigated the peripheral sensory and glandular organs of several Gephyreans, has given a detailed account of the sensory apparatus of these worms, and figures some conditions which present interesting similarities to the condition which I have found in Phascolosoma Gouldii. In case of Sipunculus nudus he finds several types of glandular organs which possess no sensory cells, and also certain organs which contain both sensory and glandular cells ; these sensory cells he describes and figures as possessing sense hairs which pass through the cuticula, and as being supplied by nerve fibers.

Technique of the Methylene-Blue Method.

The methylene-blue method was employed as follows : a considerable quantity of B. X. methylene blue was injected into the bodies of several freshly dug worms. The worms were not previously narcotized, and after injection were returned to the dishes of sea water and put away in a dark place for three and a half or four hours. Then an examination was made by cutting out small pieces from the body wall and observing them in sea water under a cover-glass. When the fresh tissue showed evidence that the right reaction had taken place, the blue color was fixed by the ammonium molybdate method of Bethé ('96). The tissue, after being imbedded in paraffin, was cut into sections from 30 μ to 50 μ in thickness, and the sections were mounted in balsam. Some of these sections were afterwards stained with alum carmine. It was necessary, in order to obtain favorable results, to use freshly dug worms. Specimens which had been in the laboratory for more than one day, even if kept in running sea water, did not, in any case, give positive results.

Summary.

1. The sensory nervous system of Phascolosoma Gouldii, posterior to the tentacles surrounding the mouth, is to be found entirely in the epidermal bodies distributed abundantly throughout the body of the worm and in the nerve fibers connecting them with the central nervous system.

2. These epidermal bodies may be grouped into two classes, and each class into two types. One class contains gland cells; the other is non-glandular. The second type of glandular organ may be distinguished from the first by the presence of intracellular sacks and canals in the gland cells. The second type of non-glandular organ is distinguished from the first by the possession of a bulb-like structure projecting above the general level of the cuticula.

3. All four types of epidermal organs possess sensory cells.

4. Nerve fibers are never found in continuity with the gland cells of either type of glandular organ, though the contrary has been several times asserted by different investigators.

5. The sensory cells of all these organs are bipolar in shape, the cell body in the non-glandular organs being larger than that in the glandular organs.

6. Each of the peripheral processes of the sensory cells ends in a delicate sensory hair, which, in some cases at least, is prolonged beyond the surface of the cuticula. In one type only, the second type of the glandular organs, the exact form of the peripheral ending was not made out.

7. The central processes of all these sensory cells enter the large nerves passing from the ventral nerve cord.

8. One type of glandular organ possesses a very remarkable structure consisting of a communicating set of intracellular canals, each canal leading from an otherwise closed pouch. All these communicating canals finally open to the surface of the worm through a common duct.

9. The peculiar sacks or pouches belonging to these glandular organs are reservoirs for the secretion from the gland cells and show much variation in size and appearance in correspondence with the phases of functional activity of the cells. The

ducts are the channels by which the secretion is conveyed to the surface of the animal. The radiating threads surrounding the sacks are probably to be regarded as continuations of the reticulum of the cell.

After the preceding part of this paper had been written and the figures sent away to the lithographer, I had an opportunity to obtain new material. In preserving this material, I took the precaution to remove the cuticula from the tissue as soon as it was placed in the fixing reagent, thus securing a very rapid penetration of the fluid. The fixing reagents employed included Hermann's and Flemming's fluids, corrosive sublimate, and Graf's chrom-oxalic mixture. For staining, a number of aniline dyes were employed, and the results obtained, besides confirming the facts already presented, furnished some additional details regarding the cells in which the intracellular sacks and canals are found. These new details have been presented fully with figures in another place [1] and will be only briefly reviewed here. Especially clear pictures were obtained from material fixed in chrom-oxalic acid and stained with the Ehrlich-Biondi triple mixture. With this stain, both when it is employed upon material fixed in Hermann or upon that fixed in chrom-oxalic, the wall of the intracellular sack is stained red, and is seen to be surrounded by a second membrane, which also takes the red color. Between the sack and this enveloping membrane stretch the delicate radiating filaments which have already been described as surrounding the sack. This membrane surrounding the sack and limiting the filaments is always present, even when the sack within has become so large as to occupy almost the whole cell, and it is as well defined as the sack wall itself. It is no mere boundary or limit of the protoplasm of the cell, as has been suggested in the first part of this paper, but a sharply defined membrane distinguished by its staining reaction from the cell protoplasm. The staining reaction of the delicate radiating threads cannot be so positively stated, for the exceeding fineness of these threads renders the determination of their color a difficult matter. In some cases

[1] *Zool. Jahrb.*, Bd. xiii, Heft i.

they were certainly stained red, but at other times the question of their color could not be decided.

The cytoplasm of the cells containing the sacks shows a variety of conditions, corresponding, I believe, with the stage of activity. Those cells in which the intracellular canals are large, and the surrounding radial zones narrow, show a coarse condition of the cytoplasmic reticulum, which stains green, while cells containing small intracellular sacks, with a broad surrounding zone of radial filaments, possess a much finer structure and show the presence of abundant red granules. The latter condition is, I believe, a young stage in the activity of the cell, the former an advanced stage.

The several canals leading from the intracellular sacks and the common duct resulting from their union are stained deep red with the Biondi-Ehrlich mixture and are seen to be enveloped in a sheath which also takes the red color. This sheath, which is directly continuous with the radial vesicle surrounding the intracellular sack, envelops each small canal from its point of union with the terminal sack and beyond the junction of the several canals forms a common sheath for the main duct. The sheath widens considerably near the mouth of the main duct, while the duct itself close to the exterior often shows a contracted lumen. Although no mention of the sheath was made in the first part of this paper, a reëxamination of the Vom Rath preparations shows that it is always present, but, owing to the lack of a differential stain, is difficult to see except with the highest powers.

By the side of the main duct, close to the point of junction of its branches, is situated a large nucleus within the sheath. The sheath broadens markedly where it encloses this nucleus. The nucleus is surrounded by a very clear, transparent, sharply limited area, which probably represents a vacuole. This nucleus within the sheath of the main duct is, I believe, always present, but may of course lie outside the section which contains the duct; for this reason it was not noted in the sections studied and drawn for this paper.

University of Minnesota, Minneapolis,
January, 1900.

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EXPLANATION OF PLATES.

The figures in Plate XXXIV are from methylene-blue preparations.   The figures in Plate XXXV, with the exceptions of 18 and 21, are from preparations made by the Vom Rath method.   Most of the drawings were made with Leitz objective 7, ocular 2, the finer details being filled in with the aid of Zeiss Apochromatic 2 mm. immersion lens and compensating ocular 6.   All figures were drawn with the aid of the Abbé camera lucida.

ABBREVIATIONS.

blb.	Bulb.		*mu. crc.*	Circular muscle.
cd. n.	Nerve cord.		*mu. lg.*	Longitudinal muscle.
cl. gl.	Gland cell.		*pap.*	Papilla.
cl. sns.	Sense cell.		*sac.*	Sack.
cta.	Cuticula.		*set. sns.*	Sense hair.
h'drm.	Hypodermis.		*vac.*	Vacuole.

EXPLANATION OF PLATE XXXIV.

FIG. 1.   Section through non-glandular organ of second type showing four sensory cells.   × 460.

FIG. 2.   Section of non-glandular organ of first type.   × 512.

FIG. 3.   Section through non-glandular organ of first type showing two sensory cells and their connection with nerve from ventral cord.   × 512.

FIG. 4.   Section of non-glandular organ of second type showing sensory hair and connection of sense cells with nerve from ventral cord.   × 460.

FIG. 5.   Section of glandular organ of first type showing five sensory cells. × 512.

FIG. 6.   Section through non-glandular organ of second type showing two sensory cells.   × 460.

FIG. 7.   Section of non-glandular organ of second type.   × 460.

FIG. 8.   Section of glandular organ of second type showing four sense cells and refractive bodies representing the intracellular sacks.   × 460.

FIG. 9.   Section of glandular organ of second type.   × 460.

FIG. 10.   Section through non-glandular organ of first type.   × 512.

FIG. 11.   Section of non-glandular organ of second type showing two sensory cells and peripheral terminations.   × 460.

FIG. 12.   Section from glandular organ of second type showing one sensory cell with its peripheral ending and intracellular sacks.   × 512.

FIG. 13.   Section through glandular organ of first type showing three gland cells and one sense cell.   × 512.

FIG. 14.   Section showing relation of main nerve to ventral cord.   These main nerves contain the central processes from the sensory cells.   × 84.

Pl. XXXIV

EXPLANATION OF PLATE XXXV.

FIG. 15. Section through glandular organ of second type showing the intracellular sacks with their communicating ducts.　× 620.

FIG. 16. Section through glandular organ of second type showing appearance of cell contents. The plane of section was not such as to show connection of intracellular sacks.　× 575.

FIG. 17. Section through glandular organ of second type to show late condition of enlarged sacks. Cell contents shown only on left.　× about 550.

FIG. 18. Optical section of bulb belonging to second type of non-glandular organ. Drawn from living tissue.　× 250.

FIG. 19. Section through glandular organ of second type representing early phase of activity.　× 550.

FIG. 20. Section through glandular organ of first type showing two nerve nuclei at base.　× 575.

FIG. 21. Portion of section of Phascolosoma Gouldii in region of retracted proboscis to show arrangement of epidermal organs.　× 33.

FIG. 22. Section through glandular organ of first type showing cells in various stages of activity.
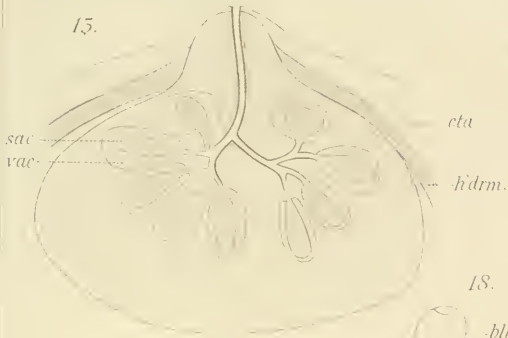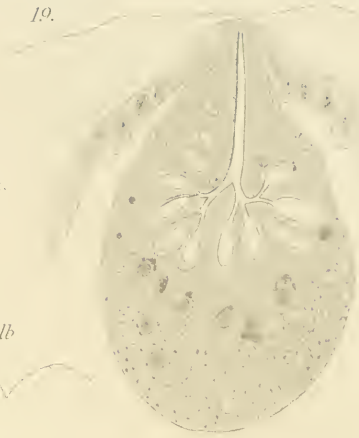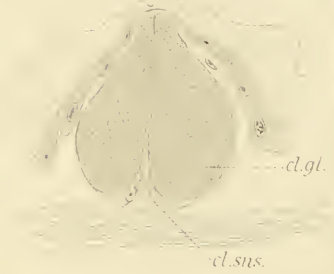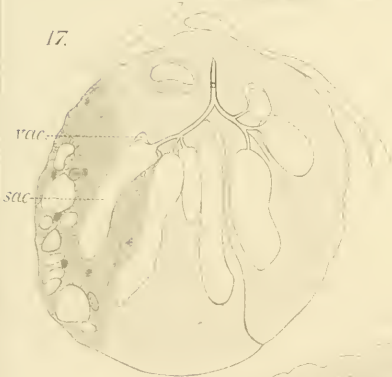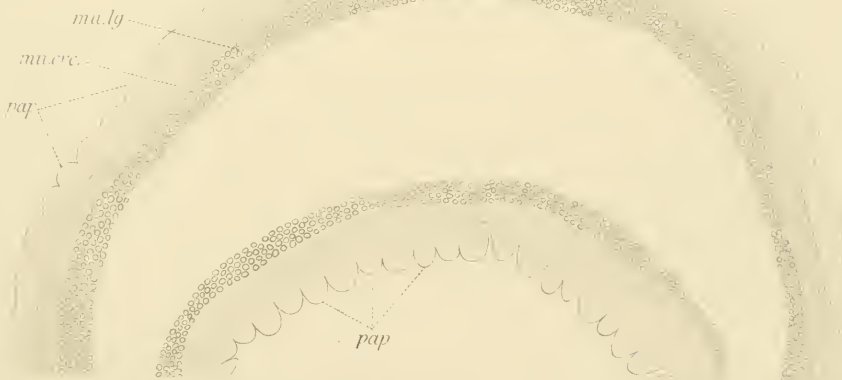
THE

CYTOGENY OF PODARKE OBSCURA VERRILL.

AARON L. TREADWELL.

CONTENTS.

THE following investigation, begun in 1891, has been carried on at the Marine Biological Laboratory at Woods Holl, Mass., in the Zoölogical Laboratory of the University of Chicago, and in the intervals of teaching at Miami University. For many helpful suggestions I am indebted to my friends, Drs. C. M. Child, E. G. Conklin, and A. D. Mead. I desire especially to acknowledge my indebtedness to Professor C. O. Whitman, without whose friendly encouragement the work would hardly

have been completed. In the preparation of my drawings I am indebted to my wife, who has finished from my camera sketches all of the figures which illustrate this paper.

Podarke obscura is a small Hesionid, abundant in various localities at Woods Holl, Mass. The species was described and named by Verrill (No. 30), who says that at night during July and August they come to the surface and swim about "in vast numbers." I have never been able to confirm the latter observation and have found only a few when towing at night, these seeming rather to be attached to bits of floating eelgrass than swimming free. In the "Eel Pond" and in "Little Harbor" they are abundant, lying in the soft, flocculent surface mud and clinging to the eelgrass a short distance from the bottom. It is almost hopeless to attempt to pick the animals from the mud, and I have found the best way to collect is to sweep through the grass with a strong net. (I used for this purpose a wooden-rimmed flour sieve.) The animals remain in the sieve and can easily be jarred into a bucket, while the finer dirt passes through with the water. They were then transferred to clean water in glass dishes in the laboratory, where with only an occasional change of water they will live indefinitely. Both in their natural environment and in captivity they seem rather sluggish, though if irritated they will swim rapidly away from the disturbing object. They will burrow quickly into any dirt or sediment that may be in the dish, but seem in no way inconvenienced if forced to live in clean water. The occurrence of bifid monsters is very common, a number of such cases having been described by Andrews (No. 1).

Specimens in captivity lay their eggs from 7.30 to 9 P.M., usually on the second or third, rarely on the first, night after they are brought into the laboratory. The eggs have no albuminous coat, and hence when extruded sink rapidly to the bottom of the dish, where, if the water be clean, they may easily be recognized and picked out with a pipette. A simpler expedient is to strain the water through a fine cloth, through whose meshes the eggs will pass, leaving the adult annelids behind.

Artificial fertilization, though tried repeatedly and at various times of the day, was never successful, unless the eggs had been laid in the normal manner by the female. Then it was perfectly possible to cut sperm from the body of the male and fertilize. Since, as already stated, the normal time of laying is from 7.30 to 9 P.M., many important early stages are passed through before daylight (the embryo begins to swim at the completion of the 64-cell stage, or about the fifth hour of development), and since the small size and opacity of the embryo make it an unfavorable object for study by artificial light, an attempt was made to delay the process of laying. Although a number of devices which had proved successful in other forms were tried, they were wholly without success. If the females are put on ice, or in a very cool place, overnight, they will hold their eggs until about daylight the next morning, but such eggs almost invariably developed abnormally; and the same result followed if for any reason the eggs were laid at other than the regular time. In one case only did I find eggs which must have been laid about 4 A.M. developing normally. It is not at all improbable that modifications of the cooling method might have proved successful, but for the purposes of this paper preserved material was so much more satisfactory than fresh that I have relied almost entirely on that, merely using the fresh, where possible, for corroboration. Cilia can of course be seen better in living than in preserved material, but the history of cells can best be made out on stained specimens where the karyokinetic figures give absolutely accurate evidence of cell origin.

When the sexual products are ripe, the sexes may be easily distinguished by the characteristic color of the ova and sperm seen through the semi-transparent body wall ; the females being a seal brown, the males a cream color. I found that the most convenient way was to isolate the sexes, which seems in no way to affect the time of egg-laying, and when laid to transfer the eggs to fresh water and fertilize with sperm cut from the body of the male. In this way the precise time of fertilization can be controlled, something which cannot always be done if the sexes are together ; and the difficulties

arising from an excessive number of sperm collected on the surface of the egg can be avoided.

While emitting the sexual products, the female usually crawls slowly along the bottom of the dish, the eggs streaming out from both sides of the body through openings at the base of the parapodia. Occasionally one will be found swimming with considerable rapidity during this process. The males are usually much more active at this time, though there is never anything like the amount of activity displayed by some other free-swimming annelids, *e.g.*, Nereis.

Material obtained and fertilized in this way was preserved at intervals of fifteen minutes for the first twelve hours, and at rather longer intervals for the later stages. There is so much variation in rate of development in the different lots, due apparently to temperature conditions, that time records are of little value, and I have not attempted to keep them after the first few divisions.

For preserving, I have found Kleinenberg's picro sulphuric (dilute) and picro acetic (made with 1% acetic) the most useful, the latter especially, when followed by Delafield's haematoxylin [1] (acidified), giving beautiful results with surface views. Flemming's fluid, though practically worthless for surface views, preserves cilia well, and gives better preservation for sectioning than the picro acetic. The specimens, preserved and stained as above, were cleared in clove oil and mounted in the same medium underneath a long cover-glass, supported at one end by a bit of capillary glass tubing. This method, for which I am indebted to my friend Dr. C. M. Child, has proved much more satisfactory than to mount in balsam, as the specimens can be rolled into any desired position and drawn with a camera as soon as they are mounted.

In the main I have followed Mead's nomenclature (No. 22) with certain modifications suggested in conference with Dr. Child, whose work on Arenicola has been carried on at the same time as this. The successive generations of " macromeres " we propose to designate by capital letters, with the

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[1] I am under great obligations to Dr. Conklin for suggestions as to the use of this method.

generation indicated by a coefficient, while the "micromeres" are indicated by small letters, each with a coefficient indicating the generation and a subscript indicating its position in the generation. Thus, A, B, C, D form the 4-cell stage. At their next division from A arises $1A$ and $1a$; from B, $1B$ and $1b$, etc. $1A$ then divides into $2A$ and $2a$, while $1a$ divides into $1a_1$ and $1a_2$.

We have continued the use of the terms "dexiotropic" and "leiotropic" to indicate the direction of the spiral cleavages, but propose the terms "dextral" and "sinistral" to designate the respective daughter-cells resulting from a spiral cleavage, reserving the terms "right" and "left" to apply to the sides of the bilaterally symmetrical body. Thus, in a dexiotropic cleavage, the upper cell when viewed from the animal pole would lie to the right, and is the "dextral" cell, the other is "sinistral," and *vice versa* in a leiotropic cleavage. In all cases the cell nearer the vegetative pole has the larger exponent, regardless of absolute size. When a cleavage is meridional, the sinistral cell receives the smaller exponent.

This method has one disadvantage, in that symmetrically placed cells which arose by a meridional division do not receive corresponding subscripts. Thus (see Pl. XXXVIII, Fig. 27), $3c_2$ and $3d_2$ divide in a meridional direction and so that their products are symmetrically placed with respect to the median plane of the embryo. After one more division their larger daughter-cells become the larval mesoblast. One of these cells is $3c_{2.1}$, and the other $3d_{2.2}$, according to the proposed system. I believe, however, the advantages of the system will outweigh any disadvantages resulting from a possible confusion. This nomenclature differs from that adopted in my preliminary paper (No. 29, *a*), but seems enough of an improvement over that to warrant the change.

Cleavage.

The eggs are small, measuring 62.9 μ in diameter, and are, in the fresh condition, very opaque. As already stated, they are not provided with any albuminous coating, and when laid

sink rapidly to the bottom of the dish. They are usually more or less irregular when first laid, but rapidly become spherical or nearly so. In some cases there has seemed to be a certain amount of axial differentiation, but these differences are not constant. A thin membrane surrounds the egg, and remains attached to it until the latest stage I have studied. Whether, as is commonly stated to be the case among annelids, it becomes the cuticle of the adult, I cannot say. It is smooth in the living egg, but becomes more or less wrinkled under the influence of reagents. Except in Fig. 1, I have not attempted to represent it in the plates.

Soon after laying, the first polar spindle appears, and the eggs remain in this condition until fertilized. A study of the maturation and fertilization stages is reserved for a future paper, and the present account begins with the first cleavage.

The first cleavage begins, with very slight variations in time, one hour after fertilization. At this time the animal pole is indicated by the position of the polar globules and by a certain amount of protoplasmic differentiation easily seen in stained material. The vegetal pole of the egg stains very slightly with haematoxylin, and has a granular appearance, while the animal half is much more homogeneous in texture and stains more deeply. The first spindle lies slightly nearer the animal than the vegetal pole. (See Pl. XXXVI, Fig. 1.)

The first cleavage furrow cuts rapidly through the egg, sinking down more rapidly at the upper than at the lower pole (*cf.* Wilson, No. 34, *d*, and Mead, No. 22), and the 2-cell stage results. (See Pl. XXXVI, Fig. 2.) Here it will be seen that the first cleavage is exactly equal. The two nuclei are directly opposite one another, there being no indication of the rotation described by Conklin in the 2-celled stage of Crepidula. A lenticular cleavage cavity is formed, which persists and becomes the large cleavage cavity of the later stages. (See Text-Figs. 1 and 2.)

Two to Four Cells. — The next three cleavages follow one another at intervals of approximately fifteen minutes, but beyond this, time records are of little value. The eggs of the same lot are usually in practically the same stage at any one

time, but owing doubtless to temperature conditions, different lots vary considerably in rapidity of development. (See Mead, No. 22, p. 269.) The 4-cell stage arises from the 2, by the equal division of both cells, there being no large D, as in other annelids. (See Pl. XXXVI, Fig. 3.) The spindles are not quite parallel with one another, so that two cells rotate upward and two downward. As a result, the familiar " cross furrow " appears. This is found at both poles, that at the upper being at right angles with the lower, and considerably shorter than it. The origin and significance of this furrow have been discussed by Conklin (No. 5, *a*), and I can add nothing to what he has said. It is of considerable *practical* importance in Podarke for purposes of orientation. The direction of this furrow at the lower pole is positively the only means of orientation before the completion of the 56-cell stage. I have taken a good deal of pains to ascertain if it remains constant in direction up to that time. There is no doubt that it does, although soon after the completion of the 64-cell stage this direction may be lost, owing to movements of the entomeres whose position determines the existence of the furrow.

The relation of the two first cleavage planes to the body axes is a point on which most investigators have laid considerable stress. In the cytogeny of annelids and mollusks two apparently distinct relations are found. In Nereis (No. 34, *d*), Crepidula (No. 5, *a*), and Limax (No. 17), for example, the second cleavage furrow is said to coincide with the future median plane, while in Amphitrite (No. 22) and Arenicola (No. 4) the quadrants are anterior, median, right, and left. This apparent discrepancy has been explained by Lillie (No. 21) and Conklin (No. 5, *a*) as arising from the fact that only a small part of the furrow, that between the entomeres, has been considered in determining the orientation. Conklin has shown that in annelids and mollusks there is no exception known to the rule that the second and fourth quartettes lie in the median and transverse planes, while the first and third lie between them. An examination also of Wilson's figures of Nereis will show that the second cleavage furrows, *if traced between the ectomeres*, lie at some distance from the median plane of the embryo.

In orienting the embryo of Podarke, it is impossible to rely on the constancy of direction of the polar furrow after the 64-cell stage. Even before they divide to form the fifth group of micromeres, shiftings in position occur (see Pl. XXXVIII, Fig. 27), and immediately after this division invagination begins. From this time on it is impossible to determine their position with any accuracy. Evidently, then, the arrangement of the ectomeres must be considered here, and that offers no exception to the rule formulated above. The second and fourth generations of micromeres lie in the median and transverse planes, the first and third lie between them. The relation of the entomeres to the ectomeres, as is shown before the latter shift their position, is exactly as in Amphitrite and Arenicola. They lie anterior, posterior, right, and left. Owing to the absence of a large D-cell, however, it is not possible to distinguish between the two ends of the second furrow.

Four to Eight Cells. — The spindles of this division appear about fifteen minutes after the completion of the last, and the cells all divide at approximately the same time. The cells are rarely in *exactly* the same stage at any one instant, and I tried to find some indication of an acceleration of development in one quadrant, such as has been described, *e.g.*, in Unio (No. 21). Such an acceleration, if present, would be of great aid in orientation, but I do not believe that any exists. In later stages, at the lower pole, the C and D quadrants divide a little more rapidly than either A or B, but precisely the opposite occurs at the upper pole (see Pl. XXXVII, Figs. 19 and 23, showing the history of the cross), and in no case was there any constant difference between C and D. Neither was it possible, by any staining reactions, to distinguish between the quadrants.

The result of this division is the formation of the first group of ectomeres, which lie on top of the macromeres, but are exactly equal to them in size, or so nearly equal that their differences lie within the limits of error of observation (Pl. XXXVI, Figs. 4 and 5). The division is dexiotropic, and it is interesting to note that here, as in Nereis (No. 34, *d*, p. 387), the spindles show an inclination from the vertical before any external trace of segmentation is visible.

The upper pole is indicated by the presence of the polar globules, which lie between the cells, almost inside the segmentation cavity (Pl. XXXVI, Fig. 5).

Eight to Sixteen Cells. — The 16-cell stage is reached by the division of each cell of the 8 in a leiotropic direction, the divisions all occurring at about the same time (Pl. XXXVI, Figs. 6 and 7). The division at the lower pole is equal and leads to the formation of the second group of ectomeres. It is of importance to note here that all of the cells of this second group are equal in size, as compared with many other annelids, in which a very large 2d, "the first somatoblast," appears. Inasmuch as the history of this cell 2d in Podarke is similar to that in other annelids, I shall also call it the "first somatoblast." At the upper pole the division is more unequal, the upper cell being the larger. (See Pl. XXXVI, Figs. 6 and 7.) Since the eight cells were all equal, it follows that now the largest cells are at the upper pole, and this relation holds until
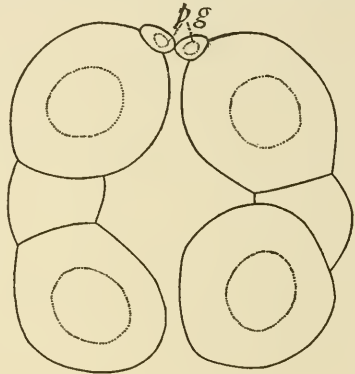
FIG. 1. — Optical section of 16-cell stage, showing large cleavage cavity. *pg*, polar globules.

the cross has been broken up into small cells (Pl. XXXVIII, Fig. 23 ; Pl. XXXIX, Fig. 37). The smaller cells, 1a₂, 1b₂, 1c₂, 1d₂, divide twice more and form the primary prototroch of the larva. I shall call them, after Mead, the *primary trochoblasts*. The segmentation cavity present as a lenticular space in the 2-cell stage is continued through the later cleavages, and is present as a good-sized cavity at this time. Its proportions a little later than this are shown in Text-Fig. 2.

Sixteen to Thirty-two Cells. — The 32-cell stage is reached by the dexiotropic division of each of the sixteen cells, the transition falling into three well marked subdivisions. *1.* The division of the macromeres to form the third group of ectomeres, which, as we shall see, are not purely ectodermal in destiny, and the division of the upper cells of the embryo giving rise to the

"intermediate girdle" cells, which occupy the spaces between the arms of the cross (Pl. XXXVI, Fig. 8). *2.* The division of the primary trochoblasts (Pl. XXXVI, Fig. 9). *3.* The division of the second group of ectomeres (Pl. XXXVI, Fig. 10). At the 32-cell stage, although well-marked size differences have appeared among the cells, the quadrants are still radially symmetrical, and it is impossible, by any differences in size or arrangement of cells, to distinguish them. The polar furrow retains its original direction and enables us to distinguish between the two cleavage planes, but no other orientation mark appears.

At the 32-cell stage the polar globules, which until this time have occupied a position at the upper pole of the egg lying on the inner face of the rounded ectomeres, pass into these latter cells, where they may be seen as small, deeply staining bodies, lying in the protoplasm of the cell (Pl. XXXVI, Fig. 11, *pg*, and Text-Fig. 4). This position they retain for some time. There seems to be no regularity in the process, the bodies sometimes passing into one and sometimes into another of the large cells. Similar observations have been recorded by Mead (No. 22) in Lepidonotus, where, also in the 32-cell stage, the polar globules pass sometimes into one, sometimes into another of the cross cells, or they may even be found in the segmentation cavity ; and in Chaetopterus, where the polar globules are ingested by the rosette cells. Grobben (No. 10) finds in Cetochilus that at least one polar globule wanders into the segmentation cavity, where it, presumably, is absorbed. Hatschek (No. 11) in Eupomatus, and Eisig (No. 8) in Capitella, have described a similar fate for the polar globules. (See p. 417.)

Thirty-two to Sixty-four Cells. — The 64-cell stage is "actual" in Podarke, and is reached by the leiotropic division of each of the thirty-two cells. Here, as before, there are three well-marked subdivisions. *1.* Thirty-two to forty cells. The fourth group of micromeres arise at the lower pole (Pl. XXXVII, Fig. 13), and four very small cells, the apical rosette, are divided off from the large cells at the upper pole (Pl. XXXVI, Figs. 11 and 12). It is important to note that the cells of the fourth quartette

are exactly equal in size; in other words, *there is no large* 4d, and so far as we can tell from observation, the cell containing the mesoderm might be either of two cells lying one at
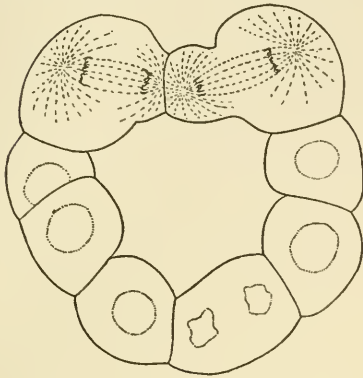
FIG. 2.—Optical section of stage shown in Fig. 11, Pl. XXXVI.

either end of the second cleavage furrow. The spindles at the upper pole are inclined downward a little from the horizontal (Text-Fig. 2). This, combined with the small size of the rosette cells, causes the latter to lie somewhat below the surface (see Pl. XXXVII, Fig. 16, and the Text-Fig. 4). Later, as a result of the pressure of the dividing cross cells, they are forced still farther below the surface. (See Text-Fig. 3.) A similar process has been described by Mead for Lepidonotus (No. 22). As a comparison of Fig. 12 (Pl. XXXVI) with Fig. 27 (Pl. XXXVIII) will show, one result of this division is further to increase the difference in size which has before been noticed between the cells of the upper and those of the lower hemisphere.

2. Forty to fifty-six cells. (*a*) A division of the trochoblasts to form four cells in each quadrant (Pl. XXXVI, Fig. 12, Pl. XXXVII, Fig. 14, $1d_{2.1}$, $1d_{2.2}$, etc.). These do not divide again, but soon develop cilia and function as the primary prototroch (Pl. XXXVII, Fig. 15, $1d_{2.1.1}$, $1d_{2.1.2}$, $1d_{2.2.1}$,

FIG. 3.—Optical section of stage shown in Fig. 15, Pl. XXXVII. *ar*, apical rosette; *pg*, polar globules.

$1d_{2.2.2}$, etc.). This band of ciliated cells is at first broken at four points, but is afterwards completed in a manner to be described later. (*b*) A division of the intermediate girdle

cells, $1a_{1.2}$, $1b_{1.2}$, $1c_{1.2}$, $1d_{1.2}$; and (*c*), a division of the third group of micromeres. See Pl. XXXVI, Fig. 12 ; Pl. XXXVII, Fig. 14, where the spindles of all these divisions are shown. *3.* The second division of the second group of ectomeres (Pl. XXXVII, Fig. 24). This is a most important division, for by it arises the first indication of a difference between the quadrants. It marks, in other words, the first appearance of bilateral symmetry. In three of the quadrants the mode of division is that shown in Pl. XXXVIII, Fig. 25 ($2b_{1.1}$, $2b_{1.2}$, $2b_{2.1}$, $2b_{2.2}$, etc.). In the fourth quadrant the case is different. The dextral cell, $2d_1$, has divided, as in the other quadrants,

with a smaller cell above. The sinistral cell, on the other hand, has divided in precisely the opposite way. The larger cell is above, and the smaller cell, which is very much smaller than any products of the division in the other quadrants, is below. This cell lies a little to the right of the second cleavage plane, and nearly over one of the fourth group of micromeres. It can easily be distinguished by its small size and deeply staining nucleus (Pl. XXXVIII, Fig. 26). Later study shows that the

Fig. 4.—Optical section of stage shown in Fig. 16, Pl. XXXVII. In this and later drawings the nuclei of the prototroch cells are stippled.

" 4 " micromere, near which it lies, contains the mesoderm. Orientation is now complete, and the quadrant in which the cell in question occurs is the " D " quadrant. A similar cell, occupying a similar position, has been described by Mead (No. 22), Lillie (No. 21), and Conklin (No. 5, *a*) in other annelids and mollusks. The polar furrow retains its original direction up to the time when this cell appears, and this enables us to orient the early cleavage stages.

At about the 40-cell stage strands of protoplasm can be plainly seen reaching across the cleavage cavity, running from

the cross and rosette cells to the upper ends of the entomeres (Text-Figs. 3 and 4). From the pointed upper ends of the entomeres in Text-Fig. 5, I believe that the connections exist also in later stages, though I have not been able to make them out on preserved material. Professor E. A. Andrews, who kindly examined some of my preparations at my request, informs me that these processes are undoubtedly protoplasmic, but that he is unable to determine from preserved material whether they are protoplasmic streamings, such as have been described in other animals (see No. 2), or remnants of the primitive continuity which has never been lost. For our present purpose the question is immaterial. Of importance is the fact that protoplasmic connections do exist between blasto-meres of relatively late cleavage stages.

The embryo is now composed of sixty-four cells, divided as follows :

1st	Quartette	32
2d	"	16
3d	"	8
4th	"	4
	Entomeres	4
		64

Three of the quadrants are exactly alike and the fourth differs from them merely in the possession of the small cell, $X_{1.2}$, which lies at what will be the posterior end of the body.

Up to this stage it is evident that Podarke agrees in the character of its segmentation with other annelids and with gasteropods and lamellibranchs. In all cases three quartettes of ectomeres arise in alternating directions. In all cases one member of a fourth quartette is budded off, and this cell is wholly or partially composed of the definitive mesoblast. Usu-ally but not always (*e.g.*, Nereis) the other three members of this fourth quartette appear. Inasmuch as they, together with the remaining macromeres, give rise to the entoderm, the dif-ference is merely that in the one case this division takes place at the surface, in the other, after invagination.

On the other hand, Podarke differs from most other annelids in two important features. The cells of the second and those

of the fourth quartettes are all equal in size, whereas in nearly all annelids there is a large 2d and a large 4d. The entomeres also are much larger in other annelids than in Podarke. These differences are important and will be discussed later. (See p. 441.)

Let me repeat that up to the time when the small cell $X_{1.2}$ appears, the polar furrow at the lower pole of the egg retains its original direction. At the 64-cell stage, however, the entomeres elongate inward to a greater or less extent, and the shiftings of position thus caused among the cells may produce a change of direction in the polar furrow. (See Pl. XXXVIII, Fig. 27.) The condition shown in Pl. XXXVIII, Fig. 27, appears only rarely, but often enough to show that the polar furrow can no longer be relied on for orientation.

Since the regular geometrical progression is now lost (some cells, *e.g.*, trochoblasts, do not divide again, while others divide repeatedly), the later history can best be treated by considering each group by itself.

First Group of Ectomeres. — At the 64-cell stage these are arranged as follows :

Trochoblasts	16
Intermediate cells	8
Cross cells	4
Rosette cells	4

The trochoblasts, as already stated, do not divide again, but form the larval locomotor organs. They become elongated as development proceeds, prominent nucleoli appear in their nuclei, and they stain with great difficulty. They are very much narrower than in Amphitrite, agreeing in this respect very closely with Lepidonotus (No. 22). (See Pl. XXXVIII, Figs. 29, 30, etc., *ap'*, etc.) Cilia appear on these cells soon after their last division, about four and a half to five hours after fertilization, and the embryos at first rotate slowly and later swim at the surface, being at first positively heliotropic. The primary prototroch thus formed is at first divided into four distinct areas, the openings between being later filled with cells having a different origin. As long as the separate cells could

be distinguished I have designated each by p', with the appropriate letter and number (Pl. XXXVIII, Fig. 25–Pl. XL, Fig. 58). In later stages, where the primary and secondary, and still later, where also the tertiary prototroch cells are not to be distinguished from one another, I have indicated the prototroch merely by p, with the appropriate letter to show to which quadrant each portion belongs.

The Intermediate Cells. — The last division of these cells was leiotropic. The next is dexiotropic and leads to the formation of four cells in each quadrant (Pl. XXXVII, Figs. 17 and 18). In three of the quadrants (a, b, and c) the lower product of the division of the dextral cell $1c_{1.2.2.2}$, etc., *completes the primary prototroch* (Pl. XXXIX, Figs. 37 and 38, ap'', etc.). They pass into the space between the sets of primary trochoblasts, become ciliated, and function as prototroch cells. I propose to call them the *secondary trochoblasts*. The other products of this division fill the space between the arms of the cross and become a part of the umbrellar ectoderm. For some few divisions I have been able to follow their history, but after the cross cells begin to divide into small cells the two sets intermingle so that it is not possible to distinguish them.

In the fourth quadrant the history of the intermediate cells is very different from that just described. Their first division into four cells is the same, but a little later the lower product of the division of the dextral cell, *i.e.*, the one corresponding to the cell which in other quadrants becomes a part of the prototroch, begins to migrate through the opening in the prototroch, and thus forms a portion of the lower hemisphere. In this migration it is accompanied by part, at least, of its sister-cell, but I think by none of the descendants of the other intermediate cells. These migrating cells push through the break in the prototroch, dividing as they go (Pl. XXXIX, Figs. 42, 44, and 45; Pl. XL, 53, 57, and 58, l_1, l_2, etc., and the cells just dorsal to them). Since the opening persists until late in the development, the process of migration lasts for a considerable time, or until the trochophore is fully formed. In Pl. XL, Figs. 53, 57, and 58, it will be seen that these cells flatten out as they pass downward, one of them, l_1 (Pl. XL, Figs. 53 and 57), elongating

very considerably. It immediately adjoins the cell $X_{3.2}$, and the two form a landmark which is unmistakable. Figs. 58 and 59 (Pl. XL) show that the cells which have migrated through the prototroch eventually comprise a large portion of the subumbrella, *fully three-fourths* of it being formed from them. The cell l_1 divides transversely. (See the products of its division, $l_{1.1}$ and $l_{1.2}$, in Pl. XL, Figs. 58 and 59.) Beyond the latter stage I have not followed it. All of these cells become excessively thin and their outlines difficult to follow, so that in the later figures I have indicated only the nuclei. The entoderm cells lie close under the ectoderm, the two being dorsally in actual contact, and it frequently becomes impossible, except in optical section, to determine whether a given nucleus belongs to ectoderm or entoderm. In Pl. XL, Figs. 58 and 59, I have intended to represent only ectoderm nuclei.

