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# CONTENTS OF VOL. VIII.

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## No. 1. — April, 1893.

	PAGES
I. WM. M. WHEELER. <i>A Contribution to Insect Embryology</i> . . . . .	1-160
II. JACQUES LOEB. <i>A Contribution to the Physiology of Col- oration in Animals</i> . . . . .	161-164
III. W. S. MILLER. <i>The Structure of the Lung</i> . . . . .	165-188
IV. ALBERT C. EYCLESHYMER. <i>The Development of the Optic Vesicles in Amphibia</i> . . . . .	189-194

## No. 2. — May, 1893.

I. J. S. KINGSLEY. <i>The Embryology of Limulus (Part II)</i> . . . . .	195-268
II. EDWIN O. JORDAN. <i>The Habits and Development of the Newt</i> . . . . .	269-366
III. WILLIAM A. LOCY. <i>The Formation of the Medullary Groove in the Elasmobranchs</i> . . . . .	367-378
IV. HOWARD AYERS. <i>Some Nerve-Muscle Experiments on the Frog</i> . . . . .	379-392

	PAGES
V. ALICE L. GAULE.	
<i>Biological Changes in the Spleen of the Frog</i> . . . . .	393-414
VI. F. MALL.	
<i>Histogenesis of the Retina in Amblystoma and Necturus</i> . . . . .	415-432
VII. S. WATASÉ.	
<i>Homology of the Centrosome</i> . . . . .	433-444

No. 3. — August, 1893.

I. HOWARD AYERS.	
<i>The Auditory or Hair-cells of the Ear and their Relations to the Auditory Nerve</i> . . . . .	445-466
II. HERBERT P. JOHNSON.	
<i>A Contribution to the Morphology and Biology of the Stentors</i> . . . . .	467-562
III. LOUISE B. WALLACE.	
<i>The Structure and Development of the Axillary Gland of Batrachus</i> . . . . .	563-568
IV. F. R. LILLIE.	
<i>Preliminary Account of the Embryology of Unio Complanata</i> . . . . .	569-578
V. EDMUND B. WILSON.	
<i>Amphioxus, and the Mosaic Theory of Development</i> . . . . .	579-638
VI. C. O. WHITMAN.	
<i>The Inadequacy of the Cell-Theory of Development</i> . . . . .	639-658

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A CONTRIBUTION TO INSECT EMBRYOLOGY.

WILLIAM MORTON WHEELER.

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TABLE OF CONTENTS.

	PAGE
Introduction .....	2
I. The embryonic development of the Locustidæ .....	3
1. <i>The oviposition of Xiphidium ensiferum, Scud.</i> .....	3
2. <i>The formation of the Xiphidium embryo and its backward passage through the yolk</i> .....	5
(a) <i>Description of surface changes beginning with the completed blastoderm</i> .....	5
(b) <i>The indusium (præoral organ) in section</i> .....	12
3. <i>The development of the embryo from the time of its reaching the dorsal surface of the yolk to revolution</i> .....	18
4. <i>Variations in the development of the indusium</i> .....	25
5. <i>The revolution of the embryo</i> .....	27
6. <i>The stages intervening between revolution and hatching</i> .....	29
7. <i>The development of Orchelimum vulgare</i> .....	35
II. Gastrulation in the Orthoptera.....	36
III. The indusium and its homologues in the Arthropoda.....	55
IV. General consideration of the embryonic envelopes and revolution of the insect embryo .....	59
1. <i>The amnion and serosa</i> .....	59
2. <i>The yolk</i> .....	64
3. <i>Blastokinesis</i> .....	67
4. <i>The elimination of the embryonic envelopes</i> .....	78

V. Neurogenesis in the Insecta .....	82
1. <i>The nerve-cord</i> .....	82.
2. <i>The brain</i> .....	99
3. <i>General remarks on the nervous system</i> .....	108
VI. The development of the reproductive organs in the Insecta.....	113
1. <i>The gonads</i> .....	113
2. <i>The male ducts</i> .....	116
3. <i>The female ducts</i> .....	119
4. <i>General considerations</i> .....	126
VII. The subœsophageal body in <i>Xiphidium</i> and <i>Blatta</i> .....	136
VIII. Technique.....	139
IX. Bibliography .....	143
Description of the plates.....	150-160

THE very primitive and synthetic character of the Orthoptera has long been recognized by systematists and comparative anatomists, but the full importance of the group from an embryological standpoint has been but little appreciated, owing to the meagre and fragmentary nature of the observations hitherto published. For this reason I have made the Orthoptera the starting point of my studies, with a view to determining their relations, on the one hand to the Apterygota and on the other to the higher Pterygote orders. Only a portion of the evidence bearing on these relationships is presented in the following paper; a number of observations on the Malpighian vessels, corpus adiposum, œnocyte-clusters and abdominal appendages will be published as separate papers.

I have devoted more attention to *Xiphidium* than to other Orthoptera, partly because the Locustidæ occupy a somewhat central position in the order, and partly because this curious form exhibits in its embryogeny better than any other insect hitherto studied, the co-existence of certain very ancient with very modern characters.

My German co-workers in the field of insect development will probably regard my treatment of the literature as rather perfunctory; but Prof. Graber, Dr. Heider and others have given from time to time such complete résumés of past and current literature that I feel justified in departing from the general custom. If I have failed to give credit where it is due,

I beg that this may be regarded as a fault of omission and not as a fault of commission.

I would express my sincere gratitude to Prof. C. O. Whitman for his kindly guidance and friendly counsel throughout the progress of my work in his laboratory at Clark University during the autumn and winter, and at the Marine Biological Laboratory during the summer months, of 1891 and 1892. I am also indebted to Mr. S. H. Scudder for the identification of several Orthoptera.