A similar migration has been recorded in Amphitrite by Mead (No. 22), but there the number of migrating cells is much less than in Podarke, and they play a much less important part in the formation of the subumbrella.

The Cross. — In the 64-cell stage the cross is composed of four equal cells, radially arranged. At the next division bilateral symmetry appears. It is interesting to note that this is accomplished by a dexiotropic cleavage ; the direction which it ought properly to assume according to the law of alternating cleavages. Fig. 16 (Pl. XXXVII) shows the beginning and Fig. 17 (Pl. XXXVII) the completion of this division. In the latter is seen, also, the beginning of the next divisions of the cross cells in two quadrants. In the latter figure, also, is shown the division of the upper (sinistral) intermediate cell in each quadrant. The result of the first bilateral division of the cross cells is shown in Pl. XXXVII, Fig. 17. Two of the cells have divided unequally, two very nearly equally, so that a vertical plane can be drawn which would cut the embryo into bilaterally symmetrical parts. This plane of symmetry coincides with the plane marked out by the small cell $X_{1.2}$, previously described at the 64-cell stage. The median plane of the embryo is now fully indicated, and orientation is complete. The constant position of the polar furrow up to

the 64-cell stage, the appearance of the cell $X_{1.2}$, and the bilaterally symmetrical cross form a series of orientation marks which are unmistakable. We may say, therefore, that in A and B quadrants the first division of the cross is very unequal ; in C and D quadrants it is nearly equal.

The Appearance of Bilaterality.

This marking out of a bilateral plane by cleavages which follow a true radial [1] type is important to my mind as indicating that the bilateral divisions have nothing to do with shiftings of material into the median plane, but rather that the bilateral and the radial are distinct in origin. The former having been, so to speak, superimposed upon the latter, the bilaterality of the organism may express itself even in the spiral cleavages.

Bilateral cleavages have not in Podarke such direct reference to the form of the body, as distinct from the head, as Conklin (No. 5, *a*) has described for Crepidula. The first bilateral *cleavage* is at the lower pole (Pl. XXXVIII, Fig. 27), but bilateral divisions appear early and are very prominent at the upper pole as well. Further, as we have seen, bilateral *symmetry* is established before bilateral cleavages appear, and is as marked at the upper as the lower pole.

Of interest in this connection are the following observations : I have seen a few (not more than three were carefully studied) cases where the cross, when formed, is radial and not bilateral. *Each* arm has three equal cells, like the anterior cross arms represented in Pl. XXXVII, Fig. 18. In these same embryos $2d_1$ and $2d_2$ divided just like the other second quartette cells, and no *small* $X_{1.2}$ appeared. In this stage, therefore, the embryo was still radially symmetrical. In one other case three cross arms were alike in having three equal cells, while the fourth was of the posterior cross-arm type. These facts are significant to my mind as indicating a reversion to a radial type of a cleavage which had secondarily become bilateral. The fact

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[1] In this and later discussions I have followed Conklin in classing both " orthoradial " and " spiral " cleavages under the general head of " radial."

that the reversion is always to the radial from the bilateral
(from the type of the posterior arm to that of the anterior)
and not in the opposite direction is suggestive. The facts are
perhaps too few in number to warrant wide inferences, but so
far as they go they seem important.

The next division of the cross cells emphasizes still more the
bilateral symmetry. The division of the basal cells in A and
B quadrants, begun in Pl. XXXVII, Fig. 17, is completed in
Pl. XXXVII, Fig. 18, and there result three subequal cells
in a row in each arm. In C and D quadrants the upper cells
divide next, but divide bilaterally, the cells sloping inward
(Pl. XXXVII, Fig. 18). In C quadrant the division is equal,
in D quadrant unequal. (See Pl. XXXVII, Fig. 19.) This
result was so unexpected that I suspected it might be an
abnormality, but in examining a large number of prepara-
tions I found always the same result.

While this last division is in progress in the basal cells of
the posterior arms the terminal cells divide, $1c_{1.1.2.2}$ usually
a little in advance of $1d_{1.1.2.2}$. The division is bilateral, and
there result two small cells, lying a little farther from the
median plane than the center of the large terminal cell. These
small cells are very little larger than their nuclei, which stain
very deeply, and they lie on top of the cross, between it and
the basal cells of the arm. They are excellent landmarks for
later study of the embryo, as it is possible at a glance to recog-
nize the C and D quadrants by means of these small deeply
staining cells (Pl. XXXVII, Fig. 20).

In Nereis, Wilson (No. 34, *d*) described cells similar to these
in origin and position, which he called, provisionally, "head
kidneys." In Amphitrite corresponding cells were found by
Mead (No. 22), from which arise the huge mucous glands of
the trochophore, and Wilson (No. 34, *f*) has since suggested
that possibly they are slime glands in Nereis also. In Podarke
the next division of the dorsal arms of the cross (Pl. XXXVII,
Figs. 20 and 21) buds off one other cell on either side. This
cell is very little larger than the first one, and the two pairs
remain in this position until very late in the development.
The last stage in which I was able to recognize them with

certainty was that represented in Pl. XXXVII, Fig. 23. I believe that they become slightly larger in the later stages and form a part of the general ectoderm of the umbrella. Once and only once did I see an indication of a division in one of these cells, $1d_{1.1.2.2.2.1}$. This I believe was abnormal. They certainly do not become slime glands (excretory glands?) in Podarke, unless the small deeply staining cells scattered over the surface have that function, for there are no such large glands as Mead has described for Amphitrite.

The anterior arms of the cross next divide meridionally. (See the division begun in Pl. XXXVII, Fig. 19, and completed in Pl. XXXVII, Fig. 20.) At their next division a number of small cells appear (Pl. XXXVII, Fig. 22). Note especially the small size of these cells and their deeply staining nuclei. To avoid tedious description I have indicated by junction lines in Pl. XXXVII, Fig. 22, the origin of many of these cells, and have put their divisions in the table, p. 438. In Pl. XXXVII, Figs. 18–23, the outline of the cross is indicated by the heavy line.

Beyond the stage represented in Pl. XXXVII, Fig. 23, I have not attempted to carry the lineage of the anterior (ventral) portion of the umbrella. The cross cells become inextricably confused with the descendants of the intermediate group, and, with possible exceptions, all contribute to the formation of the ventral ectoderm. The exceptions are these. I believe that some of the very small cells are pushed into the cleavage cavity and finally absorbed. Immediately after they are formed they sink below the surface, lying near the bottom of the rather thick ectoderm. A little later dark bodies are seen lying inside the segmentation cavity, and later still they touch the entoderm (see Text-Fig. 8, *ec?*). The point needs reinvestigating before it can be considered absolutely proven, but I believe the fate of the cells is as I have described. A similar process occurs in Crepidula (No. 5, *a*), where, however, the cells are thrown outside the body, and more recently Miss Langenbeck (No. 20) has described, in Microdeutopus, cells which are taken into the segmentation cavity. In Lepidonotus, Mead (No. 22) states that the polar globules pass into the

cross cells (see above, p. 417), and he figures them in Figs. 93
and 105.   In Podarke, as already stated, the polar globules have
a similar fate, but long before the stage corresponding to
Mead's Fig. 105 they have completely disappeared.   On the
other hand, these small cells derived from the ectoderm migrate
inward and occupy a position very similar to that of the "polar
globules" of the latter figure.   This leads to the suggestion
that possibly both processes may occur in Lepidonotus also,
and that the small cell figured in Fig. 105 is derived from the
ectoderm.

In the dorsal arm of the cross, the cells $1c_{1.1.2.1.2}$ and
$1d_{1.1.2.1.1}$ next divide (Pl. XXXVII, Fig. 20), and this is
followed by the division of $1c_{1.1.2.1.1}$ and $1d_{1.1.2.1.2}$.   (See
Pl. XXXVII, Figs. 21 and 22, and table, p. 438.)   The large termi-
nal cells of the dorsal arms divide considerably later, the divi-
sions being nearly equal.   Figs. 22 and 23 (Pl. XXXVII) show
the division in C quadrant.   (See also Pl. XXXIX, Fig. 42,
$1d_{1.1.2.2.2.2}$.)   Later than this I have not attempted to follow
the cells in detail.   They all enter into the formation of the
ectoderm on the dorsal side of the umbrella, in which I have
been able to make out no specialized organs.   Ventrally there
are two "eye-spots" and the large "problematic" organs
which develop from either the ventral cross cells or from the
intermediate cells.   It is impossible to tell from which.

The Rosette Cells. — At the 64-cell stage these are four in
number, lying between the cross cells and considerably elon-
gated inward.   (See Pl. XXXVII, Figs. 16, 17, and 18,
and Text-Figs. 3 and 4.)   Soon after the stage represented
in Pl. XXXVII, Fig. 17, one of them, invariably the one of
the A quadrant, comes to the surface and divides equally
(Pl. XXXVII, Fig. 18, *ar*).   Next, the one in the D quadrant
divides (Pl. XXXVII, Fig. 20, *dr*).   Later, the two remaining
cells divide, and the resulting plate of eight cells is shown
with stippled nuclei in Pl. XXXVII, Figs. 22 and 23.   These
cells differ so little in size from those around them that the
two are hard to distinguish, but I think the arrangement
given in Pl. XXXVII, Fig. 23, is correct.   The cells elongate
slightly inward (Text-Figs. 5 and 6, *ar*).   From them arises the

tuft of apical cilia which from now on is a prominent feature of
the larva.   I think that all of the eight cells become ciliated.

  The Second Quartette. — At the 64-cell stage there are sixteen
of these cells, four in each quadrant, and a difference between
the quadrants has shown itself by the peculiar division of one
cell, leading to the formation of $X_{1.2}$, already mentioned.
Since the fate of the second group of micromeres in the
dorsal quadrant is different from that in the others, it will be
advantageous to consider it by itself, and turn first to the
second quartette in quadrants A, B, and C.

  The first division concerns the upper dextral and lower
sinistral cell (Pl. XXXVIII, Fig. 29).   This is followed some-
what later by a division of the upper sinistral and lower
dextral cells (Pl. XXXIX, Fig. 37).   These divisions follow
the law of alternation of cleavages only in the dextral cells,
the sinistral dividing in precisely the same direction as their
preceding division.   Neither can the divisions be called bi-
lateral, since the cells are not bilaterally arranged ; and,
indeed, from the 64-cell stage on, while some of the divisions
of cells which lie near the median plane may be bilateral,
the majority are not.   They develop so as to produce in the
end an embryo with bilaterally symmetrical organs ; but data
concerning the precise direction of each spindle, except in so
far as they bear on the identification of cells, seem to me of
little value.   Cleavages may follow the law of alternation, or
they may not, and neither case in itself is of any importance.
The divisions of the dextral cells are of interest, for by them
are produced three cells, which correspond to the "secondary
trochoblasts" of Amphitrite, the cells $2a_{1.1.1}$, $2a_{1.1.2}$, $2a_{1.2.1}$,
etc. (Pl. XXXIX, Fig. 38).   In Amphitrite, however, the
division of the upper cell is equal, while in Podarke it is very
unequal (Pl. XXXIX, Figs. 37 and 38).

  In each quadrant, then, there are three cells, two large and
one small, corresponding in origin to the secondary trocho-
blasts of Amphitrite.   (See Pl. XXXIX, Figs. 37, 38, 41, etc.,
$ap_1{'''}$, $ap_2{'''}$, $2a_{1.1.1}$, $bp_1{'''}$, $bp_2{'''}$, $2b_{1.1.1}$, $cp_1{'''}$, $cp_2{'''}$, $2c_{1.1.1}$.)
Fig. 56 (Pl. XL) shows the relation of these cells to the rest
of the prototroch : $cp_1{'''}$, $cp_2{'''}$, $2c_{1.1.1}$.   While, however, $2c_{1.1.1}$,

and $2c_{1.1.2}$ (cp_1''' and cp_2''') have elongated considerably, their
nuclei have become swollen and contain prominent nucleoli,
and the whole cell stains with difficulty, — agreeing in this
respect with the cells of the primary prototroch, — the small
cell $2c_{1.1.1}$ remains very small and with a very deeply stain-
ing nucleus. Later, as a result of the shifting of cell areas,
this small cell is shoved out of the prototroch ring, and, I
believe, forms a part of the ectoderm of the subumbrella,
though its later history is difficult to follow. This process
occurs in all three quadrants. Compare Pl. XL, Fig. 56, an
embryo of 12 hours 35 minutes, with Pl. XXXIX, Fig. 48,
an embryo of 24 hours ($2b_{1.1.1}$ and $2c_{1.1.1}$). These figures
show two stages in the shoving of this small cell out of the
prototroch ring. Later embryos show it lying entirely below
the prototroch. The other two cells form a part of the com-
pleted prototroch, though they acquire their cilia very late.
In the stage represented in Pl. XXXIX, Fig. 48, they are
still without cilia. A little later they push up into the proto-
troch ring and all the cells elongate still more, this latter
process coinciding with the closure of the dorsal interruption.
They stain very poorly, so that their outlines are impossible
to follow in later stages.

The other divisions of the second quartette are as indicated
in Pl. XXXVIII, Fig. 36; Pl. XXXIX, Figs. 41, 42, 46;
Pl. XL, Figs. 50, 51; and in the table, p. 438.[1] One cell is, how-
ever, of especial interest. This is the cell $2b_{2.2.1}$ shown in
Pl. XXXIX, Fig. 41. Here it is seen at some distance from
the edge of the blastopore. It divides once only. (See the
products of the division in Pl. XL, Fig. 51.) While the corre-
sponding cell in quadrant A divides vertically and equally
(Pl. XXXIX, Fig. 41), this division is horizontal and unequal,
a small cell being budded off dorsally. The ventral product,
$2b_{2.2.1.2}$, does not divide again at the surface. Gradually the
cells between it and the blastopore, — descendants of $2b_{2.2.1}$,
— invaginate to form a part of the stomodaeum, and this

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[1] In Pl. XXXIX, Fig. 46, I have indicated by the numeral on each cell the
share which the second and third quartettes take in the composition of the ven-
tral ectoderm.

cell, $2b_{2.2.1.2}$, approaches closer and closer to the blastoporic margin, and finally invaginates. (See Pl. XXXIX, Figs. 43, 44; Pl. XL, Figs. 49, 50, 51, 52, 54, 55.) In the stage represented in Pl. XXXIX, Fig. 47, it lies underneath the surface in the wall of the stomodaeum, where its nucleus is indicated by the dotted line. It later divides, but I have not been able to follow its subsequent history farther than to say that it forms a part of the stomodaeal wall.

The history of the second quartette cells in the dorsal quadrant is of especial interest for purposes of comparison, since in many annelids and mollusks they give rise to a large part or all of the ectoderm of the body, and in Amphitrite and Arenicola, at least, the paratroch of the larva is formed from descendants of this group. I shall follow Wilson and Mead in giving to these cells the especial name " X." For the sake of clearness I have enclosed the X-cells in a heavy outline.

The first divisions of X have already been described, the small $X_{1.2}$ arising in the 64-cell stage. (See Pl. XXXVIII, Fig. 26, where I have labelled the figure to correspond with Mead's nomenclature for the X-cells.) The next divisions occur in $X_{1.1}$ and X_2 (Pl. XXXVIII, Fig. 30, and Pl. XXXIX, Fig. 39), and these are soon followed by a division of X_3 (Pl. XXXVIII, Figs. 31 and 32), where the completion of this division is shown. $X_{1.1.1}$ next divides (Pl. XXXIX, Fig. 39), and there results a larger cell above, $X_{1.1.1.1}$, and a smaller, $X_{1.1.1.2}$, below (Pl. XXXIX, Fig. 40). At nearly the same time $X_{1.2}$ divides (Pl. XXXVIII, Fig. 35, and Pl. XXXIX, Fig. 39). In Pl. XXXIX, Fig. 40, is shown the division of $X_{2.1}$, and $X_{2.2}$, and $X_{1.1.2}$. Pl. XXXIX, Fig. 42, shows the division completed. $X_{1.1.1.1}$ next divides and at about the same time division occurs in $X_{2.1.1}$. These divisions are shown as completed in Pl. XXXIX, Fig. 42. These cells with their descendants now become difficult to distinguish from the cells of the third quartette near them, and as nothing of importance was to be gained from such a study, I have not attempted to carry them farther. $X_{1.1.2.1}$ and $X_{1.1.2.2}$ next divide equally (Pl. XXXIX, Fig. 43), and $X_{2.2.1}$ (Pl. XXXIX, Fig. 44), very unequally, the latter budding off a small cell to the left of

$X_{2.2.2}$. Later, $X_{1.1.2.1.1}$ and $X_{1.1.2.1.2}$ divide very unequally, each budding off a small cell downward, and at the same time $X_{3.1}$ sends a small bud upward (Pl. XXXIX, Fig. 45, and Pl. XL, Fig. 53). These small cells, formed from $X_{3.1}$ and $X_{1.1.2.1.1}$, have very deeply staining nuclei, and are excellent landmarks. The general rotation, which all the dorsal cells undergo, affects these latter cells so that the small cell, $X_{1.1.2.1.1.2}$, comes to lie underneath $X_{3.2}$, while its sister-cell lies to its right (Pl. XXXIX, Fig. 48). Meanwhile the small cell, $X_{1.2.2}$, has migrated inward, and forms a part of the wall of the proctodaeum, while its sister-cell lies at the edge of the blastopore. This afterwards divides again (Pl. XXXIX, Fig. 46).

As a result of the divisions described above, the condition of the X-group is as shown in Pl. XXXIX, Fig. 47. At the dorsal edge of the blastopore are three cells, one of which is dividing. $X_{2.2.2}$ divides next, unequally and in a meridional direction. The smaller product lies to the outside, and later $X_{1.1.2.2.1}$ buds off a small cell dorsally, which lies just below $X_{3.2}$. These cells occupy this position for some time, but subsequently divide, and the ventral edge of the X-group is composed entirely of small cells. (See Pl. XL, Fig. 59.)

In Pl. XXXIX, Fig. 47, $X_{3.2}$ is shown very much flattened and elongated, having remained undivided from the stage shown in Pl. XXXIX, Fig. 39. Together with the ectoderm cells which have migrated from the upper hemisphere, it has become excessively thin and transparent, but can always be recognized by means of its large vesicular nucleus and prominent nucleolus (Pl. XL, Fig. 57). As already stated, it and the "l_1" cell just above it are excellent landmarks in following the shiftings of position which the dorsal ectoderm cells undergo. Finally l_1 divides, and immediately afterwards $X_{3.2}$ divides bilaterally. Pl. XL, Fig. 58, shows the spindle of this division with the products of the division of l_1 lying just above it on either side. The daughter-cells of $X_{3.2}$ elongate considerably, and push apart (Pl. XL, Fig. 59). They may be recognized in later stages lying in this position, one on either side of and dorsal to the protodaeum. Each soon buds off a small cell downward and outward. In a considerably

later stage I have seen, occupying the position of these cells on either side, a row of two or three small cells, which apparently have come from a division of $X_{3.2.1+}$ and $X_{3.2.2+}$, but I am unable to give any positive statements about their origin or destiny. They have an appearance which strongly suggests teloblasts, but I do not know if they have that character. Indeed, the cells $X_{3.2.1}$ and $X_{3.2.2}$ (Pl. XL, Fig. 59) look very like teloblasts, but if they are their teloblastic growth must begin much later, for none of the ectoderm anterior to them has, in the stage figured, come from their division. Their later position also resembles very much that of the paratroch cells figured by Mead (No. 22) in Amphitrite, but in the latest stages I have seen no cilia are found on them.

I have described these divisions of the X-cells at some length because of their value for purposes of comparison with other annelids. Amphitrite and Arenicola (No. 4) are the only annelids where the exact origin of the paratroch is known. Podarke, as said before, probably has no paratroch, but so important an organ might, on one theory of cleavage, be supposed to be present as an ancestral rudiment ; and I was interested to follow the cleavages in the three genera to see if any similarities appear. The divisions of the X-cells in Podarke beyond the stage of Pl. XXXVIII, Fig. 30, have no similarities whatever to those of Amphitrite (No. 22) nor to those of Arenicola, as I learn from a comparison with figures kindly given me by Dr. Child.

I have already spoken of the way the dorsal ectoderm cells flatten out and spread apart. It is as if the cells from the upper hemisphere spread out like a fan, with their center near the upper edge of $X_{3.2}$, and carried the X-cells with them around towards the ventral side. The direction in which the forces which produced this shifting must have acted is shown in Pl. XL, Fig. 59, by the elongated cells $X_{3.2.1}$ and $X_{3.2.2}$.

The break in the prototroch remains open much later than in Amphitrite, and is represented as just closing in Pl. XL, Fig. 58. Whether or not this be regarded as the cause, the result is that a much larger portion of subumbrellar ectoderm

comes from these cells than from the corresponding cells in Amphitrite. In fact, *a very limited portion of the subumbrella is formed from the X-cells,* as is shown in Pl. XXXIX, Fig. 48 ; Pl. XL, Figs. 58 and 59. To this point, which is mainly of importance in a discussion of homologies, I shall return. (See p. 467.)

As a result of this migration and expansion of the dorsal ectoderm cells, the X-group are forced ventrally and finally concresce on the ventral surface (Pl. XXXIX, Figs, 46–48). I am not absolutely certain that some of the cells figured as lying just outside the X-group may not really belong to the latter. The descendants of the third group of micromeres lie close to the X-group on either side, and so many shiftings occur that it is impossible to follow every cell. For our present purpose the precise history of every cell is not absolutely necessary. The point of importance is that the X-cells surround the proctodaeum and, if it be safe to rely on analogy, make up the budding zone of the larva. I have thus far been unable to rear larvae, though they will live in confinement for as many as ten days. Probably connected with the lack of proper food (for the small amount of yolk in the egg must be rapidly used up) is an exceedingly slow rate of development during the time that they will live in captivity. Similar difficulties with other annelids have been described by Hatschek (No. 11) in Eupomatus, von Drasche (No. 7) in Pomatoceros, and Mead (No. 22) in Lepidonotus. Beyond a slight increase in length, practically no growth takes place after the second day. In Amphitrite, Mead has proved that all of the body behind the first segment arises from descendants of 2d. For reasons above mentioned, I am unable to make any positive statements about their fate in Podarke, but in view of their position I think the presumption is strong that here also the body ectoderm arises from 2d.

Of interest in this history of the X-cells in Podarke is the fact that their divisions are not bilateral, so that until at least a very late stage the cells are not symmetrically placed with regard to the sagittal plane. Pl. XXXIX, Fig. 46, for example, shows a marked asymmetry. This asymmetry is partly due to

asymmetrical divisions, and partly due to shiftings of positions which the cells undergo. In Pl. XXXIX, Fig. 42, for example, the cells $X_{1.1.1.1}$ and $X_{2.1.1}$ are seen to lie close against the prototroch cells in C and D quadrants respectively. Just to the left of $X_{2.1.1}$ are two small cells, $2a_{2.1.1}$ and $2a_{2.1.2}$ (Pl. XXXIX, Fig. 43). When the cell areas shift so that the prototroch cells take their final position, I believe that the cell $2a_{2.1.1}$ is pushed up between $X_{2.1.1}+$ and the prototroch (Pl. XXXIX, Figs. 44 and 45), while, on the other hand, the cell $X_{1.1.11}.+$ is pushed up between the prototroch cells (Pl. XXXIX, Fig. 45 ; Pl. XL, Fig. 56). The result of this movement is that on the left side the X-cells occupy a broader area, when seen from below (Pl. XXXIX, Fig. 48), than do those of the right of the median plane. In much later stages (Pl. XL, Figs. 57 and 58) a small cell lies wedged in between the cells of the prototroch in the C quadrant. This cell, I believe, is a descendant of $X_{1.1.1.1}$, which has been separated from the X-group by the migrating cells from the upper hemisphere. In later stages the large $X_{3.2}$ divides symmetrically (Pl. XL, Fig. 58), but I have been able to discover no other symmetrical divisions. Neither could I find any divisions, such as Mead has described in Amphitrite, which, though themselves asymmetrical, had direct reference to the symmetry of the trochophore.

The Third Quartette.—At the 64-cell stage these are eight in number, two in each quadrant. We have already seen that the next division establishes bilateral symmetry at the upper pole. Symmetry also appears at the same time at the lower pole as a result of the peculiar division of two of the third group and one of the fourth. Leaving the latter for the present with merely the remark that it is 4d and contains the mesoderm, let us notice the division of the third quartette cells (Pl. XXXVIII, Figs. 26 and 27). Here $3d_2$ and $3c_2$, lying one on either side of 4d, are dividing in a bilaterally symmetrical fashion. (The second line of cleavage in the specimen from which Pl. XXXVIII, Fig. 27, was drawn had changed its direction. This is rare in this stage and I have never found it in earlier stages before $X_{1.2}$ is formed.) The outer product of the division in both

cases is the smaller and may be neglected in the further description. The inner products show from the first a tendency to invaginate (see Pl. XXXVIII, Fig. 28), where their nuclei lie beneath the surface. Later each cell divides dorsally a small cell, coming to the surface to divide (Pl. XXXVIII, Figs. 35, $3d_{2.1}$ and $3d_{2.2}$, and 36, $3c_{2.1.2}$ and $3d_{2.2.2}$). Their nuclei immediately start to invaginate again. Finally each nucleus comes to the surface and a very small cell is budded off (Pl. XL, Figs. 50 and 51), and all four of the cells thus formed invaginate to give rise to a large part of the *larval mesoblast*, which is entirely the functional mesoblast of the larva. As we shall see immediately, the larval mesoblast is completed by the addition of other cells from another quadrant.

Of the other products of 3d and 3c little need be said; $3d_1$ and $3c_1$ divide meridionally soon after the first division of $3d_2$ and $3c_2$ (Pl. XXXVIII, Fig. 28, $3d_1$), and a number of later divisions have been noted. (See Pl. XXXIX, Figs. 40, 42, 44, 46, and table, p. 438.) They form a portion of the ventral ectoderm of the trochophore, take part in the closure of the blastopore, and make up a part of the proctodaeal wall. (See Pl. XXXIX, Fig. 46.)

The first division of the third group of micromeres in the A and B quadrants is shown in Pl. XXXVIII, Fig. 28, where $3a_2$ and $3b_2$ are dividing meridionally. A little later $3a_1$ and $3b_1$ divide also meridionally (Pl. XXXVIII, Figs. 34 and 35). The next division in A quadrant is of cell $3a_{2.2}$ (Pl. XXXIX, Fig. 41). This divides unequally, sending a small cell upward, $3a_{2.2.1}$ (Pl. XL, Fig. 50), while the larger lower portion begins to invaginate (Pl. XL, Figs. 50 and 51). This cell, $3a_{2.2.2}$, passes into the segmentation cavity and becomes *larval mesoblast*. Originally situated at the left of the median plane, it passes forward and lies directly in this plane. Its next division is equal. (See Pl. XL, Fig. 52, and Text-Fig. 7.) From this time on its products lie symmetrically on either side of the median plane. A corresponding cell in B quadrant divides at about the same time as this and apparently invaginates (Pl. XL, Figs. 49 and 51). I at first supposed that this also becomes *larval mesoblast*. Careful examination, however, fails to show

any trace of this cell lying in the segmentation cavity, and all the larval mesoblast of the anterior end of the body, as is unmistakably shown by the specimens, comes from the cell $3a_{2.2.2}$. I have been unable to trace the cell $3b_{2.2.2}$ with any accuracy after the stage shown in Pl. XL, Fig. 51, but from its position I suppose that it aids in the formation of the stomodaeum. The entoderm cells have divided repeatedly, and at this time are no larger than the cells at the edge of the blastopore, so that it is difficult to distinguish between the two sets.

The further history of the other descendants of 3a and 3b, so far as I have followed it, is given in Pl. XXXIX, Figs. 46 and 47, and in the table, p. 438. Detailed description would be profitless. They form a part of the stomodaeum and the general ectoderm of the subumbrella.

Larval Mesoblast.

This arises in Podarke, as we have seen, from descendants of 3d, 3c, and 3a. The two former are symmetrically arranged from the beginning, while the latter only become so placed after invagination and division. It is important to note that this division, which occurs in $3a_{2.2.2}$, *after* its invagination, corresponds to the division which in the other cells gave rise to the small cell ventrally, and which takes place at the surface. In both cases both products of the division become mesoblast, so that all the cells of the larval mesoblast belong to the same generation. I have indicated these cells as *right*, *l.m.r.*, *left*, *l.m.l.*, and *median*, *l.m.m.* After the next division, immediately to be described, the smaller product of the posterior larval mesoblast, when seen from the side, lies over the large $4d_2$ cells (Pl. XL, Fig. 56), while the others have spread farther apart in the cleavage cavity.

The larger products of the posterior larval mesoblast divide next, and there is formed a band of three cells on either side of the median line (Pl. XL, Figs. 52 and 54, and Text-Fig. 7). Since the posterior end of each band lies very close to the definitive mesoblast (colored a deeper pink in the figures), the effect is that of two well-developed mesoblast bands, lying in the

usual position in the segmentation cavity, and they would undoubtedly be described as such by any one who saw merely the preparation figured in Pl. XL, Fig. 54. Very soon, however, the larval mesoblast cells begin to migrate (Pl. XL, Figs. 53, 55, 56). In Pl. XL, Fig. 56, is shown the position of the posterior larval mesoblast, while the anterior, at this stage, is composed of four cells on a side. The cells elongate and become the larval musculature. They are especially well developed in the region of the prototroch, under which the long, spindle-shaped cells may easily be recognized in later stages. (See Text-Fig. 8.)

Comparative. — A larval mesoblast was first discovered by Lillie (No. 21) in Unio, where it arises asymmetrically in the A quadrant only, from the second group of micromeres, $2a_2 +$. This migrates so as to lie symmetrically in the cleavage cavity, and by its division are formed cells which become metamorphosed into the "myocites" and larval adductor muscles, which are functional only during larval life. Later Conklin (No. 5, *a*), in Crepidula, found larval mesoblast arising from the second quartette in three quadrants, A, B, and C. In the left-wound gasteropods, Physa and Planorbis, which have the "reversed" type of cleavage, Wierzejksi (No. 31) and Holmes (No. 15, *b*) found it arising from the third quartette in B and C quadrants ; in these reversed cleavages, therefore, symmetrically arranged. In annelids, thus far, the structure in question has been found only in Aricia, Capitella, and Podarke. In Aricia, Wilson (No. 34, *g*) finds two cells arising symmetrically from either the second or third quartette. Through lack of material he was unable to discover its exact origin. In Capitella, Eisig (No. 8) finds it arising from the ventral products of the second division of 4d, thus corresponding in origin to cells which in other annelids become entoderm. (See p. 451.) To this point I shall return later.

From the figures which Hatschek gives for Eupomatus, it seems to me extremely probable that a larval mesoblast is present there also, although the point is not one to which Hatschek paid any attention. His figures (Pl. XXXIX, Fig. 42 to Pl. XL, Fig. 49) show scattered muscle cells in the upper hemisphere of

the larva, which could hardly have come from the feebly developed mesoderm bands at the posterior end of the body. The same suggestion would apply to Pomatoceros, as described by von Drasche (No. 7), where Pl. XXXVII, Fig. 20, shows a muscle cell just underneath the apical pole, and the small compact mass of seven mesoblast cells, showing no trace of differentiation, quite at the other end of the larva.

The cell origin of the larval mesoblast in the forms where it has been thus far described is as follows :

Unio	$2a_{2 \cdot 1}$ +.
Crepidula	2a, 2b, 2c.
Physa . . ' . . .	3b, 3c.
Planorbis	3b, 3c.
Aricia	2nd or 3rd quartette.
Capitella	$4d_{2 \cdot 2}$, $4d_{1 \cdot 2}$.[1]
Podarke	$3a_{2 \cdot 2 \cdot 2}$, $3c_{2 \cdot 1 \cdot 2}$, $3d_{2 \cdot 2 \cdot 2}$.

Fourth Quartette. — At the 64-cell stage the fourth group of micromeres has just been formed, and, so far as one can tell with the microscope, are all exactly alike. Very soon, however, as we have seen, one of the cells divides bilaterally, thus aiding in the establishment of the bilateral symmetry of the body. These cells, like those of the third quartette just adjoining them, early show a tendency to invaginate, and they divide at the same time as the latter, each budding off ventrally and posteriorly a very small cell (Pl. XL, Fig. 51). The two then invaginate and can be seen through the transparent X-cells lying just underneath the surface. It is important to notice, however, that at the same time that these cells invaginate a gastrulation begins, and the entoderm cells push in at the same time as the cells $4d_1$ and $4d_2$. (Since until the stage of Pl. XL, Fig. 57, the mesoderm is not fully differentiated, I have retained the designation $4d_1$, etc., for the descendants of 4d in the earlier figures. The small cells which enter into the archenteric wall I have indicated by *en* in Pl. XL, Figs. 51–57.) This accounts for the fact that the descendants of 4d undergo such extensive shiftings of position without at the same time

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[1] Here, as elsewhere in the comparative portion of this paper, I have modified the nomenclature to conform with that adopted for Podarke. (See also p. 465.)

losing their connection with the wall of the archenteron. The two small cells described as budded posteriorly from $4d_1$ and $4d_2$ form a part of the wall of the archenteron. Since the greater
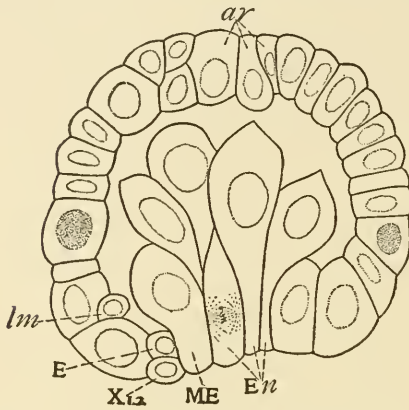
Fig. 5.— Beginning of entodermal invagination. *En*, entoderm; *lm*, larval mesoblast; *ME*, 4d before the final separation of mesoderm from entoderm; *E*, the first entoderm cell budded off from $4d_2$. $X_{1.2}$, the "anal" cell.

part of 4d gives rise to the definitive mesoblast, it is therefore, to use Conklin's term, a mesentoblast cell.

The cells $4d_{1.1}$ and $4d_{2.1}$ at first lie in the wall of the archenteron, though they protrude considerably into the segmentation cavity. Consequently, in a section passing a little to one side of the sagittal plane, they seem to lie wholly in this cavity (Text-Fig. 6). Each buds off a second small cell (Pl. XL, Fig. 56), and the larger portion is the definitive mesoblast, M_1 and M_2. Considerably later (Fig. 57, Pl. XL, and Text-Fig. 8) each divides equally. Only until this last division do these cells entirely lose their connection with the archenteric wall. Text-Fig. 8 is an optical section of the specimen drawn in Fig. 57, and shows that the descendants of 4d are still connected with the entoderm. I have actual

Fig. 6. — Later stage of invagination. *a*, archenteric cavity.

sections of this stage which show exactly the same thing. The products of the last division migrate rapidly through the segmentation cavity, retaining approximately their original

direction, at right angles to the prototroch, and eventually lie on either side the ventral surface, just underneath the adoral zone of cilia (Pl. XL, Fig. 59). This is the latest stage to which I have carried the mesoderm cells in what I am positive were normal embryos. In older embryos I have found a large pole cell occupying a position like the posterior one of the two cells figured in Fig. 59, while a row of small cells extended anteriorly from it. The difficulties experienced in getting larvae to develop after the third day have as yet prevented me from securing any complete details of the later history of these cells, and since the study of the trochophore development has been taken up by another worker, I have thought it best to leave the subject at this point. In calling these cells the germ bands, I am not relying entirely on analogy with other annelids, although the resemblance is close, but on the fact that both ectoderm and entoderm are fully differentiated at this stage, and these cells, lying in the segmentation cavity, are the only source from
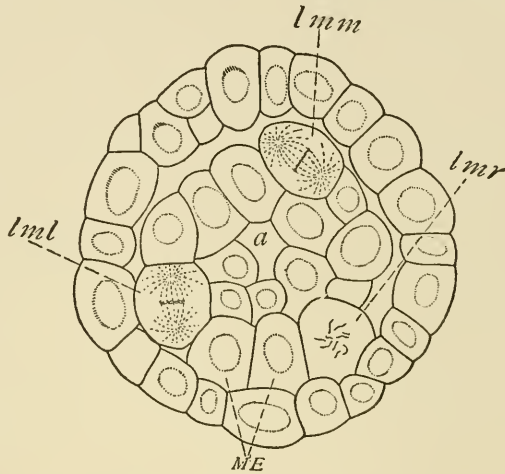
FIG. 7. — Horizontal optical section through Fig. 52, Pl. XL, taken at level of larval mesoblast. *lmr, lml, lmm,* right, left, and median larval mesoblast. Other letters as before.
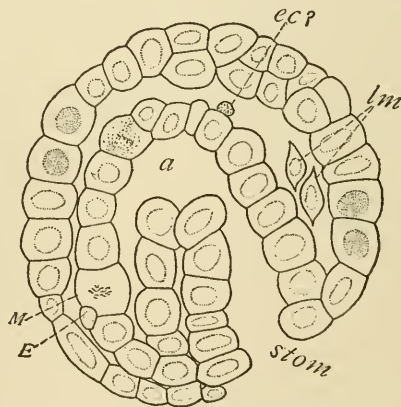
FIG. 8. — Sagittal section through stage of Fig. 57, Pl. XL. *ec,* ectodermal cell; *M,* definitive mesoblast, in its first division after eliminating all the entodermal elements; *stom,* stomodaeum.

which mesoderm can arise, unless we assume an ectodermal origin, which is not probable. Hence I have no hesitation in saying positively that they are the germ bands.

The second pair of small cells, like the first, enter into the wall of the archenteron.

Comparative. — In all annelids, gasteropods, and lamellibranchs thus far studied, with one exception, the definitive mesoblast arises from 4d. The exception is Capitella, where, according to Eisig, 4d contains only a little larval mesoblast, while the greater part of the cell is ectodermal and enters into the ventral plate. The definitive mesoblast in this form arises from 3c and 3d. (See p. 451.) In Nereis, Wilson (No. 34, *d*) described small cells budded off ventrally from $4d_1$ and $4d_2$, which he at first called "secondary mesoblast," and supposed they lay on the dorsal wall of the archenteron. In Aricia only one of these cells appears on either side. Later, however, Wilson has shown (No. 34, *g*) that the so-called secondary mesoderm cells do not form mesoderm at all, but really become a part of the wall of the archenteron. In Amphitrite (No. 22), Polymnia [1] (No. 34, *d*), and Arenicola (No. 4) this entodermal portion is lacking in the cell 4d. In Amphitrite, Mead described a very small cell at the anterior end of the germ band, which he suggests may be a reminiscence of the superficial budding which takes place at the surface in other forms, and Wilson has definitely homologized the two sets of cells. Inasmuch, however, as these cells always remain in the mesoderm, such a comparison seems to me doubtful, and, if *vestigial at all*, they represent rather some mesodermal structure. As I have pointed out on p. 472, the small size of cells when first formed is no proof of their vestigial character, since they may subsequently undergo any amount of growth. In Clymenella, Mead (No. 22) described the first division of $4d_1$ and $4d_2$ as equal instead of the very unequal division of Nereis or Aricia. Wilson (No. 34, *g*) has suggested that the posterior portion of each of these cells is entoderm. While this is possible, it has not been proved, and hence the structure is of little value for comparison.