## I. THE EMBRYONIC DEVELOPMENT OF THE LOCUSTIDÆ.

### 1. *The Oviposition of Xiphidium ensiferum*, Scud.

*Xiphidium ensiferum*, Scudder, a very common Locustid in Wisconsin and the neighboring states, deposits its eggs in the silvery napiform galls produced by *Cecidomyia gnaphaloides* (and perhaps allied species) on the low willows that abound in the marshy lands and along small water courses. I have found the insect ovipositing from the middle of August to the middle of September. It thrusts its ensate ovipositor in between the imbricated scales of the gall and places its eggs singly or in a more or less even row with their long axes directed like the long axis of the gall. The eggs are completely concealed by the scales, the overlapping edges of which spring back to their original positions as soon as the ovipositor is withdrawn. The number of eggs deposited in a gall varies greatly: sometimes but two or three will be found; more frequently from fifty to one hundred; in one small gall I counted 170 and I have opened a few which contained more. Sometimes as many as ten eggs will be found under a single scale; when this is the case, the eggs adhere to one another and are more or less irregularly arranged, as if two or three insects had in succession oviposited in the same place.

The *Cecidomyia* galls vary considerably in shape: some are long and more or less fusiform, others are spheroidal. In the former variety the scales are pointed and flat, while in the latter they are rounded and have their median concave portions less closely applied to the convex surfaces of the scales

which they overlap. These differences materially affect the eggs, for many of those thrust in between the closely appressed scales of the spindle-shaped galls are so much flattened as to be incapable of developing; on the other hand the eggs deposited in the more spacious interstices of the globular galls are usually in no wise injured. The two forms of gall do not always occur in the same locality and may be the productions of two distinct species of *Cecidomyia* or of one species on different willows. The Locustids, however, seem to show no preference for the globular galls.

The galls of *Cecidomyia*, being essentially stem-galls, do not drop to the ground in the autumn like the various leaf-galls on the willows, but persist through several seasons. Although the insects are not averse to ovipositing in the fresh galls, they nevertheless seem to prefer these blackened and weather-beaten specimens, probably because their scales are more easily forced apart.

I have called attention to the fact ('90<sup>b</sup>) that *X. ensiferum* departs widely in its habits of oviposition from its congeners, several of which are known to lay their eggs in the pith of easily penetrated twigs, like the species of the allied genus *Orchelimum*. *X. ensiferum* has evidently found it of great advantage to make use of the galls so abundant in its native haunts. So recent may be the acquisition of this habit, that on further investigation some females may, perhaps, even now be found to have a tendency to oviposit, like *Conocephalus ensiger*, between the root-leaves and stems of plants, or even in the plant tissues. It still occasionally happens that the eggs are run through or into the tissues of the gall-scales, and not loosely deposited. The fact that the insects have not yet learned to distinguish the kind of gall best adapted to their purposes, lends some support to the view that it is not so very long since *X. ensiferum* agreed with its congeners in habits of oviposition.<sup>1</sup>

<sup>1</sup> In the vicinity of Worcester, Mass., I found galls very similar to those formed on the Wisconsin willows. They contained a few slender yellow eggs, smaller than those of *X. ensiferum*. As this species does not occur in New England I conclude that these eggs were probably deposited by the very common *X. fasciatum*, De Geer.

2. *The Formation of the Embryo and its Backward Passage Through the Yolk.*

a. SURFACE CHANGES.

The sub-opaque, cream-colored egg of *Xiphidium* is elongate oval, 3–5 mm. long and 1 mm. broad through its middle. One of its poles is distinctly more attenuate than the other, and there is a faint curvature in the polar axis which causes one side of the egg to be distinctly convex and the other distinctly concave. The broader pole is the posterior, and is the first to leave the vagina during oviposition; the attenuate pole is, therefore, the anterior. In the galls the eggs stand with their attenuate poles pointing upwards. The convex face of the egg is the ventral, the concave face the dorsal region. Inasmuch as the egg undergoes no change in shape during development, it is easy to orient the embryo in its different stages. This is of considerable importance, as will appear from the sequel.

The yolk is pale yellow and very similar in constitution to the yolk of other Orthopteran eggs. It is enclosed by a thin leathery chorion which suddenly becomes transparent on immersion in alcohol. When dry it is white, and the creamy color of the egg is due to the yellow yolk shining through. As in *Blatta*, the chorion is the only envelope of the freshly laid egg; what I described in a former paper ('90b) as the vitelline membrane is in reality comparable to a "Blastodermhaut" as I shall point out.

The chorion varies somewhat in thickness at different points in the egg, being  $11\mu$  towards the middle and  $19\mu$  at the poles. It is quite elastic and when cut curls in at the edges. Its inner surface is very smooth, while outwardly it is covered with round or oval projections which measure about  $3.7\mu$  in diameter. They are flattened at their summits and are placed so closely together that only narrow channels run between them and give the chorion the appearance of being covered with a fine net of nearly uniform meshes. On closer examination it is seen that the projections are arranged in hexagonal groups. These are very distinct at either pole but fade away

on the median portions of the egg till they become very difficult to resolve. They evidently coincide with the areas covered by the polygonal cells of the follicular epithelium.

No traces of micropyles could be found. Their absence in *Xiphidium* is of interest, since Leuckart ('55) long since described and figured them in several European Locustidæ (*Meconema*, *Decticus*, *Locusta*, *Ephippigera*). In these genera they consist of funnel-like perforations on the ventral surface of the chorion either near the anterior pole or nearer the middle of the egg.

The preblastodermic stages were not studied. They probably resemble the corresponding stages of *Blatta*, of which I have given a detailed account in a former paper ('89).

When fully formed the *Xiphidium* blastoderm, like that of *Blatta*, consists of a thin sheet of cells, that have in part reached the surface from the interior of the egg, and are in part derived from these centrifugal cells by tangential division after their arrival at the surface. Numerous cells—the future vitellophags—are to be found at different points in the yolk. Whether they are derived from the incompleting blastoderm by centripetal division, or are inhibited before reaching the surface, my limited observations will not permit me to decide.

The cells forming the blastoderm are polygonal, much flattened and of uniform size and distribution. Those on the center of the convex, or ventral face of the egg soon begin to change their dimensions; from being broad and flat, they become more nearly cubical, their lenticular nuclei again assuming the spherical or oval shape which they had in preblastodermic stages. These changes take place over a limited and somewhat oval area and result in the formation of the ventral plate. The few eggs that I have been able to find in the very first stages after the completion of the blastoderm leave me in some doubt as to the exact process whereby the embryo is established. I am satisfied, however, that the thickening and narrowing of the individual blastodermic cells does not take place simultaneously over the whole ventral plate area, but that there appear, as in the crustacean egg (*c.g.* *Astacus*, *Homarus*), several discrete centres about which the

cells are at first more closely aggregated. The spaces between these centres are subsequently filled in by tangential cell-divisions. Of such centres I can distinguish four: two of them, the precursors of the procephalic lobes, are paired, while the other two form respectively the growing caudal end of the ventral plate and what I shall call the indusium.<sup>1</sup> The indusial centre, which does not make its appearance till a short time after the other centres are formed, does not join the body of the embryo till after the spaces between the procephalic and caudal centres are filled in. This is distinctly seen in Fig. 1 (Stage A) where the somewhat T-shaped embryo is already established and distinctly marked off, at least posteriorly, from the undifferentiated blastoderm. The nuclei of the blastoderm are as yet no larger than the nuclei of the ventral plate. Numerous caryokinetic figures in all parts of the embryo bear witness to active cell proliferation. No such figures were to be seen in the extra-embryonal blastoderm during and after this stage. The ventral plate including the indusium is scarcely a fifth as long as the egg, being much smaller in proportion to the size of the yolk than in some other Orthoptera (*Blatta*, *Gryllotalpa*).

The blastopore is seen in the stage figured as a very narrow but distinct groove extending from the oral region to the caudal end of the embryo, where it bifurcates before its termination. The infolded cells give rise to the mesoderm and also, I believe, to the entoderm.

In *Xiphidium* the three folds that form the amnion and serosa arise like their homologues in *Blatta*. The first appears as a crescentic duplication surrounding the caudal end; thence it grows forward and after enveloping the whole postoral portion of the embryo coalesces with the two head-folds, each of which arises from the edge of a procephalic lobe. The progress of the anal fold is shown in Fig. 2 (Stage B) Pl. I. Although agreeing in its main features with what has been described for most insect embryos, the process of envelope-

<sup>1</sup>In a preliminary note ('90<sup>c</sup>) this structure was called the præoral plate (Præoralplatte). Many reasons have led me to abandon this term together with others referring to the parts of the organ in its subsequent development.

formation in *Xiphidium*, is, nevertheless, peculiar in two respects: first, the envelopes are so closely applied to the germ-band that in surface view their advancing edges can be detected only with difficulty, though they may be distinctly seen in sections; second, the point of closure of the envelopes is situated further forward on the head than in *Blatta*, *Hydrophilus*, *Doryphora*, etc. This I infer from an embryo, which I figure (Fig. 15, Pl. II.) Here the cells and nuclei of the amnion and serosa have become much larger than the cells and nuclei of the embryo. The edges of the folds are unusually distinct and enclose a circular space through which the oral and præoral regions are clearly visible. On the median anterior edge of the head the amnion and serosa are completely interrupted. In no other insects have I found the envelopes lacking on the anterior edge of the head in so late a stage. This fact is probably significant when taken in connection with changes about to occur in front of the head.

The wide procephalic lobes are succeeded by the strap-shaped body. In this a number of segments have made their appearance. These are in order from before backwards: the mandibular (*md. s*), the first maxillary (*mx. s<sup>1</sup>*), the second maxillary, (*mx. s<sup>2</sup>*) the three thoracic (*p. s<sup>1</sup>*-*p. s<sup>3</sup>*), and the first abdominal (*a. s<sup>1</sup>*). Further back lies a small segment which is incompletely constricted off from the first abdominal and which I take to be the proliferating terminal segment, or telson. The seven segments depicted in the figure are undoubtedly definitive segments. The manner of their appearance will be clear from a glance at Fig. I. In A the ligulate part of the germ-band is seen to be faintly constricted at its base into two segments with indications of a third. In B, a slightly later stage, four definitive postoral segments are present, but a portion of the germ-band still remains unsegmented. This is, however, soon broken up into segments and we reach the stage in Fig. 15, Pl. II. It will be observed that the embryos in Fig. I are in many respects older than that in Fig. 15, Pl. II. The antennæ have made their appearance and the amnio-serosal fold has closed completely. These embryos prove several points:—first, that the wave of metameric segmen-

tation passes from before backwards dividing the germ-band into 7 or 8 segments; second, that these segments are the definitive segments and not macrosomites, or complexes of definitive segments; and third, that there is considerable variation in the time when segmentation sets in. To these points I may add a fourth: segmentation appears first in the ectoderm and only somewhat later in the mesoderm.

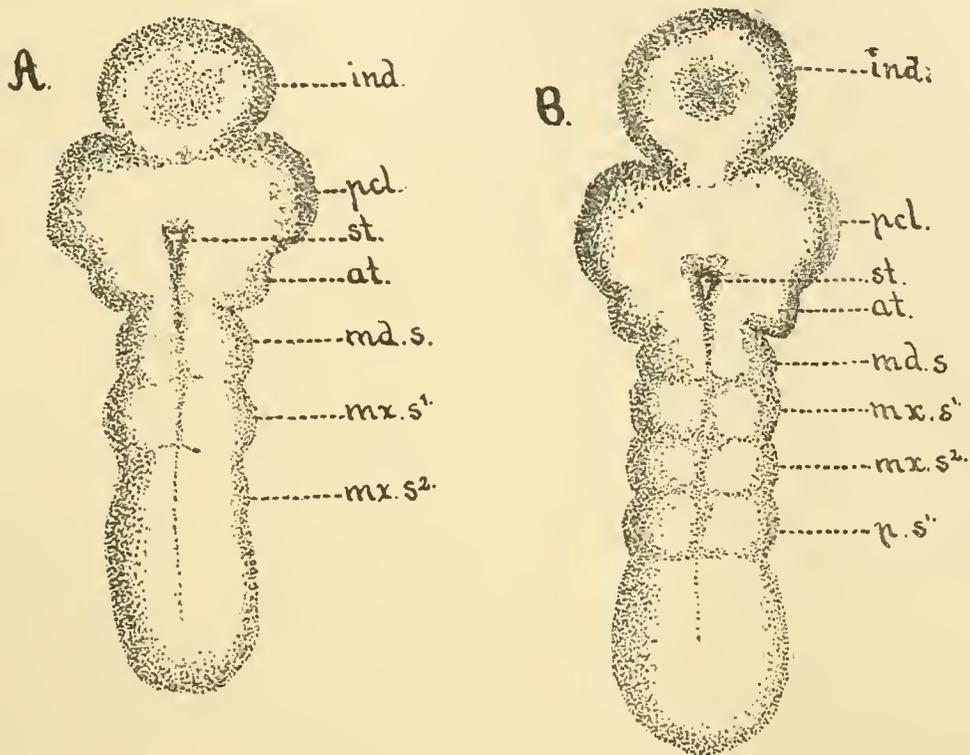


FIG. I.