[1] See, however, Wilson (No. 34, *g*), p. 12 of reprint.

Among the mollusks Conklin (No. 5, *a*) has shown that 4d in Crepidula is more than half entoderm. It is not impossible that a portion of this cell in Unio may have a similar fate, and a close relation between mesoderm and entoderm has been described for Cyclas (No. 28) and Patella (No. 26), though the precise cell origin of the mesentoblasts in the latter cases was not determined. These observations seem to indicate a lack of uniformity in the mode of origin of the mesoblast and also show an *apparently* close relation between mesoblast and entoblast. To this point I shall return later. (See p. 449.)

The other members of the fourth quartette apparently invaginate. (See their small superficial area in Pl. XXXVIII, Figs. 33 and 35.) In Pl. XXXVIII, Fig. 34, the nucleus of 4a shows underneath the 2a cells. In some cases I was unable, in the stained specimens, to see the outlines of the cells. This fact led me to the erroneous statement in my preliminary paper (No. 29, *a*) that they invaginate before dividing. As a matter of fact, the cells come to the surface and divide equally (Pl. XXXVIII, Fig. 36), and then immediately invaginate, entering into the wall of the archenteron.

The Fifth Quartette. — A fifth series of four cells arising from the entomeres has been called the fifth quartette of micromeres by other workers, and in my preliminary note (No. 29, *a*) I emphasized the fact that in Lepidonotus, as in Podarke, such a quartette appears. The term, however, is misleading, since the cells thus formed are entodermal in destiny; and we must regard this division as corresponding to the division which, in other forms, takes place after invagination. The only difference between the two cleavages is that one takes place at the surface and the other below it. The one is as truly a fifth quartette of micromeres as the other.

This division in Podarke is shown in Pl. XXXVIII, Fig. 33, occurring in C and D quadrants, and later it appears in quadrants A and B (Pl. XXXVIII, Fig. 34). The eight cells thus formed, together with six derived from the division of three members of the fourth quartette, make up an invaginating plate of fourteen cells, which rapidly invaginates, its cells meanwhile dividing again; and the alimentary canal of the embryo is

quickly mapped out. The details of this process I am unable
to give, for it is impossible on surface views to see the outlines
of the cells; and on account of the small size of the embryos,
sections are very unsatisfactory objects for study, the difficulties
in the way of accurate orientation being too great. I can only
say that the alimentary canal is rapidly formed, being practi-
cally completed by the twenty-third hour of development. As
in Nereis (Nos. 34, *d* and *g*), the posterior ectoderm cells bud
off some small cells, which enter into close relations with the
entodermal portion of 4d. Some stages in the invagination and
hollowing out of entoderm cells are shown in Text-Figs. 5, 6,
7, and 8.

The stomodaeum, as already stated, is formed by cells from
both the second and third quartettes. The invagination which
some of these cells undergo is shown most clearly by the aid of
the landmark furnished by the cells $2b_{2.2.1.2}$. already described
on p. 420. The anterior portion of the blastopore becomes the
mouth, and its sides are closed by cells from both the second
and third quartettes. The proctodaeum arises at the posterior
end of the blastopore, which I do not believe ever completely
closes. Thus both mouth and anus arise from it (*cf.* Conn,
No. 6). The cell $X_{1.2.2}$, as already stated, early invaginates to
form a part of the proctodaeal wall, but the exact origin of the
other cells which invaginate I was unable to determine. From
their position in earlier stages, I believe that the cells $3c_{2+}$ and
$3d_{2+}$ are the ones most deeply concerned, but the small size of
the cells and the shiftings of position which they undergo make
it impossible to be certain. (See Pl. XXXIX, Fig. 46.)

The trochophore is now fully formed. The apical tuft of
cilia still remains at the anterior end of the umbrella, and about
midway between this and the prototroch is a second tuft. (See
Pl. XL, Fig. 60.) The prototroch is now a complete ring, the
origin of its cells having already been described. An adoral
zone of cilia extends from the mouth to the anus (" Neurotro-
choid " of Eisig), but no paratroch is present. On the ventral
surface of the umbrella are the two orange-colored "eye-spots,"
and the large clear spaces, which are undoubtedly similar in
origin and function to the "frontal bodies" of Nereis and the

"problematic bodies" of Amphitrite. In Nereis, Wilson sup-
poses each to arise from a single cell, which becomes vacuolated
and probably has a glandular function. In Podarke there are
two on a side, closely crowded together just in front of the eye-
spot, and a fifth very small one on the median line in front.
They stain very deeply with haematoxylin, an outer portion
(Wilson's "duct") staining much more deeply than the inner.
From the number of nuclei surrounding each "body" in the
early stages, I think they are formed from more than one cell.
Their staining reactions indicate that they have a glandular
function, and they are apparently the only glands in the body.
In the case of these glands, as well as in that of the so-called
"slime glands" in other annelids, I agree with Eisig (No. 8),
that they probably have an excretory rather than a slime secret-
ing function, the necessity for the one and not for the other
being apparent.

A marked feature of the larva of thirty hours and later is a
circular shelf, which extends around the cavity of the archen-
teron and, when seen from the side, apparently divides it into
distinct compartments (Pl. XL, Fig. 60). Careful observa-
tion shows, however, that this is really a broad, very thin shelf
of tissue. The opening through it is at first at the center of the
ectoderm cavity; later it lies at the dorsal side of the enteron.
Just above it is a tuft of long cilia, a modification of the general
ciliation of the archenteron. (See Pl. XL, Fig. 60.) The first ap-
pearance of this structure is at about twenty-three hours, when a
slight constriction appears at about the middle of the archenteron.
Partly by the swelling out of the archenteron above and below it,
and partly by an active growth of the cells involved, is formed a
circular shelf of tissue extending out into the cavity of the ali-
mentary canal. In embryos of about thirty hours, if the speci-
men be rolled so that the proctodaeum looks upward, careful
focusing through the proctodaeal opening shows this circular
shelf running entirely around the archenteron, and extending
about one-half the way across its cavity. The organ at this
stage bears a very striking resemblance to the velum of a
medusa. Later it becomes very thin and vacuolated, and the
details of its structure are difficult to make out. The original

opening, as I believe, moves backward to the position described above, and I think other openings break through it, but of this I am not certain. When first formed, the partition is ciliated above and below. I do not know how long that condition lasts. (See Pl. XL, Fig. 60.) The section passed a little to one side of the central opening, which therefore does not show in the drawing.

The suggestion has been made that this partition represents the first septum, but I do not believe that is so. So far as I can discover, only entoderm cells enter into it. It seems to me rather to lie at the boundary line between "stomach" and "intestine" of the larva, and to be merely an exaggeration of the constriction found at that point in other annelid larvae. (See No. 7, Pl. XXXVIII, Fig. 26.)

As already stated on p. 435, the wall of the archenteron swells out so as to lie close under the ectoderm. Since dorsally both layers are very thin and transparent, it becomes difficult, on surface views, to make out the outlines of the cells. Indeed, it frequently is impossible, except by rolling the specimen so as to get an optical section, to determine to which layer a given nucleus belongs. Ventrally (Pl. XL, Fig. 60), the ectoderm is much thicker.

At this point the cell lineage naturally ends, and the study of the trochophore begins. Since the problems of the latter subject are so different from those of the former, it has seemed best to reserve the metamorphosis of the trochophore for another paper.

Comparison with other Annelids having "equal" Cleavage. — The most complete account of the cleavage of an equally segmenting annelid previously published is that of Mead (No. 22) on Lepidonotus. To Dr. Mead's generosity I have been indebted for preparations of the later stages. A few observations have also been made on the cleavage of *Hydroides dianthus* and *Sthenolais picta*, both of which resemble Podarke in their mode of cleavage. I have not as yet carried the development of these forms far enough to justify a detailed account of the process, but enough has been seen to show as I pointed out in a preliminary paper (No. 29, *a*) that bilateral

symmetry appears at practically the same time here as in Podarke, and that there is as much differentiation in the ovum as in any annelid with the unequal type of segmentation. The theoretical bearings of this fact I shall discuss later.

Table of Cleavages.

I have followed all divisions up to about 140 cells ; later than this only especial groups were followed. All of these divisions are indicated in the accompanying tables. The well-marked stages of the earlier cleavages are denoted by the numbers at the top of the column, but after eighty cells these numbers must be regarded as only approximate. Each table gives the divisions of a single quadrant and begins with the 4-cell stage. The direction of each division is indicated by the straight line / inclined to the right for a dexiotropic, to the left for a leiotropic, vertical for a meridional, and horizontal for a horizontal cleavage. If it be remembered that the line always has the direction of the boundary plane between the two cells, I think the table will be readily understood. When the products of a division are equal, that is indicated by the sign of equality. If unequal, by the sign of inequality, placed across the direction line. A plus (+) sign after a cell number indicates that it has been seen to divide again, but its divisions were not followed.

In all cases the daughter-cells resulting from a division are put in the column indicating the number of cells in the whole embryo immediately after the division of these cells. Thus, at the 16-cell stage, 2A divides dexiotropically and unequally, the larger cell lying below. At the completion of this division the whole embryo contains twenty-four cells. The smaller cell, 3a, does not divide again, until about forty cells, and when this division is completed the whole embryo contains fifty-six cells, etc.

PART II. GENERAL CONSIDERATIONS.

Axial Relations.

The axial relations of the first cleavage planes have been described above (p. 405). Those of the trochophore agree with Amphitrite. The mesoblast bands occupy from the first their definitive position at right angles to the prototroch. Although the X-cells, as in Nereis, occupy a final position much farther from the prototroch than they were at first, the cells between them and the prototroch have a very different origin from the cells in a similar position in Nereis, and I have seen no evidence for a shifting of the neural axis, such as has been described by Wilson (No. 34, *d*). The descendants of the $X_{3.2}$ group form a row of large cells across the body which look like teloblasts, but I have no evidence that they really have that function. (See p. 423.) I do not think that all of the ventral ectoderm of the trochophore arises from the X-cells. All the evidence indicates that some of the other second, and probably to a limited extent the third, quartette cells have this fate. (See Pl. XXXIX, Fig. 46.)

Types of Cleavage.

In Podarke, as in other annelids, and in gasteropods and lamellibranchs, two types of cleavage, the radial and the bilateral, have been distinguished, the former appearing first, and being gradually displaced by the latter as development proceeds. The subject has been thoroughly discussed by Wilson (No. 34, *d*), Mead (No. 22), and Conklin (Nos. 5, *a* and 5, *b*), and I need not go over the ground again. As I have said in another place (see p. 470), the most reasonable explanation of these cleavages to my mind is that they were primarily mechanical, and that morphogenetic processes have been secondarily moulded upon the primitive forms. The first of these causes leads to the radial or spiral divisions which appear first, and the second to the bilateral divisions which have a direct reference to the bilaterality of the future body. I agree perfectly with

$A \neq$

$1a \nearrow$

$1a_1 \uparrow$

$1a_{1.1} \nwarrow$

$1a_{1.1.1} \neq$ — — — $\begin{cases} 1a_{1.1.1.1} \\ 1a_{1.1.1.2} \end{cases}$ rosette.

$1a_{1.1.2} \nearrow$ — — — $1a_{1.1.2.1} \nearrow$ — — — $\begin{cases} 1a_{1.1.2.1.1} + \\ 1a_{1.1.2.1.2} \nwarrow \end{cases}$ — — — $\begin{bmatrix} 1a_{1.1.2.1.2.1} + \\ 1a_{1.1.2.1.2.2} \end{bmatrix}$

$1a_{1.1.2.2} \neq$ $\begin{cases} 1a_{1.1.2.2.1} \nwarrow \cdots \begin{cases} 1a_{1.1.2.2.1.1} + \\ 1a_{1.1.2.2.1.2} + \end{cases} \\ 1a_{1.1.2.2.2} \neq \cdots \begin{cases} 1a_{1.1.2.2.2.1} \nwarrow — — — \begin{bmatrix} 1a_{1.1.2.2.2.1.1} \\ 1a_{1.1.2.2.2.1.2} \end{bmatrix} \\ 1a_{1.1.2.2.2.2} + \end{cases} \end{cases}$ Ectoderm of umbrella.

$1a \uparrow$

$1a_{1.2} \neq$ — — — — — $1a_{1.2.1} \neq$ — — — — $\begin{cases} 1a_{1.2.1.1} \\ 1a_{1.2.1.2} \end{cases}$ Ectoderm of umbrella.

$1a_{1.2.2} \nearrow$ — — — — $\begin{cases} 1a_{1.2.2.1} \\ 1a_{1.2.2.2} \end{cases}$ Secondary trochoblast.

$1a_2 \neq$ — — — — $\begin{cases} 1a_{2.1} \neq \\ 1a_{2.2} \neq \end{cases}$ — — — $\begin{cases} 1a_{2.1.1} \\ 1a_{2.1.2} \\ 1a_{2.2.1} \\ 1a_{2.2.2} \end{cases}$ Primary trochoblasts

$2a \neq$ — — — — — $2a_1 \nwarrow$ — — — — — $\begin{cases} 2a_{1.1} \nearrow \\ 2a_{1.2} \swarrow \end{cases}$ — — $\begin{cases} 2a_{1.1.1}\ \text{Ectoderm of subumbrella.} \\ 2a_{1.1.2} \end{cases}$ Tertiary trochoblasts. $\begin{cases} 2a_{1.2.1} \end{cases}$ $2a_{1.2.2}$ Ectoderm of subumbrella.

$2a_2 \nwarrow$ — — — — $\begin{cases} 2a_{2.1} \neq — — — \begin{cases} 2a_{2.1.1} + \\ 2a_{2.1.2} + \end{cases}\ \text{Ectoderm of subumbrella.} \\ 2a_{2.2} \nwarrow — — \begin{cases} 2a_{2.2.1} \neq — — — \begin{bmatrix} 2a_{2.2.1.1} \\ 2a_{2.2.1.2} \end{bmatrix}\ \text{Ectoderm of subumbrella.} \\ 2a_{2.2.2} + — — — — \end{cases} \end{cases}$

$1A \neq$

$2A \uparrow$

$3a \nwarrow$ — — — — $3a_1 \neq$ — — — — $\begin{cases} 3a_{1.1} + \\ 3a_{1.2} + \end{cases}$ Ectoderm of subumbrella.

$3a_2 \neq$ — — — — $\begin{cases} 3a_{2.1} \\ 3a_{2.2} \nearrow — — — — 3a_{2.2.1} \end{cases}$ stomodaeum wall. $3a_{2.2.2}$ larval mesoblast.

$3A \nwarrow$ — — — — $4a \neq$ — — — — $\begin{cases} 4a_1 + \\ 4a_2 + \end{cases}$ Entoderm.

$4A \neq$ — — — — $\begin{cases} 5a + \\ 5A + \end{cases}$

no. of cells	4	8	16	24	28	32	40	56	64	80 to 100	100 to 150	150 and over

$1b1.1.1. \neq$

$\left[\begin{array}{l} 1b1.1.1.1 \\ 1b1.1.1.2 \end{array}\right]$ rosette.

$1b1.1. \nwarrow$

$1b1.1.2. \nearrow$

$1b1.1.2.1. \nwarrow \cdots \left\{\begin{array}{l} 1b1.1.2.1.1. + \\ 1b1.1.2.1.2. + \end{array}\right.$

$1b1.1.2.2. \# \cdots \left\{\begin{array}{l} 1b1.1.2.2.1. \nwarrow \\ 1b1.1.2.2.2. \nwarrow \end{array}\right. \left\{\begin{array}{l} 1b1.1.2.2.1.1. + \\ 1b1.1.2.2.1.2. + \\ 1b1.1.2.2.2.1. + \\ 1b1.1.2.2.2.2. + \end{array}\right\}$ Ectoderm of umbrella.

$1b1. \nwarrow$

$16 \nwarrow$

$1b1.2. \neq$

$1b1.2.1. \neq \left\{\begin{array}{l} 1b1.2.1.1. + \\ 1b1.2.1.2. + \end{array}\right.$ Ectoderm of umbrella.

$1b1.2.2. \left\{\begin{array}{l} 1b1.2.2.1. + \\ 1b1.2.2.2. \end{array}\right.$ Secondary trochoblast.

$1b2. \neq \left\{\begin{array}{l} 1b2.1. \# \\ 1b2.2. \# \end{array}\right. \left[\begin{array}{l} 1b2.1.1. \\ 1b2.1.2. \\ 1b2.2.1. \\ 1b2.2.2. \end{array}\right]$ Primary trochoblasts.

$B \neq$

$2b. \neq \left\{\begin{array}{l} 2b1. \nwarrow \\ 2b2. \nwarrow \end{array}\right.$

$2b1. \nwarrow \cdots \left\{\begin{array}{l} 2b1.1. \nwarrow \\ 2b1.2. \nwarrow \end{array}\right.$

$2b1.1. \nwarrow \cdots \left\{\begin{array}{l} 2b1.1.1. \cdots \text{ Ectoderm of subumbrella.} \\ 2b1.1.2. \end{array}\right.$ Tertiary trochoblasts.

$2b1.2.1. \\ 2b1.2.2. \cdots$ Ectoderm of subumbrella.

$2b2.1. \# \cdots \left\{\begin{array}{l} 2b2.1.1. \\ 2b2.1.2. \end{array}\right.$ ectoderm of subumbrella.

$2b2.2. \nwarrow \left\{\begin{array}{l} 2b2.2.1. \wedge \cdots \left\{\begin{array}{l} 2b2.2.1.1. + \text{ Ectoderm of subumbrella} \\ 2b2.2.1.2 \end{array}\right. \\ 2b2.2.2. \neq \cdots \left\{\begin{array}{l} 2b2.2.2.1. \\ 2b2.2.2.2. \end{array}\right\} \text{ stomodaeal. wall.} \end{array}\right.$

$1B \neq$

$2B \nwarrow$

$3b \nwarrow \left\{\begin{array}{l} 3b1. \neq \cdots \left\{\begin{array}{l} 3b1.1. + \\ 3b1.2. + \end{array}\right\} \text{ Ectoderm of subumbrella.} \\ 3b2. \neq \left\{\begin{array}{l} 3b2.1. \neq \cdots \left[\begin{array}{l} 3b2.1.1. \\ 3b2.1.2 \end{array}\right] \begin{array}{l} \text{Ectoderm.} \\ \text{and} \\ \text{stomodaeum.} \end{array} \\ 3b2.2. + \end{array}\right. \end{array}\right.$

$3B \nwarrow$

$4b \# \cdots \left\{\begin{array}{l} 4b1+ \\ 4b2+ \end{array}\right.$

$4B \neq \cdots \left\{\begin{array}{l} 5b+ \\ 5B+ \end{array}\right.$ entoderm.

$C \neq$

$1C \downarrow$

$1c_1 \downarrow$
- $1c_{1.1} \uparrow$
  - $1c_{1.1.1} \neq$ — $\left\{ \begin{array}{l} 1c_{1.1.1.1} \\ 1c_{1.1.1.2} \end{array} \right\}$ rosette.
  - $1c_{1.1.2} \uparrow$
    - $1c_{1.1.2.1} \neq$
      - $1c_{1.1.2.1.1} \neq$ — $\left\{ \begin{array}{l} 1c_{1.1.2.1.1.1} + \\ 1c_{1.1.2.1.1.2} + \end{array} \right.$
      - $1c_{1.1.2.1.2} \uparrow$ — $\left\{ \begin{array}{l} 1c_{1.1.2.1.2.1} + \\ 1c_{1.1.2.1.2.2} + \end{array} \right.$
    - $1c_{1.1.2.2} \uparrow$
      - $1c_{1.1.2.2.1}$
      - $1c_{1.1.2.2.2} \uparrow$ — $\left\{ \begin{array}{l} 1c_{1.1.2.2.2.1} \\ 1c_{1.1.2.2.2.2} \neq \left\{ \begin{array}{l} 1c_{1.1.2.2.2.2.1} + \\ 1c_{1.1.2.2.2.2.2} + \end{array} \right. \end{array} \right.$

Ectoderm of umbrella.

- $1c_{1.2} \neq$
  - $1c_{1.2.1} \neq$ — $\left\{ \begin{array}{l} 1c_{1.2.1.1} + \\ 1c_{1.2.1.2} + \end{array} \right.$
  - $1c_{1.2.2} \uparrow$ — $\left\{ \begin{array}{l} 1c_{1.2.2.1} + \\ 1c_{1.2.2.2} \end{array} \right.$ secondary trochoblast.

$1c_2 \neq$
- $1c_{2.1} \neq$ — $\left\{ \begin{array}{l} 1c_{2.1.1} \\ 1c_{2.1.2} \end{array} \right.$ Primary
- $1c_{2.2} \neq$ — $\left\{ \begin{array}{l} 1c_{2.2.1} \\ 1c_{2.2.2} \end{array} \right]$ trochoblasts.

$2C \neq$

$2c_1 \neq$
- $2c_{1.} \uparrow$
  - $2c_{1.1} \uparrow$ — $\left\{ \begin{array}{l} 2c_{1.1.1} \text{ ectoderm. of subumbrella.} \\ 2c_{1.1.2} \end{array} \right.$ Tertiary trochoblasts.
  - $2c_{1.2} \uparrow$ — $\left\{ \begin{array}{l} 2c_{1.2.1} \\ 2c_{1.2.2} : \text{ Ectoderm of subumbrella.} \end{array} \right.$
- $2c_{2.} \uparrow$
  - $2c_{2.1} \neq$ — $\left\{ \begin{array}{l} 2c_{2.1.1} + \\ 2c_{2.1.2} + \end{array} \right.$
  - $2c_{2.2} \downarrow$ — $\left\{ \begin{array}{l} 2c_{2.2.1} \downarrow \\ 2c_{2.2.2} \neq \end{array} \right.$ — $\left\{ \begin{array}{l} 2c_{2.2.1.1} + \\ 2c_{2.2.1.2} + \\ 2c_{2.2.2.1} + \\ 2c_{2.2.2.2} + \end{array} \right\}$ Ectoderm of subumbrella.

$1C.$

$3c \uparrow$
- $3c_1 \neq$
  - $3c_{1.1} \neq$ — $\left\{ \begin{array}{l} 3c_{1.1.1} + \\ 3c_{1.1.2} + \end{array} \right.$ Ectoderm of subumbrella (and Proctodaeal wall?)
  - $3c_{1.2}.$
- $3c_2 \neq$
  - $3c_{2.1} \uparrow$ — $\left\{ \begin{array}{l} 3c_{2.1.1} + \\ 3c_{2.1.2} \downarrow \end{array} \right.$ — $\left\{ \begin{array}{l} 3c_{2.1.2.1} + \\ 3c_{2.1.2.2} + \end{array} \right\}$ Larval mesoblast.
  - $3c_{2.2} +$ ectoderm of subumbrella.

$2C \uparrow$

$4c \neq$ — $\left\{ \begin{array}{l} 4c_1 + \\ 4c_2 + \end{array} \right\}$ Entoderm

$3C \uparrow$

$4C \neq$ — $\left\{ \begin{array}{l} 5c + \\ 5C + \end{array} \right.$

Cell-lineage chart (column headings, left to right): number of cells — 4, 8, 16, 24, 28, 32, 40, 56, 64, 80 to 100, 100 to 150, 150 and over.

$D\neq$

1d lineage (ectoderm of umbrella, migrating cells, primary trochoblasts):

$1d\,⋎$
- $1d_1\,⋎$
  - $1d_{1.1}↰$
    - $1d_{1.1.1.}\neq$ — $\begin{cases}1d_{1.1.1.}\\1d_{1.1.2.}\end{cases}$ rosette.
    - $1d_{1.1.2.1}↰$
      - $1d_{1.1.2.1.}⋎$ — $\begin{cases}1d_{1.1.2.1.1.}\neq\\1d_{1.1.2.1.2.}\neq\end{cases}$ — $\begin{cases}1d_{1.1.2.1.1.2.1.}+\\1d_{1.1.2.1.1.2.2.}+\end{cases}$; $\begin{cases}1d_{1.1.2.1.2.1.}+\\1d_{1.1.2.1.2.2.}+\end{cases}$
      - $1d_{1.1.2.2.}↰$ — $\begin{cases}1d_{1.1.2.2.1.}\\1d_{1.1.2.2.2.}↰\end{cases}$ — $\begin{cases}1d_{1.1.2.2.2.1.}\neq\end{cases}$ — $\begin{cases}1d_{1.1.2.2.2.1.}+\\1d_{1.1.2.2.2.2.2.}+\end{cases}$
  - $1d_{1.2.}\neq$
    - $1d_{1.2.1.}\neq$ — $\begin{cases}1d_{1.2.1.1.}+\\1d_{1.2.1.2.}+\end{cases}$
    - $1d_{1.2.2.}\neq$ — $\begin{cases}1d_{1.2.2.1.}\\1d_{1.2.2.2.}\end{cases}$ migrating cells from upper hemisphere. Figs. 45, 57, &c.

ectoderm of umbrella. (right-hand bracket label)

- $1d_2\neq$
  - $1d_{2.1.}\neq$ — $\begin{cases}1d_{2.1.1.}\\1d_{2.1.2.}\end{cases}$
  - $1d_{2.2.}\neq$ — $\begin{cases}1d_{2.2.1.}\\1d_{2.2.2.}\end{cases}$ Primary trochoblasts.

1D → 2d / 2D:

$1D\neq$

$2d\neq$ (Subumbrellar ectoderm):
- $2d_1\,↰$
  - $2d_{1.1.}(x_3)↰$
    - $x_{3.1}↰$ — $\begin{cases}x_{3.1.1.}+\\x_{3.1.2.}\end{cases}$ — $\begin{cases}x_{3.2.1.}\\x_{3.2.2.}\end{cases}$
    - $x_{3.2.}\neq$
  - $2d_{1.2.}(x_2)↰$
    - $x_{2.1.}⋎$ — $\begin{cases}x_{2.1.1.}⋎\\x_{2.1.2.}+\end{cases}$ — $\begin{cases}x_{2.1.1.1.}+\\x_{2.1.1.2.}+\end{cases}$
    - $x_{2.2.}\neq$ — $\begin{cases}x_{2.2.1.}⋎\\x_{2.2.2.}\end{cases}$ — $\begin{cases}x_{2.2.1.1.}+\\x_{2.2.1.2.}+\end{cases}$; $\begin{cases}x_{2.2.2.1.}+\\x_{2.2.2.2.}+\end{cases}$

Subumbrellar ectoderm. (right bracket label)

- $2d_2\,⋎$
  - $2d_{2.1.}(x_{1.1})\neq$
    - $x_{1.1.1.}\,⋎$ — $\begin{cases}x_{1.1.1.1.}\\x_{1.1.1.2.}+\end{cases}$ — $\begin{cases}x_{1.1.1.1.1.}\\x_{1.1.1.2.2.}\end{cases}$
    - $x_{1.1.2.}\neq$
      - $x_{1.1.2.1.}\neq$ — $\begin{cases}x_{1.1.2.1.1.}↰\\x_{1.1.2.1.2.}⋎\end{cases}$ — $\begin{cases}x_{1.1.2.1.1.1.}\\x_{1.1.2.1.1.2.}\end{cases}$; $\begin{cases}x_{1.1.2.1.2.1.}\\x_{1.1.2.1.2.2.}\end{cases}$
      - $x_{1.1.2.2.}\neq$ — $x_{1.1.2.2.1.}↰$ — $\begin{cases}x_{1.1.2.2.1.1.}\\x_{1.1.2.2.1.2.}+\end{cases}$; $x_{1.1.2.2.2.}⋎$ — $\begin{cases}x_{1.1.2.2.2.1.}\\x_{1.1.2.2.2.2.}\end{cases}$
  - $2d_{2.2.}(x_{1.2})\neq$
    - $x_{1.2.1.}\neq$ — $\begin{cases}x_{1.2.1.1.}\\x_{1.2.1.2.}\end{cases}$ Proctodaeal wall.
    - $x_{1.2.2.}$

Ectoderm and proctodaeal wall.

2D → 3d / 3D:

$2D↗$

$3d↰$
- $3d_1\neq$ — $\begin{cases}3d_{1.1.}+\\3d_{1.2.}+\end{cases}$
- $3d_2\,⋎$ — $\begin{cases}3d_{2.1.}+\\3d_{2.2.}↰\end{cases}$ — $\begin{cases}3d_{2.2.1.}\\3d_{2.2.2.}\end{cases}↰$ — $\begin{cases}3d_{2.2.1.}\\3d_{2.2.2.}\end{cases}$ Larval mesoblast.

3D → 4d / 4D:

$3D↰$

$4d\neq$
- $4d_1\,⋎$ — $\begin{cases}4d_{1.1.}⋎\\4d_{1.2.}\ \text{Entoderm.(mesentoderm).}\end{cases}$ — $\begin{cases}4d_{1.1.1.}+\ \text{Mesoderm.}\\4d_{1.1.2.}\ \text{Entoderm.(mesentoderm)}\end{cases}$
- $4d_2\,⋎$ — $\begin{cases}4d_{2.1.}↰\\4d_{2.2.}\ \text{Entoderm.(mesentoderm)}\end{cases}$ — $\begin{cases}4d_{2.1.1.}\ \text{Mesoderm.}\\4d_{2.1.2.}\ \text{Entoderm.(mesentoderm)}\end{cases}$

$4D\neq$ — $\begin{cases}5d+\\5D+\end{cases}$ Entoderm.

Conklin that both have become hereditary, which would explain their similarity in all these forms.

That the two types are not sharply separated but overlap is shown by the fact that the first indication of bilateral symmetry arises by a spiral cleavage, and is due to size differences of cells and not to any *reversed* direction of cleavage. (See Pl. XXXVII, Fig. 17.) Of value in this connection are the observations recorded on p. 415, showing cases of reversion to a radial type. The next divisions are bilateral (see Pl. XXXVIII, Fig. 27), showing divisions of $3c_2$ and $3d_2$. Hence in Podarke the transition is not the sudden one described for Nereis, but is gradual, as would be required by our hypothesis. Mead and Conklin have already pointed out the difficulties in the way of accepting Wilson's explanation of the cause for the time of appearance of bilateral symmetry, and to their conclusions I can add the facts in the development of Podarke, where bilaterality appears early, and no equalizing division is necessary for 2d.

The bilateral cleavages appear, as might be expected, at different times and in different ways in different animals. For example, in Crepidula, Conklin has shown that the primitive radial symmetry is preserved in the anterior quadrants after it has disappeared in the posterior ones, *e.g.*, the arms of the cross. In Podarke it is the cross which very early shows bilateral symmetry (see Pl. XXXVII, Fig. 17), so that the relation of bilateral cleavages solely to the processes which lead to the formation of the body is not as close here as in other forms (*cf.* Conklin, No. 5, *a*, p. 185). Indeed, I see no reason why they should, for, as I understand it, neither the trochophore nor its hypothetical ancestor, the trochozoön, has a radial, but rather a bilateral organization. The eye-spots, the glands, in short, *all* the structures of the annelid trochophore are bilateral and not radial. Hence the same causes which would transform the spiral cleavages into bilateral *body-forming* ones, would operate in producing the cleavages which lead to the formation of the *head*. What these causes are we do not know, other than that they must be mainly intrinsic.

Cell Mechanics.

In view of the number of observations which have been recorded by Mead (No. 22), Conklin (No. 5, *a*), Jennings (No. 16), Wheeler (No. 32), and others, showing that none of the laws of cell division, as formulated especially by Hertwig (No. 12), are of universal or even of general application, further additions to the list may seem superfluous, and I will mention but one case which shows that the karyokinetic spindle does not invariably lie with its long axis in the direction of greatest protoplasmic mass, or of least resistance. When the entoderm cells begin to invaginate, they become elongated, and their lower ends are very much compressed by the pressure of surrounding cells. (See Text-Fig. 5.) During this process the cells divide. Now it is evident that if division took place at right angles to the greatest elongation of the cell, or in the direction of greatest pressure, a two-layered entoderm would result. A two-layered entoderm would be of no especial use to the animal ; what is needed is a larger area of absorptive surface, and that can only be obtained by a division in the opposite direction ; in other words, in a direction such that the spindle lies in the line of greatest pressure, and at right angles to the greatest elongation of protoplasm. I have repeatedly seen spindles lying in these cells during the invagination process, and invariably they occupy the direction shown in Text-Fig. 5. Many other examples of this nature might be found in the cleavage of Podarke, emphasizing the fact that, as Conklin pointed out, protoplasm is " not soapsuds or oil emulsion," and that dominating the developing embryo is some force vastly more powerful in its influence on the embryo than any merely mechanical forces, and providing that every spindle shall take the direction necessary in order that a bilaterally symmetrical organism may result. If there is no especial reason why it should be otherwise, the spindle may lie in the long axis of the cell, or successive spindles may alternate in direction ; but if in order that a perfect organism shall develop the spindles need to take some other position, that position will be assumed. Not only is this force manifest in the direction

of divisions, but in the shifting of cell areas which occurs throughout the ontogeny. In an elaborate work on Ascaris, Zur Strassen (No. 36) has proposed a theory that attractions (cytotropisms) exist between groups of cells, and that these mutual attractions bring about the shifting of areas, which is a marked feature of the Ascaris embryo. Aside from the fact that there is no positive evidence of the presence of this cyto-tropism, the theory of Zur Strassen is hardly an explanation ; for, supposing that these attractions do exist (and no convincing evidence is given for their presence), their source and nature remain as mysterious as before. The fact cannot be too strongly emphasized that the embryo from the beginning is a complete organism, not a collection of more or less independent units, the "cells," and that controlling the whole development is some force the nature of which we do not know, but which provides that from a given ovum a given organism shall result.

Equal and Unequal Cleavage.

It has happened that most of the work on annelid cytogeny previously published has been done on annelids with the unequal type of cleavage, and the development of Podarke, therefore, is of especial interest as affording a means of comparison between the equal and unequal types. As we have seen, up to the 56-cell stage, all the quadrants of Podarke are alike, the only indication of a differentiation being the constant position of the polar furrow. Since the two ends of this furrow are, so far as we can tell with the microscope, alike, the possibility remains that they are *actually* alike, and that it is a matter for later determination whether one or the other shall be the posterior end. That this is not the case is, I think, shown by the regular appearance of definite organs at definite times and places in the later development. The small cell, $X_{1.2}$, appears first as an orientation point, and this is followed by the bilateral division of 4d and the regular series of events leading to the bilaterally symmetrical embryo. This indicates to my mind that the polarity of the ovum and of the early cleavage stages is as great as in any animal with the

unequal type, even though this polarity may not be expressed
so clearly in the form of cleavage. The alternatives to this
position would be the theory that differentiation came in sud-
denly at the 64-cell stage, or that it, so to speak, accumulated
very slowly, only attaining a visual importance at that stage.
Of the three, I believe the first is correct. It is difficult to
understand why closely related animals (or, for that matter,
animals not closely related) should vary in the amount of polar-
ity possessed by their ova at the beginning of development,
and I believe a very different principle must be invoked to
explain the difference. This question of differentiation in the
trochophore of Lepidonotus, which also has an equal cleavage,
has been discussed by Mead (No. 22, p. 278) ; and what he
has said there applies equally well to Podarke. At the time
Dr. Mead's paper was written there was still doubt whether
the trochophore of Lepidonotus had a radial or a bilateral sym-
metry, inasmuch as the origin of the mesoblast was not known.
As stated in a previous paper (No. 29, *a;* see also p. 436), there
is no doubt that Lepidonotus has a bilaterally symmetrical
cross and a small $X_{1.2}$ cell, exactly as in Podarke ; and it is
very probable that the history of the *definitive* mesoderm at
least is essentially the same in Lepidonotus as in Podarke.

According to Mead, the important question to be settled by
a study of equal cleavage is whether one of the two cells in
equal is homologous with the larger in unequal cleavage. In
the paper just cited, I have expressed my belief that it is.
Later study has led me to somewhat different conclusions con-
cerning homologies from those held by Professor Mead (see
pp. 469); and to avoid misunderstanding it may be desirable to
change the terminology, although the essential meaning re-
mains the same. I believe that one area in the annelid with
equal cleavage is homologous with a corresponding area in an
annelid with unequal, and that there is as much differentiation
in the one case as in the other, though, as I shall try to show
later, the cells are probably not homologous.

What is the meaning of unequal cleavage ? The old explana-
tion was that it is due to the effects of yolk, either actually
present or working through heredity. Rabl (No. 27, *b*), in

his work on Planorbis, describes a series of mollusks in which
at the 4-cell stage are found all gradations from four equal
cells through others with an accumulation of yolk in three,
then in two, and finally in one cell, the yolk-laden cells being
larger than the others.   From these observations Rabl argues
that the primary cause of a difference in size among cleavage
blastomeres is an accumulation of yolk in one cell, or a group
of cells ; this mass of yolk causing the cell which contains it
to be larger than the others.   If a difference in size appears
between micromeres above and macromeres below, it indicates
that there is a good deal of yolk in the egg.   If, on the other
hand, this size difference appears in the 4-cell stage, there was
not necessarily either more or less yolk involved, but its dis-
tribution is different.   As far as I can discover, however, Rabl
makes no attempt at explaining why yolk should have this
unequal distribution.

If the problems of equal and unequal cleavage concerned
only the first three divisions of the ovum, an explanation of
this sort might suffice ; but the most superficial observation
will show that it does not apply at all in later stages.   Here
cells of the same size develop equally or unequally, apparently
without any reference whatever to the presence of yolk, and
solely with reference to the needs of the developing embryo.
Examples of this sort are numerous in the development of
Podarke.   At the 32-cell stage the cells at the upper pole are
a very little larger than those at the lower, but at the next
division the very small apical rosette cells appear at the upper
pole, while at the lower the division is nearly equal.   It cannot
be maintained that there is more yolk in the cross cells than in
the cells which later become entoderm.   Again, the cell $2d_{2.2}$
certainly contains no more yolk than the corresponding cells in
the other quadrants, but its division, as we have seen, is much
more unequal than theirs.   Examples of this sort might be
multiplied indefinitely.

Within certain limits yolk does undoubtedly modify the form
of cleavage.   No one would dispute, I think, that the mero-
blastic cleavage of the egg of the teleost or the bird is caused
by an accumulation of yolk at the lower pole, and the large

size of the macromeres in, *e.g.*, Crepidula is to a certain extent
due to the yolk which they contain.   But even in these cases
size differences, plainly not referable to yolk, appear among
the blastomeres, and when we attempt any general survey of
the cleavage phenomena the principle wholly fails.