A and B. Isolated embryos of *Xiphidium* in successive stages of metamerization. *ind.*, indusium; *pcl.*, procephalic lobe; *st.*, stomodæum; *at.*, antenna; *md. s.*, mandibular segment; *mx. s<sup>1</sup>*, first; *mx. s<sup>2</sup>*, second, maxillary segment; *p. s<sup>1</sup>*, prothoracic segment.

The indusium (Fig. 15, *p. o.*) is still only a rounded thickening of the blastoderm. Its small deep cells are continuous through a zone of larger cells with the relatively very large and flat elements of the primitive cell-layer. Two broad and flat commissures appear to connect the organ with the procephalic lobes. Thus a small space containing a few larger cells is enclosed between the indusium and the head of the embryo. This space (*y*), seen as a clear spot in surface view, lies at the breach in the envelopes. In many embryos the indusium is

united with the head of the embryo (Fig. I, A and B) before the stage of Fig. 15 and soon after this stage is, I believe, normally united with it. This union is probably purely mechanical—the organ remaining at its place of origin on the surface of the yolk, while the embryo lengthens till its head unites with the posterior end of the organ. This union is of brief duration as is seen in Fig. 3 (Stage C).

During this stage the caudal tip of the embryo shows a tendency to bury itself in the yolk. The amnion and serosa; hitherto closely applied to each other, now separate at the caudal end, where, as I have said, they first arose as a crescentic fold. Soon the tendency to enter the yolk becomes more pronounced so that the tail curls back and leaves the ventral face of the egg. Meanwhile the remainder of the embryo moves down the ventral face a short distance, thus pushing its tail still further into the yolk and causing the separation of the envelopes to advance still further headwards. The indusium does not accompany the embryo in this movement, but remains nearly or quite stationary; consequently the head gradually separates from the organ till it is connected only by means of a slender band of cells in the median line. (Fig. 3 and Fig. 16.) This link soon ruptures and the indusium is set adrift from the embryo, or, more precisely, the embryo is set adrift from the indusium. (Fig. 4, Stage D.) In profile the embryo now resembles the small letter j, —the dot being supplied by the isolated indusium.

Important changes begin to affect the indusium during or more frequently just after its separation from the embryo. The closely packed cells at the periphery, as indicated by their nuclei, begin to arrange themselves radially (Fig. 16). Some of the large nuclei of the serosa may be seen encroaching on the edges of the disk from all sides, leaving only the median portion free. Sections show that the organ is now forming an amnion like that of an embryo. In the middle of the disk appear several shrunken but distinctly defined nuclei which are proved by focusing to be confined to the surface of the organ.<sup>1</sup>

<sup>1</sup> Only four of these peculiar bodies are represented in the figure (*nn*); there were several others in the same preparation, but for the sake of clearness I have omitted them in the drawing.

The serosal fold continues to advance from all sides till the organ is entirely covered. Viewed from its ventral surface the egg now has the appearance of Fig. 4 (Stage D). Here the indusium is cordate in outline and somewhat larger than usual. Of the abdomen only the two basal segments still remain on the ventral face of the egg; the remaining segments curl back into the yolk.

During this and the two preceding stages the cephalic and thoracic appendages have become distinctly established as rounded lateral outgrowth of their respective segments. The antennæ (*at*) originate as lobular outgrowth from the posterior edges of the procephalic lobes. They are distinctly postoral in origin. The margins of the triangular oral orifice are somewhat swollen; the anterior edge, where the labrum is about to appear, is cleft in the median line (Fig. 16). The three thoracic segments are very slightly or no broader than the two maxillary segments. The appendages of these five segments are also alike in size, shape, and position. In very early stages of other insect embryos, even before the amnion and serosa are fully formed, the thoracic become broader than the maxillary segments, and the legs, as soon as they appear, may be readily distinguished from the two pairs of maxillæ by their greater size and prominence. The Locustid embryo, therefore, has even a stronger tendency to revert to annelid-like or myriopod-like ancestors than is apparent in any of the other insects whose ontogenies have been investigated.

The mandibular segment of *Xiphidium* like that of other insects, is somewhat retarded in its development. Between this and the antennary segment careful study of sections and surface preparations reveals the presence of another segment, shown very distinctly in outline in Fig. 16 (*tc. s.*). This is no other than what I have called the intercalary segment in *Doryphora*. It is the tritocerebrum of Viallanes ('90<sup>a</sup>, '90<sup>b</sup>).

The embryo continues to move back into the yolk, following the curved path established by the inflexion of the posterior segments till its tail is finally arrested by striking the serosa on the dorsal surface. At this time the embryo has the form of an arc subtending the dorsoventral diameter of the egg.

Returning to consider the indusium, we find that it begins to increase in size before the embryo's head leaves the ventral face. The organ stains much less deeply, and even in surface view its expansion may be seen to be due to a flattening of its component cells. In Fig. 5 (Stage E) is represented an embryo merged in the yolk up to the first maxillary segment. The indusium extends around on either side nearly to the middle of the lateral face of the egg. Either the transition of the embryo takes place rapidly or the organ changes very gradually, for the latter is in about the same stage after the embryo has become established on the dorsal surface. The manner in which the expansion of the indusium is brought about will be apparent when I come to describe its structure in sections.

#### b. THE INDUSIUM IN SECTION.

As will be seen from the preceding account, the indusium is simply a circular thickening of the blastoderm, situated in the median line, between and a little in front of the procephalic lobes. It does not arise as a part of the ventral plate but as a separate centre which is at first merely a cluster of blastoderm cells that have changed from the pavement to the cubical or columnar type. This centre is further increased in breadth and thickness by caryokinesis. In the earliest stages examined, sections of the organ show the same cell-structure as sections of the procephalic lobes.

Median longitudinal sections of the embryo in Stage C are interesting as showing the relations of the indusium to the embryo and its envelopes. I reproduce such a section in Fig. 21, Pl. III. Here the organ (*p. o.*) appears as a large flattened cell-aggregate somewhat thinner in the centre than nearer its periphery. Owing to the shape of the mass, the median cells, as indicated by their nuclei, are arranged with their long axes perpendicular to the flat outer surface of the organ, while the cells of the thickened lateral portions become gradually more oblique till those on the extreme periphery assume the same position as the serosa cells (*s.*). The nuclei are most frequently

situated at the inner ends of the cells so that masses of enucleate protoplasm are left at the surface. Posteriorly the organ is linked to the embryo by means of a few flattened cells. In the section two of these cells are seen at *s* differing in no wise from the serosal elements (*s.*) in front and on either side of the organ ; the upper cell passes directly into the serosa covering the embryo, while the lower abuts on the cells that form the transition from the ectoderm to the amnion. The ectodermal layer of the embryo (*ec.*) is nearly as thick as the indusium and of similar cytological structure. The beginning of the stomodæal invagination is shown at *o*.

The next section figured (Fig. 17 Pl. II) is from an indusium in a somewhat younger stage than that represented in surface view in Fig. 2. Being transverse the section shows an evenly convex outer surface, continuous with the surface of the serosa (*s.*) enveloping the yolk. The cell-contours are still visible and show that the cells constituting the median portion of the organ are polygonal. The nuclei of these elements are spherical or oval and contain one, or more rarely, two nucleoli besides the usual chromosomes. In the peripheral ring-shaped thickening the cells (*d.*) are larger and pyramidal or fusiform in outline, while their nuclei differ in no wise from the nuclei of the median cells. The serosal cells stain more deeply than the cells of the organ, as may be seen at *s* where a single cell overlaps the edge of the disk. This depth of color is apparently purely optical, being due to the greater size and flatness of the serosal nuclei. The walls of both the small polygonal and larger pyramidal elements fade away towards the surface, where the bodies of the different cells become confluent to form a homogeneous mass.

In this surface-mass of protoplasm which takes the normal pink stain in borax carmine, are to be found several of the peculiar nuclei, mentioned above as distinctly discernible from the surface (Fig. 16). They differ markedly in structure and appearance from the normal nuclei in the inner portions of the indusium as will be seen by comparing the cells of Fig. 24 with those in Fig. 23, both of which figures were drawn with a high power. The normal cells (Fig. 23) have spherical or

oval, evenly rounded nuclei with one or two nucleoli and their chromatin is distributed in what I take to be the typical resting reticulum. The caryolymph, or Kernsaft, is faintly stainable. On the other hand, in the nuclei of Fig. 24 the nuclear wall is very irregular, the caryolymph much more limpid and refractive and the chromatic reticulum has coarser meshes. The chromatic nodes of the reticulum are larger than in Fig. 23 and seem to be applied to the indentations of the nuclear wall. Nucleoli appear to be absent. These specialized nuclei also vary greatly in size. In a series of sections it is easy to find nuclei intermediate between the two extremes here described, being evenly rounded but with colorless caryolymph and coarse chromatic reticulum. A cluster of four such nuclei is shown at *m*<sup>2</sup> Fig. 17. These intermediate forms, occurring as they usually do, between the normal and the modified nuclei may be taken to indicate that the nuclei of the extreme types are genetically connected. Some of the normal nuclei probably leave their respective cells in the median portions of the organ and move up into the syncytial protoplasmic layer, undergoing the modification in structure during their emigration. When they have reached their destination they are perhaps broken down and converted into protoplasm. Certain it is that later no traces of them are to be found in the indusium. I do not believe that I am here considering collapsed and distorted caryokinetic figures, as these delicate structures are quite faithfully preserved in eggs killed by means of heat. The distorted nuclei are not confined to the indusium but occur also in the ectoderm of the embryo itself.

When the organ has reached the state just described it usually separates from the head of the embryo; it may, however, remain attached for some time longer. Like the embryo it is now an isolated body lying on the yolk; but unlike the embryo it is still only a part of the serosal envelope (which is itself only the extra-embryonal portion of the blastoderm). The serosa is a closed sack enveloping the whole yolk and the indusium is simply a swelling at one point on its inner face. (Fig. II, A.) The process of envelope formation which now begins in the indusium is much less clear than the cor-

responding process already completed in the embryo. From among the numerous preparations which I have made I select for illustration one (Fig. 18) which seems to show the process clearly. In surface view the organ would appear as in Fig. 3. The spreading of the serosal cells over the edges of the disk from all sides is now seen to be due to a process of induplication, or folding. The circular fold is, of course, cut in two places in the median transverse section figured. It advances in such a manner as to leave the outer face of the indusium evenly rounded and undisturbed, the upper surface of the fold usually forming a continuous line both with the outer surface of the serosa and with the median still uncovered portion of the organ. The fold continues to advance from all sides till the layers of which it consists meet and become confluent in essentially the same manner as the folds that form the amniotic and serosal layers over the embryo proper. We now have three layers of cells. (Fig. 19.) The outermost layer, *s*, is the serosa which has everywhere the same structure and evenly envelops the whole egg, having been separated first from the embryo and now by a similar process also from the indusium (Fig. II, B). The innermost layer consists of the unchanged greater portion of the organ. The median layer, to judge from its component cells, seems to be derived exclusively from cells of the original body of the organ and not from the serosa. This layer is, therefore, like the amnion of the embryo proper, structurally more closely related to the body it envelops than to the serosa. Fig. 18 favors this conclusion, which presupposes that only the outer half of the circular fold is derived from the serosa, for in this section the lower and thicker layer of the fold on either side certainly consists of cells derived from the body of the organ. Even before the layers are fully formed the edges of the two-layered organ are sharp and somewhat irregular (Fig. 18), not rounded like the edges of the embryo when its amnion is completed. The whole organ still has essentially the same form that it had in the stage represented in Fig. 17.