What seems to me the most probable explanation was first
advanced by Lillie (No. 21).   In a discussion of this subject he
says (p. 45) : "Unequal cleavage is conditioned by the constitu-
tion of the segmenting ovum, and always means the precocious
localization of an organ or set of organs in the larger cell."
This principle was subsequently elaborated by Conklin (Nos.
5, *a* and 5, *b*), who added the qualification that the size of a
blastomere stands in direct relation to the size and the time of
formation of the part to which it gives rise, and he explained
many of the phenomena in the development of Crepidula by
the aid of this principle.

Turning now to equal cleavage, it might be supposed that
this form of division was due to a lack of differentiation in the
early stages, and, so far as I know, this has been the usual
explanation given for it.   Rabl (No. 27, *a*) explained the differ-
ence between the unequal cleavage of a mollusk and the equal
cleavage of an ascidian by supposing that in the former there is
a greater differentiation of parts, manifesting itself in size dif-
ferences among the blastomeres, which does not appear in the
latter.   In the ascidian this differentiation begins much later,
and only then do size differences make their appearance.   Rabl
believed that an early differentiation was of distinct advantage
in the struggle for existence, and that it probably had appeared
in the mollusks on that account, while for some reason it was
delayed in the ascidian.

From the facts given in the earlier part of this paper, there
can be, I think, no doubt that in the embryo of Podarke there
is as great a differentiation, appearing as early as in any form
with the equal type.   The polar differentiation is obviously as
well marked.   A bilateral arrangement of parts, foreshadowed
by the constant relation of the polar furrow to the body axes,
is evidently present, and the only doubt would be whether in
the 4-cell stage there may not be two possibilities of orientation,

one of which the egg chooses before it develops much farther. We might assume that in the later cleavages a differentiation suddenly appears, *e.g.*, at the 64-cell stage, so that from that time on bilateral divisions occur. I know at present of no way by which we could prove or disprove this supposition. From a careful study of the egg, however, I do not believe that such is the case. At the 64-cell stage and later, marked differentiations are evident ; but it is to my mind much more reasonable to suppose that a differentiation has been present from the beginning than that it suddenly appears at this stage. I do not mean by this that the material is completely marked off in the unsegmented ovum, but that in Podarke, as in Amphitrite, a gradual differentiation appears as development progresses, and that the process is essentially the same in the two cases.

The important differences to be explained between the cleavage of Podarke and forms with equal cleavage are these : 1. In Amphitrite, Nereis, Arenicola, etc., D is larger than either A, B, or C ; in Podarke all four cells are equal. 2. In Amphitrite, etc., the first generation of ectomeres is smaller than the cells below them ; in Podarke they are all alike. 3. In Amphitrite, etc., 2d is much larger than either of the other three members of the same quartette ; in Podarke they are all of the same size. 4. In Amphitrite, etc., 4d is much larger than 4c, 4b, or 4a ; in Podarke again they are of the same size.

I have given above my reasons for assuming that lack of differentiation is not responsible for these size differences, and in two previous articles (Nos. 29, *a* and 29, *b*) I have attempted to explain them by applying the principle of "precocious segregation" proposed by Lillie and modified by Conklin. In other words, I believe that the equal cleavage of Podarke is due to the fact that in the cell D there is accumulated a smaller amount of material destined to produce certain definite parts of the body than in those forms with the unequal type.

From the cell D first arises 2d, which in Amphitrite gives rise to the entire ectoderm of the body behind the first somite,

and to a considerable portion of the ectoderm of the head. Essentially the same fate has been described for it in Nereis and Arenicola. In Podarke the cell 2d divides at first very much as in the other forms, but from the first, as was to be expected from its small size, a much smaller amount of the trochophore arises from it than from the corresponding cells in the other genera. In the later stages, when the trochophore is completed and begins to elongate, only a narrow band of ectoderm near the posterior end has arisen from 2d, all of the dorsal ectoderm in front of this having been derived from cells which migrated through the dorsal break in the prototroch (Pl. XL, Fig. 59). Not only in amount, but in rate of development, is 2d in Podarke less than in the other forms. Making all allowances for the slow development due to unfavorable conditions, there is, I think, no doubt that Podarke develops the products of these cells, *viz.*, dorsal ectoderm of the larva and trunk ectoderm, more slowly than does Amphitrite. If study of the pelagic larvae should subsequently prove that this slow development is really due to the unfavorable conditions surrounding the embryos in confinement, the other part of the argument, that only a very small portion of the body is composed of these cells, still retains its force ; but the rapid development of the entoderm cells, when compared with Amphitrite, would indicate, I think, that the enfeebling force of environment is really not responsible for the slow development of ectodermal tissue.

Again, 4d is large in unequal, small in equal cleavage. This fact I believe to be correlated with the small amount and slow development of mesoderm derived from 4d in Podarke when compared with other forms. As already described (see p. 431), the germ bands derived from 4d arise very late in the development, and their cells divide slowly, so that at a time when these bands in Amphitrite contain a great many cells there are in Podarke only two on a side. This slow development of mesoderm is also characteristic of Lepidonotus (No. 22). Further, in Podarke the greater part, if not all, of the mesoderm of the trochophore is composed of the larval mesoderm arising from the third quartette of ectomeres ; hence the definitive mesoderm

appears late in the development, and in accordance with our theory its Anlage in 4d is small when formed. So far, then, our principle holds good for 4d.

We have already seen that the larval mesoderm of Podarke arises asymmetrically, and that 3b does not contribute to its formation. Objection might be made to the above theory on the ground that, if it were true, since 3b does *not* contain larval mesoblast, it should be smaller than 3a, which does. I believe, however, that 3b contributes a larger amount of material to the stomodaeum than does 3a. As described on p. 426, a cell very similar in size to the mesoblast arising from 3a is formed at about the same time as the latter in 3b, and I at first supposed that it contributed to the mesoblast formation. Careful examination, however, failed to show any trace of it in the segmentation cavity, and I believe that its fate is as above described; so that a larger part of the stomodaeum arises from 3b than from 3a.

We have now accounted for the small size of 2d and 4d, and this explanation carries with it the explanation for the equality of D with the other cells in the 4-cell stage. There remains to explain the reason for the equality between micromeres and macromeres in the 8-cell stage. This may be due in some measure to the small amount of yolk, but the comparatively large size of the micromeres is, I believe, correlated with the unusually large development of the umbrella as compared with the subumbrella of the trochophore, and with the fact that a large portion of subumbrellar ectoderm really comes from the first quartette of micromeres. (See p. 413.) In discussing this point in a previous paper (No. 29, *b*) I overlooked the fact that the prototroch cells of Amphitrite are relatively much larger than those of Podarke, and I argued that the relatively larger cross cells in the latter would account for the relatively larger size of the first group of micromeres. The larger size of the prototroch cells in Amphitrite would probably compensate for the small cross, and so the argument loses its force; but the further consideration, that a very large part of the subumbrellar ectoderm in Podarke arises from these cells, is in itself a sufficient explanation for their size. We have already seen that the

amount of ectoderm arising from these migrating cells in Amphitrite is comparatively very small.

Among the mollusks, I believe that the evidence at our disposal sustains the theory. In Crepidula the peculiarities of the 8-cell stage are probably to be explained by the presence of abundant yolk in the macromeres, but the fact that D is no larger than A, B, or C is due to the slow development of the characteristic products of that cell — the shell gland and the mesoblast. In Unio the principle certainly applies. Unfortunately, we know too little about the later larval stages of other mollusks, whose cell lineage has been studied, to draw any very positive conclusions. In Umbrella (No. 14), D is smaller than A, B, or C. Heymons did not follow the history of the descendants of this cell very carefully, but describes the shell gland as arising very late. If analogy can be depended upon at all, this gland arises from 2d and our theory holds.

The application of this principle to later stages demonstrates, as I believe, its correctness. To mention but a single example : Perhaps the most noticeable feature of the development of the cross in Podarke is the way the anterior arms divide up into very small cells, while the cells of the posterior ones remain large. This is, I believe, correlated with the development of the large, thin-walled dorsal ectoderm, while the ventral ectoderm of the umbrella is much thicker and gives rise to the eyespots and the frontal (excretory ?) bodies, the former having a very much greater superficial extent than the latter. It is probable also that some cells of the latter are thrown off and absorbed.

Equal cleavage, then, is not, I believe, the expression of a lack of differentiation in the ovum and early embryo, but is due to the fact that a certain amount of formative material, which in other genera is sufficient, when accumulated in a single cell, to increase noticeably the size of that cell, is here present in so small a quantity that no size differences appear. This small amount of formative material is correlated either with the small size of the organ to which it gives rise, or with the late development of that organ.

Mesoblast and Larval Mesoblast.

As already stated in the descriptive portion of this paper, a double origin of mesoblast seems to occur in a number of annelids and mollusks, the phenomenon apparently being more widespread in the latter than in the former group. To the one tissue Lillie gave the name "larval mesoblast," because of its purely larval function. The cells composing it early lose their connection with one another, and wander through the segmentation cavity, where they become elongated and form the larval muscles. The term "mesenchyme" has been so generally applied to cells having this structure and position that Lillie uses the name here and supposes that these cells are phylogenetically related to the mesenchyne cells of ancestral forms which originally arose from ectoblast, but in this case are massed into a single cell.

The second source of mesoblast in Unio is at the posterior end of the body, arising from the cell 4d, and differs from the first in that its cells are grouped into definite germ bands, and give rise to the mesoderm of the adult body.

As we have already seen, while the definitive mesoblast of annelids and mollusks usually arises as in Unio from the cell 4d, an additional *larval* mesoblast having a different origin but similar function in each case has been described for Physa, Planorbis, and Crepidula, among the mollusks, and for Aricia and Podarke among the annelids. Conklin (No. 5, *a*, p. 151) has stated his belief that the larval or primary mesoblast is phylogenetically the older, and indicates a primitive radial origin for this layer, while it has been replaced in the ontogeny by the secondary or bilateral mesoblast, arising from 4d.

Professor Wilson, as a result of renewed investigation on platodes[1] (see p. 462), has eliminated some apparent inconsistencies between these forms and the annelids, lamellibranchs, and gasteropods, and has shown that Lang was probably correct in his derivation of the mesoderm in polyclades from the second and third quartettes of micromeres. In an exceedingly interesting and suggestive article Professor Wilson has proposed the

[1] See also Appendix to Literature.

theory that this larval mesoblast (or, as he calls it, "ectomeso-blast," because of its origin from ectoderm) is a distinct tissue from the definitive "entomesoblast" (so called because it arises from entoderm), and is homologous with the mesenchyme of the turbellarian ancestors of the annelids, while the mesoderm from which the adult structures arise is phylogenetically younger and is represented prophetically in the ontogeny of such a form as Discocoelis by the peculiar bilateral division of the cell 4d. The ectomesoblast and entomesoblast are therefore phylogenet-ically of different origin, a point previously urged by Meyer (No. 23).

Professor Wilson has certainly made out an attractive theory, and the history of the two sorts of mesoderm in Podarke, espe-cially the long-continued connection of the mesoblast with the entoderm, at first sight lends confirmation to his view. For reasons given below, however, I am not inclined to accept it as stated, and propose instead a suggestion which seems to me more in accordance with the facts.

I believe that no hard-and-fast distinction can be made between the two kinds of mesoblast. This belief is based on the following facts :

In Nereis, Wilson (No. 34, *d*) has shown that cells from the anterior ends of the germ bands early separate from the bands and pass forward into the segmentation cavity, where they give rise to the larval musculature, which corresponds exactly in structure and function with the larval mesoblast of Unio or Podarke.

In Lumbricus, also, Wilson (No. 34, *a*) has shown that previous writers were in error in supposing an origin of mesenchyme distinct from mesoderm, and that the mesen-chyme of the embryo is really formed from the anterior cells of the germ bands which have migrated forward and taken on a mesenchyme structure. Similar observations have been recorded by Hatschek (No. 11) and Von Drasche (No. 7). (See, however, p. 428.)

To these observations must be added those of Wilson on Hydroides (No. 34, *b*): "In its earliest recognizable condition the mesoblast band consists of a group of three or four cells,

wedged into the angle between the posterior part of the archen-
teron and the ectoblast.   Beside this, there are a number of
scattered cells lying in the narrow cleavage space.   The mesen-
chyme cells appear to graduate both in form and position into
those of the germ bands.   The latter appear about the time of
gastrulation as two bilateral masses of cells that are pushed
into the cleavage cavity near the blastopore.   Some of these
appear to pass forward and give rise to the mesenchyme ; the
remainder form the secondary mesoblast bands.''   Here again
we have a larval mesenchyme which apparently arises from the
anterior ends of the germ bands, and its cells must be homol-
ogous with those of the definitive bands.

In the most recent paper on annelid cytogeny, Eisig (No. 8)
has described a very different origin of mesoblast from what is
found in any other annelid.   Instead of arising from 4d, the
definitive mesoblast (coelomesoblast) arises from 3c and 3d (it
would therefore be " ectomesoblast " in Wilson's sense), while
the larval (paedomesoblast) arises from 4d, and not from the
portion of 4d which in Nereis or Podarke gives rise to meso-
blast, but from the portion which in those forms becomes a part
of the entoderm wall.

Capitella, from the large proportion of abnormal eggs found
in the tubes, seems to have been an especially unfavorable form
for the study of cytogeny.   It is difficult to believe that one of
Professor Eisig's scientific attainments could have committed
so serious an error, but these results are so very different from
anything else that has been described, that even though bearing
in mind Eisig's strictures on '' Die Thatsachen nicht achten-
den Verallgemeinerungen,'' one may be pardoned, for the pres-
ent at least, doubt whether normal specimens were studied.
To   these differences, which are mainly of importance in a dis-
cussion of cell homology, I shall return later (see p. 464).   Of
importance in this connection is the fact that, according to
Eisig, the two sorts of mesoderm are not absolutely distinct,
but that cells may migrate out from the '' Coelomesoblast '' and
become '' Paedomesoblast.''   Eisig believes that the two kinds
of tissue may have originally come from a common Anlage.
Eisig further argues that neither can properly be said to arise

from ectoderm nor entoderm, but rather from cleavage cells,
inasmuch as at the time of formation there has been no differ-
entiation into germ layers, and he accepts Meyer's theory that
the mesoblast bands represent a gonad tissue, from which may
arise by differentiation all the mesoderm of the body, including
new germ cells.

From these facts it seems to me, if we accept Professor
Wilson's theory, we are bound to believe in two non-homolo-
gous sets of larval mesenchyme ; the one arising from ecto-
derm, found, for example, in Podarke, and the other arising
from the anterior ends of the germ bands and found in Nereis
and Lumbricus.   These two sets apparently do not, as a rule,
exist together, only one case, that of Capitella, having been
described.   On the other hand, no one has proved, so far as
I know, that no " mesenchyme " arises from the germ bands
in cases where a larval mesenchyme exists.   Either, then,
we must assume two sets of non-homologous organs having
exactly the same structure and function, and in at least one
case existing side by side, or (and this seems to me the more
reasonable assumption) we must regard both mesenchyme and
mesoderm as morphologically the same tissue, apparent differ-
ences in their mode of origin being of no significance.   If, as
I believe, the trochophore represents an ancestral stage in the
phylogeny of the annelids, the mesodermal structures found in
it undoubtedly represent the mesoderm of the ancestral form.
Whether the origin of metamerism was due to a process of
budding, or to growth and subsequent metamerization, is not
essential for the present discussion.   In either case, as Wilson
has pointed out (No. 34, *c*) (it is only fair to say that the fact
was used as an argument against the ancestral significance of
the trochophore), the trochophore at the present time is more
than a mere ancestral stage, for it contains in a concentrated
form the Anlage of the whole future body.   Mead has proved
that in Amphitrite all the ectoderm for the body behind the
first septum arises from a group of cells which surround the
proctodaeum of the young trochophore and are descended from
a single cell, the first somatoblast, and probably the same thing
occurs in Podarke.   The grouping of the mesoderm at the

posterior end of the body is also a familiar feature of annelid ontogeny.

Now, as there is no need to assume a phylogenetically new formation of ectoderm for the body as distinct from the head, so I see no need for assuming a new formation of mesoderm. This is evident in Nereis or Lumbricus, and I see no reason why a difference should be made in the case of Aricia or Podarke. In the former case there has simply been a more complete concentration of mesoderm than in the latter. If the facts bear on the point at all, they tend to show an origin of mesoderm from ectoderm rather than from entoderm, though this concentration of material must have led to such changes that we are hardly justified, from the present position of the mesoderm, in drawing any conclusions as to its primary mode of origin.

I believe, therefore, that the mesoderm cells collected at the posterior end of the trochophore larva, and which we know as the definitive mesoblast, represent neither entodermal evaginations nor gonad tissue, but that they represent the mesoderm of the body, which is morphologically continuous with that of the head (as we know it is in Nereis or Lumbricus), and which has been concentrated at this point to provide for the needs of the elongating body. In some cases this concentration is less complete than in others, so that some forms seem to have a double origin of mesoblast, but this (apparent) double origin in no way interferes with the morphological unity of the tissue. Whether the mesoderm arose originally from ectoderm or from entoderm or from neither, we are not at present in a position to say.

Rabl's theory of the mesoderm (No. 27, *c*) seems to have rested too much on an error of observation (Hatschek's description of the mesoblastic teloblasts in Amphioxus; see also Minot's criticism, No. 24, p. 155), and the "Coelomtheorie" of the Hertwigs (No. 13) makes, as I believe, too sharp a distinction between mesoderm and mesenchyme. In the introduction to his paper on Nereis, Wilson hopes, by a study of cytogeny, to break away from the deadlock of opinion concerning the mesoderm, but as I understand his conclusions on p. 393,

he secured no evidence bearing on the primitive mode of origin of this layer. In his later paper (No. 34, *g*) Professor Wilson has suggested, as above stated, that the definitive mesoblast came from entoderm, while the secondary is of ectoblastic origin. The conclusion follows that the mesoblast of the turbellarian is not homologous with the definitive mesoblast of higher forms, but only with the larval.

If we once assume that the mesoderm in different groups must have exactly the same cellular origin in order to be homologous, we land ourselves in an inextricable maze of difficulties. Especially is this true if, in addition to the strictly embryological data, we take into consideration the facts of regeneration and bud development. The literature of this subject is too familiar to need citing. (See, however, Nos. 34, *e*, and 18.) Eisig (No. 8) devotes a considerable amount of space to this question, and since any general review of the various theories would be largely a repetition of what he has said, I shall not attempt it here. Meyer (No. 23) had previously maintained that the definitive mesoderm represents gonad tissue, while the mesenchyme had a different origin. Eisig accepts this position not only for the first-named tissue, but extends it so as to include the mesenchyme as well, and supposes that both sets of mesoderm are really gonad cells which may be mixed with any other cells without losing their characteristic qualities or affecting the character of the latter. From these may arise all the other cells of the body, though in the actual ontogeny of the individual the outer and inner layers having been provided for already, they develop only into the middle layer.

As said above, the accumulation of mesoblast at the posterior end of the body seems to me a secondary condition, connected with the need of supplying material for the elongating embryo, and I see no evidence that it has now, or ever has had, a gonad structure. The changes which must have occurred since the layer was first differentiated are so great that it does not seem to me we are justified in claiming positively any especial mode of origin for it.

We must, I think, go back to the position taken originally

by Wilson (No. 34, *c*), and which, I judge, he has subsequently abandoned, that, "We must primarily take anatomy as the key to embryology and not the reverse. Comparative anatomy, and not comparative embryology, is the primary standard for the study of homologies." The mesoblast of the mollusk or the annelid is a single continuous structure, not a mixture of non-homologous structures, and the mesoblast of the platode is homologous, in the proper use of the word "homology," with the whole and not with a part of it. Whether this conclusion would apply to more widely separated groups might perhaps be doubted, for the mesoderm apparently has very diverse modes of origin (see Montgomery, No. 25), but in forms as closely related as the annelids, mollusks, and platodes I believe that it is correct ; and, as said before, coenogenetic modifications must have been so great that we are hardly at liberty to assume that the diverse modes of origin are proofs of the non-homology of this layer in other cases.

Cell and Regional Homologies.

One of the most important problems connected with the study of cell lineage, especially in its bearing on the meaning of cleavage, is the question of cell homology. The wonderful agreements which exist between the cleavage stages of annelids, lamellibranchs, gasteropods, and platodes, when viewed in the light of widely accepted homologies between the larvae and adults of these groups, have led most workers to propose for cleavage stages an homology as complete and accurate as for the adult organs. And, indeed, if a blastomere of an early cleavage stage in a gasteropod is identical in position and origin with a blastomere in an annelid, and the descendants of each give rise to an organ which can be considered homologous in the two cases, the cell homology would certainly be proved. As Conklin has said, " I believe there is no escape from the conclusion that the protoblasts of homologous organs are as certainly homologous as are the organs to which they give rise ; that the protoblasts of homologous layers are as surely

homologous as are these layers, and that the protoblasts of homologous regions are as much homologous as are those regions"; and, "We therefore reach the conclusion that, in related organisms with determinate cleavage, homologies may be predicted of single cells, whether they be protoblasts of the nervous system, the excretory system, or the locomotor apparatus; of the ectoderm, the mesoderm, or the entoderm; of the right or left, the anterior or posterior portion of the body."

If this be true, cleavage similarities have as great a phylogenetic value as larval or adult similarities, but I shall try to show that the number and completeness of these resemblances have been greatly overestimated; and that the second of the above propositions by no means follows from the facts at our disposal.

The first suggestion I have been able to find along this line is that of Rabl (No. 27, *a*): "Ueberhaupt scheint es nach allen bisherigen Beobachtungen nicht unwahrscheinlich zu sein, dass jede mehr oder weniger scharf umschriebene Thiergruppe ein gemeinsames für alle Glieder dieser Gruppe giltiges Furchungs-Schema besitze, und dass es daher durchaus nicht undenkbar sei, dass man künftig einmal aus der grösseren oder geringeren Uebereinstimmung im Furchungsprozesse auf eine engere oder weitere Verwandtschaft zweier oder mehrerer Thierformen werde schliessen können." Inasmuch, however, as Lillie (No. 21) has shown that many of Rabl's observations were incorrect, this hypothesis must be considered as a more or less fortunate guess than as following logically from his results.

At the time when Professor Wilson's work on Nereis appeared, practically the only papers available for comparison were those of Lang on Discocoelis (No. 19), Blochmann on Neritina (No. 3), Whitman on Clepsine (Nos. 33, *a* and *b*), and von Wistinghausen on Nereis (No. 35). In addition, Wilson referred to the then unpublished observations of Conklin on Crepidula. While the polyclade agreed with the annelid and mollusk in the most remarkable fashion in the details of its cleavage, it differed very decidedly in at least one important particular, *viz.*, the origin of the mesoblast.

These differences and the conclusions Wilson draws from

them are as follows : " The general later history of the blasto-
meres thus formed is as follows : In the polyclade the first
group of micromeres gives rise to the entire ectoblast, the
second and third groups to the mesoblast, the macromeres to
the entoblast.   In the mollusk and annelid, on the other hand,
the second and third groups of micromeres give rise to ecto-
blast, like the first set, and the mesoblast arises subsequently.
This remarkable divergence between the polyclade on the one
hand and the mollusk and annelid on the other is a fact of
capital importance, for it proves that cells having precisely the
same origin in the cleavage, occupying the same position in
the embryo, and placed under the same mechanical condi-
tions, may nevertheless differ fundamentally in morphological
significance."

Extending the comparison in the mollusks, Wilson describes
the peculiar cross figured by Blochmann in Neritina, and com-
pares it with the similar structure found in Nereis.   Making
all due allowances for Blochmann's error, which Conklin had
corrected, he finds that the two structures are composed of
cells of a very different generation, and occupy a very differ-
ent position with reference to the axis of the body, in the
annelid, from what they do in the mollusk.   Wilson's conclu-
sion is that these structures are analogous and not homologous,
and their origin is in some way connected with mechanical
conditions of cleavage.

Lastly, Wilson shows that the velum of the mollusk and the
prototroch of the annelid have a very different cell origin, but
is careful to insist that this fact does not, in his mind, invali-
date the idea of an homology between the completed organs.

Wilson decides, at the end of his discussion, that it is neces-
sary to be very cautious about drawing morphological conclu-
sions from the comparative study of early cleavage stages.
Blastomeres having precisely the same mode of origin and
precisely the same spatial relations to the rest of the embryo
are by no means necessarily equivalent, either physiologically
or morphologically, and the early cleavage stages in themselves
have little morphological value.   The respective value of the
blastomeres must be determined by their ultimate fate, and

this is an indispensable datum for the study of comparative embryonic anatomy. *The fundamental forms of cleavage are primarily due to mechanical conditions, and are only significant morphologically in so far as they have been secondarily remodelled by processes of precocious segregation.* (The italics are in the original.) Since, however, the facts seemed to him to show that this process has taken place to a greater or less extent, Wilson closes his paper with a plea for the study of cell lineage as the best means by which we may hope to obtain a firm basis for a comparison of the germ layers.

In his paper on Unio (No. 21), written two years later, Lillie, while recognizing the fact that difficulties exist in the way of the assumption, argues for an homology of cells, basing this argument on the origin of the mesoblast from a corresponding cell in a wide range of annelids and mollusks, and on the similar origin and fate of three quartettes of ectomeres in all these forms. Lillie thus argues for a cell homology. His definition of this term, as I understand it, is that two cells whose products are homologous must be themselves homologous, even though they have a different origin in the cleavage. Lillie calls attention to one important difference between Nereis and Unio, *viz.*, that a cell which in Nereis is a stomatoblast, in Unio gives rise to the larval mesoblast. His explanation of this difference is that the form of cleavage being constant, whenever a new organ appears, it must appear in some cell already present, the formation of a new cell being an impossibility. Hence this larval mesoblast, being an ectodermal structure, must make use of a cell which in other cases remains in the outer germ layer.

In his lecture on "The Embryological Criterion of Homology" (No. 34, *e*), Wilson, while recognizing the "marvelous agreements" in the cytogeny of related forms, argues that as we extend the comparison "the contradictions reach a climax." The facts of regeneration, also, show important differences from embryological processes, differences which seem to invalidate the whole germ-layer theory. The conclusion which Wilson reached was that, for the study of homologies, adult structures are of much more value than embryological, since

homologous adult parts may arise from non-homologous Anlagen ; and, conversely, cleavage cells having the same origin give rise to non-homologous organs. While most of his lecture deals with embryonic structures which appear after the cleavage, it is, I think, evident that Professor Wilson intended to discredit the study of cytogeny as a means of determining homologies.

Mead (No. 22) devoted considerable space to a discussion of this question of cell homology, which he defined as follows : " Essential similarity in origin and fate will be considered a sufficient criterion of the homology of cells, as it is, by common consent, of tissues and organs." Mead first shows that in general the mode of origin and the fate of the quartettes of ectomeres are the same in all annelids and mollusks. Then, examining the cleavage more in detail, he finds that in Amphitrite and Clymenella the primary and secondary trochoblasts are cell for cell the same ; and, further, in Scolecolepis, which has a suppressed trochophore, these very cells are diminutive, as might be expected from a non-functional organ. This prototroch formation is very different from the process as described for Nereis, but Mead gives reasons for believing that Wilson's description was erroneous.

Mead further shows that the objection · raised by Lillie to cell homology because of the varying fate of cell $3a_{2.2}$ is not valid, because in neither case was the whole of this cell involved. In both cases it divides horizontally, and while the lower portion in Nereis becomes stomatoblast, in Unio it is the upper portion which becomes larval mesoblast.

Another case of cell homology is the apical rosette, which probably has exactly the same origin and fate in all the annelids studied, and in Crepidula among the mollusks. The slime glands of Amphitrite also are homologous in position, and probably in fate, with the " head kidneys " of Nereis.

The fate of the remaining cells of the upper hemisphere was, according to Mead, so uncertain that he did not attempt to follow homologies, and the same was true of the lower hemisphere, except, in a general way, the somatoblast 2d, and, more especially, one of its descendants, the small cell, $X_{1.2}$, which

apparently has the same origin and fate in Nereis, Amphitrite, Unio, and Clymenella.

The most complete homology, according to Mead, is shown in the origin of the mesoderm. This always arises leiotropically at the ideal 64-cell stage, and is one of the fourth generation of micromeres given off from the cell D. A serious discrepancy here and a strong argument against cell homology was the origin of the mesoblast in Discocoelis, where, according to Lang (No. 19), although the general form of the cleavage is the same as the annelid, the mesoderm arises not from 4d, but from the second and third group of micromeres, the cell 4d forming a part of the entoderm. Lang's figures, however, show a bilateral division of 4d very different from the division of the other members of this quartette, and Mead has suggested that possibly there was an error in the interpretation, and that mesoblast may arise from this cell in the platode as well as in the annelid.

In his "Embryology of Crepidula," published a little earlier than Mead's paper, and in a subsequent lecture (No. 5, *a* and *b*), Conklin has extended the comparison between annelids and mollusks still farther. After mentioning the similarities above noted, he says: "To this list of resemblances between the annelid and the mollusk, which I can confirm in the case of the gasteropod, I have been able to add the following: The rosette series of the gasteropod is exactly like the cross of the annelid in origin, position, and probably in destiny. The intermediate girdle cells of the annelid are like the cross of the gasteropod in origin, position, and destiny (at least in part). The differences, therefore, between the annelidan and molluscan cross which Wilson emphasizes are not real ones. The trochoblasts of the annelids and gasteropods are precisely similar in origin and destiny, *at least in part*." (The italics are mine. See p. 466.) "In some annelids (Amphitrite, Clymenella, Arenicola) the prototroch is completed by cells of the same origin as in Crepidula and Neritina. The differences which Wilson points out between these two structures do not therefore exist. In both annelids and mollusks the prototroch lies at the boundary between the first quartette on one side and the second and third

on the other.   In both there is found a preoral, adoral, and a
postoral band of cilia.   In the gasteropod the apical cells give
rise to an apical sense organ, such as is found in many annelid
trochophores.   The supraoesophageal ganglia and commissure
apparently arise from the same group of cells in annelids and
gasteropods.   The fourth quartette in annelids and gasteropods
contains mesoblast in quadrant D, but is purely entoblastic in
quadrants A, B, and C.   A fifth quartette is formed in gas-
teropods and some annelids (Amphitrite) and consists of ento-
blast only.   In the gasteropod, larval mesoblast arises from the
same group of ectoblast cells as in Unio, differing, however, in
this regard, that it is found in quadrants A, B, and C, whereas
in Unio it is found in quadrant A only.   To this list of accurate
resemblances in the cleavage cells may be added the fact that
among annelids and mollusks the axial relations of all the
blastomeres (except possibly the four macromeres) are the
same.

"The cause of such resemblances, like the cause of determi-
nate cleavage and of the constancy of specific characters, must
be found in protoplasmic structure, and I cannot escape the
conviction that these likenesses belong to the same category
with the fundamental resemblances between gastrulae, larvae,
and adults.   Whatever criterion of homology one may adopt, —
whether similarity of origin, position, history, or destiny, or all
of these combined, — certain of these resemblances in cleavage
bear all the marks of true homologies."

The polyclade cleavage offered difficulties which were great,
"perhaps irreconcilable," but Conklin argues that, since we
find no perfect homology between adult structures, we need
not expect to find perfect homology between cleavage stages.
Professor Conklin's conclusions may, I think, be fairly given in
the following passage (No. 5, *a*, p. 198): "If organs which are
homologous among annelids and mollusks, such as the proto-
troch, the apical sense organ, the stomodaeum, and the ventral
plate, can be traced back in their development to certain indi-
vidual cells of similar origin, position, size, and history, are not
these cells truly homologous?   If not, where in this develop-
mental process shall we say that homologies begin?"

Conklin further distinguishes between cell and regional homology, the latter appearing, for example, between annelids and gasteropods on the one hand, and the ctenophores on the other. Further, if I understand him correctly, although he gives no formal definition, he would distinguish between complete and incomplete cell homology. I am able to see very little distinction to be logically made between an incomplete and a regional homology, and it seems to me a clearer definition of cell homology is to be desired. If cell homology is the resemblance which exists between two organs whose Anlagen are merely *similar* and not *identical* in origin, I have no objection to the use of the term in recent writings ; but it seems to me very little distinction can be made between this and regional homology, and I see no reason why, using this definition, all homologies are not cell homologies. The phrase " incomplete cell homology " is to me a contradiction of terms.

I have no desire to split hairs over a mere definition, and it may be that the term is convenient and should be retained (for example, to designate an homology between two organs, not determined by adult structure alone, but by a similarity in cellular origin as well). At the same time it seems to me desirable to emphasize the distinction between the complete and the incomplete, as the hasty reader of many recent cell-lineage papers would, I think, be in danger of carrying away too exaggerated a notion of the number and importance of the former. To this point I shall return.

Wilson, in a paper already quoted, has reinvestigated the platode cleavage, and has shown that although the cleavage in its general character agrees perfectly with the annelid and gasteropod, yet the mesoblast, precisely as described by Lang for Discocoelis, arises from the second and possibly, to a limited extent, from the third, quartette of ectomeres. While this breaks down Mead's criticism of Lang, it at first sight seems to revive the old difficulty in the way of accepting homologies between the two groups. In view of the recent discovery of larval mesoblast in annelids, gasteropods, and lamellibranchs, however, Wilson believes that the apparent contradiction is in reality new proof of the homology as showing

an homology between the *larval mesoblast* of the annelid and mollusk, and the *adult mesenchyme* of the platode. (See the section on the mesoderm, p. 449.)

Another difficulty in the way of cell homology has been the cleavage of Polychoerus, which, according to Gardiner (No. 9), is of the bilateral type. With the aid of Leptoplana and Disco-coelis for comparison, Wilson [1] has reëxamined Polychoerus, and finds that it has the true spiral type of division and is thus brought into line with the other genera.

In the light of these facts Wilson has abandoned the position taken in his lecture and has gone back to that of his first paper on Nereis, though his views are even more extreme than in 1892. His conclusions may be summarized in the following extracts (No. 34, *g*): "The phenomena shown in the history of the micromere quartette in annelids, platodes, and mollusks render it highly probable, if they do not actually demonstrate, that development may exhibit ancestral reminiscence as clearly in the cleavage of the ovum as in the later formation of tissues and organs. These facts may well give us hope that when the comparative study of cell lineage has been carried farther, the study of the cleavage stages may prove as valuable a means for the investigation of homologies and of animal relationships as that of the embryonic and larval stages.

"These facts seem on the whole to emphasize the importance of cell formation in development, and it would be difficult to explain ancestral reminiscence [*e.g.*, the small "entero-blasts" of Aricia, which he regards as ancestral rudiments, and the larval mesenchyme of Unio or Crepidula] in cell lineage under any view which does not recognize in cell outlines the definite boundaries of differentiation areas in the developing embryo."

Eisig's study of Capitella cleavage brings to light many similarities with the cleavage of other annelids, especially with Nereis (No. 34, *d*), which he used most frequently for comparison, and he finds that in general the cleavages are remarkably alike in the two cases; so that "Die Parallele, welche

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[1] I am indebted to Professor Wilson's courtesy for permission to quote this, as yet unpublished, observation.

Conklin mit Recht als wunderbar bezeichnet, wird nun aber durch mehrere Nachweise dieser meiner Arbeit noch erheblich gesteigert."

The origin of the mesoderm from a cell of the third quartette is not an argument against this position, because the mesoderm cells are not differentiated but are undifferentiated cleavage cells, corresponding with germ cells of the lower animals, and may be mixed with (beigemengt) various cleavage cells without affecting the inherent quality of the latter. That the mesoderm is mixed with a macromere, or a micromere, is shown by the fact that the whole micromere or macromere never passes into the mesoderm, but rather buds off a daughter-cell from which the mesoderm arises. Inasmuch as this mixing does not affect the character of the mother-cell, its homology with other cells in other groups which do not happen to contain mesoderm is not affected.

I may remark, in passing, that this conception of the wandering character of the mesoderm cells would, if accepted, remove one of the strongest arguments for cell homology. The constant position of the mesoblast pole cells and their origin from 4d have been the strongest argument in support of the theory.

Lastly, in a short preliminary, Child (No. 4) has taken a definite stand against cell homology, basing his claim on the absence of a prototroch in Sternaspis, — where, however, it seems to me, his criterion of homology is too severe, — and on a comparison of the paratroch of Arenicola with that of Amphitrite, the origin being very different in the two cases. It is only fair to say, however, that the origin of the prototroch is the same in both.

The development of Podarke not only has furnished no new evidence for complete cell homology, but, on the contrary, shows that many which have been assumed by previous writers are not of wide application. A comparison of the various details will be instructive.

1. *The Apical Rosette.* — The cells composing this have the same origin, and probably have the same fate, in a number of different annelids and mollusks. The apical tuft of cilia is, in

many cases at least, carried by them. Whether they divide to form eight cells, or remain four in number, does not affect the homology, which in this case is probably complete.

The other cells of the upper hemisphere have undoubtedly the same general fate, but no comparison can with our present knowledge be made between them. The mucous glands in Amphitrite have the same cell origin as the large glands in Nereis, but the comparison does not extend farther. If we believe in ancestral rudiments in cleavage, we may maintain that the small cell on each dorsal arm of Podarke, which corresponds in position to this gland, is really a rudiment of it become functionlesss in the latter genus. It certainly does not give rise to a gland, but I believe that it afterwards enlarges and enters into the general ectoderm of the head. The cross cells differ from their first formation in the number and arrangement of cells, so that no comparison can be instituted.

2. *The Trochoblasts.* — Mead[1] has considered the precise similarity between the formation of these cells in Amphitrite and Clymenella, the similarity extending not only to the primary but to the secondary trochoblasts. In Arenicola, also, the prototroch is formed as in Amphitrite, and thus far the homology holds. As stated above, however, the Podarke prototroch arises in a different way. The comparison can best be seen from the table.

	PRIMARY TROCHOBLASTS.	SECONDARY TROCHOBLASTS.
Amphitrite ⎫ Arenicola ⎬ . . . . . . ⎧ 1a 2.1.1 Clymenella ⎭	1a 2.1.1 1a 2.1.2 etc., in all 1a 2.2.1 four quadrants. 1a 2.2.2	2a 1.1.1 2a 1.1.2 in A, B, and C 2a 1.2.1 quadrants.
Podarke.	The same as the others.	1a 1.2.2.2 in A, B, and C 2a 1.1.2 quadrants. 2a 1.2.1

In the description of the trochoblasts, I have distinguished between "tertiary" and "secondary." For convenience both are here grouped under "secondary."

Thus, in Podarke, three out of the twenty-five cells which make up the completed prototroch are different from those

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[1] See Appendix to Literature.

found in the other cases mentioned. This difference is slight, but enough to disprove a complete homology.

In Nereis, according to Wilson, while the cells corresponding to the primary trochoblasts of the other genera appear in each quadrant, the upper sinistral one is pushed out of the prototroch ring, and the completed organ is composed of only twelve cells. The origin of the secondary trochoblasts was not determined.

Mead has thrown doubt on the accuracy of these observations and a reinvestigation of the subject is perhaps to be desired. I can discover no internal evidence of their incorrectness. If true, they strengthen the position here taken and show a lack of complete cell homology between the prototroch of Nereis and the other annelids.

Conklin also has argued for a very complete homology between the trochoblasts of Crepidula and those of annelids, but a comparison of his description of the velum of Crepidula with the annelid prototroch seems to me not to bear out the precise similarity which he maintains. There are in Crepidula four cells corresponding in origin to the four primary prototroch cells of Annelida. Of these, *two* — those of the ventral surface (quadrants A and B) — enter into the formation of the velum, *but the posterior ones do not*, unless the whole head vesicle is a part of the velum, for they lie in front of the functionless posterior branch of the velum, which Conklin considers the homologue of the velum in other mollusks and the prototroch of annelids, and behind the functional anterior branch which he considers a new formation. The velum is completed in the ventral portion at least by cells which have the same origin as the secondary trochoblasts of Amphitrite, but proportionally more cells from the second quartette come in, and it is not impossible (p. 134) that some of the third quartette cells may also form a part of the prototroch. The organ occupies a corresponding position in annelids and in mollusks, and I have no doubt that it is homologous in the two cases. The close correspondence in the mode of origin is additional evidence for this homology, but the point I wish to emphasize here is that this homology is not a complete homology, nor does it seem to

me as close as Professor Conklin would maintain, nor as close as one would infer from his statement (p. 194, etc.).

3. *The X-Cells.* — In the lower hemisphere the cell 2d has been cited as an example of cell homology, for from it arise the ectoderm of the trunk, the ventral plate, and the growing point. Within the limits of the cell the most important larval organ is the paratroch. The cell origin of this is known only in Amphitrite and in Arenicola, but it is not the same in the two cases. In Podarke, as already stated, I have been unable to find any paratroch, and the comparisons which I have made between the divisions of X in Podarke and those in the other two annelids show absolutely no similarities.

A further point in the development of Podarke is the relatively small amount of dorsal ectoderm arising from 2d. As shown in Pl. XL, Fig. 59, this forms only a comparatively narrow band around the proctodaeum, all the rest of the dorsal ectoderm having arisen from cells which migrated through the dorsal break in the prototroch. Either, then, we must assume that 2d is not completely homologous in Podarke and in Amphitrite, or we must assume that the portion of the trochophore lying just behind the prototroch is not homologous in the two cases. Of the two, the former seems the more reasonable.

The small cell, $X_{1.2}$, arises in the same manner and occupies the same position in all the forms studied. Its fate is unknown, except for the general statement that it forms a part of the proctodaeal wall. The constant position of this small cell must have some meaning, although I am not able at present to say what that is. It may be another case of complete homology, and for the sake of the argument we may assume that it is, though it should be remembered this has not been proved.

4. *The Mesoblast.* — There remains one more cell for which a complete homology is assumed in the various forms, and that is the cell 4d, which, with but one exception, contains the definitive mesoblast.

This cell arises from the posterior macromere D, and, so far as its origin is concerned, is alike in all cases. That it is qualitatively different is shown, I think, by the most recent

investigations. The following table is not intended to be a complete list of papers where the origin of this cell has been described, but contains the most recent.

<div align="center">

4d contains

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Nereis . . .	Mesoderm and a little entoderm.
Aricia	" " "
Polymnia . . .	" only (?).
Amphitrite . . .	" only.[1]
Arenicola . . .	" "
Unio	" and " secondary mesoderm " (entoderm ?).
Umbrella . . .	" " "
Planorbis . . .	" " "
Crepidula . . .	" and a comparatively large amount of entoderm.
Podarke . . .	" and a little entoderm.
Capitella . .	Larval mesoderm and ventral ectoderm.

Here, leaving out of consideration, for the moment, the case of Capitella, we must assume either that the cell 4d is not completely homologous in Podarke and in Amphitrite or in Unio and Crepidula, or we must assume that the mesoblast of the one form is partly homologous with the entoderm of the other ; and here again my preference is for the former assumption. If Capitella be as described by Eisig, and we claim cell homology for 4d, we are driven to the assumption that the mesoblast of Nereis is homologous with the ventral plate of Capitella, and the larval mesoblast of Capitella is homologous

[1] Professor Wilson has shown that it is not impossible that small cells budded off from the primary mesoblasts in Amphitrite, Unio, Umbrella, Planorbis, and Physa are really entoblasts and not mesoblasts. In Amphitrite, however, these cells do not arise in the same position as in Aricia or Nereis, and they remain at the anterior end of the mesoderm band ; their fate is therefore very different in the two cases. Heymons is positive that in Umbrella these cells are mesoblast, and in Planorbis they lie "in the cleavage cavity " (No. 15, *a*), hence have the proper position at least for mesoderm. In a personal communication (1899) Dr. Holmes assures me that there is no doubt of the mesoblastic fate of these cells. For these reasons I have thought best to regard all these cells as mesoderm until further study shall show that they are not. In Clymenella, also cited by Professor Wilson, we have no evidence that the cells in question do not become mesoderm, and the first division of the primary mesoblasts, as described by Mead, may separate off an entodermal portion or it may be the first division toward the formation of the mesoderm bands. At present we are unable to say which view is correct, and the observations are of little use in this discussion.

with the entodermal portion of 4d in Nereis ; while the defini-
tive mesoblast of Capitella is homologous merely with the
larval mesoblast of other forms.   As already stated on p. 451,
I can hardly believe that these were normal cleavages.   If
normal, they put an end to all questions of cell homologies,
complete or incomplete.

The larval mesoblast is an organ whose exact origin is known
in a number of groups.   It may arise from the second quar-
tette (Unio and Crepidula), from the third (Podarke, Physa,
Planorbis), or, *if Eisig be correct*, from the fourth (Capitella).
Its exact origin is probably not the same in any two of these
cases.   It may arise asymmetrically, later becoming symmet-
rical (Unio and Podarke), or it may be symmetrical from the
beginning (Crepidula and Planorbis).   Evidently no complete
cell homology can be demonstrated here, and we are given two
alternatives : either this larval mesoblast is not homologous
in all cases, or it arises from cells which are not completely
homologous.   I prefer the latter assumption.

Other organs of the embryo are the proctodaeum, stomo-
daeum, and supraoesophageal ganglia.   While these are un-
doubtedly homologous organs, their precise cell origin is
different in the different cases.

I have described these facts at some length, because it
seemed to me that the more we investigate the subject the
more difficulties do we find in the way of complete cell homolo-
gies, at the same time that marvelously close resemblances are
brought to light.   I admit fully the justice of the position taken
by Conklin (see above, p. 465, first quotation), but I maintain that
with the single exception of the apical rosette (and the cell $X_{1.2}$?)
no organ of the annelid or mollusk has been traced back to
a similar cell in enough cases to establish cell homologies or
to justify Conklin's second statement (see above, p. 466, " We
therefore," etc.).

This insistence upon a distinction between complete and
incomplete cell homology may seem a quibble, but I believe
the distinction is real, and that if we could prove complete cell
homology, we would be led logically to a very different theory of
development from what we must adopt if we regard homologies

as incomplete or regional. If cleavage had the differential
and determinate character urged by Wilson and Conklin, I do
not see why all differential cleavages in gasteropods and anne-
lids, or at least in two genera of annelids, should not show
complete cell homologies. The number of divisions after dif-
ferentiation might vary, depending on physiological needs of
the organism, but *up to the stage of complete differentiation* I
do not see why, on this theory, any incompleteness in the
homology should appear. Conversely, if complete cell homolo-
gies do exist between the different groups, then the cleavage
must have a differential and mosaic character. The evidence
is, I believe, against both assumptions.

If cleavages are differential and have a phylogenetic signifi-
cance, why should the mesoderm in Nereis not arise by the
same number of divisions as in Podarke, since the two genera
are pretty closely related? Why should Arenicola completely
separate its mesoderm with the formation of 4d, while Aricia
requires one more division, and Nereis three or four, to com-
plete the separation between mesoderm and entoderm? Exam-
ples of this sort might be multiplied, showing, I believe, very
grave difficulties in the way of cell homologies and determinate
cleavage.

What explanation are we to give, then, for these remarkable
resemblances? Are we to regard them as mechanically pro-
duced, and dependent upon conditions of pressure, temperature,
etc.? Certainly not. I believe that these resemblances are
due to homologies having perhaps a phylogenetic significance,
but to homologies which are not bounded by definite cell areas.
I believe that the prototroch, the paratroch, the supraoeso-
phageal ganglia, the mesoderm, are to be regarded as homolo-
gous structures, and that the fact that they do not lie within
corresponding cell walls in the different cases is an indication
that we are here dealing with a regional as distinct from a cell
homology.

Since the ancestral form made use of a certain cleavage pat-
tern, secondarily moulding morphogenetic processes on cleav-
ages which were primarily mechanical, we would naturally
expect its descendants to follow in general the same cleavage

design.   Thus homologous organs, arising in corresponding portions of the embryo (as they naturally would in closely related forms) *might* arise in exactly the same cell, and cell homologies would appear ; but these homologies are secondary and accidental, and found in no wide range of forms.

The fact that homologous organs have such a similar mode of origin in the cleavages of annelids and gasteropods, while not proving cell homology, is I think additional evidence in favor of the near relationship of the groups, as indicating not only an homology between corresponding portions of the adult, but between corresponding portions of the embryo as well.

The proper solution of the problem, it seems to me, lies along the line suggested by Whitman (No. 33, c), where he argues that we must regard the developing embryo as a complete organism possessed of a definite organization from the very beginning, and that the fact that this becomes split up into more or fewer cells does not affect this complete individuality.   The embryo, like the adult, is an individual, not a collection of individuals, and it is governed by inherent force of its own, not by the mutual interaction of a number of forces. " Organization precedes cell formation and regulates it, not the reverse."   " The structure which we see in a cell mosaic is something superadded to organization, not itself the foundation of organization."   " The organization of the egg is carried forward to the adult as an unbroken physiological unity, or individuality, through all modifications and transformations " (No. 33, c).   The difficulties I have pointed out in the way of cell homologies, as well as the facts of post generation in mutilated embryos, seem to me to strengthen this position.

I believe that, as originally stated by Wilson (No. 34, d), " The fundamental forms of cleavage are primarily due to mechanical conditions, and are only significant morphologically in so far as they have been secondarily remodeled by processes of precocious segregation "; and that the homologies which are found between different embryos are none the less homologies because they are not bounded by definite cell walls ; nor does the fact that in some cases cell walls *do* bound homologous areas, have more than an accidental significance.

This position is not, I think, inconsistent with the explanation given before for the distinction between equal and unequal cleavage. I do not believe that the accidental accumulation of a certain amount of material in any particular cell necessarily implies that that material is, at the time of localization, differentiated. As a physiological rather than a morphological process, the egg has cleaved into a number of cells, and the organism, making use of these cleavage products, has stored in each variable amounts of material according to the needs of that particular region of the body. If this be true, there would follow a different explanation for the small size of some cells than the one given by Professor Wilson. He suggests that the small size of many cells in the cleavage stages indicates, like the degenerate organs of adults, degenerated structures which were once functional, but have in the phylogeny of the group become so functionless that they are now represented in the ontogeny merely by rudiments. (While this caution would possibly not apply to the small "mesentoblasts" of Nereis or Podarke, yet it should be remembered that mere size is in itself no indication of a degenerate condition. Cells which are very small when first formed may increase in size later and make up an appreciable portion of the embryo.)

If organs which are to appear early, or make up a large portion of the embryo, may be and are represented in the ontogeny by large cells, the converse of this proposition is correct, and the small size of many cells is due to the fact that the portion of the organism which is to arise from them is either small when formed, or appears late. (See above, p. 448.)

On this supposition portions of the embryo which are small are regarded as having a smaller amount of material set apart for them in the cleavage stages, and would thus be represented by smaller cells. Since these small cells are rarely the same in number (*cf.* the number of "mesentoderm cells" in the various cases), and their behavior is different in different forms (*cf.* the anomalous division of the cells corresponding to the prototroch in Chaetopterus, No. 22, Figs. 131 and 132), the rudimentary character of the cells must be regarded as individual

variations, to be explained by the above assumption and not as an ancestral rudiment.

The most complete examples of rudimentary cells are not explained by either of the above assumptions. These are the small cells described by Conklin and Miss Langenbeck, and tentatively by myself in Podarke, as thrown off from the outside of the body, or absorbed into the segmentation cavity. These are hardly ancestral rudiments, and yet their fate is difficult to explain on the theory of precocious segregation of formative material. I can offer no satisfactory explanation of the phenomena.

OXFORD, OHIO, March 2, 1899.

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NOTE. — Since this paper left my hands, March 2d, 1899, several important papers on cell lineage have appeared. See Appendix to Literature. Nothing in these papers has, however, led me to modify any of the views stated in the above.

VASSAR COLLEGE, January 5, 1901.

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DESCRIPTION OF PLATES.

In the following drawings no attempt has been made to represent the actual appearance of the cells, but only to indicate their size and position and the direction of the karyokinetic spindles. A peculiar feature of Podarke is the large size of the nuclei with their prominent chromosomes, the latter being especially prominent after staining with haematoxylin. These characters are not exaggerated in the drawings.

EXPLANATION OF PLATE XXXVI.

FIG. 1.   First cleavage spindle, *pg*, polar globules.

FIG. 2.   Two-cell stage from above.

FIG. 3.   Four-cell stage from above.

FIG. 4.   Eight-cell stage from the side.

FIG. 5.   Eight-cell stage from above.

FIG. 6.   Eight, forming sixteen cells.   Formation of second quartette.

FIG. 7.   Sixteen cells from the side.

FIG. 8.   Sixteen, forming twenty-four cells.   Formation of third quartette and intermediate cells.

FIG. 9.   First division of primary trochoblasts.

FIG. 10.   Twenty-eight to thirty-two cells.   Division of second quartette.

FIG. 11.   Thirty-two to forty cells from above.   Formation of apical rosette.

FIG. 12.   Forty cells forming fifty-six.   Second division of trochoblasts and first division of intermediate cells.
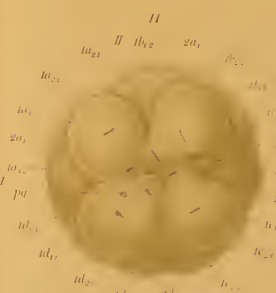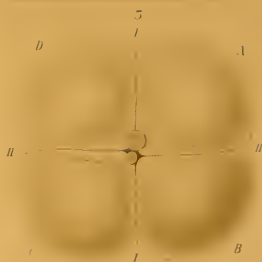
EXPLANATION OF PLATE XXXVII.

FIG. 13. Thirty-two cells forming forty, from below. Formation of fourth quartette.

FIG. 14. Forty cells forming fifty-six, from the side. Second division of the trochoblasts, first division of the intermediate cells, and first division of third quartette.

FIG. 15. Fifty-six-cell stage from the side. Completion of the final division of the primary trochoblasts.

FIGS. 16–23. Successive stages in the history of the cross. ap' to dp', primary trochoblasts. ap'' to dp'', secondary trochoblasts. ar to dr, rosette cells.

FIG. 24. Second division of second quartette. Formation of $X_{1\cdot2}$.
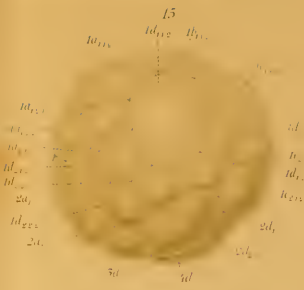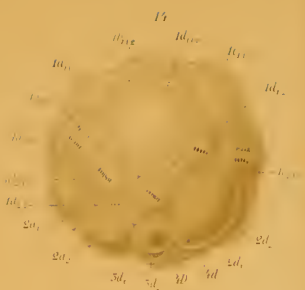
EXPLANATION OF PLATE XXXVIII.

Fig. 25. Completed division of second quartette in quadrant B.

Fig. 26. Completed division of second quartette in quadrant D, showing $X_{1 \cdot 2}$. Bilateral division of 4d, $3c_2$, $3d_2$.

Fig. 27. Bilateral division of 4d, etc., from below.

Fig. 28. Third division of second quartette cell and first division of $3a_2$ and $3b_2$.

Fig. 29. Side view of preceding stage, showing A quadrant.

Fig. 30. Same as 29, showing D quadrant.

Figs. 31 and 32. Division of X_3.

Figs. 33 and 34. Formation of fifth group of micromeres.

Fig. 35. Division of $X_{1 \cdot 2}$ and $3c_{2 \cdot 1}$. Note the small superficial area of 4a, 4b, and 4c.

Fig. 36. First division of 4a, 4b, and 4c.

Pl. XXXVIII.
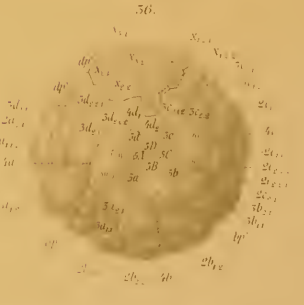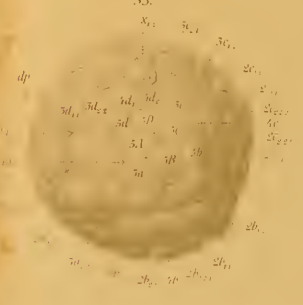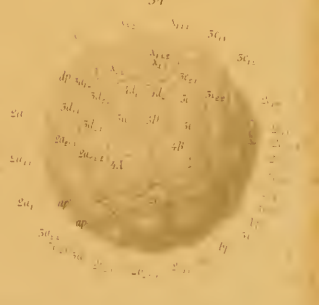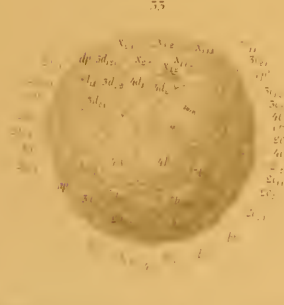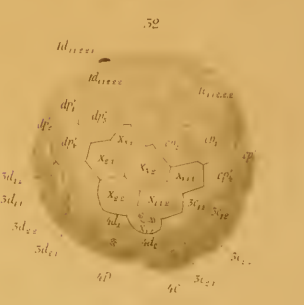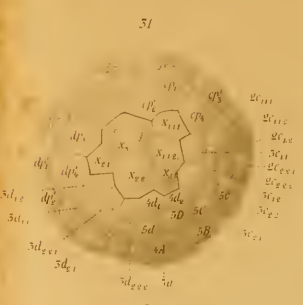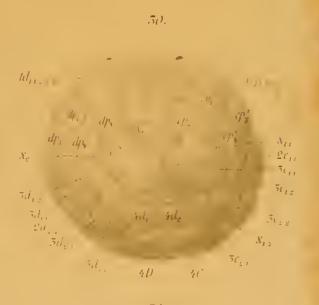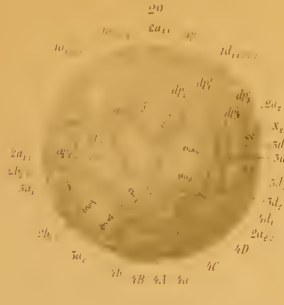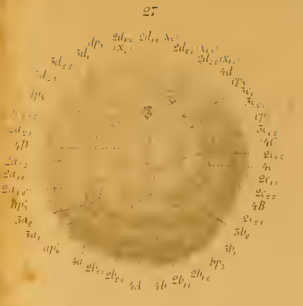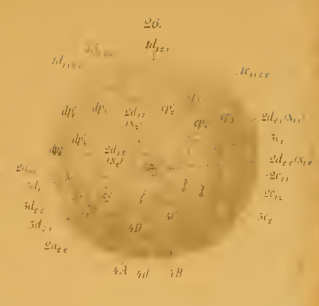
EXPLANATION OF PLATE XXXIX.

Figs. 37 and 38.   Formation of tertiary trochoblasts, ap'''_1, ap'''_2.

Figs. 39 and 40.   Divisions of X-cells.

Fig. 41.   Stage of Fig. 38 from below.   Note divisions of $3a_{2\cdot2}$, ap''' to d'''', tertiary trochoblasts.

Fig. 42.   Completion of division of X-cells, begun in Fig 40.

Fig. 43.   Beginning of closure of the blastopore, and division of X.   *Blp.*, blastopore.

Fig. 44.   Division of $X_{2\cdot2\cdot1}$ and beginning of migration of "1" cells from the upper hemisphere.

Fig. 45.   Division of X-cells and further migration of "1" cells.

Fig. 46.   Further closure of the blastopore and division of $X_{1\cdot2\cdot1}$.

Fig. 47.   Completed closure of the blastopore.   *Proc.*, proctodaeum.   *Stom.*, stomodaeum.

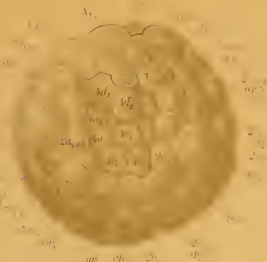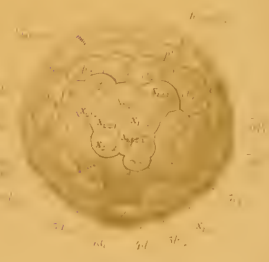Fig. 48.   A little later than 47.   Complete concrescence of the X-cells on the ventral surface.

Pl. XXIV.
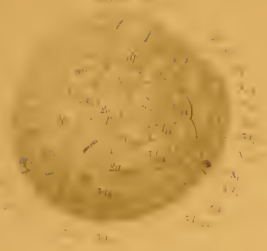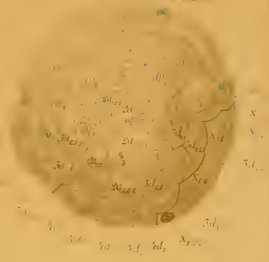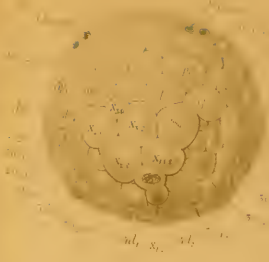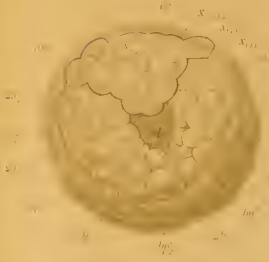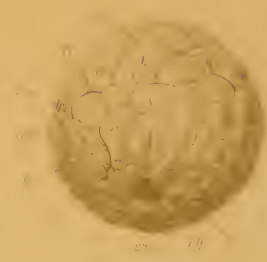
EXPLANATION OF PLATE XL.[1]

FIG. 49. Beginning of invagination of $3a_{2.2}$ (*l.m.m.*).

FIGS. 50 and 51. Division of $4d_1$ and $4d_2$ and of larval mesoblast. *l.m.m.*, median larval mesoblast; *l.m.r.*, right larval mesoblast; *l.m.l.*, left larval mesoblast.

FIG. 52. Second division of larval mesoblast.

FIG. 53. Final separation of definitive mesoblast and migration of larval mesoblast. Note migration of cells from upper hemisphere.

FIG. 54. Completion of divisions of Fig. 53.

FIG. 55. Next division of *l.m.m.* *l.m.r.* and *l.m.m.* have divided.

FIG. 56. Stage of Fig. 54 from the side.

FIG. 57. First division of definitive mesoblast. Note migration and large size of "1" cells.

FIG. 58. Completion of division of l_1 and division of $X_{3.2}$.

FIG. 59. Stage of forty hours from below. *M.*, mesoblast bands.

FIG. 60. Optical sagittal section of stage of forty hours. *en.*, entoderm; *ec.*, ectoderm; *ad.*, adoral zone; *ap.*, apical cilia; *pr.ap.*, preapical cilia; *pr.*, prototroch.

[1] In Figs. 49 and 50, $2b_{2.2.1}$ should read $2b_{2.2.1.2}$.

Pl. XL.

ON THE OSTEOLOGY OF THE PIGEONS
(COLUMBÆ).

Dr. R. W. SHUFELDT.

INTRODUCTION.

DURING the past twenty years I have carefully examined and compared the skeletons of upwards of twelve hundred species of birds, and read a great deal upon what has been printed on the osteology of this interesting group of vertebrates. Many families and genera are represented by the material I have examined, the most of it being from the representatives of the United States avifauna. My observations have been written out and illustrated, the whole making a manuscript work of about twenty-five hundred pages, with over five hundred figures.

In November, 1899, Professor C. O. Whitman of the University of Chicago, who at that time was making some very interesting observations upon pigeons, asked me if I could not furnish him with a memoir upon the osteology of that group (*Columbæ*) for the JOURNAL OF MORPHOLOGY. As my chapter in the aforesaid manuscript work on the skeletology of the pigeons had never been published, I soon ascertained from him that it would be quite acceptable for the purpose he had proposed. To copy the original chapter was by no means a light task, but it was cheerfully undertaken, and in the most painstaking manner completed, by my wife, who is ever ready to assist me in my work in such matters. My sincere thanks are extended to her here for the promptness with which the assistance was rendered, especially as it came at a time when a very formidable work of mine now in press was engaging my entire attention. Notwithstanding this, by the aid of my camera and drawing pen I reproduced all of the original plates and figures. Our labor will not have been in vain, however,

if this brief account of the osteology of the group proves
to be of any service to those laboring upon the structure of
birds.

R. W. SHUFELDT.

WASHINGTON, D.C., Dec. 22, 1899.

COLUMBÆ.

SUPERFAMILIES.	FAMILIES.	SUBFAMILIES.
Didoidea	Dididæ	
Columboidea	{ Geouridæ Carpophagidæ Columbidæ	{ Didunculinæ

Existing pigeons the world over form a very well circum-
scribed suborder of birds, for the most part quite homogene-
ous in their morphology.   In studying their osteology I have
read the various accounts extant upon the remains of the dodo
(*Didus ineptus*); skeletons of *Didunculus strigirostris* (U. S.
Nat. Mus. Coll.), of *Pterocles arenarius* (U. S. Nat. Mus. Coll.),
and sterna of *Syrrhaptes* (for which I have to thank Professor
A. Newton, F.R.S.) have also been examined by me.   Much
of the literature and figures have also been looked over, and
the skeletons of a great many genera and species of existing
Columbæ examined and compared.   Our United States pigeons
are all members of the family *Columbidæ*, and consequently
are all true columbine birds.

They represent the genera *Columba* (with three species),
Ectopistes (one species), *Zenaidura* (one species), *Zenaida* (one
species), *Engyptila* (one species), *Melopelia* (one species), and
Columbigallina, Scardafella, Geotrygon, and *Starnœnas*, all with
one species each.   Skeletons of most of these genera are at
hand at the present time, but I am obliged to rely upon the
works of others, of which there are a number of very reliable
ones, for the osteology of *Columba*.   I have by me, however,
skeletons of the common domesticated pigeons, but they vary
greatly in their characters, as has been shown by Darwin and
others.[1]

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[1] Mr. J. S. Singley ,of Giddings, Texas, has furnished me with some good
osteological material for the United States pigeons, especially in the genera

Professor Huxley, it is known, made a group, *Alectoromorphæ*, to include the gallinaceous birds, including in it the *Pteroclidæ*, while another group, the *Peristeromorphæ*, was made to contain the true columbine forms, including the extinct dodo (*Didus*). Of these two groups Professor Huxley has said : " The relations of the *Peristeromorphæ* with the *Alectoromorphæ* are very close.   On the other side they seem to be allied with the owls and the vultures " (*P. S. Z.*, 1867, pp. 459, 460).[1]

Newton, in speaking of the characters common to the gallinaceous birds and the pigeons, has said: " But peradventure the real lesson taught by this aggregation of common characters is rather the retention of the union of the *Gallinæ* and *Columbæ* into a single group, after the fashion of bygone years, under the name, however meaningless, of *Rasores*.   Failing that, the general resemblance of most parts of the osteology of the Sand grouse to that of the pigeons, so well shown by M. Milne-Edwards, combined with their pigeon-like pterylosis, inclines the present writer to group them as a suborder of *Columbæ ;* but the many important points in which they differ from the more normal pigeons, especially in the matter of their young being clothed with down, and their colored and speckled eggs, must be freely admitted (art. " Ornithology," *Encycl. Brit.*, 9th ed., Vol. XVIII, p. 46).

Another classificatory view of this group has been taken by Coues, who in the last edition of his " Key " remarks that " the *Columbæ*, as above indicated, are intended to be made conformable to Huxley's *Peristeromorphæ* plus *Pterocletes*. Assuming the imperfectly known extinct dodo, *Didus ineptus*,

[1] In characterizing his *Peristeromorphæ*, and referring to the *sternum*, Professor Huxley says, " The *sternum* has two posterior notches, the inner pair of which may be converted into foramina.   The external processes thus formed are, as in the *Alectoromorphæ*, much shorter than the internal lateral processes " (*loc. cit.*, p. 459).   Evidently it is meant here the "*four* posterior processes," and the words *external* and *internal* have through a *lapsus calami* been transposed.

to have been a modified columbine, and considering the *Ptero-cletes* to represent rasorial modification of the columbine series, the order *Columbæ* may be separated into THREE groups, or suborders, DIDI, PTEROCLETES, and PERISTERÆ, the first two certainly, and the last probably, of a single family. The *Peristeræ* alone are American " (p. 562).

By the American Ornithologists' Union they are considered to be an ORDER, *Columbæ*, which is the arrangement also adopted by Mr. Ridgway in his Manual. Finally, Fürbringer makes his "Columbiformes" an intermediate suborder standing between his orders, Alectorornithes and Coracornithes, classifying them thus :

$$\text{Im. S. O. Columbiformes} \begin{cases} \text{G. s. str. Pterocletes. F. Pteroclidæ.} \\ \text{G. s. str. Columbæ} \begin{cases} \text{F. s. str. Dididæ.} \\ \text{F. s. str. Columbidæ.} \end{cases} \end{cases}$$

They constitute in the present volume my suborder, *Columbæ*.

Ectopistes affords us a good average columbine type, and from its skeleton we may study the osteological characters generally met with in the family.

The brain-case in the *skull* is comparatively capacious, being rounded for the most part above and behind, while its basal floor is nearly horizontal. It projects backwards considerably, being situated largely posterior to the orbital cavities and the quadrates. In outline the *foramen magnum* is cordate, and the condyle small, sessile, and notched posteriorly. The occipital ridge is fairly well marked, and it passes across the median, not very conspicuous supraoccipital prominence. Viewed superiorly, the interorbital area of the skull is markedly broad, being gently and uniformly convex from the parietal region to the mandibular base. These superficies are smooth and show no sutural traces. For the most part, the entire periphery of an orbit is sharp and unbroken in outline, being continuous behind with the anterior edge of the down-pointing, spiculiform sphenotic process. The apex of this latter is well removed from the apex of the much aborted, stumpy squamosal process. Either orbit is here a capacious cavity, while the mesial septum between them is quite deficient posteriorly and above. This vacuity merges with the large common

foramen, giving exit in life to the optic nerves, but not so with the equally extensive aperture above for the nerves of the first pair. So much for these interorbital fenestræ. At the upper part of an orbit the groove for the olfactory nerve is seen to be single, and anteriorly it enters a very small circumscribed foramen above the pars plana. The pars plana and the very large *lacrymal bone* fuse completely together, and to some extent with the frontal bone above them. This creates an unusually broad, entire inter-orbito-narial partition. Upon its outer border the lacrymal is broadly notched ; below it meets the zygomatic bar, and in front it is seen to be wedged in between the nasal and frontal, this wedge being another considerable part of the bone. In the cranium of this Passenger pigeon the *tympanic cavities* are large, very open and exposed, and either one of them facing downwards, forwards, and upwards. The anterior apex of the basitemporal gives but slight protection to the mesial common aperture of the Eustachian tubes. Close to and upon either side of this opening we are to note an aborted *basipterygoid process*, and neither of these latter meet the pterygoid bone opposite them.[1]

The rostrum in this pigeon is thickened, rounded below, and gradually tapers to a sharp point anteriorly, being fused in the latter locality with the lower margin of the mesethmoid, which here extends forwards considerably beyond the pars plana upon either hand. The Passenger pigeon has no *vomer*, and Parker, in his *Morphology of the Skull*, says, " In the Pigeons and Sand grouse the vomer is absent " (p. 263); but T. J. Parker, in his *Zoötomy*, declares for *Columba livia* that "the *vomer* [is] a small median bone lying between the palatines at the anterior end of the parasphenoid." Perhaps the vomer is sometimes present and sometimes absent, and I am inclined to think that this may apply also to some of the *Tetraonidæ*, as declared by me in a former memoir.

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[1] This fact rather surprises me, as heretofore I have always been under the impression that the *basipterygoid processes* among the *Columbidæ* were fully functional, and extensively articulated with the pterygoids. Not so, however, in this specimen (No. 18520, Coll. U. S. Nat. Mus.). It will be interesting to examine more specimens of *Ectopistes* on this point.

Owing to the considerable size of the gaping aural entrances, the basitemporal region is much contracted, but the basitemporal bone itself is more ample, as we find that in the embryos it extends out laterally to assist in forming the antero-inferior parietes of the tympanic walls upon either side.

The auditory cavities and the ossified stapes, as well as the cranial cavity proper, all present their usual ornithic characters, departing in no very marked manner from ordinary birds. All these cranial structures just named remind us more or less of the corresponding ones in the skull of the fowl.

A *pterygoid* is a short, thickish bone, somewhat twisted upon itself, and presents upon its midshaft (opposite the corresponding basipterygoid process) an inconspicuous, low and rounded eminence. In other species the summit of this is faceted. The *palatines* do not appear to articulate with each other in the middle line, but are accurately moulded upon the side of the rounded rostrum, upon which they glide in life, — the mesial lamina being extended forwards as a pointed process, also in the closest contact with the rostrum. This is the spine that has been by others erroneously termed the vomer among the *Pici*.

The postero-external angles of the palatines are very much rounded off, and upon the whole these bones are but feebly developed. Anteriorly, the prepalatine portion of either one is carried forwards as an exceedingly slender rod, widely separated from the fellow of the opposite side.

This very narrow prepalatine part fuses anteriorly with the nether surface of the lengthy maxillo-palatine of the same side, and fails to reach the premaxillary. All pigeons, so far as I am at present aware, are typically *schizognathous* birds, and the *nasals* are extremely peculiar bones. For, in addition to being acutely forked, the premaxillary process is markedly long and slender, running almost to the apex of the beak, closely moulded upon the underside of the coössified, slender nasal processes of the premaxillary. The delicate "external process" of a nasal is also rather long, and fuses with the upper edge of the maxillary process of the premaxillary of the same side. On top of the skull, in the middle line, the nasals unite with each

other, and spread out over the upper surface of the broad abut-
ment afforded by the ethmoid ; but they do not cover it entirely,
as the supero-lateral margins of this bone are seen upon either
side in the "nasal slit." The fused *premaxillaries* present
long and slender processes ; the narial apertures (or bony nos-
trils) being long, capacious clefts between them laterally (see
Figs. 1, 3, and 4, Pl. *A, px.*). They fuse also with the bones
with which they come in contact, as the nasals and the maxil-
laries. Faint sutures, however, may often be discerned among
them, even in fully adult pigeons.

A *maxillary* is completely absorbed or fused with the pecul-
iar *maxillo-palatine* process that it gives rise to anteriorly ;
while its backward-extending rod for the zygoma is curved
downwards and is exceedingly slender. A considerable inter-
val divides the *maxillo-palatines* from each other in the middle
line, and either one of these elements is found to be an elon-
gated osseous roll, *spongy within*, perfectly smooth *without*, and
fitting snugly in among the maxillary proper, the palatine, and
the nasal process of the premaxillary, with all of which it fuses
in the adult bird. Extending backwards and downwards from
the maxillary bone, the especially slender zygomatic bar has
upon its proximal end a minute peg-like process with which to
articulate with the outer side of the quadrate. Both *jugal* and
quadrato-jugal elements are found in this hinder end of the
zygoma, and their suture lines may often be made out even in
skulls of adult birds.

Either *quadrate* has a double mastoidal head ; two elongated
trochleæ upon the condylar process, that are placed lengthwise
directly in a transverse line with respect to each other ; and
finally the orbital process is well developed. Several of these
bones at the base of the skull are pneumatic, and their air holes
are easily made out in each case. In the quadrate it is situated
on its posterior aspect, directly between the mastoidal heads ;
in the pterygoid it is on the anterior side of the bone, close to
the quadratal head ; and in the palatine it is on the outer sur-
face of the bone and close to the pterygoidal head. Other
parts of the skull in *Ectopistes* are also pneumatic.

Coming to the *mandible*, it is seen to be of the V-shaped

pattern, with the symphysis short and feeble. The anterior moiety of the bone is bent downwards somewhat abruptly, and the ramal sides, from the angle thus formed to the symphysis, are shallow and weak, while the posterior halves are double the height and double the thickness transversely. The superior ramal margin of either limb is rounded off as it passes over the angular bend at the middle of the bone ; and below this point, on the side of the ramus, we note the ramal vacuity, this being much farther forward than we see it in most birds. In a great many pigeons this fenestra is closed in. The mandibular ends are thickened, with their inturned processes short and blunt, while posteriorly their hinder parts are completely and squarely truncated from above, downwards and forwards.

The hyoidean arches are seen to be very slenderly constructed in the *Columbidæ*, and to this *Ectopistes* forms no exception. Rather longish and acute, the *glosso-hyal* is seen to be preformed entirely in cartilage, as are indeed the slender and projecting *cerato-hyals* of the anterior cornua. The two *basi-branchials* are short, delicate rods of bone, and not fused together, where the anterior ends of the even more slender *cerato-branchials* articulate. *Epi-branchials* are much shorter, but have about the same caliber as the cerato-branchials. They are tipped with cartilage behind, as is also the second basi-branchial. Mr. T. J. Parker, in his description of the hyoid arches of *Columba livia* (*Zoötomy*, p. 198), found those parts essentially the same as I have described them here for *Ectopistes*, and this account will probably practically agree for all of our United States pigeons. These arches do not depart much from the galline type of structure.

Of the Skull in Zenaidura macroura.

Although agreeing closely with *Ectopistes*, the skull of this species presents us with some few points of difference. The interorbital septum is usually deficient along the track of the first pair of nerves, often as far as the pars plana. Both sphenotic and squamosal are much reduced, and differ from each other but little with respect to size.

Basi-pterygoidal processes are well developed and functional; the palato-pterygoidal articulation is very firm. An interpalatine spur is present, and a prepalatine passes along the outer side of the corresponding maxillo-palatine rather than beneath it, as it does in the Passenger pigeon. *Zenaidura* has its maxillo-palatines compressed from side to side, firm, and very slightly inclined to be scroll-like. A zygomatic bar is almost of hair-like caliber, and its quadrato-jugal end meets the outstanding process of the quadrate and its fore-part rather than its side.

Agreeing with all of our *Columbidæ*, we find a slight, rounded elevation on the superior aspect of the maxillary process of a premaxillary at a point about opposite the anterior apex of the maxillo-palatine (see Figs. 3 and 4, Pl. *A*).

The *mandible* appears invariably to lack the ramal vacuity at the side of either ramus (numerous skulls have been examined by me for this character).

Engyptila albifrons has a skull and associated parts very similar to that we find in *Zenaidura;* the "nasal slits," however, are almost entirely filled in by the swelling of the bones upon either side of them, — the mesethmoid, frontals, and lacrymals.