It will be convenient to name the different layers of cells, thus far distinguished. For the amnion of the embryo proper

I shall retain the old name; the corresponding envelope of the indusium and the body of the organ will be designated as the outer and inner indusium respectively.

In by far the greater number of cases the process of

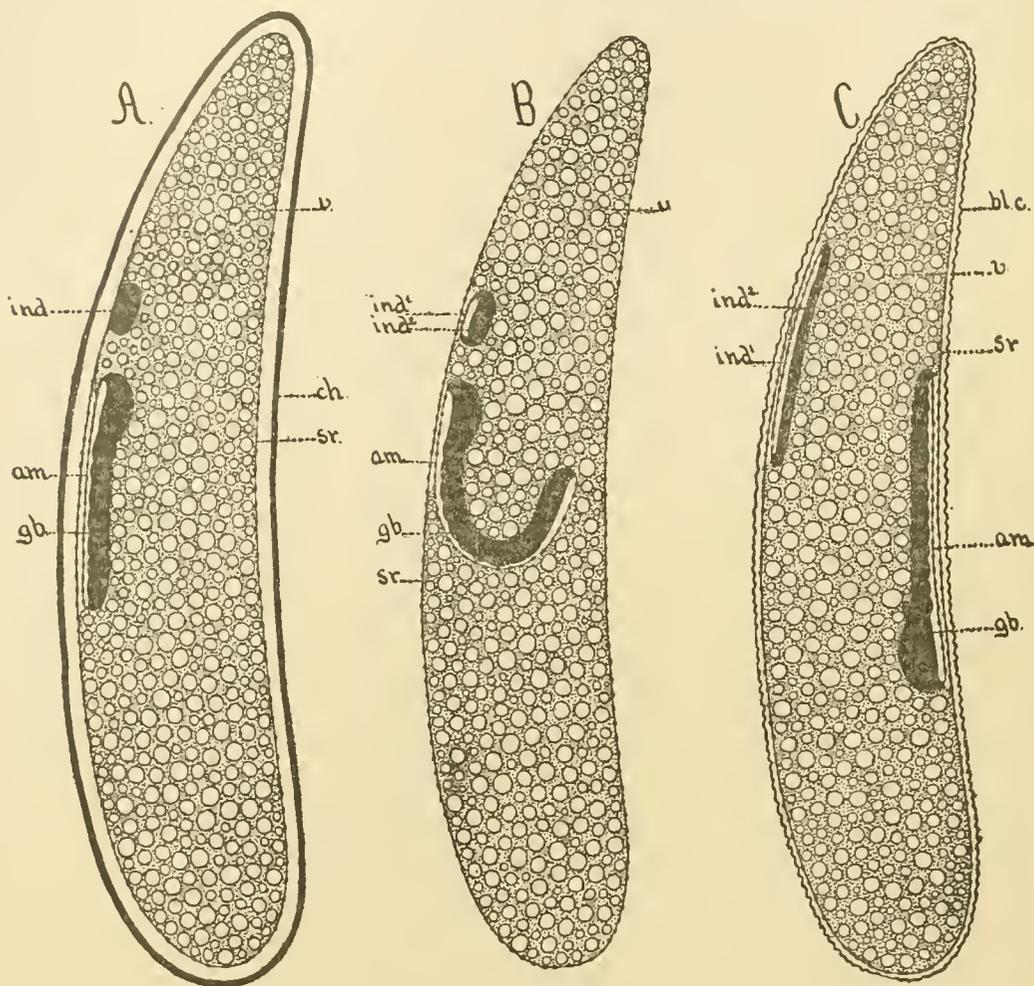


FIG. II.

Diagrams illustrating the movements and envelopes of the *Xiphidium* embryo. *A*, after the closure of the amnioserosal folds; *B*, during the embryo's passage to the dorsal surface; *C*, just after the straightening of the embryo on the dorsal surface. *ind.*, indusium — afterwards forming *ind*<sup>1</sup>, the inner, and *ind*<sup>2</sup>, the outer indusium; *ch.*, chorion; *sr.*, serosa; *am.*, amnion; *gb.*, germ-band; *v.*, yolk; *bl. c.*, Blastodermhaut.

envelope formation over the indusium is much obscured by rapid slurring. In fact the whole process has frequently the appearance of being due rather to a shifting and migration of cells than to the formation of true folds. The cells of the serosa seem to creep over the disk while the cells forming the

edge of the organ itself appear to creep along under and a little in the rear of the advancing serosal elements. I cannot here go into greater detail without unduly increasing the number of my figures. Nor is it necessary, since it will, I believe, be