Melopelia leucoptera presents in its skull also only differences of the most trifling character as compared with the two just-named pigeons. The skull in this form exhibits a very complete state of pneumaticity.

Aside from their diminutive size, the skulls of *Scardafella inca* and *Columbigallina passerina* agree in all important particulars with the characters as seen in *Zenaidura*. They are seen, however, to be comparatively narrower between the upper margins of the orbits in the frontal region, and there is a well-marked median furrow passing longitudinally here. The orbital peripheries are very slightly tilted upwards.

When we come to the so-called "quail-doves" one would rather be inclined to look for at least some little variation in the pattern of the skull as compared with the more typical pigeons, but such can hardly be said to be the case. *Starnœnas cyanocephala* possesses a skull very much like what we

find in *Ectopistes* and *Zenaidura*. Upon the superior aspect, though, it reminds us more of what we see in the little Ground pigeons named above, especially in the frontal region; while the cranial base is more horizontally disposed than it is in *Ectopistes*, the entire foramen magnum and more being in this plane.

Another character is seen upon lateral aspect of the skull where the apices of the sphenotic and squamosal processes tend to fuse together. This reminds us of the state of affairs in many of the *Tetraonidæ*. It is a galline character. Among the *Columbidæ* the *sclerotals* of the eyes are of median size in so far as their width is concerned, but, owing to their being longer than usual, they are comparatively fewer in number. They overlap each other to some degree. I count but eleven of them in an eye of the Blue-headed quail-dove.

Of the vertebral column in the pigeons, and the remainder of the trunk skeleton: Ectopistes has eighteen vertebræ between skull and pelvic sacrum ; there are fourteen more fused together in the sacrum ; six free ones in the skeleton of the tail, and to all these must be added the rather large, flat, and subtriangular *pygostyle*. In the cervical region the " odontoid process " of the second vertebra is prominent, and the cup of the *atlas* nearly entirely absorbed. Barring the first and the last two cervical vertebræ, the vertebrarterial canal is complete throughout this division of the spinal column ; it is not so, however, in the case of the carotid canal, where in no instance does it ever get to be more than a groove. In the tenth cervical it is most nearly closed. From the third to the tenth inclusive the parial parapophyses are long and spiculiform ; and from about the fourth to the ninth inclusive the vertebræ themselves are lengthy, especially the fifth, sixth, and seventh. Their postzygapophyses are lengthened, and this leaves large openings among them leading into the spinal canal, on the dorsal aspect, in the articulated column. Two pairs of ribs are to be found in the cervical division of the column, — a tiny free pair having both capitula and tubercula on the thirteenth vertebra, and a larger free pair on the fourteenth ; these last possess epipleural appendages. The three leading dorsal

vertebræ fuse together to form one bone, and it has shortish lamelliform hypapophyses beneath its center. All its ribs connect in the usual manner by hæmapophyses with the sternum, as do the next succeeding pair which come from the fourth and which is at the same time the only free dorsal vertebra. These dorso-vertebral ribs have coössified unciform processes that are rather broad and flat, and the last one may be fenestrated. A pair of ribs come also down from the first sacral vertebra, but they do not support epipleural appendages, nor do their hæmapophyses quite reach the costal borders of the sternum. In the specimen at hand there is also to be found a very small pair of true "floating ribs"; they are immediately behind the articulation of the sacral-vertebral rib and its hæmapophyses, one upon either side.

Regarded as a whole, the *pelvis* of *Ectopistes* is seen to be broad and comparatively shallow, and although the ilia are in contact with the sacrum for their entire lengths, the union is a very feeble one, ordinary maceration being often sufficient to detach the ossa innominata. The sacrum is broad, roughly lozenge-shaped, with a low "crista" anteriorly, and the inter-diapophysial foramina very small, being confined to a single row upon either side of the middle line. Concaved in front for their pre-acetabular parts, the ilia are widely separated in that region, while their posterior margins lie roughly in the same curve with the line of the last sacral (or uro-sacral) vertebra.

Viewed laterally, we are to note the triangular notch on the hinder pelvic margin, indicating the division between the ilium and ischium. The post-pubic element is long and narrow, of nearly uniform caliber, and projects considerably behind. Distally the "foot" of the ischium rests upon it, and the *obturator space* or *cleft* above is a long, narrow vacuity, which in front opens into the small oval obturator foramen. There is no evidence whatever of the presence of a pre-pubis. The ischiac foramen and the acetabulum with its anti-trochanter are also both small, and indeed this group of openings on the side of the pelvis are comparatively closely huddled together and of little size. Upon ventral aspect, we find in front the pair

of free sacral ribs already described, and behind them the apophyses of three more vertebræ are thrown out against the under surfaces of the ilia as supporting braces. The eighth sacral also has its processes moderately modified, as compared with the others, for struts to the acetabulæ; and their outer extremities dilate, and are coincident with the lateral salient angles of the sacrum, of which they form a part.

Pneumatic fossæ are seen upon this ventral aspect, near the hinder borders of the ilium, upon either side, and I miss these air holes upon any part of the external surface of the pelvis where they usually occur in birds.

The middle vertebra of the tail, or, more correctly speaking, the third one of these segments, has the longest transverse processes, and these projections in the other vertebræ become gradually shorter as we proceed either forwards or backwards. They are entirely aborted in the last one, which, however, has the longest chevron bone, there being but two of these latter apophyses. The neural spines are quite lofty throughout this series of six caudal vertebræ in *Ectopistes*, and of its pygostyle I have already spoken above.

Taken as a whole the body of the sternum is concaved dorsally, and correspondingly convexed upon its pectoral aspect. The deep carina runs the entire length of this sternal body, being sharp on its concaved anterior border, and exhibiting a broadly rounded carinal angle below. A thickened welt in it passes down from the coracoidal grooves to spread out and be lost before reaching the long margin of the keel.

The anterior border of the sternum is much thickened, especially in the middle line, within which a single pneumatic foramen is seen. The costal border, upon either side, or so much as intended for the hæmapophysial facets, is concave and limited in length. Either costal process leans backwards somewhat and is quite prominent.

In front of the costal process, on a line with their bases, the coracoidal grooves are seen to be well marked; standing between them, in the middle line, the short stumpy spine of a manubrium projects forwards. Although the true body of this sternum is quite narrow for its mesial part, the bone

gains breadth by its spreading xiphoidal processes. Of these, the larger and stouter pair are the external ones, which have pointed free ends directed backwards and inwards. They spring from the middle of the side of the bone. The internal xiphoidal processes are considerably shorter, far more slender, and have transversely dilated ends, and they s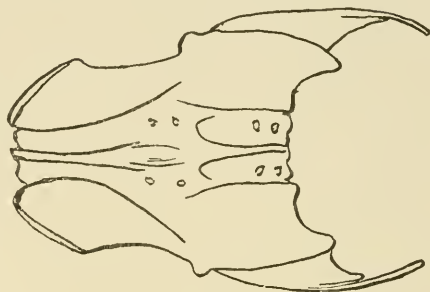pring from the hinder third of the bone. Mid-xiphoidal process is of a quadrilateral outline, with its posterior-external angles somewhat produced. These processes form a pair of notches upon either side of the carina; the large antero-external pair are widely open behind, and for the rest subelliptical in outline; while the small postero-internal pair, also of subelliptical outline, are nearly closed in behind. In several particulars the sternum reminds us of the galline pattern of the bone.[1]
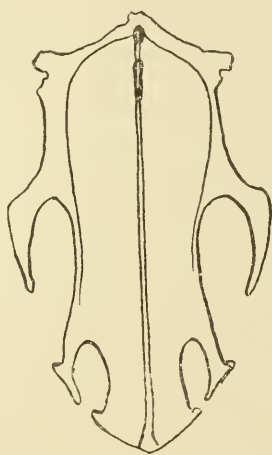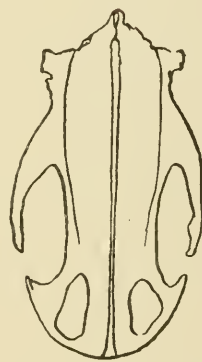

Fig. 1

Fig. 2.

Fig. 3.

Fig. 1. — Dorsal aspect of the pelvis of *Engyptila albifrons;* adult ♂; natural size.

Fig. 2. — Pectoral aspect of the sternum of the Passenger pigeon (*Ectopistes migratoria*); natural size.

Fig. 3. — The same view of the sternum of the Mourning dove (*Zenaidura macroura*). These bones give a good idea of the form of the pelvis and the sterna as they occur among the ordinary pigeons generally.

[1] Some instructive figures of the sterna of various pigeons appear in Pl. XXIII of A. and E. Newton's "Osteology of the Solitaire" (*Pezophaps solitarius*) in the *Phil. Trans. Royal Society of London*, 1869, pp. 327–362. The outlines of the sterna of the following species are given: *Columba livia, Didunculus strigirostris, Leucosarcia picata, Patagiœnas caribbœa, Chamœpelia trochila, Geopelia striata,* and *Goura coronata.* A sternum of *Columba livia* is given that has

Passing now to the shoulder-girdle we find all of its parts very well developed.

A *coracoid* has a length of shaft above the average ; and it is straight and subcylindrical in form. As usual its sternal extremity is dilated, especially to its outer side, while on the other hand the " coracoidal head" is not much enlarged in this pigeon. The scapular process is conspicuous, and in addition to giving room for the articulation of the scapula, it curves, as a flattened lamina of bone, inwards and then forwards to come near in contact with the os furcula. This latter element of the girdle is very slenderly constructed ; it being of the typical U-shaped pattern, with the limbs of nearly uniform caliber. Its free ends above are but very moderately enlarged, and the hypocleidium may be said to be entirely absent. Either head, upon its outer aspect, develops a feeble shoulder to rest in articulation against the head of a coracoid. Below, the os furcula is connected with the keel of the sternum by ligament, a mesial band passing between two bones.

The glenoid cavity, as usual, is formed by the coracoid and scapula, in the proportion of two-thirds contributed by the former, to the remaining one-third of the latter. A *scapula* is of the usual cimeter-form pattern, — the straight variety ; its anterior moiety is thickish and narrow, being expanded and flattened posteriorly, where it is obliquely truncated from within backwards and outwards to a point. In the natural skeleton this apex about reaches to the front border of the last dorsal rib.

With regard to *the remainder of the trunk skeleton* in *Zenaidura macroura*, as compared with what has just been described for *Ectopistes*, we find but few and comparatively unimportant differences. Among the most evident of these are those seen in the sternum, where the external-xiphoidal processes are more slender and curved, and do not present the distal truncation seen in the Passenger pigeon. The internal-xiphoidal processes in *Zenaidura* unite by their postero-internal

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internal-xiphoidal fenestræ and external-xiphoidal processes. *Leucosarcia picata* appears to have the pattern of the bone much as we find it in *Starnœnas*. *Goura* has quite a unique sternum for a pigeon, so far as I am at present aware.

angles with the postero-external angles of the mid-xiphoidal prolongation, thus creating fenestræ instead of notches.

Columbia livia also has a sternum of this pattern (Parker, Newton), but it is not invariably inherited by the various forms of domestic pigeons, as in the skeleton of an "Archangel" before me I find that the internal-xiphoidal processes do not unite with the mid-xiphoidal piece.[1]

Nearly all of the *Columbidæ* show a distinct, mesial, forward-projecting process on the anterior border of the sternum, which, in most species, is quite as conspicuous as the manubrium below it.   It is present in both *Zenaidura* and *Ectopistes.*[2]

Zenaidura has slenderer scapulæ than we see in the Passenger pigeon; their posterior moieties not being evidently truncated, and they are narrower and more pointed.

The characters seen in the vertebral column, pelvis, ribs, etc., of *Ectopistes* are essentially repeated with great exactness in such a species as *Engyptila albifrons*, and the same may be fairly said for the sternum and shoulder-girdle.   An interesting point, however, presents itself in the two skeletons of *Engyptila* at hand, for in the male bird the sternum is widely *two-notched* upon either side of the carina, — the female having had a sternum the internal xiphoidal processes of which unite behind with the mid-xiphoidal piece and thus create fenestræ, as in *Zenaidura*.   The union is very complete and firm and is important as going to show that the "fenestration," or "notching," of the xiphoidal portion of the sternum in pigeons counts as nothing for a character.   From this it will be seen that it varies even in the same species.

As figured by the Newtons, the sternum of *Patagiœnas caribbœa* possesses the anterior pair of notches, but it is only upon the left side posteriorly that a very small foramen is seen to exist.   In other words, the internal xiphoidal processes have almost merged with the mid-xiphoidal prolongation in this species (*Phil. Trans.*, 1869, Pl. XXXIII, Fig. 173).

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[1] Mr. Schollick, of the United States Museum, kindly furnished the skeleton of this domestic pigeon.

[2] See memoir on the *Accipitres* (in MSS.), and the description of a somewhat similar process on the sterna of the several species of *Falco*.

In a specimen of *Melopelia leucoptera* before me these pos-
terior sternal fenestræ are of unequal size, and the anterior
" notches " are situated well forwards, the external xiphoidal
prongs being curved, rather short, and slender. This speci-
men also has its last cervical vertebra fused with the anterior
dorsal one.   The species has a delicately constructed skeleton,
and the fusion of the ilia with the pelvic sacrum is very feeble,
the bones being easily separated by maceration.   Otherwise
the trunk skeleton of this form presents the usual columbine
characters.

Among the dwarfs of the family, as in *Scardafella inca* and
Columbigallina passerina, we find the same salient characters
present as were described in the trunk skeleton of *Ectopistes.*
In the first-mentioned species the corpus sterni is strikingly
narrow, and the external xiphoidal processes of the bone very
slender and spreading, helping to form very large anterior
xiphoidal notches.   These characters are also enjoyed to nearly
the same extent by the little Ground dove.   In both these
diminutive forms the os furcula hangs high and is almost of
thread-like proportions in the size of its limbs.   We must
also notice in the sternum that the anterior carinal angle is
not rounded off as it is in *Ectopistes* and *Zenaidura,* but truly
angulated.

Some notable differences, however, are to be observed when
we come to examine the trunk skeleton of *Starnœnas cyano-
cephala,* for this pigeon has nineteen vertebræ between the skull
and pelvis, fifteen in the pelvic sacrum, and only five free caudal
vertebræ, not including the pyzostyle.   There are four dorsal
vertebræ, the leading three being fused into one bone.   Its
pelvis has the general columbine pattern, but the sternum
of this bird is, comparatively speaking, enormous in size.   It
extends so far backwards that the extreme posterior xiphoidal
tip is, in the naturally articulated skeleton, in a vertical line
below the first caudal vertebra.   The keel to this bone is also
of an equal length and is not lacking in goodly depth.   The
corpus sterni is narrow, being nearly of uniform width for its
entire extent.   Both internal and external xiphoidal processes
are slender and produced ; the posterior notches being cleft-like,

and the anterior external pair being found far forwards and of great size. This bone faintly calls to mind the sternum as it is seen in *Tinamus*.

Of the skeleton of the limbs: Returning to our skeleton of *Ectopistes*, we now come to regard the osteology of its *pectoral extremity*. We note first that the brachium is somewhat shorter than the anti-brachium, which latter in turn is not as long as the skeleton of manus. *Humerus* then is comparatively short and straight, with a *triangular* radial crest, and a very jutting ulnar tuberosity, and this last over-arches a capacious pneumatic fossa, at the base of which are to be seen the little groups of pneumatic foramina. The *radius* is nearly straight, but the *ulna* is considerably bowed, and shows the papillæ adown its shaft for the insertion of the quills of the secondary feathers. *Carpus* is composed, proximally, of the two usual ossicles, *radiale* and *ulnare*, and the *ulnare* is crowded well in over to the anconal side of the limb. The terminal finger-joints are stout and long, with their salient edges sharp and pronounced. Especially is this the case with the long distal digit of index phalanx, which exhibits marked excavation down its ventral surface, thus giving its borders this distinct prominence. The flat and expanded proximal digit of index is entire, presenting no fenestræ in its expanded portion. It develops a conspicuous process posteriorly; and the little free digit behind it of the middle finger also has a stumpy process upon its posterior margin. There are a number of points in the pectoral limb of this pigeon that call to my mind the limb as we find it in *Geococcyx*.

Passing to the *pelvic limb* of *Ectopistes* we see its composition stamped with all the most usual characters of the ordinary members of this class of vertebrates.

The individual long bones are somewhat slender, but in their lengths appear to be in proportion to the size of their owner. I fail to find any evidences of pneumaticity present, though more material may show this. *Femur* has a straight, subcylindrical shaft, with its sharp trochanter raised moderately above the proximal summit of the bone. Its head is small, sessile, and placed at right angles to the shaft. A very

minute *patella* is present at the front of the knee-joint, and
it may exist in two pieces, mere ossific granules in the tendon
of the extensor femoris.

The *tibio-tarsus* of this limb has also nearly a straight shaft,
of a subcylindrical form.   Its pro- and ecto-cnemial processes
are not markedly produced, while distally its condyles are far
more prominent on their anterior aspect than they are behind.
Above them, in front, we note the usual little osseous bridge
beneath which in life pass certain tendons of the muscles of
the leg.

The fibula is long and very slender, and articulates in the
usual manner with the femur and the tibia.   Behind the internal
condyle of the tibio-tarsus there is to be seen quite a sizable
bony *sesamoid*, being of an elongated, concavo-convex form.

Coming to the *tarso-metatarsus*, it is to be noted that it has
a length equaling rather more than half the length of the tibio-
tarsus, for the condyles of which latter it offers upon its proxi-
mal summit two very decided excavations for articulation.

Its hypotarsus behind is jutting, short, and not especially
produced down the shaft.   Most conspicuous mesially, it is for
the most part situated to the outer side of the bone, and
exhibits one complete perforation at its middle for the pas-
sage of a tendon, and externally at least two very distinct
groovelets for the same purpose.   To the hinder side of the
hypotarsus the shaft is thin antero-posteriorly and is there
pierced by a foramen.   A section made of the shaft of this
bone just above the accessory metatarsal would show nearly an
elliptical figure.   The facet for articulation of this accessory
metatarsal is distinctly excavated, and the segment itself situ-
ated rather low upon the shaft is proximally twisted upon itself,
thus bringing its distal trochlea into the transverse position.
Thus *hallux* is brought low down, and its basal joint is the
longest of any in the foot.   These podal joints of pes are upon
the usual ornithic plan of arrangement, and they present no
strong characters for us to seize upon.   For any one of the
anterior toes its basal joint is the longest, while as we proceed
towards the ungual ones they become, in any case, a mite
shorter.

Upon the limbs of the skeleton of other pigeons found in our United States avifauna with what has just been described for *Ectopistes*, we find the differences to be but slight and hardly worthy of special record.   The same general columbine characters run through them all.   Perhaps it is *Starnœnas* that departs the most from this general set of characters of the extremities, and in it the tendency is towards the gallinaceous types, — rather tetraonine than otherwise.   Its wing, in so far
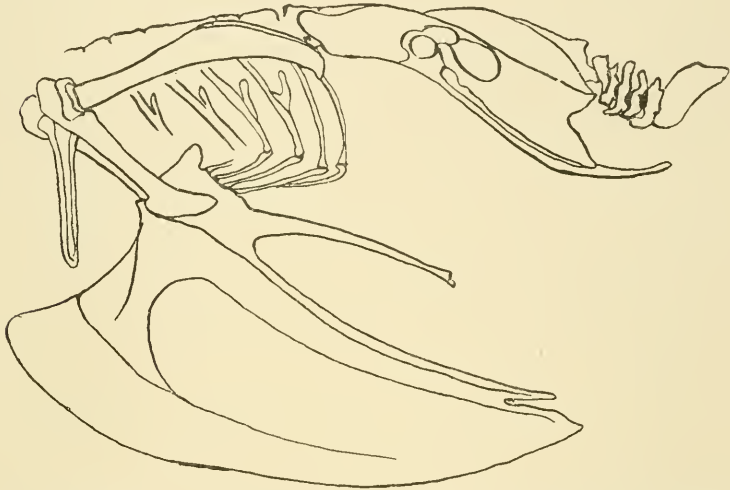

FIG. 4. — Line-sketch of the left lateral aspect of body-skeleton of the Blue-headed quail-dove (*Starnœnas cyanocephala*), natural size, by the author from specimen No. 33834 of the Collections of the United States National Museum, and designed to show the unusually large sternum in representatives of this subfamily of pigeons, — the *Starnœninœ*.

as its skeletal structure is concerned, again reminds me of the wing of *Geococcyx*, with a tincture of the fowl in it.

In the *pelvic limb* of *Starnœnas cyanocephala* the trochanter of the femur is unusually conspicuous and lofty ; the patella consists of one large, well-formed sesamoid, and usually a separate tiny ossific granule to its inner side ; the pro- and ectocnemial processes or crests of tibio-tarsus are considerably more prominent than in any of the other pigeons ; while, finally, two or more ossified pieces may be found in the "tibial cartilage" behind the distal end of the tibia.   Otherwise the skeleton of the leg of this species is completely columbine in character.

With respect to the pneumaticity of the long bones of the limbs of the pigeons we have had under consideration in this chapter, I would say the *humerus* is the only bone of the pectoral extremity that enjoys this condition, and the air is admitted to the shaft of it in all our *Columbidæ*, — so far as my observation goes.

The *femur* I am more doubtful about, for in some species it has the appearance of being pneumatic, while in others it is most assuredly not so. *Melopelia leucoptera* seems to belong to the first-mentioned class, while the bone is undoubtedly non-pneumatic in the Ground and Inca doves.

Principal Osteological Characters of the United States Columbidæ.

1. Completely schizognathous birds, with elongated narial apertures in the skull, which are not separated by an osseous septum nasi.
2. A large lacrymal present which fuses extensively with the pars plana, thus forming an unbroken plate.
3. Large vacuities in the anterior wall of the brain-case, the lower one of which merges with a big one in the interorbital septum.
4. Zygoma very slender.
5. Basipterygoid processes present which may (in all save *Ectopistes?*) or may not articulate with the short pterygoids — the latter not in contact in the middle line anteriorly.
6. Palatines very slender, with their laminæ somewhat reduced, and with their postero-external angles completely rounded off.
7. Maxillo-palatines antero-posteriorly elongated, internally spongy, and fused with the prepalatines, the maxillary, and the premaxillary.
8. The premaxillary process of either nasal bone carried far forwards, beneath the osseous culmen.
9. Sphenoidal rostrum sharp in front, thick and rounded behind.
10. Vomer may or may not be present (?). Huxley figures *Columba palumbus*, and says, " The vomer is very slender " (*P. Z. S.*, 1867, p. 434). Parker says the pigeons are without a vomer.
11. Quadrates typically ornithic, and with two transversely disposed facets for articulation with mandible.
12. Mandible V-shaped, its symphysis short and feeble ; articulatory ends transversely truncated posteriorly, from above downwards and forwards ; ramal vacuity may (*Ectopistes*) or may not (*Starnœnas*) be present.
13. Eighteen (*Ectopistes*) or nineteen (*Starnœnas*) vertebræ in the spinal column between the skull and pelvis. Three leading dorsal vertebræ fuse together to form one bone (*Ectopistes*), and with it may

fuse the ultimate cervical (*Melopelia*). Five (*Starnœnas*) or six (*Ectopistes*) free caudal vertebræ. A good-sized pygostyle present. Pelvis broad and shallow ; no prepubic spine present.

14. Os furcula U-shaped, without hypocleidium, and very slender.

15. Sternum large, with very deep carina ; two pair of flaring xiphoidal processes, usually making the bone four-notched, but the posterior or more inconspicuous internal pair of xiphoidal processes may unite by their extremities with the mid-xiphoidal prolongation and thus create fenestræ behind. Manubrium small, corpus sterni often narrow for its entire length. Usually four articular facets upon either costal border.

16. The *humerus* is straight, pneumatic, and its radial crest is triangular in form. The *radius* is straight and the *ulna* is bowed.

17. Trochanter of *femur* elevated above the summit of the shaft. *Patella* may be very small and in two pieces, or it may be larger with a single minute piece near it (*Starnœnas*). Ossific centers in tibial cartilage.

18. Hypotarsus of *tarso-metatarsus* of short cubical form, and is both pierced and grooved for the passage of tendons. *Hallux* on a level with the other toes, and its metatarsal peculiarly twisted. Phalanges of pes 2, 3, 4, 5 for the 1–4 toes, respectively.

Affinities of the Pigeons.

Our suborder of *Columbæ* in the United States contains but one family — the *Columbidæ*. Whether the quail-doves of the genus *Starnœnas* should constitute a subfamily of the *Columbidæ* can only be settled when we are in possession of a full knowledge of their anatomy. So far as the osteology of *Starnœnas cyanocephala* goes, it would seem to indicate that a subfamily line separates it from our other pigeons. One of the best-established facts in ornithology is that the *Columbidæ* are nearly related to the great gallinaceous group of birds ; so then the nearest relatives they have in our avifauna are the *Tetraonidæ*, especially the grouse. Then beyond them the *Cracidæ* and turkeys. Huxley has said, as we have already noted elsewhere, that "on the other side they seem to be allied with the owls and vultures." Such affinities, however, must be quite remote. There is no question about the links that connect the columbine types with the grouse and ptarmigans (*Lagopus*), for they are most perfectly seen in the

Sand grouse, holding, as these latter do, a morphological position directly between them. The plovers are not so far off in another direction, and *Tinamus* and *Hemipodias* have also distant claims to kinship. The extinct dodo and the existing *Didunculus* of Samoa are included in the suborder. Fossil remains of pigeons have not as yet been found in this country, though those of turkeys have.

Taken as a whole, the suborder *Columbæ* can easily be divided into two superfamilies — the *Didoidæ*, represented by the extinct dodo (*Didus ineptus*) of the family *Dididæ*, and the *Columboidea*, to contain all modern pigeons and such fossil ones as may come to light exhibiting the typical columbine characters in their remains.

The *Columboidea* fall naturally into three families — the *Gouridæ*, the *Carpophagidæ*, and the *Columbidæ;* and this last, in my opinion, contains as a subfamily the Samoan toothbilled pigeon, the well-known *Didunculus strigirostris.* A complete skeleton of this form I have carefully compared with the skeletons of a variety of pigeons, and am thus enabled to present the following :

Notes upon the Osteology of Didunculus strigirostris.

Apart from the *skull*, this bird has the skeleton of a pigeon that is subtypical but in a very few points. All of the cranial portion of the skull would answer for any ordinary *Columba;* the principal differences being that in *Didunculus* the interorbital septum is entire, and the apices of the post-frontal and squamosal processes of the skull are united by an osseous bridge, agreeing with what is seen in many of the true gallinaceous fowls (*Melagris*, for example).

It is in the facial portion of the skull and in the lower mandible that *Didunculus* exhibits the greatest differences as compared with other pigeons, and even here the traces of columbine structure are plainly to be seen. The slender zygomatic bar still meets the posterior border of the nasal bone at its middle, while the latter has simply become much broadened and shorter in comparison.

Schizorhinalism is evident, as in true pigeons, only somewhat

masked by the extraordinary upper mandible proper. This has the form of the beak of a small raptorial bird, its distal apex being hooked and sharp, and at its base pushed into, as it were, the frontal region of the skull. Indeed, it is the beak of a hawk engrafted upon the face of a dove. From the anterior portions of the palatines at the base of the skull, backwards, the characters are of any ordinary pigeon. Turning to the *mandible*, we find it much thicker and stronger than it is in the *Columbæ* generally ; it is nearly of uniform depth throughout, with a strong symphysis of a very strigine look.

The articular ends agree with some pigeons, but the ramal vacuity does not exist, and this is present in *Ectopistes* and others. In the dodo the ramal vacuity was very small and inconspicuous.[1]

The osseous *hyoidean apparatus* of *Didunculus* is extremely slender and agrees with the corresponding parts as seen in *Columba livia*.

As for the balance of the skeleton of this Samoan pigeon, it is simply after the general columbine type, a slight departure alone being seen in the sternum. This has a great open notch of an elliptical outline upon either side of the keel, — there being no smaller pair of notches posterior to these, as in most all pigeons. But, nevertheless, by holding the sternum of *Didunculus* up to the light, a thinning of the bone over the usual sites for these latter, as found in other representatives of the suborder, is evident.

In their excellent memoir cited in note below, Newton and Gadow have shown that *"Columba, Phaps,* and *Didunculus* differ from the others (*Treron, Carpophaga, Goura, Pezophaps,* and *Didus*) in having only twelve true cervical vertebræ,[2] two short,

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[1] For the best accounts of the osteology of *Didus ineptus* see the memoir by Professor Owen (*T. Z. S.*, vi and vii) ; Fürbringer's *Morphologie und Systematik der Vögel* (taf. xxi und xxii, pp. 778–781) ; Brown's *Thier-Reich : Vögel* (p. 950) ; but especially the memoir by Sir Edward Newton and Dr. Hans Gadow, entitled *On Additional Bones of the Dodo and Other Extinct Birds of Mauritius obtained by Mr. Theodore Sauzier* (*T. Z. S.*, London, vol. xiii, pt. vii, p. 296).

[2] These authors consider that "cervico-dorsal vertebræ are those which carry movable short ribs," and that " the other neck-vertebræ are true cervical vertebræ ; with the exception of the atlas and the epistropheus, they all possess a transverse foramen and immovable rib-rudiments."

four sternal, and one almost sternal pair of ribs, because their fifteenth or first anchylosed vertebra (instead of the sixteenth or second anchylosed vertebra) carries the first pair of sternal ribs."

It is very likely that *Didunculus strigirostris* is as nearly related to the genus *Columba* as it is to any other one of the minor groups of pigeons.

Concluding Remarks.

Since this memoir was presented for publication in the winter of 1899, an important work upon birds has appeared. I refer to the *Hand-List of Birds* compiled by Dr. R. Bowdler Sharpe, for a copy of which I have to thank him and the trustees of the British Museum. In Vol. I of that excellent work (pp. 51–92) we find upwards of six hundred species of existing pigeons enumerated, and not a few extinct forms. As a group, Dr. Sharpe places this large assemblage of birds next after the Pteroclidiformes (Order IV) and immediately before the Opisthocomiformes (Order VI), and classifies them as follows:

ORDER (V).	SUBORDERS.	FAMILIES.	SUBFAMILIES.
COLUMBIFORMES.	1. Columbiæ.	1. Treronidæ.	1. Treroninæ.
			2. Ptilopodinæ.
			3. Carpophaginæ.
		2. Columbidæ.	4. Columbinæ.
			5. Macropygiinæ.
			6. Ectopistinæ.
		3. Peristeridæ.	7. Zenaidinæ.
			8. Turturinæ.
			9. Geopeliinæ.
			10. Peristerinæ.
			11. Phabinæ.
			12. Geotrygoninæ.
			13. Calœnadinæ.
		4. Gouridæ.	
		5. Didunculidæ.	
	2. Didi.	6. Dididæ.	

All the forms in the second suborder of this arrangement are now extinct, including as it does only the two genera

Pezophaps and *Didus.*   It represents quite a natural taxonomy, and essentially agrees with what I have proposed at the beginning of the present memoir, that is, when we consider that Dr. Sharpe's suborders are coequal with my superfamilies, and he has included in his classification all of the known families and subfamilies in the world's avifauna.   I am inclined to think — that is, judging from osteological premises alone — the latter two groups, namely, the families and the subfamilies, can be somewhat restricted or reduced.   In any event, the main osteological characters I have given in this memoir for the species of existing pigeons will, in all essential particulars, apply to any representative of that group in any part of the world.   Osteologically they are quite a homogeneous assemblage of birds.

EXPLANATION OF PLATE *A.*

Hyoid arches and various views of the skull of *Columbia livia;* about twice natural size. After W. K. Parker.

FIG. 1. Basal view of skull.

FIG. 2. Rear view of skull.

FIG. 3. Upper view of skull.

FIG. 4. Lateral view of skull.

FIG. 5. Superior view of the hyoid arches.

So., superoccipital; *m.o.f.,* middle occipital fontanelle; *e.o.,* exoccipital; *o.c.,* occipital condyle; *b.o.,* basioccipital; 9, anterior and posterior condyloid foramina; 8, foramen for vagus nerve; *i.e.,* opening for internal carotid artery; *bt.,* basitemporal; *q.,* os quadratum; *qj.,* quadratojugal; *bs.,* basisphenoid; *pg.,* pterygoid; *pa.,* palatine; *ao.,* antorbital; *po.,* prevomer; *n.,* nasal; *px.,* premaxillary; *ar.,* articular; *sq.,* squamosal; *p.,* parietal; *f.,* frontal; *fo.,* postfrontal; *l.,* lacrymal; *as.,* alisphenoid; *ps.,* presphenoid; *pe.,* perpendicular ethmoid; 1, olfactory groove; 2, optic foramen; *ag.,* angular; *sag.,* surangular; *d.,* dentary; *prf.,* upper prefrontal; *s.o.b.,* superorbital ridge; *ch.,* connate ceratohyal; *bh.,* basihyal; *uh.,* urohyal; *ppt.,* posterior pterygoid process.

EXPLANATION OF PLATE *B.*

Fig. 6.   Left lateral view of the skeleton of *Didunculus strigirostris.*   Reduced about one-third.   Specimen No. 17793, Coll. U. S. National Museum.

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PHOTOGRAPHS OF THE EGG OF ALLOLO-
BOPHORA FŒTIDA. II.

FURTHER NOTES ON YOLK-NUCLEUS AND POLAR RINGS.

KATHARINE FOOT AND ELLA CHURCH STROBELL.

In 1898 Van Bambeke (30) published an important con-
tribution to the problem of the yolk-nucleus. He adduces
most convincing evidence that the yolk-nucleus substance,
after reaching its stage of disintegration (*désagrégation, mor-
cellement*), becomes directly metamorphosed into deutoplasmic
(fat) granules; these in turn being sacrificed to the formation
of the *sphères vitellines.* " On peut dire que l'apparition des
granules adipeux est le signal de celle des sphères vitellines.
Grâce à l'appoint fourni par ces granules, le cytoplasme ovu-
laire mieux nourri, devenu plus actif, sécrète ou élabore les
matériaux qui constituent les sphères vitellines," p. 550.

The author's arguments and figures presented such a strong
case, with conclusions so opposed to our earlier interpretation
of the phenomenon in the egg of Allolobophora — where we
claim that the yolk-nucleus substance contributes to the for-
mation of the polar rings — that we were led to reinvestigate
the problem, collecting fresh material for this purpose in the
summer of 1899. A comparison of Van Bambeke's Pl. XXIII
with the photos of the young oöcytes of our Pl. XLI will show
that the denser areas in the cytoplasm of our sections are mor-
phologically identical with the substance Van Bambeke calls
corps vitellin (yolk-nucleus). *Cf.* also Photo 90, Pl. XLV (a
reproduction of Van Bambeke's Fig. 9, Pl. XXV), with our
photos of scattered and peripheral archoplasm, Pl. XLI, Photos
11–23. Further, his fat (deutoplasmic) granules are undoubt-
edly the same substance as our osmophile granules.[1] These

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[1] The term "deutoplasmic" was used to designate these granules in an earlier
paper (9).

latter blacken intensely with osmic acid, and their morphological similarity will be recognized by a comparison of Van Bambeke's Fig. 4, Pl. XXVI,[1] with our Photos 41–44, Pl. XLII.

One of the conditions necessary to meet the demands of Van Bambeke's theory as to the destiny of the yolk-nucleus is that the deutoplasmic granules must not be present until the yolk-nucleus has disintegrated, as their formation is dependent upon this disintegration. "Deuxième stade — Désagrégation de ce corps vitellin. Troisième stade — Métamorphose graisseuse des éléments figurés issus de cette désagrégation " (30), p. 512.

Van Bambeke's figures illustrate this point, as they show no indications of the presence of deutoplasmic granules until *after* the disintegration of the yolk-nucleus.

This condition is not met in the egg of Allolobophora. The deutoplasmic (osmophile) granules are present long before the disintegration (scattering) of the yolk-nucleus, and they can be demonstrated during every stage of the development of the egg. We find them in nearly all the cells of the ovary, from the small cells near the proximal end, which show the first indication of a yolk-nucleus (Photos 28, 29, Pl. XLII), to the large oöcytes, first order, at the distal end (Photos 42–44, Pl. XLII).

If their presence can be thus demonstrated, where a *disintegrating* yolk-nucleus is out of the question (Photos 28–35, Pl. XLII), does it not suggest that the two phenomena are independent of each other and have no causal relation ? Furthermore, similar osmophile granules can be demonstrated in the tiny cells surrounding the ovary (Photos 49, 50, Pl. XLII), in the egg membrane itself or a capillary space within it (Photos 44 and 48), and in the spaces between the eggs (Photos 46, 47).

Van Bambeke's method of identification of the fat granules is their reaction to osmic acid and their solubility in turpentine. " La nature graisseuse des granules se traduit par leur coloration noire au contact des liqueurs à l'acide osmique, et par leur solubilité dans la térébenthine " (30), p. 546.

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[1] See Photo 96, Pl. XLV, for reproduction of this figure.

The osmophile granules in these early stages show precisely the same reaction to Van Bambeke's test as is shown by the granules in the older oöcytes (Photos 41–44, Pl. XLII), which are beyond question the same structures as those described and figured by Van Bambeke (*cf.* Photo 96, Pl. XLV). A detailed account of the reaction of these granules to turpentine and xylol was given in an earlier paper (11).

In Pholcus, Van Bambeke finds that the fat granules disappear at a definite stage — that they are transitory formations — "que leur nombre diminue et enfin qu'ils disparaissent, par une digestion intra-cellulaire, dans les oöcytes plus âgés" (30), p. 554. "Toutefois, on peut considérer les premiers, c'est-à-dire les granules adipeux, comme n'ayant qu'une existence *transitoire*,[1] leur mission étant de fournir des matériaux nutritifs au cytoplasma et de le rendre ainsi plus apte à élaborer, à sécréter les secondes; (the vitelline spheres) les sphères vitellines représentent, par conséquent, le vrai vitellus nutritif" (30), pp. 554, 555.

In the egg of Allolobophora they are not transitory formations; their presence can be demonstrated during all stages of the growth of the egg, the maturation, fertilization, and cleavage (Pl. XLII, Photos 60–62, Pl. XLIII, and Photo 82, Pl. XLIV).

Is there a structure in this egg homologous to the vitelline spheres of Van Bambeke? Photo 96, Pl. XLV, is a reproduction of Van Bambeke's Fig. 4, and represents, besides the fat granules, the first appearance of the *sphères vitellines* of Pholcus. A comparison of these spheres with Photos 56–59, Pl. XLIII, and Photo 78, Pl. XLIV, indicates that the hyaline globules of Allolobophora *may* be the homologue of the *sphères vitellines*. These hyaline globules, which appear, however, at a much later stage than the spheres of Pholcus, show no evidence of being formed at the expense of the deutoplasmic granules. The eggs found in the cocoons are less rich in osmophile substance than the eggs at the distal end of the ovary (*cf.* Photos 41–44, Pl. XLII, with Photos 60–62, Pl. XLIII, and Photo 82, Pl. XLIV). We interpret this as

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[1] The italics are ours.

indicating that the nutritive substance in the cocoon obviates the necessity of the accumulation in the egg itself of so large an amount of deutoplasm as is found in the ovarian egg. In Allolobophora the diminution of the osmophile substance appears to be correlated with the albumen of the cocoon rather than with the development of any one special structure in the egg.