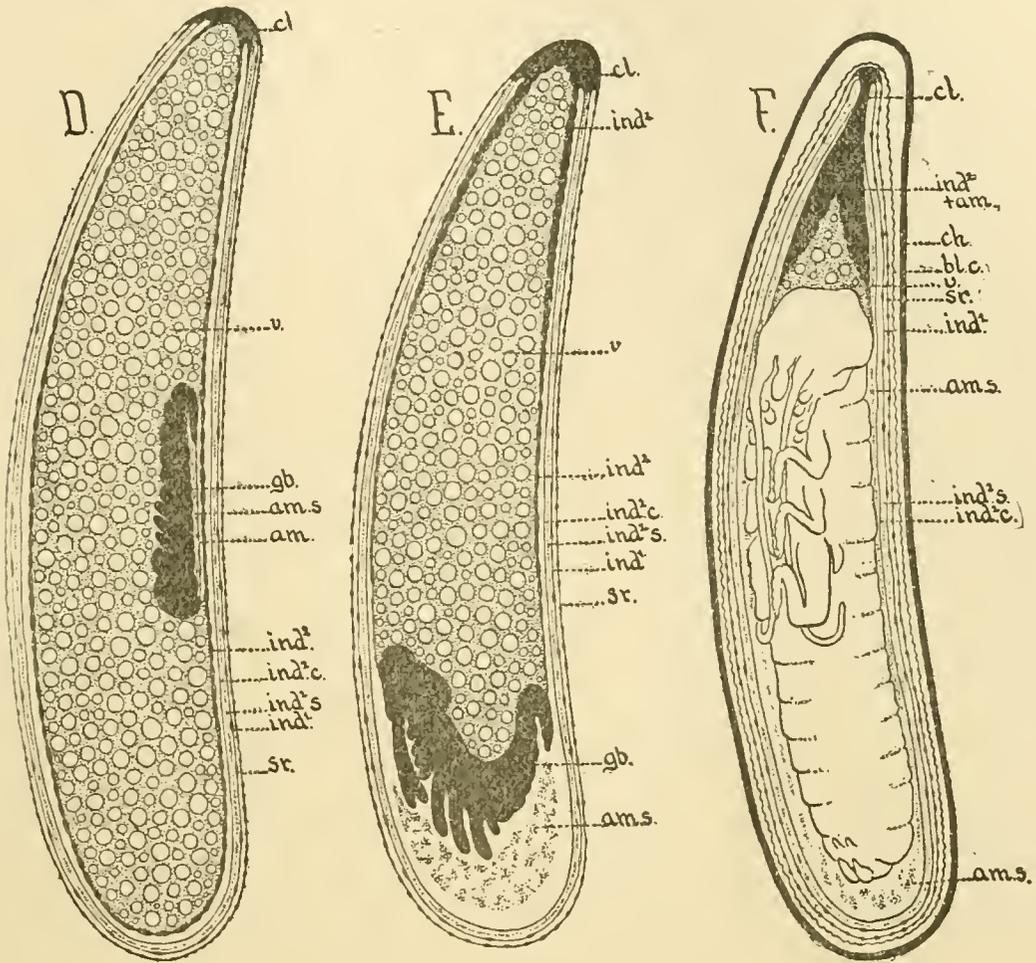


FIG. II.

Diagrams illustrating the movements and envelopes of the *Xiphidium* embryo. *D*, the stage of the shortened embryo on the dorsal yolk; *E*, embryo returning to the ventral surface; *F*, embryo nearly ready to hatch. *ch.*, chorion; *bl. c.*, Blastodermhaut; *sr.*, serosa; *ind*<sup>1</sup>, outer indusium; *ind*<sup>2</sup>, inner indusium; *ind*<sup>2</sup> + *am.*, inner indusium and amnion fused; *am.*, amnion; *ind*<sup>2</sup> *c.*, cuticle of the inner indusium; *ind*<sup>2</sup> *s.*, granular secretion of the inner indusium; *am. s.*, amniotic secretion; *v.*, yolk; *cl.*, columella; *gb.*, germ-band.

acceded that the process briefly described in the above paragraph, though now occurring in comparatively few embryos, is very probably the more primitive process, whereas the slurring observed in so many cases is to be attributed to an unquestionably rudimental condition of the organ.

By the time the folds have closed over the indusium the abdomen of the embryo has sunk into the yolk to a considerable extent, presenting in surface view the appearance of Fig. 4. The organ seems to undergo no further change till the embryo has almost left the ventral face of the egg. Then, as we have seen, it begins to increase by spreading. An early stage in this process is shown in section in Fig. 20. No change is perceptible in the serosa, which is now independent of the organ; the outer indusium ( $am^1$ ) is much attenuated, as may be seen by comparison with Fig. 19. Its cells have assumed the same shape and dimensions as those of the superjacent serosa; only along the edges of the disk, where the outer becomes continuous with the inner indusium, or body of the organ, do the cells still retain their original shapes. In the body of the organ the cells are arranged in two irregular rows, whereas in the previous stage (Fig. 20) there were three. This diminution in the number of cell-rows is the result of horizontal spreading, a process which also accounts for the stretching of the outer indusium as indicated by the flatness of its cells. At  $nn$  is seen one of the large modified nuclei, which has persisted unusually late.

In Fig. 22 I give a section of the indusium seen in surface view in Fig. 5. The spreading of the cells has progressed till the organ lies like a saddle on the ventral face of the egg, covering nearly half of its circumference. The serosal layer ( $s$ ) is, of course, unaffected. The outer indusium ( $am^1$ ) is stretched to such an extent that its cells are united only by an exceedingly thin and in many places, almost imperceptible layer of protoplasm. The inner indusium now consists of a single row of cells, instead of two rows as in the preceding stage. It is in about the same state of tension as the outer layer in Fig. 19.

### 3. *The Development of the Embryo from the Time of its Reaching the Dorsal Yolk to Revolution.*

In the foregoing paragraphs the development of the embryo was traced to Stage E, when the germ-band hangs festoon-like

in the yolk with its cephalic amnion applied to the ventral serosa and the amnion overlying its terminal abdominal segments applied to the serosa covering the dorsal yolk. No sooner has the caudal end become fixed than the head is detached from the ventral face of the egg and the embryo swings back, straightens out, and becomes applied full length to the dorsal serosa. The movements whereby this condition is attained resemble the movements of a leech in passing from one side of a test-tube to the opposite surface; holding fast to the glass by means of the oral sucker, the tail is stretched out till it reaches the opposite surface, when the anterior end is loosened and the body drawn over. There is, however, a marked difference between the embryo and the leech since the body of the former is not contracted during its transition.

Fig. 5 represents a rather rare condition in that the procephalic lobes lie at the same level and are symmetrically disposed with respect to the long axis of the egg. More frequently the germ-band is twisted during its transition so that one of the procephalic lobes reaches further forward than the other on the surface of the yolk. Sometimes it is the left lobe which extends further forward but more frequently it is the right. The twist in the germ-band occurs in the thoracic or abdominal region, more often in the former, the abdomen being nearly straight. I take this twisting of the embryonic axis to indicate that the germ-band executes a screw-like movement while penetrating the yolk, and I believe it to be perfectly normal, having observed it in the majority of embryos. Traces of this twisting are clearly discernible even in embryos which have almost straightened on the dorsal surface.