The decrease in the number of osmophile granules occurs between the germinal vesicle stage (eggs at distal end of ovary) and the metaphase of the first maturation spindle (eggs in the freshly deposited cocoons). We hope to be able to find these intermediate stages with a view to tracing the diminution of the osmophile substance, for we are inclined to think that the storing of the osmophile granules in the ovarian egg may be for the use of the egg during this period.[1]

We have been unable to determine the origin of the deutoplasmic (osmophile) granules. If they are secreted by the egg itself, should they not be more numerous in normal eggs, where presumably the functional activity is most pronounced? This, however, is not the case. If their origin is due directly to any substance outside of the egg, should they not be more numerous at the periphery? This again is not the case. The deutoplasmic granules are relatively more numerous in pathological eggs, and in these cases they appear to be formed at the expense of the entire cytoplasm, and not alone of the yolk-nucleus substance. In studying eggs in varying stages of degeneration, the amount of osmophile substance in relation to the cytoplasm increases as degeneration progresses, and finally we have a stage where the *entire cytoplasm* of the egg has become osmophile. We do not feel justified in asserting that this substance is not the same as that forming the normal deutoplasmic granules, for the steps between the two extremes are so gradual that it is impossible to draw a line at any point between the two. One of the first indications of an abnormal egg appears to be an increase in the number of osmophile granules, and a further stage of degeneration is evidenced by aggregations of the granules or their fusing into homogeneous masses (Photo 45, Pl. XLII), the number of

[1] Since writing the above we have secured these stages.

these masses increasing with the progress of degeneration. We interpret the normal condition as that shown, for example, in Photos 41–44, Pl. XLII, in which relatively minute osmophile granules are quite evenly distributed throughout the cytoplasm.   At the proximal end of the ovary often a majority of the eggs contain an abnormal amount of osmophile substance massed as in Photo 45, Pl. XLII.   This condition is in keeping with the fact that in most cocoons the number of degenerating eggs is far in excess of the normal eggs—sometimes in so large a proportion as twenty-five to one. These degenerating eggs can be readily recognized under the dissecting microscope, and when an advanced stage of degeneration is reached the eggs fall to pieces or collapse as soon as they are dropped into fixative.   If in these cases of degeneration we have an exaggerated expression of the formation of the normal osmophile (deutoplasmic) granules, then this egg supports Van Bambeke's theory to the limited extent that some cytoplasmic constituent becomes metamorphosed into osmophile granules.   What the substance is we are unable to determine; but we do not believe that the entire yolk-nucleus is sacrificed to the formation of these granules, as Van Bambeke claims for the egg of Pholcus.   We are convinced that *in the egg of* Allolobophora *the yolk-nucleus persists and finally a part of it contributes to the formation of the polar rings.*

A comparison of the photos of Pls. XLI–XLIV with the lithographic plate of an earlier paper (9) will demonstrate that these photos taken directly from the preparations support the interpretation then published, that "the polar rings and the so-called yolk-nucleus are one and the same substance."   At that time one of us differentiated this substance from the rest of the cytoplasm with Lyon's blue.   Its distribution is very variable, this variability often bearing a definite relation to special fixatives, and a differentiation in color is possible only where the substance is aggregated into definite masses.   This was accentuated in the above-mentioned paper (9) as follows : "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,[1] 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 equal throughout the cytoplasm (p. 7). *The process* (of staining) *must be carefully watched under the microscope, for the lithium carmine in both the spindle fibers and cytoplasmic network may be replaced by the Lyon's blue"* (p. 4).[2]

Photos 1–23, Pl. XLI, show successive stages of the growth and the varying distribution of the yolk-nucleus, from its first appearance in the tiny cells of the ovary until its final distribution throughout the cytoplasm of the large oöcytes, first order, at the distal end of the ovary. During all these stages it is morphologically an accumulation of granules. When the granules are scattered through the cytoplasm they are very difficult to demonstrate; but when they are aggregated into more or less definite masses they can be readily differentiated. We interpret Photos 51–59, Pl. XLIII, as showing later phases of the same granular substance. In Photo 55 part of it is aggregated into masses, which resemble the ovarian eggs of Photos 21 and 23, Pl. XLI. Through all these stages the substance is morphologically the same (granular); but it has not been possible always to reproduce this feature in the prints. These photos (51–59, Pl XLIII) show that this substance, which in the younger eggs (51–54) is more evenly scattered through the cytoplasm, aggregates towards the periphery (55–57) as the egg develops, finally leaving the central part of the egg relatively free from the dark granular substance. This feature is clearly shown in the fifteen serial sections of Pl. XLIV (Photos 63–77). We have thirty-three

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[1] This point is illustrated in Photo 55, Pl. XLIII, and in many photos of an earlier paper (12).

[2] In view of this specific statement of the need of exercising great caution in the use of Lyon's blue, to avoid staining other structures in the cell, it is interesting to note Wilson's criticism published in his work on the cell. "To identify as 'archoplasm' everything that stains with Lyon's blue is indeed a broad use of the term" (31, '96), p. 121. Later this was repeated by one of his pupils. "Foot calls everything in the cell which stains with Lyon's blue, archoplasm." Calkins (4), p. 728.

serial sections of this egg; but the fifteen reproduced are enough to show the peripheral arrangement of the archoplasm at this stage, *i.e.*, mature egg, with the pronuclei at the early stages of formation (*cf.* Photos 57, 58, Pl. XLIII, for same stage of development at a magnification of 660 diameters; the latter (Photo 58) is a section of the periphery). In Photo 59, Pl. XLIII (vignetted section), we have a little later stage, when the archoplasm is no longer distributed around the *entire* periphery of the egg, but is beginning to concentrate at two nearly opposite areas, preparatory to forming the two polar rings. One of these areas is shown in Photo 59, and successively later stages in Photos 78–80, Pl. XLIV. The pronuclei gradually increase in size during these stages. The most common form of the completed polar ring is shown in Photos 82–84, Pl. XLIV, but a comparison of these with Photos 80, 81, and 85 will show that this form is not constant. (The granular structure of the archoplasm is shown in Photo 84, Pl. XLIV; see explanation of plate.) The tiny granules demonstrated throughout the cytoplasm in the stained preparation of Photo 83 resemble morphologically the accumulated mass of granules forming the polar rings.

In Molgula, Crampton (6) finds that the yolk-nucleus, which is typical of one stage of the egg's development, becomes the yolk-spheres (deutoplasmic spheres) of a subsequent stage, his results in the main features supporting Van Bambeke's observations in Pholcus. A comparison of Crampton's figures (1–7) with photos of the young oöcytes of Pl. XLI will show that the yolk-nuclei in Molgula and in Allolobophora are morphologically alike. The identity of the two substances cannot be insisted upon, however, because of the unusual reaction to hæmatoxylin, Crampton describes as typical for the yolk-matrix in Molgula. He says: "It is important to note that *the yolk-matrix fails to stain with hæmatoxylin*" (6), p. 32. In Allolobophora the yolk-nucleus stains *intensely* with hæmatoxylin, and thus is in accord with its reaction in other forms (Van Bambeke (30), pp. 521, 527). The yolk-spheres in Molgula show a striking morphological resemblance to the deutoplasmic (osmophile) granules in Allolobophora,

but their chemical reaction is quite different, for Crampton finds in Molgula that "the chemical reaction of the yolk-spheres to dyes and other tests are *precisely the same*[1] as those of the disintegrating yolk-matrix"[2] (6), p. 42.

An examination of the unstained preparations of Allolobophora (Pl. XLII) will show that the chemical reaction of the deutoplasmic (osmophile) granules is very different from that of the yolk-nucleus. The latter fails to blacken with osmic acid, even after many hours' immersion in a one per cent solution, whereas an immersion of fifteen or even five minutes is sufficient to blacken the osmophile granules. The yolk-nucleus invariably stains intensely with hæmatoxylin, while the osmophile granules *very rarely* react to this stain.

The homogeneous masses of osmophile substance (Photo 45, Pl. XLII), indicative of a pathological condition, suggest the "great yolk-plates" of Lumbricus (Calkins (3)). As stated in an earlier paper (9), "in normal eggs I find no structures answering to the 'great yolk-plates' described and figured by Calkins for Lumbricus." In his book on the cell, Wilson (31) reproduces Calkins's figures, and in his last edition (1900) describes Calkins's "great yolk-plates" as "fragmentation of yolk-nucleus." Is this a correction of Calkins's interpretation? If this can be supported by a further correction of Calkins's microchemical analysis and of his assertion that these bodies are "great yolk-plates," "homogeneous" in appearance, and not granular, like the yolk-nucleus, then Calkins's Fig. 5 (see reproduction, Photo 88, Pl. XLV) might be interpreted as representing a fragmentation of the yolk-nucleus, and thus be compared to our Photos 19, 21, or 23, Pl. XLI. Calkins's (3) text, however, prohibits this comparison. "Next the disintegrating masses *lose their granular structure* and become large and homogeneous, forming the yolk-plates of the egg," p. 226. "In Lumbricus the yolk-nucleus disintegrates

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[1] The italics are ours.

[2] Their similarity in response to dyes seems to have one exception. "Corrosive sublimate affected the yolk so that it retains the iron-hæmatoxylin," p. 30. "It is important to note that the yolk-matrix fails to stain with the hæmatoxylin," p. 32.

and the parts become *homogeneous in appearance and then enlarge to form the great yolk-plates,"* p. 228.[1]   In Allolobophora the yolk-nucleus substance retains its granular form at this stage and shows the same chemical reaction.   In view of Calkins's text the bodies in question can be compared only to the mass of osmophile substance of Photo 45, Pl. XLII, or to a pathological condition which is less usual, in which dense homogeneous chromatophile masses are scattered throughout the cytoplasm (Foot (9)).   Photo 87, Pl. XLV, is a reproduction of Calkins's Fig. 4, which he describes as "later stage of disintegration."   Wilson applies this interpretation to Calkins's Fig. 5 (Photo 88, Pl. XLV), Calkins himself describing this as a "nearly ripe egg showing yolk-plates." If his interpretation of these bodies is correct, they should be found in later stages of the egg, *i.e.*, during its maturation, its fertilization, and in the mature (ripe) egg.   This is not the case, however, for Allolobophora.   In normal eggs there is nothing that can be interpreted as the "great yolk-plates" which Calkins describes for Lumbricus.

Origin of the Yolk–Nucleus (Archoplasm).

The weight of authority is in favor of a nuclear origin.   In the egg of Allolobophora we have been unable to discover any decisive evidence on this point, and we must therefore retain the neutral attitude taken in an earlier paper of avoiding any assumption as to its origin, evidence being given to show only that it was neither of chromatin nor nucleolar substance.   It is first seen close to the nuclear wall, and this proximity to the nucleus is the *only* evidence we can discover in favor of a nuclear origin.   The fact that in such small cells as those shown in Photos 1, 3, and 6, Pl. XLI, there is no continuity between the yolk-nucleus and any nuclear constituent is the sole evidence we have of a cytoplasmic origin.[2]

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[1] Italics are ours.

[2] We call attention to this here because in spite of the care taken to emphasize these points in an earlier paper (9) they have been misinterpreted.   Crampton (6), p. 47, says: "Foot asserts that the appearances described by Calkins are pathological, that in Allolobophora the yolk-nucleus is of cytoplasmic origin,

Crampton (6) has applied Miescher's interesting artificial digestion experiments to the egg of Molgula, with a view to sustaining his conclusion that the yolk-nucleus owes its origin to albuminous granules within the nucleus. "In order to place beyond question the identification of the yolk-matrix and nuclear granules as the only purely albuminous constituents of the cell at this stage, artificial digestion was resorted to," p. 38; "it seems an almost unavoidable conclusion that the nuclear granules are either solid masses which pass out from the nucleus to form the yolk-matrix, or they are semi-fluid albuminous substances which are able to pass readily out through the nuclear wall, and which in preserved material are coagulated by the fixing reagents," p. 40.

We have applied this method of artificial digestion to the yolk-nucleus in Allolobophora, but in spite of repeated trials we have been unable to obtain the results Crampton gets in Molgula.[1] The yolk-nucleus in Allolobophora is especially favorable for these experiments, for it stains intensely with iron-hæmatoxylin, and its presence can be demonstrated as long as any trace of the substance is left in the cell.

The ovaries were fixed in corrosive sublimate, sectioned (5 μ) and fastened to the slide with the hot-water method. Before placing them in the digestion fluid they were immersed for thirty minutes in a dilute solution of iodine, in order to remove all trace of corrosive sublimate. Experiment proved this to be a necessary precaution, for the sections not treated by the iodine showed no response to the pepsin, even after six hours' immersion. While in the digestion fluid the temperature was kept at 40° C. by means of the water bath.[2]

After examining several preparations before and after digestion, we were convinced of the inconstancy of the action of the

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arising from the same substance as archoplasm." Wilson (31), p. 121 : "Foot maintains that the yolk-nucleus in Allolobophora is not of nuclear but of 'archoplasmic' origin, though no relation between it and an attraction sphere is established."

[1] We used "Pure Golden Scale Pepsin," manufactured by the New York and Chicago Chemical Company, following the formula given by Crampton (1% pepsin in 1/5% HCl).

[2] We are greatly indebted to Dr. R. W. Tower for kindly directing these experiments.

digestion fluid. For example, in one section of an ovary we would find that in some cells only the chromatin had disappeared; in others the cell membrane or nuclear membrane, or both, would succumb first to the action of the pepsin, and some of the cells would disappear completely; in others part of the cytoplasm was lost, while the membrane of the cell and nucleus remained intact. As a rule, the nucleoli and archoplasm (yolk-nucleus) show the strongest resistance to the action of the digestion fluid, this possibly being due to their relative density.

To test the suspicion that these results might be caused by maceration due to the warm hydrochloric solution, a slide was subjected to the same technique, omitting the pepsin, but no effect was produced upon the preparation, even after six hours' treatment. In order to ascertain as nearly as possible the exact effect of the digestion fluid, we adopted the following method: After mounting an unstained preparation in xylol, xylol balsam, or glycerine,[1] we selected a small oöcyte showing cytoplasm, yolk-nucleus, and nucleolus; and this we photographed at a magnification of 660 diameters, the same cell being photographed again after treatment with the digestion fluid. By a persistent repetition of this method we reached a pretty definite conclusion as to the inconstancy of the action of the pepsin and how far this inconstancy invalidates it as a precise test.

At first we selected and photographed more than one cell in each preparation, but the unequal rapidity of the action of the pepsin made this impracticable, for one cell often entirely disappeared before the other showed any response to the digestion fluid. After photographing the unstained cell, the slide was put in the digestion fluid at a temperature of 40° C. and examined every thirty minutes (under a 2 mm. immer. lens), comparing the cell with the negative. It is thus possible to follow each step in the process, and to determine positively just which structures show the least resistance to the digestion fluid, and also possibly whether digestion is the only

[1] The action of the digestion fluid was not impeded by previous mounting in glycerine, xylol, or xylol balsam.

factor that takes part in the process.   Photo 24, Pl. XLI, shows
a very young oöcyte from an unstained preparation, before
treatment with the digestion fluid.   The yolk-nucleus (archo-
plasm), the cell membrane, the cytoplasm, and the nucleolus
are shown.   After securing a satisfactory negative of this cell,
the preparation was put for two hours in the digestion fluid
following the method above described.   It was then stained
with iron-hæmatoxylin, mounted in balsam, and the same cell
again photographed (Photo 25).   A careful comparison of the
two photographs will show that the cell membrane has com-
pletely disappeared and nearly all of the cytoplasm; some of
the yolk-nucleus is also lost, but a large part of it still remains.

Photo 26, Pl. XLI, is a section through an older oöcyte
showing archoplasmic masses (yolk-nucleus) scattered through-
out the cytoplasm.   This was photographed from an unstained
preparation before treatment with the digestion fluid.   It was
then placed in the warm digestion fluid for two hours, stained,
mounted, and re-photographed (Photo 27).   These two photos
(26, 27) illustrate again the digestibility of the cytoplasm and
cell membrane, and show that the archoplasm is one of the last
constituents in the cell to yield to the action of digestion fluid.
Though many of the archoplasmic masses still remain intact
(Photo 27), both the chromatin and nuclear membrane have dis-
appeared, leaving no trace of a nucleus.   It is possible that this
entire section of the nucleus may have dropped out, but varying
stages of disintegration of nuclei can be seen in the preparation.

In a third preparation nothing was left of the photographed
cell after an hour and ten minutes in the digestion fluid.   In
a fourth preparation, after thirty minutes, the cell membrane
and chromatin had disappeared, all other constituents remain-
ing intact.[1]   After forty additional minutes no perceptible
change had occurred ; after one hour additional (total, two
hours and ten minutes), the nuclear membrane and part of the
cytoplasm had disappeared ; the yolk-nucleus, nucleolus, and
chromatin were left untouched.

[1] This disappearance of the chromatin might be explained on the ground that
albumen is a constituent of chromatin.   Fischer (7), p. 75, Wilson (31), 1900,
pp. 41 and 332.

In a fifth preparation nothing was left of the photographed cell after thirty-five minutes in the digestion fluid. We have frequently repeated the above method, with the following results : As a rule, the cell membrane is the first to disappear, then the nuclear membrane with part of the cytoplasm, and the contents of the nuclei, with the exception of the nucleolus. In many eggs the large nucleolus is all that is left to mark the presence of the germinal vesicle.

The yolk-nucleus and nucleoli appear to be the last to succumb to the action of the digestion fluid, but whether this power of resistance is due to their chemical constitution or merely to their relative density we cannot say.[1]

The Specific Nature of Archoplasm. — The specific nature of archoplasm claimed by Boveri has been subjected to repeated attacks. In some cases, however, these attacks lose their force, for the author figures a substance in the cell which morphologically resembles archoplasm and which he dignifies with a special name. A few authors stoutly maintain the specific nature of archoplasm, a few retain the term "archoplasm," but avoid defining it ; while the expressions of others on the subject are so contradictory that they create a problem as obscure as the one under discussion. This substance, which appears to be fluid in the living egg (Foot (9), pp. 7, 8) and granular after fixation, is in such intimate combination, after certain fixatives, with the substance which forms the network, that one is led to question the individuality of the two substances. We are convinced, however, that in this egg the individuality of each substance can be demonstrated, though in many cases the granular archoplasm is so massed that the cytoplasmic network is completely obscured. Our preparations (Photos 51–53, Pl. XLIII) show

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[1] These results are quite opposed to Crampton's (6). He finds that the nucleolus "is partially dissolved by artificial digestion," p. 39, and that the yolk-matrix (yolk-nucleus) disappears by digestion, while the cytoplasm remains intact. "With the other stains confirmatory results were obtained, all agreeing in demonstrating the more or less complete removal of the albuminous yolk-matrix and nuclear granules, according to the length of time the section was digested, and showing furthermore the indigestibility of the cell body," p. 39. Crampton's preparations were digested "from one to two hours."

areas of cytoplasm which appear to be entirely free from
archoplasm, these areas showing a network structure which is
typical (at least after fixation).   This appears to indicate
that the archoplasm is not necessary to the formation of
the typical network and is thus an individual constituent,
which may combine more or less intimately with the other
constituents of the cytoplasm.   We claim merely to be able
to demonstrate a morphological dissimilarity of the two sub-
stances (as they appear in fixed material), and we hope to
illustrate with our complete series of photos the presence of
the archoplasm during all stages of the development of the
egg, and each step of the final aggregation of part of it to
the poles of the mature, fertilized egg.   The continuity of
the substance is so complete that we do not feel justified in
giving it another name at any one stage of the development
of the egg (Pls. XLI, XLIII, XLIV).

Technique. — The technical manipulation of the cocoon
eggs has been described in an earlier paper (10).   The
ovaries were vivisected and dropped immediately into the
fixing fluid, the time required for the removal of the ovaries
from the living worm averaging about five minutes.   The
fixative for each subject is noted under the explanation of
the photographs.   '

Study of the Living Ovaries. — Since first studying the
living ovarian eggs (1894) the observations have been repeatedly
renewed, these proving a valuable control for fixed material,
but beyond this being of little practical value.   Even with
the aid of a Zeiss 2 mm. immer. lens, eggs are very rarely
found in which the yolk-nucleus can be recognized.   In many
of the smaller cells, however, a differentiated area can be
distinguished close to the nucleus, and this corresponds in
size and position to the archoplasm (yolk-nucleus) seen in
fixed material.   Munson (24), Pl. XIII, Fig. 16*a*, shows a
differentiated area in the living ovarian egg of Limulus,
which he describes as granular, but this area in Allolobophora
appears to be clear and homogeneous.   In the living egg the
membranes of the egg and of the germinal vesicle can always
be seen distinctly, but a nucleolus is the only constituent of

the germinal vesicle that can be clearly differentiated. When a nucleolus is not visible the germinal vesicle looks somewhat opaque, but homogeneous, like fine ground glass. The germinal vesicle is always spherical, and its contact with the cytoplasm complete, while the cytoplasm is in close contact with the cell membrane. This has made us very cautious in drawing conclusions from fixed material showing a distorted germinal vesicle or cytoplasm torn away from the cell membrane, for a study of the living egg will readily demonstrate that this condition is an artefact. The osmophile granules also show conspicuously in the living egg, and agree *both in size and position* with those seen in fixed material. They appear in the substance between the cells and in the small cells (oögonia?) near the proximal end of the ovary. Besides the osmophile granules there are clearer and denser areas, but no evidence of a network, the general effect being homogeneous. In the cytoplasm there is no evidence of hyaline globules such as those described for the later cocoon stages (11 and 12), and if the substance is present in the living ovarian egg, it is not in the form of globules. It is very difficult to obtain a good fixation of the oöcytes, first order (at the distal end of the ovary); nearly all the fixatives which give satisfactory results in the later stages and in the younger ovarian eggs distort the structures in the oöcytes, first order, shrinking the germinal vesicle and tearing the cytoplasm from the egg membrane.[1] The defense of any structures seen in fixed material, at one stage of development of the egg, on the ground that this stage has been subjected to the same technique used for an earlier or later stage, would be quite worthless in the case of Allolobophora, where the only safe criterion is a comparison at each stage of the fixed with the living material.

Homologues. — The question as to what extent the archoplasm of this egg can be homologized with substances in other cells offers an interesting problem. The homology of the yolk-nucleus to substances in other cells has been suggested by various authors, — Nussbaum (28), ('82) ; Balbiani (2), ('93);

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[1] Carnoy and Lebrun (5) have experienced a like difficulty for the oöcytes, first order (first spindle stage), of Ascaris. "Nous ne connaissons pas encore un

Meves (20), ('94); Foot (9), ('96), and others. These homolo-
gies are emphasized, extended, and most suggestively pre-
sented, in Prenant's classic work, *Sur le Protoplasma Supé-
rieur* (*Archoplasme, Kinoplasme, Ergastoplasme*) (29), ('98, '99).
The homology of the yolk-nucleus in oögenesis to the Neben-
kern of spermatogenesis is perhaps the most familiar. As early
as 1882 Nussbaum (28) identified the Nebenkern of a variety
of gland cells with the Dotterkern of eggs and the Nebenkern
of the spermatocytes. On Pl. XLV we have reproduced (from
the works of various authors) figures which appear to show a
special cytoplasmic substance that resembles the archoplasm
in the egg of Allolobophora. We realize the superficial nature
of this morphological comparison, and we therefore shall avoid
making any assumptions, but hope to aid in the solution of
the problem by presenting these likenesses in a convenient
form for the cytologist of broader experience to consider.

Photo 86, Pl. XLV. A reproduction of Auerbach's (1)
Fig. 6^d — *Paludina vivipara.* " Intermediäre Proliferation der
primären Samenzellen. Eine ebensolche ist durch Furchung
in vier Tochterzellen geteilt; in letzteren ist noch während
ihres Zusammenhangs die Nebenkernbildung weit vorge-
schritten. Vergr. 1000" (*cf.* Photos 1–6, Pl. XLI). Auerbach
regards the Nebenkern as a differentiation and concentration
of the general cytoplasm : " Die Zellsubstanz erfährt eine
allmählich vor sich gehende innere Differenzierung, die zur
Bildung eines Nebenkerns führt," p. 443. " Auch hier
entsteht ein solcher auf dem früher geschilderten Wege durch
Verdichtung des Cytoplasma," p. 491. The author's Neben-
kern contributes to the formation of the spindle, and he regards
it as the homologue of Kinoplasm. " Auch in seiner weiteren
Funktion erweist sich der Nebenkern als ein Kinoplasma in
demselben Sinne wie dasjenige der Pflanzen," p. 445.

Photos 97, 98, Pl. XLV, reproductions of Meves's (20)
Figs. 26 and 28 (*Salamandra maculosa*). " Spermatogonien des
erwachsenen Salamanders," Fig. 26 (Photo 97). " Körner-
kranz," Fig. 28 (Photo 98), " Körnermassen zu einem Haufen

bon fixateur des œufs d'Ascaris à cette période, et Dieu sait si nous en avons
essayés," p. 79.

vereinigt." The author homologizes these masses of granular substance to the yolk-nucleus of authors and to the attraction sphere, pp. 143, 144 (*cf.* photos of the young oöcytes of Pl. XLI). In a later paper (21) he names the substance *Idiozom*, declining to adopt any of the names used for homologous structures (for example, Archoplasm, Nebenkern, and Centrodeutoplasm), because none of these reach the limits he has defined for Idiozom. Meves's results are supported by the observations of Carl and Georg Niessing (26 and 27). "Meves hat schon mehrfach beweisende Beobachtungen vorgeführt, ebenso mein Bruder und ich, welche das Vorhandensein einer besonderen specifischen Substanz in der Sphäre und damit die Existenz dieser selbst zeigten" (27), p. 105.

Among the more recent papers, Gurwitsch (13) has given strong evidence in support of Meves's identification of Idiozom and yolk-nucleus. "Wir können somit mit gutem Rechte den Dotterkern der unreifen Säugethiereier dem Idiozom der Samenzellen vollständig homologisiren. Sowohl die männlichen als die weiblichen Genitalzellen haben somit die Eigenthümlichkeit, dass die Centralkörper derselben von einer deutlich differenzirten, anscheinend in ihrer Zusammensetzung sehr constanten und von dem umgebenden Cytoplasma scharf abgegrenzten Hülle umgeben sind" (p. 381). "Es wäre gewagt, in anbetracht der scharf umgrenzten Rubinfärbung des Idiozoms, von einer specifischen färberischen Reaction der Substanz derselben zu sprechen und jede entsprechend gefärbte Substanzanhäufung im Eicytoplasma für einen Bestandtheil oder Product des Idiozoms zu erklären" (p. 386). "Bei der Mitose entsteht aus dieser Plasmaanhäufung sicher ein theil, wahrscheinlich auch die ganze achromatische Figur" (p. 389).

Photo 93, Pl. XLV. Reproduction of Murray's (25) Fig. 21 (Arion), resting spermatocyte. The author concludes "that the Nebenkern is the attraction sphere of the spermatocyte," p. 431; and in reference to its origin he says: "The observations recorded above are so little in favor of the nuclear nature of the Nebenkern that it would be preferable to drop the term altogether. Some purely descriptive name, such as archoplasm mass or attraction sphere (although this is also open

to objection), should be adopted till our knowledge of these structures is more advanced," p. 434.

Photos 100 and 102, Pl. XLV. Reproductions of spermatocytes, first order of Pentatoma. Montgomery (22), Figs. 98–100. These figures show Meves's idiozom substance clearly defined from the rest of the cytoplasm, and a comparison of Photos 1–6, Pl. XLI, supports Meves's ((20), p. 143, 144) conclusions as to the homology of this substance to the yolk-nucleus. Photo 103 is a reproduction of Montgomery's Fig. 195, illustrating the persistence of the substance to the metaphase of the first division (*cf.* Photo 52, Pl. XLIII). Many of his figures of Pl. V show its continued presence in the spermatocytes, second order. It seems to us that this substance, so clearly shown by Montgomery to persist during these stages, can be homologized to the archoplasm (yolk-nucleus) of Allolobophora.

Photo 104, Pl. XLV. Reproductions of Hermann's (15) Fig. 13. "*Proteus anginneus.* Spermatocyt im Ruhestadium mit Archoplasma-kugel." The dense filaments within the archoplasmic mass the author terms "Archoplasmaschleifen," and identifies them with the Nebenkern of some authors, p. 585.

Photo 99, Pl. XLV. Reproduction of Hermann's (16) Fig. 6. *Scyllium catulus* — spermatid. This is one of many figures in which is clearly differentiated the author's "Archoplasmaanhäufung (eine stärker granulirte, sich dunkel tingirende Protoplasmamasse ")" (*cf.* Photos 1–6, Pl. XLI). On p. 282 he says : "Technisch ist die Archoplasmaanhäufung gerade in den Selachierspermatiden sehr leicht nachzuweisen, vor allem an Material, welches mit Bena'scher Lösung fixirt und hierauf einer Doppelfärbung mit Hämatoxylin und Saffranin unterworfen würde ; schon bei Anwendung ganz schwacher Systeme (Leitz Obj. 3) wird man das schwarzgefärbte Archoplasma neben dem rothen Kerne mit aller Deutlichkeit wahrnehmen."

Photo 95, Pl. XLV. Reproduction of part of Calkins's (4) Fig. 9. "Prophase of division" (in *Noctiluca miliaris*). "The sphere is dividing." Of this sphere Calkins says : "The sphere

in Noctiluca apparently corresponds to Boveri's archoplasm. It is a persistent cytoplasmic structure, of a definite size and shape in resting and active cells, and appears to consist of a specific substance. Astral rays and central spindle are formed from its substance, p. 727. These observations support Ishikawa's (17) ('94) earlier interpretation of an archoplasmic spindle."   Later he says, " Here both the central spindle fibers and the radial fibers are formed out of archoplasm " (18), ('99).

Photo 94, Pl. XLV.   Reproduction of v. Lenhossék's (19) Fig. 1.   " Nervenzelle mittlerer Grösse (45 μ) aus dem spinalganglion des Frosches," showing the " Plasmaschollen " (Nissl's granules). The author regards these as aggregations of the cytoplasm, "bloss um koncentrisch verlaufende Verdichtungen in der wabenartigen Struktur des Protoplasmas handelt," p. 351.   A comparison of this figure with photos of scattered archoplasm in Pls. XLI and XLIII recalls Prenant's (29) conclusions as to the homology of these structures to kinoplasm (archoplasm).   " S'il y a ou non dans la cellule nerveuse une substance qui satisfait aux conditions requises d'un kinoplasme.   Pour le dire tout de suite, la substance chromatophile de Nissl nous paraît remplir ces conditions," p. 179.   Prenant supports Marinesca's interpretation of this substance and accepts his term " Kinetoplasma," p. 188.

Photo 89, Pl. XLV. Reproduction. Henneguy (14), Fig. 288. " Spermatocyte de *Pyrrhocoris apterus* . . . montrant des filaments de kinoplasma plus fortement colorés . . . que le reste de la cellule." Henneguy identifies this substance as the Nebenkern of spermatocytes.   "Ces éléments forment le Nebenkern dans les spermatocytes des générations ultérieures. . . . Ces éléments figurés représentent le kinoplasma de Strasburger dont on peut ici suivre l'évolution, et qui restent différenciés du trophoplasma depuis les premiers spermatocytes jusqu'à la fin du développement du spermatozoïde," p. 380 (*cf.* photos of scattered archoplasm of Pl. XLI and Photo 55, Pl. XLIII).

Photos 91, 92, Pl. XLV.   Reproductions of Morgan's (23) Figs. 18 and 41 — unfertilized eggs (Arbacia, Sipunculus),

after treatment with sodium chloride. The preparations show aggregations of the author's "cyanoplasm." These aggregations are described as "collections of granules of deeply staining cyanoplasm." Later, however, Morgan states that "the granular effect is due only in part to granular structure. The cyanoplasm appears to be made up of groups of alveoli. Possibly it is only a network. . . . It corresponds probably to Boveri's archoplasm, and if so the latter cannot be looked upon as a specific substance in the cell. . . . An accumulation of deep blue cyanoplasm is present both in *normal* eggs and in those from the salt solution around the polar spindle. . . . This cyanoplasm forms the clear polar area of the living egg." A comparison of these figures with our Photos 51–59, Pl. XLIII, indicates that these aggregations of cyanoplasm are comparable to the aggregations of archoplasm in the egg of Allolobophora. Whether the latter are artefacts we can hope to decide only by a careful comparison of the distribution of the substance after a variety of fixatives. A study of artificial (perhaps exaggerated) aggregations of the substance should aid the cytologist in determining whether such aggregations are merely condensations of the network substance, or whether they demonstrate the presence of an individual constituent of the cytoplasm. Our preparations indicate that a network can be formed without any contribution from the archoplasm, and that therefore the two substances represent individual constituents of the cytoplasm. See p. 529.

Photographic Technique. — Plates, Carbutt (New Process or Carbutt B – 16) and Hydrochinon developer, according to the formula recommended for these plates. When necessary, the negatives were intensified. Our method of focusing has been described in an earlier paper (12).

To ascertain at a glance whether the focus has slipped during the interval of rest[1] a very simple device has been found helpful. After determining the exact focus desired for the detail to be photographed, select at least two microsomes

[1] "Let the microscope stand undisturbed for at least ten minutes before pulling down the camera, and never attempt to take a photograph unless the focus has held absolutely true during this interval" (12), p. 613.

on slightly different planes, noting the effect raising or lower-
ing the micrometer screw has on each microsome. Thus the
slightest slip of the focus can be detected at a glance, and
the relative sharpness of the two microsomes will indicate in
which direction it has changed. A further aid in settling any
doubt as to whether the focus has slipped during the inter-
val of rest is to glance at the preparation through a stronger
and weaker focusing lens — *e.g.*, if you have focused with a
— 7.D., and the preparation looks sharper through a — 8.D.,
the focus needs to be slightly lowered. If, on the contrary,
the image appears sharper through a —6.D., the focus needs
to be slightly raised.[1] The stronger lens shows a lower plane,
the weaker lens a higher plane. These relations are reversed,
however, in the negative, *i.e.*, the stronger lens shows a higher
plane, and the weaker lens a lower plane.

The results of our experiments with different combinations
of lenses and camera draw may be of some practical value,
although some of the experiments have not been repeated
often enough to warrant our offering them as infallible
guides.

For the photos taken at a magnification of about 660 diam-
eters, we use a Zeiss 2 mm. immer. 140 aperture, projection
ocular 4, diaphragm at 0 and 21½ inch camera draw (measuring
from stage of microscope to plate-holder). For a magnifica-
tion of about 1000 we extend the draw to 29¾ inches. For
the photos taken at a magnification of 286 diameters, Zeiss
4 mm. apo., the collar at the lowest point and a thin cover-
glass, the camera draw and projection ocular being the same
as for the 660 magnification. With a 2 mm. immer. projec-
tion ocular 4, the same focusing lens has given a satisfactory
focus, whether the diaphragm of the projection ocular 4 is at
0 or 10. The magnification of the latter is, however, so much
less, that it is much more satisfactory to use a 4 mm. dry and
the 29¾ inch draw (× about 450), for the 4 mm. dry has greater
depth.

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[1] The focus should be corrected by hair-breadth turns of the micrometer screw
without looking through the eye-piece, for when watching the preparation it is
difficult to avoid turning the screw beyond the point required.

The simplest method of determining the proper focusing lens is to photograph a minute cytological detail, using a focusing lens of medium strength, for example, — 5.D. If your negative shows too high a focus (*i.e.*, too high a plane), you need a weaker lens; if too low a focus, a stronger lens, and successively stronger or weaker lenses can be placed on the eye-piece, until one shows the exact focus reproduced by the negative, that being the lens you should have used. A readjustment of the focus with the right lens will give an exact reproduction of the finest details. With this method it is necessary to take only one photograph in order to determine the proper focusing lens, instead of taking several, as suggested in an earlier paper.

The spherical lenses are inexpensive and a series from — 1.D. to — 10.D., will be found useful.

PAPERS REFERRED TO.

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EXPLANATION OF PLATE XLI.

The photos of this plate illustrate successive stages of the growth and distribution of the yolk-nucleus (archoplasm) in the ovarian cells, from its first appearance in the smallest cells near the proximal end of the ovary to its distribution in the large cells at the distal end of the ovary — *i.e.*, oöcytes, first order. All the photos are taken at a magnification of 660 diameters (Zeiss 2 mm. immer. lens). All the preparations except Fig. 20 are stained with iron-hæmatoxylin. Where it is stated that the fixation was followed by osmic acid, this was done to demonstrate the presence of osmophile granules, and after these were studied in the unstained preparations and sometimes photographed, the preparations were stained in iron-hæmatoxylin. In the plates we shall use the term "archoplasm" to designate the substance known as yolk-nucleus. In several of the original velox prints one or more details have been slightly strengthened with a lead-pencil, in order to insure a more satisfactory reproduction. If any of our readers should wish to compare the reproductions with the original velox prints, one or more may be obtained on request.

Photo 1, section ($2\frac{1}{2}\mu$) of three tiny oöcytes showing the archoplasm close to the nuclear membrane. The nucleolus is shown in only one of the cells. Fixative, corrosive sublimate.

Photo 2, section ($2\frac{1}{2}\mu$) of a small oöcyte with archoplasmic masses distributed through the cytoplasm. Fixative, Rabl's picro-sublimate.

Photos 3 and 4, sections ($2\frac{1}{2}\mu$) of young oöcytes. The archoplasm of photo 3 is in closer proximity to the nuclear wall than that shown in Photo 4. Fixative, corrosive sublimate.

Photo 5, section (3μ) of a small oöcyte. Archoplasm massed near the nucleus. Fixative, corrosive sublimate followed by osmic acid.

Photo 6, section ($2\frac{1}{2}\mu$) of two small oöcytes. Archoplasm closely attached to nuclear wall. Fixative, corrosive sublimate.

Photo 7, section (5μ) of a larger oöcyte. Archoplasmic masses throughout the cytoplasm. Fixative, corrosive sublimate.

Photo 8, section ($2\frac{1}{2}\mu$) of a young oöcyte. Peripheral archoplasmic masses. Fixative, picro-sublimate, followed by osmic acid.

Photos 9 and 10, sections ($2\frac{1}{2}\mu$) of growing oöcytes. Masses of archoplasm scattered through the cytoplasm, nearly encircling the nucleus. Fixative, corrosive sublimate.

Photos 11 and 12, sections ($2\frac{1}{2}\mu$) of growing oöcytes. Archoplasm massed around the periphery of the cell. Fixative, picro-sublimate, followed by osmic acid.

Photo 13, section ($2\frac{1}{2}\mu$) of a growing oöcyte. Masses of archoplasm scattered through the cytoplasm. Fixative, corrosive sublimate.

Photo 14, section ($2\frac{1}{2}\mu$) of a growing oöcyte. Archoplasm massed at the periphery of the cell and scattered through the cytoplasm. Fixative, picric acid.

Photo 15, section ($2\frac{1}{2}\mu$) of a growing oöcyte. Archoplasm scattered through the cytoplasm. Fixative, corrosive sublimate.

Photo 16, section ($2\frac{1}{2}\mu$) through the cytoplasm of a growing oöcyte. Scattered archoplasmic masses and many of the osmophile granules. These granules

did not stain with the hæmatoxylin, but retained enough of the osmic to leave a brownish yellow tint which photographs sharply. Fixative, picro-acetic, followed by osmic acid.

Photo 17, section ($2\frac{1}{2}\mu$) of a growing oöcyte. Archoplasmic masses aggregated near the nucleus, which is somewhat shrunken. Fixative, picric acid.

Photo 18, section ($2\frac{1}{2}\mu$) of a growing oöcyte, near the distal end of the ovary. Small archoplasmic masses scattered through the cytoplasm. Fixative, corrosive sublimate.

Photo 19, section ($2\frac{1}{2}\mu$) through the cytoplasm of a growing oöcyte, near the distal end of the ovary. Archoplasm massed at the periphery of the cell and scattered through the cytoplasm. Many of the osmophile granules can be seen (*cf*. Photo 16). Fixative, picro-nitric, followed by osmic acid.

Photo 20, section ($2\frac{1}{2}\mu$) through a growing oöcyte, near the distal end of the ovary; preparation unstained. The archoplasmic masses retained enough of the picric acid to give a sharp differentiation in the photograph. In this preparation the osmophile granules are black, due to the subsequent treatment with osmic acid. Fixative, picro-nitric, followed by osmic acid.

Photos 21 and 23, sections ($2\frac{1}{2}\mu$) of a large oöcyte, first order, at distal end of the ovary. The archoplasmic masses are quite evenly distributed through the cytoplasm. Fixative, Rabl's picro-sublimate.

Photo 22, section ($2\frac{1}{2}\mu$) of two growing oöcytes, near distal end of the ovary. A large part of the archoplasm aggregated at the periphery. Fixative, Rabl's picro-formalin.

Photo 24, section (5μ) of a very young öocyte, showing an archoplasmic mass attached to the nuclear membrane. The chromatin which was present in the nucleus is not in focus, the nucleolus alone being represented in the photo. Preparation unstained and mounted in glycerine. Fixative, corrosive sublimate.

Photo 25. The same cell shown in Photo 24, after the preparation had been immersed for two hours in artificial digestion fluid, stained with iron-hæmatoxylin and mounted in balsam. The photo shows that by the action of the pepsin the cell has lost its membrane and cytoplasm, but nearly all the archoplasm remains intact.

Photo 26, section (5μ) of a young oöcyte, with archoplasmic masses scattered through the cytoplasm. Preparation unstained and mounted in xylol. Fixative, corrosive sublimate. Many of the details in this photo were strengthened with a pencil.

Photo 27. The same cell shown in Photo 26, after the preparation had been immersed for two hours in artificial digestion fluid, stained with iron-hæmatoxylin and mounted in balsam. The photo shows that the cell and nuclear membranes have disappeared, also a large part of the cytoplasm, while many of the archoplasmic masses still remain intact.

Pl XLI.
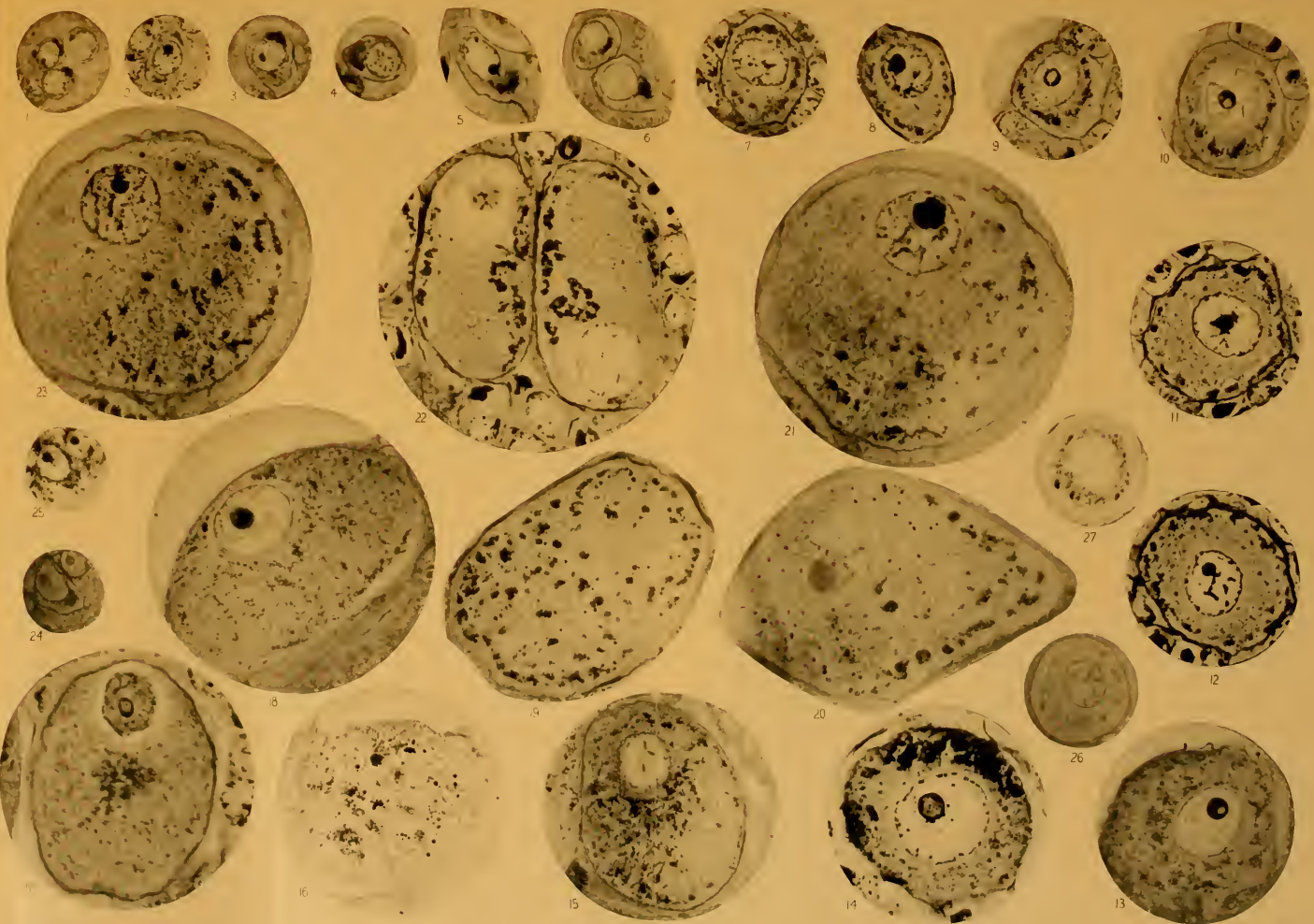
EXPLANATION OF PLATE XLII.

The photos of this plate show osmophile granules in the ovarian eggs — from the smallest cells near the proximal end of the ovary (cells in which the first trace of archoplasm is seen) to the large oöcytes, first order, at the distal end of the ovary. These granules are also shown in the large and capillary spaces between the oöcytes, in the membrane or capillary space within the membrane surrounding the eggs, and in the cells around the ovary. All the photos except No. 37 were taken from unstained preparations, 'and the magnification is the same for all, with one exception, No. 38 When using a fixative not containing osmic, the ovaries after fixation were always put in this acid for a few minutes, in order to blacken the deutoplasmic (osmophile) granules. These granules are sharply differentiated in the unstained preparations, as no other structure in the cell shows this reaction to the osmic. They can be readily seen in cells where it is impossible to identify them after staining. Where the archoplasm is differentiated, it is due to a yellowish tint produced by the fixative.

Photo 28, section ($2\frac{1}{2}\mu$) of two very young oöcytes, near the proximal end of the ovary, each showing osmophile granules. The archoplasm in these cells is at the same early stage of development shown in the stained preparation of Photo 1, Pl. XLI. Fixative, corrosive acetic, followed by osmic acid.

Photo 29, section ($2\frac{1}{2}\mu$) of three young oöcytes, each showing one or more osmophile granules. The archoplasm in these cells resembles closely that of the stained preparation of Photos 1 and 2, Pl. XLI. Fixative, Graf's picro-formalin, followed by osmic acid.

Photo 30, section ($2\frac{1}{2}\mu$) of a young oöcyte. One osmophile granule at the periphery of the small mass of archoplasm, which is faintly differentiated even in the unstained preparation. For a sharp differentiation at this stage, see Photos 3, 4, and 6 of the stained preparations of Pl. XLI. Fixative, Hermann's fluid.

Photo 31, section ($2\frac{1}{2}\mu$) of a young oöcyte. One osmophile granule nearly in the center of the small mass of archoplasm, which is in contact with the nuclear wall (*cf.* Photos 3, 4, and 6, Pl. XLI, for stained preparations of archoplasm at this stage). Fixative, Hermann's fluid.

Photo 32, section ($2\frac{1}{2}\mu$) of a young oöcyte. Two large osmophile granules. The cell contains the amount of archoplasm usual at this stage of development (*cf.* with stained preparations shown in Photos 3, 4, and 6, Pl. XLI). Fixative, Hermann's fluid.

Photo 33, section ($2\frac{1}{2}\mu$) of young oöcyte. Two osmophile granules nearly in contact. See Photos 3, 4, and 6 for stained archoplasm at this stage. Fixative, Hermann's fluid.

Photo 34, section ($2\frac{1}{2}\mu$) of young oöcytes, showing osmophile granules and archoplasm. In the largest cell there are four osmophile granules, in the smallest three, and in the cell on the right there are two. A dark print was made of this preparation, in order to show the archoplasm in the two cells on the right. The archoplasm in the largest cell is not unlike that shown in Photo 5, Pl. XLI. Fixative, Plat-acetate-formalin, followed by osmic acid.

Photo 35, section ($2\frac{1}{2}\mu$) of a young oöcyte. Five osmophile granules can be seen in the section, though the archoplasm is still in close contact with the

nuclear wall, and shows no indication of disintegrating (*cf.* Photos 4 and 6, Pl. XLI, for stained archoplasm at this stage). Fixative, Hermann's fluid.

Photo 36, section (2⅓ μ) of a young oöcyte, showing osmophile granules surrounded by archoplasm; three to the right, and one above the nucleus. These granules are completely obliterated after the archoplasm is stained (*cf.* Photo 37). Fixative, Hermann's fluid.

Photo 37, the same cell shown in Photo 36, after staining with iron-hæmatoxylin. The archoplasm is deeply stained and completely obliterates the osmophile granules, which were sharply differentiated in the unstained preparation.

Photo 38, section (3 μ) of a young oöcyte. The archoplasm still showing no signs of disintegration. Two widely separated osmophile granules, only one of them in contact with the archoplasm. Fixative, chromo-acetic, followed by osmic acid. × 930 diameters.

Photo 39, section (2½ μ) of a growing oöcyte. This large cell shows an increase in the amount of archoplasm and in the number of osmophile granules, the intermediate stages showing a gradual increase of both. There are archoplasmic aggregations on the periphery and throughout the cytoplasm. For the varying distribution of the archoplasm at this stage, see Photos 13, 14, and 15, Pl. XLI. Fixative, Hermann's fluid.

Photo 40, section (2½ μ) of a growing oöcyte. The archoplasm is scattered through the cytoplasm, and has increased in amount with the growth of the egg. There are also more osmophile granules present at this later stage of development. For the varying distribution of the archoplasm at about this stage, see Photos 17, 18, 19, 20, and 22, Pl. XLI. Fixative, Hermann's fluid.

Photo 41, section (2½ μ) through the cytoplasm of a large oöcyte, first order, at the distal end of the ovary. In these eggs the archoplasm is much more evenly distributed, and the number of osmophile granules has increased enormously, but it is a question whether they have increased more than the archoplasm or other constituents of the cell. For an example of the distribution of the archoplasm at this stage, see Photos 21 and 23, Pl. XLI. Fixative, Hermann's fluid.

Photos 42, 43, and 44, sections (2½ μ) of oöcytes, first order, at the distal end of the ovary. These eggs are further examples of what we interpret as the normal size and distribution of the osmophile granules at this stage of development. Their increase is scarcely out of proportion to the growth of the rest of the cytoplasm. In degenerating eggs, however, the osmophile substance is largely in excess of all other constituents of the cell (*cf.* Photos 21 and 23, Pl. XLI, for stained archoplasm at this stage). Fixative, Photo 42, Hermann's fluid; Photo 43, corrosive sublimate, followed by osmic acid; Photo 44, picro-nitric, followed by osmic acid.

Photo 45, section (2½ μ) of a degenerating oöcyte, first order, at the same stage of development shown in Photos 42 and 44. This egg was filled with masses of osmophile substance such as we see in the photo, and the rest of the cytoplasm is degenerating; it has shrunken away from the cell membrane, and not only the archoplasm, but all parts of the cytoplasm appear to have been sacrificed to the formation of these large masses of osmophile substance. Fixative, corrosive sublimate, followed by osmic acid.

Photo 46, section (2½ μ) through a space between three large oöcytes. The two black masses to the left are sections of blood which was tinted yellow by the fixative. The rest of the black masses, as well as the granules, are intensely black

in the preparation, *i.e.*, osmophile substance. This channel can be traced to a large degenerating oöcyte, which is composed almost entirely of osmophile substance. A small portion of such an egg is seen in Photo 50, and on its right, degenerating cells, showing like masses of osmophile substance. Fixative, Hermann's fluid.

Photo 47, section ($2\frac{1}{2}\mu$) of a capillary space between two eggs, showing osmophile granules in the fluid substance of these spaces. The black masses cannot be interpreted as blood, for in no case has the blood (in either the large or small vessels) blackened with osmic. These capillary spaces can be traced to larger ones, and these in turn to degenerating eggs (*cf.* Photo 46). Fixative, Rabl's picro-sublimate, followed by osmic acid.

Photo 48, section ($2\frac{1}{2}\mu$) of a portion of the periphery of a large oöcyte at the distal end of the ovary. This part of the egg is in contact only with the epithelial covering of the ovary. The osmophile substance appears to be in a capillary space within this covering. This same condition is seen in Photo 44. Fixative, picro-nitric, followed by osmic acid.

Photo 49, section ($2\frac{1}{2}\mu$) of one of the tiny cells which surround the large oöcytes at the distal end of the ovary. It contains two relatively large osmophile granules, and there is a third in the small cell to the right. These cells contain osmophile granules and also masses of a more deeply stained substance which closely resembles the archoplasm of the oöcytes. Three such masses are present in this section. Fixative, acetic alcohol, followed by osmic acid.

Photo 50, section ($2\frac{1}{2}\mu$) of three small degenerating cells and a part of a degenerating egg to the left. Fixative, 10% formalin, followed by osmic acid.

Pl. XLII.
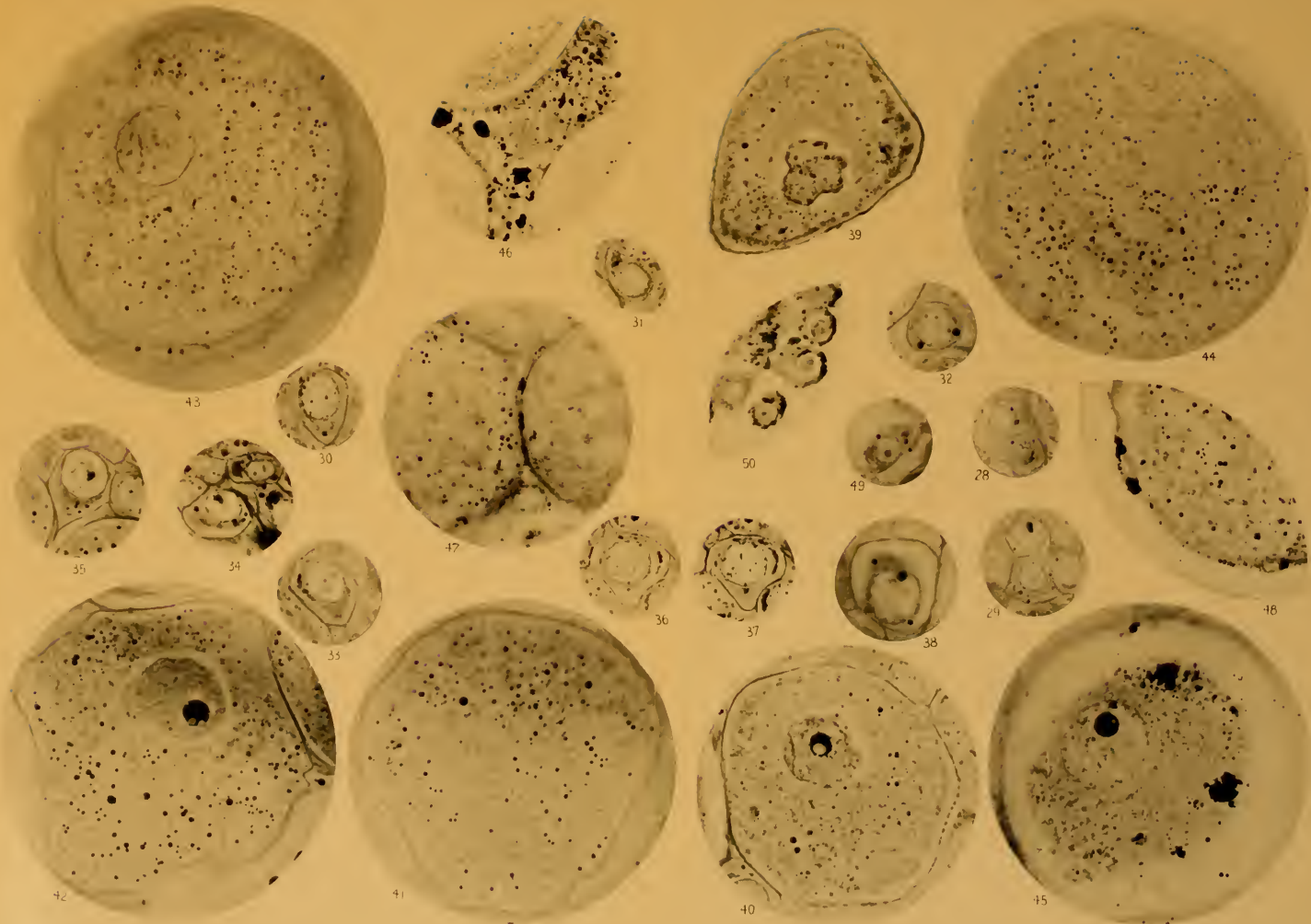
EXPLANATION OF PLATE XLIII.

The photos of this plate show different stages of the egg found in freshly deposited cocoons. The archoplasm in all cases is composed of masses of granules; but it is impossible to reproduce this structure satisfactorily without sacrificing a proper reproduction of the rest of the cytoplasm. Photo 84 has been printed with the aim of showing the structure of the archoplasm in the polar rings, and this photo must be referred to as illustrating the finer structure of the archoplasm in both the ovarian and the cocoon eggs.

Except Photo 62, all the photos in this plate were taken at a magnification of 660 diameters.

Photo 51, section (3μ) through the cytoplasm of an unfertilized oöcyte, first order. The first maturation spindle is at the stage shown in the egg of Photo 52. The preparation is unstained, but the fixative has tinted the archoplasm yellow. This print has been made very dark in order to show the two substances in the cytoplasm, the dark granular archoplasm and a faintly differentiated, relatively homogeneous substance which is much more difficult to stain. This is the substance which in some cases forms the network or rays, but we do not feel justified in asserting how much of this condition is due to fixation. The dark printing of the archoplasm has obliterated most of the osmophile granules which were black in the preparation (*cf.* Photo 60 for osmophile granules at this stage of development of the egg). Fixative, Hermann's fluid without acetic acid.

Photo 52, section (3μ) of an unfertilized oöcyte, first order. To avoid obliterating the centrosome the archoplasm here was not printed so dark as in Photo 51. Only two of the eleven chromosomes are clearly shown in this section. The chromosomes, archoplasm, and centrosome were tinted yellow by the fixative. Only a few of the larger osmophile granules are shown (*cf.* Photo 60 for these). Fixative, Hermann's fluid without acetic acid. Preparation unstained.

Photo 53, section (3μ) through the cytoplasm (near periphery) of an unfertilized oöcyte, same stage of the egg as shown in Photos 51 and 52. The preparation was stained in iron-hæmatoxylin and the granular archoplasm sharply differentiated. Fixative, 1% osmic acid in 70° alcohol.

Photo 54, section (3μ) of a fertilized oöcyte, first order. The apex of the fertilization cone is seen near the center of the section. The spindle is probably not normal, for in all other preparations it has reached the anaphase when the fertilization cone is present. The preparation suggests that the cytoplasm has reached the stage of development necessary to the appearance of the fertilization cone, but the spindle is retarded; it is not radial and has not reached the periphery. The curved chromosome seen on the right is an exception. This preparation shows aggregations of granular archoplasm through the cytoplasm. See Photos 51 and 53 (*cf.* Photo 61 for osmophile granules in eggs at this stage of development). Fixative, corrosive sublimate.

Photo 55, section (3μ) of a fertilized oöcyte, second order. Part of the sperm sphere is shown near the center of the section and just above it a cross-section through the head of the sperm, which at this stage is a short thick rod. Archo-

plasmic masses are scattered through the cytoplasm and on the periphery, and smaller archoplasmic aggregations surround the sphere. This section shows that much of the archoplasm which in the earlier stages is distributed through the cytoplasm (Photos 51–54) is beginning to aggregate at the periphery, leaving the rest of the cytoplasm relatively free from the substance (*cf.* these masses of archoplasm with those in the ovarian egg, Photos 21 and 23, Pl. XLI). Fixative, corrosive sublimate.

Photo 56, section (3μ) through the cytoplasm of an egg at the same stage of development as that of Photo 55; this section, however, is nearer the periphery. It shows the same archoplasmic aggregations, but the configuration of the cytoplasm is quite different, due undoubtedly to the difference in fixation. The cytoplasm of Photo 56 resembles the living egg more closely, though the hyaline spheres are larger than is usual at this stage. This suggests that the first effect of the fixative on the cytoplasm may be to stimulate it to a more rapid development. Fixative, Hermann's fluid without acetic acid.

Photos 57 and 58, sections (3μ) through the cytoplasm of two eggs at the same stage of development, *i.e.*, fertilized mature eggs. The section of Photo 58 is very near the periphery, showing the aggregations of archoplasm, which in Photo 57 are at the outer edge of the section. These eggs represent a later stage of development than those shown in Photos 55 and 56; the second polar body has been formed, and the chromosomes and sperm rod have reached the vesicular stage. Fixative of Photo 57, picro-acetic, and of Photo 58, Flemming's fluid. The picro-acetic does not preserve the form of the hyaline globules, which can be seen distinctly in the living egg at this stage (*cf.* Photos 63–77, Pl. XLIV, for fifteen serial sections of an egg at about the same stage of development, *i.e.*, pronuclei just beginning to form).

Photo 59, vignetted section (3μ) of an egg, showing a later stage of development, the pronuclei having increased in size and the archoplasm no longer scattered around the entire periphery of the egg, is beginning to concentrate at two areas, preparatory to forming the two polar rings. One of these areas is shown in the photo. Successively later stages are shown in Photos 78, 79, and 80, Pl. XLIV. The hyaline globules in this section are larger than those seen in the living egg at this stage. Fixative, Flemming's fluid without acetic acid.

Photo 60, section (3μ) through the cytoplasm of the same egg shown in Photo 51. This photo was taken to show the osmophile granules at this stage. We have had to make a very light print of this, sacrificing a sharp differentiation of the archoplasm, to avoid obliterating the granules. Near the center of the section, the sphere at the lower pole of the first maturation spindle is faintly indicated. In many eggs at this stage there are more osmophile granules than we have in this section. The rapidity with which they fade depends upon the time the egg is left in osmic acid, and the length of time the sections have been kept before examination. As this preparation was not examined for several months after fixation, many of the smaller granules have undoubtedly faded (*cf.* Photo 61). Fixative, Hermann's fluid without acetic acid. Preparation unstained and mounted in glycerine.

Photo 61, section (3μ) of a fertilized oöcyte, first order, at the same stage of development as the egg of Photo 54. Near the center of the photo is shown a slight indication of a cross-section through the fertilization cone, near its apex.

This photo was taken to show the osmophile granules in eggs at this stage, and all other constituents of the cell were sacrificed to the demonstration of these granules. Fixative, 5% osmic with acetic acid.

Photo 62, section (3μ) of a fertilized oöcyte, second order, at the same stage of development as that shown in Photo 55. The rod sperm and its sphere could be seen in this section, but they are scarcely indicated in the photo. These had to be sacrificed in order to bring out the osmophile granules. Fixative, chromo-acetic, followed by osmic acid. Preparation unstained and mounted in glycerine. × 500.
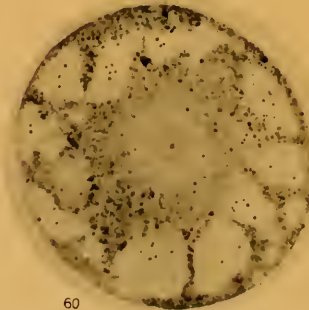
Pl. XLIII.
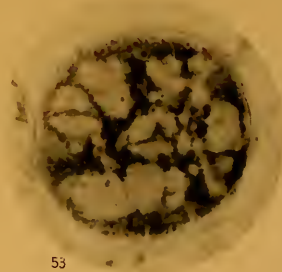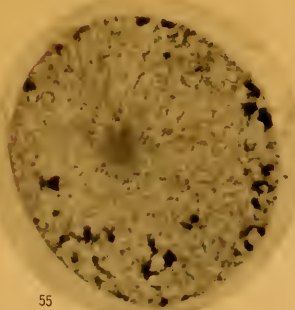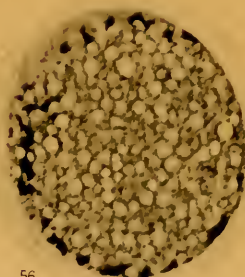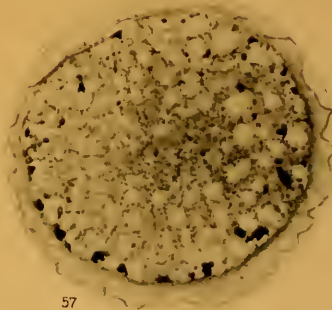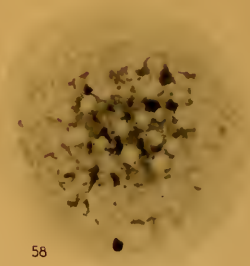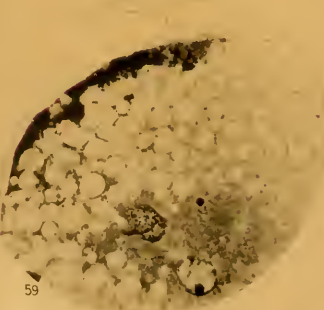
EXPLANATION OF PLATE XLIV.

The photos of this plate are from preparations of eggs found in cocoons where the slime tube is still intact or is just beginning to degenerate.

Photos 63–77, × 286 diameters.

Photos 78–85, × 660 diameters.

All the preparations were stained with iron-hæmatoxylin, except No. 82, which is unstained.

Photos 63–77, fifteen serial sections $(3\,\mu)$ of a mature egg, the pronuclei in early stages of formation. At this stage archoplasmic masses are distributed around the entire periphery of the egg, later accumulating at two definite areas and forming the polar rings (Photos 78–85). Fixative, chromo-acetic, followed by osmic acid. × 286 diameters.

Photo 78, section $(3\,\mu)$ of a mature egg. The peripheral archoplasm has concentrated at nearly opposite poles; but the polar rings are not yet complete and the pronuclei have not reached more than half their maximum size. The conditions surrounding the pronuclei are probably abnormal; but the rest of the cytoplasm closely resembles that seen in the living egg at this stage. Just to the left of the upper thickening of the right polar ring, several hyaline globules appear to be fixed in the process of fusing. This has been seen repeatedly in the living egg (*cf.* Photo 59, Pl. XLIII, for an intermediate stage between this egg (78) and Photos 63–77). Fixative, Flemming's fluid without acetic acid.

Photo 79, section $(3\,\mu)$ of an egg at about the same stage of development shown in Photo 78. The pronuclei have increased in size and small masses of archoplasm surround them. The configuration of the cytoplasm of the two sections is quite different; the egg of Photo 79 having been fixed in corrosive sublimate and the egg of Photo 78 in Flemming's fluid without acetic acid (*cf.* Photo 84 for the granular structure of the archoplasm of the polar rings).

Photo 80, section $(3\,\mu)$ of an egg at a slightly later stage of development than that of 79 ; the pronuclei have increased in size, although they have not yet reached their maximum growth, and the archoplasm of the polar rings has aggregated into more compact masses. The polar rings not being at exact opposite poles, only a small piece of the left ring is in the section. A few small masses of granular archoplasm are near the pronuclei, and archoplasmic granules can be seen scattered through the cytoplasm. Fixative, corrosive sublimate (*cf.* Photo 84 for the granular structure of the archoplasm of the polar rings).

Photo 81, vignetted section $(3\,\mu)$ of an egg at nearly the same stage of development as that of Photo 80. In this egg the pronuclei appear to have reached their maximum growth (they are not shown in this section). This photo is one of a number taken to show that the form of the rings is not constant — a comparison of the rings of Photos 80, 81, 83, and 85 will illustrate this point which was made in an earlier paper.[1] Fixative, chromo-acetic, followed by osmic acid (*cf.* Photo 84 for the granular structure of the polar rings).

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[1] " No two rings of either pole have been found exactly alike. The light areas in their center differ in size and shape, and the number and form of the smaller light areas, which occur through the mass of the rings, are by no means constant. The outer edges of the rings are never exactly alike in any two specimens," Foot (8).

Photo 82, vignetted section (3μ) of an egg at about the same stage of development as that of Photo 81, one polar ring shown in the section. This photo was taken to show osmophile granules in the cytoplasm and in the polar rings. Many of them have undoubtedly faded, as the preparation was not examined for many months after fixation. In fresh material many more are present. Preparation unstained and mounted in glycerine. Fixative, Hermann's fluid.

Photo 83, the same section shown in Photo 82 after the preparation had been stained with iron-hæmatoxylin. A close examination of the two sections will show that both have been taken at nearly the same focus; the largest osmophile granules of Photo 82 can be seen in this stained preparation, but the smaller ones (especially those in the polar rings) are obliterated by staining the archoplasm around them. In the stained preparations they are no longer black, but have retained a brownish yellow tint, which makes a sharp photo. A close comparison of the two sections will show that many of the small granules not blackened by the osmic in Photo 82 have stained intensely with the hæmatoxylin. They resemble the granules which have aggregated to form the polar rings (*cf.* Photo 84 for the granular structure of the polar ring).

Photo 84, vignetted section (3μ) of an egg with the first cleavage spindle at the metaphase. The section passes through one polar ring near the periphery of the egg. The granular form of the archoplasm which is aggregated as a polar ring is clearly shown in this photo. Fixative, chromo-acetic.

Photo 85, vignetted section (3μ) of an egg at the same stage of development shown in Photo 81. This section through one polar ring further illustrates the inconstancy of the form of the polar ring. (See under Photo 81.) Fixative, corrosive sublimate, followed by platinum chloride.
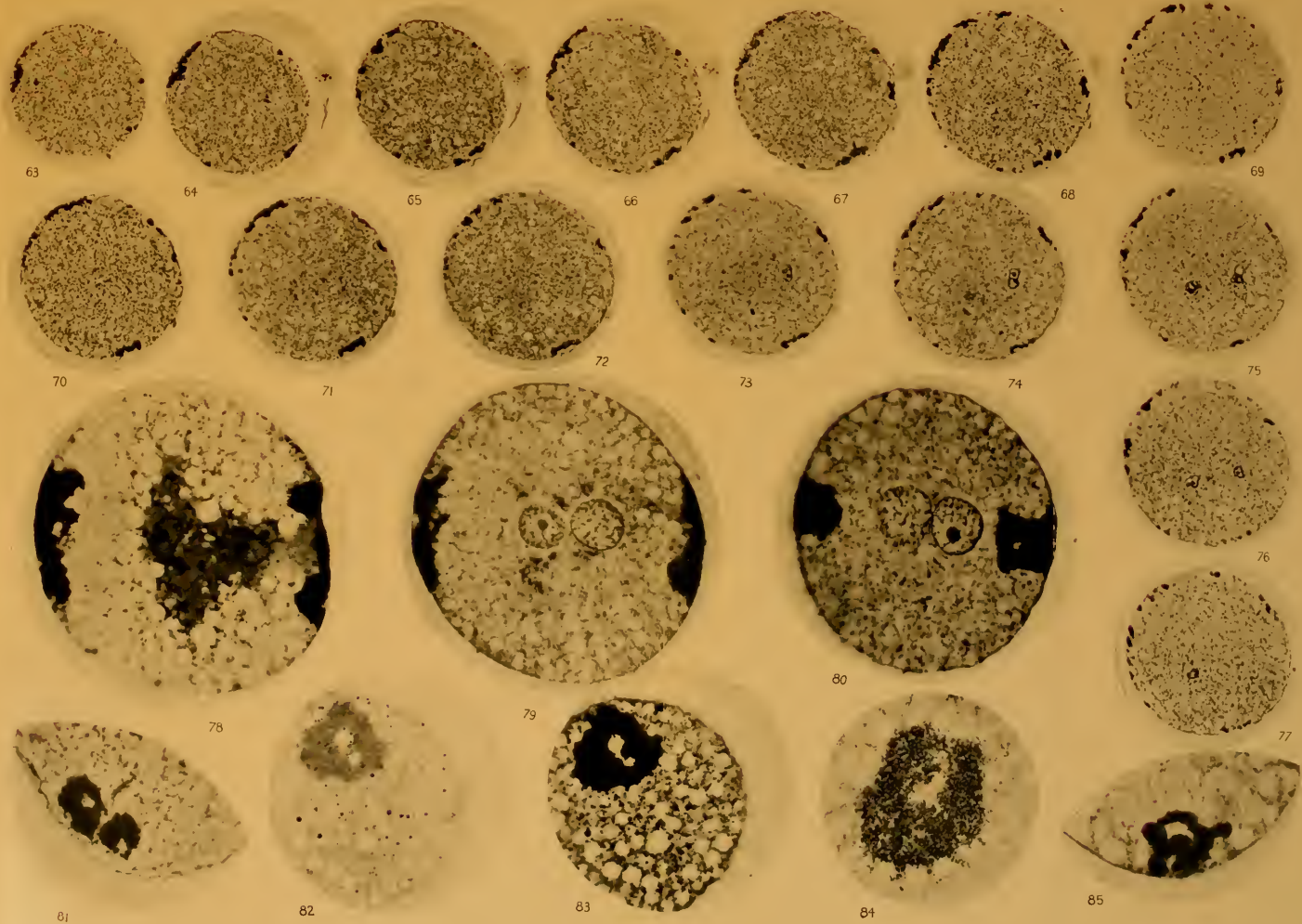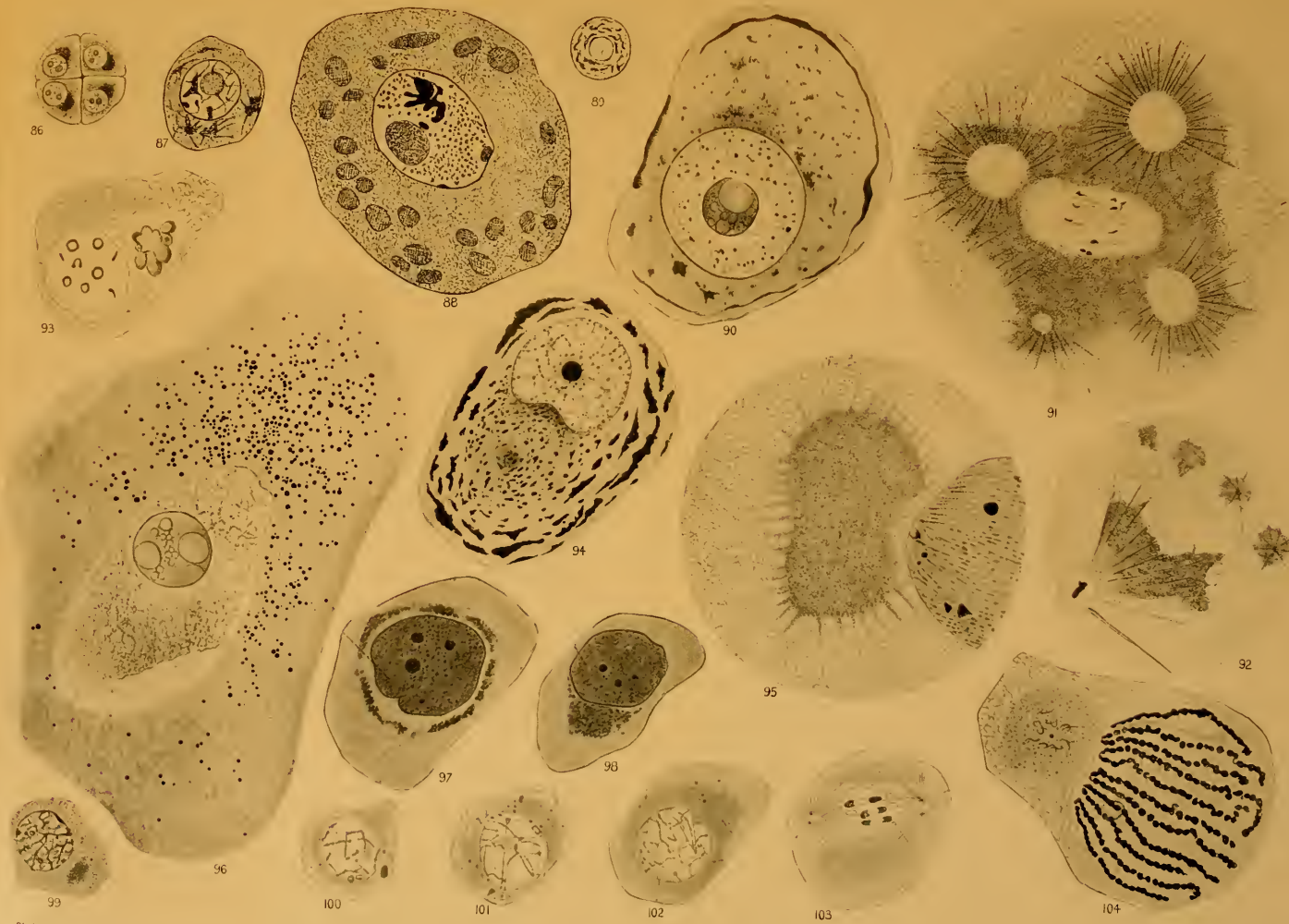
Pl. XLIV.

PLATE XLV.

Reproduction of figures of various authors referred to in text.

Pl. XLV.