As a consequence of the passage of the embryo through the yolk in the manner above described, the germ-band has shifted its position from the median convex ventral to the median concave dorsal surface of the yolk, so that it is now reversed: originally its head pointed to the tapering anterior pole, now it lies with its head directed towards the blunt posterior pole of the egg. The amnion, of course, accompanies and remains in close contact with the ventral surface of the embryo during all this time.

During or more frequently at the close of the embryo's migration the primary serosa secretes from its whole outer surface a thin chitinous cuticle. In my preliminary notes ('90<sup>b</sup>, '90<sup>c</sup>) I wrongly designated this cuticle as the vitelline membrane, an error which is, to a certain extent, pardonable, inasmuch as the layer in question is structurally exactly like the vitelline membranes of other insects. But it certainly cannot be homologized with these membranes since it is secreted during a comparatively advanced stage by an embryonic cell-layer, the serosa, and not by the surface protoplasm of the unsegmented egg.

As soon as the embryo has taken up its position on the dorsal surface, the yolk segments; each vitellophag appropriating as many of the yolk-bodies as the radiating filaments of its cytoplasm can hold together and fashion into a rounded mass. Apparently the process is delayed in order that the passage of the embryo through the yolk may be facilitated, for obviously the embryo will move more easily over a prescribed path through a mass of small mobile particles than between large masses formed by the aggregation of such particles. The yolk-masses, at first very distinctly marked, soon fuse with one another so that their boundaries can be traced only by reference to their centres, which coincide with the nuclei of the vitellophags.

After leaving the ventral face of the egg the embryo increases greatly in length. Just before burying its tail in the yolk and while still completely on the ventral surface it measured only .7 mm.; now it measures 1.7 mm. This increase in length, as will be inferred from the foregoing description, is due to two causes: an intercalation of new segments in front of the anal plate to complete the abdomen, and a stretching of the segments thus arising.

A glance at Fig. 6, which represents an embryo in the stage of its greatest elongation on the dorsal surface, shows that many important changes have taken place since it left the ventral surface. The cephalic and thoracic appendages have assumed a more definite character. The labrum (*lb.*) has suddenly appeared, the first and second maxillæ (*mx*<sup>1</sup>, *mx*<sup>2</sup>) have

each become trilobed, while the metathoracic leg ( $p_3$ ) already exhibits unmistakable traces of its characteristic thickening in the larva and imago. The pleuropodia ( $pl. (ap^1)$ ) stand out clearly from the edges of the first abdominal segment. Shining through the stretched ectodermal layer of the abdominal segments may be seen the paired mesodermal somites ( $coe.$ ), or mesomeres. The anal plate with its pair of cerci ( $cc. (ap^{11})$ ), and the anus are definitely established. A faint neural furrow runs from the mouth to the anus, and in the thoracic region faint metameric indications of the ganglia are apparent. All these important changes have taken place within the yolk during the transition of the embryo. This renders their study on hardened material very difficult, for although the embryo may be dissected away from the yolk, it is so much curved that it can be mounted only in pieces, and the yolk is at this period so difficult to cut that only fragmentary series of sections can be obtained.

One of the most interesting changes undergone while the embryo is still in the yolk is the appearance of the labrum. In Fig. 6 (Stage F) the labrum is a distinctly unpaired circular appendage. But that it has a paired origin I infer from a transverse section, part of which is represented in Fig. 35. This passes just in front of the mouth of an embryo but little older than Stage E. The appendage ( $lb.$ ) is here seen to be distinctly bilobed although it does not yet project beyond the general level of the head. This bilateral condition is speedily slurred over and the organ grows into an unpaired and in most embryos perfectly circular disk overhanging the mouth. Very rarely, as in Fig. 7 it may show traces of its paired origin even during later stages.

Let us return to the indusium which we left as a thin round plate gradually spreading over the yolk just beneath the ventral serosa. The outlines of this plate are not always circular but exhibit traces of lobulation (Fig. 5). The spreading is at first uniform along its whole circumference so that the organ soon assumes the shape of a circular scroll clasping the egg. Its lateral edges approximate on the dorsal surface just over the ventral face of the embryo but are temporarily arrested

in their growth before they unite. The anterior and posterior edges, however, continue to advance without interruption, so that the disk if spread out on a plane surface would in its successive stages represent a series of ellipses with constant short axis but continually increasing longitudinal axis. In this manner the disk grows towards either pole while enveloping the egg laterally. The edges of the organ continue to approximate on the dorsal surface but stop growing just before they meet. Hence, when the egg is viewed from the dorsal surface a long, narrow slit is seen extending nearly its entire length and separating the dorsal edges of the organ. It is not till the anterior and posterior edges have nearly or quite reached their respective poles that this slit closes with the fusion of the edges of the organ. The raphe is at first so weak that the edges may be broken apart by slight pressure with the needles, but it soon becomes permanent and the egg is now completely enveloped by two further membranes—the inner and outer indusia. Before the fusion of these two membranes the amnion of the embryo was in contact with the serosa but now that the edges of the indusia have worked their way in between the serosa and amnion, the latter comes to lie in contact with the inner indusium. Henceforth the serosa is excluded from taking any part in the development of the embryo; both its position and function are now usurped by the inner indusium.

One is enabled to follow the different stages in the progress of the indusium, from its disk-like condition on the ventral yolk to the complete union of its dorsal-growing edges, by means of a peculiar secretion of its inner layer. This is a brownish or blackish granular substance, probably some urate, which appears to be secreted by all the cells of the inner indusium and which gives the organ the appearance of a large brown blotch in a stage a little older than E. At first pale and hardly perceptible, this spot gradually deepens in color till its advancing edges become distinctly outlined on the underlying yolk. A clear idea of the closure of the edges may be obtained from Fig. III, A–C. The dark granular secretion is shown in Fig. 6 at *enwl*.

Soon after the union of the edges of the outer and inner indusial layers a chitinous cuticle is secreted by the outer surface of the latter. This cuticle is thicker and seems to be of a deeper hue than the cuticle secreted by the serosa. It

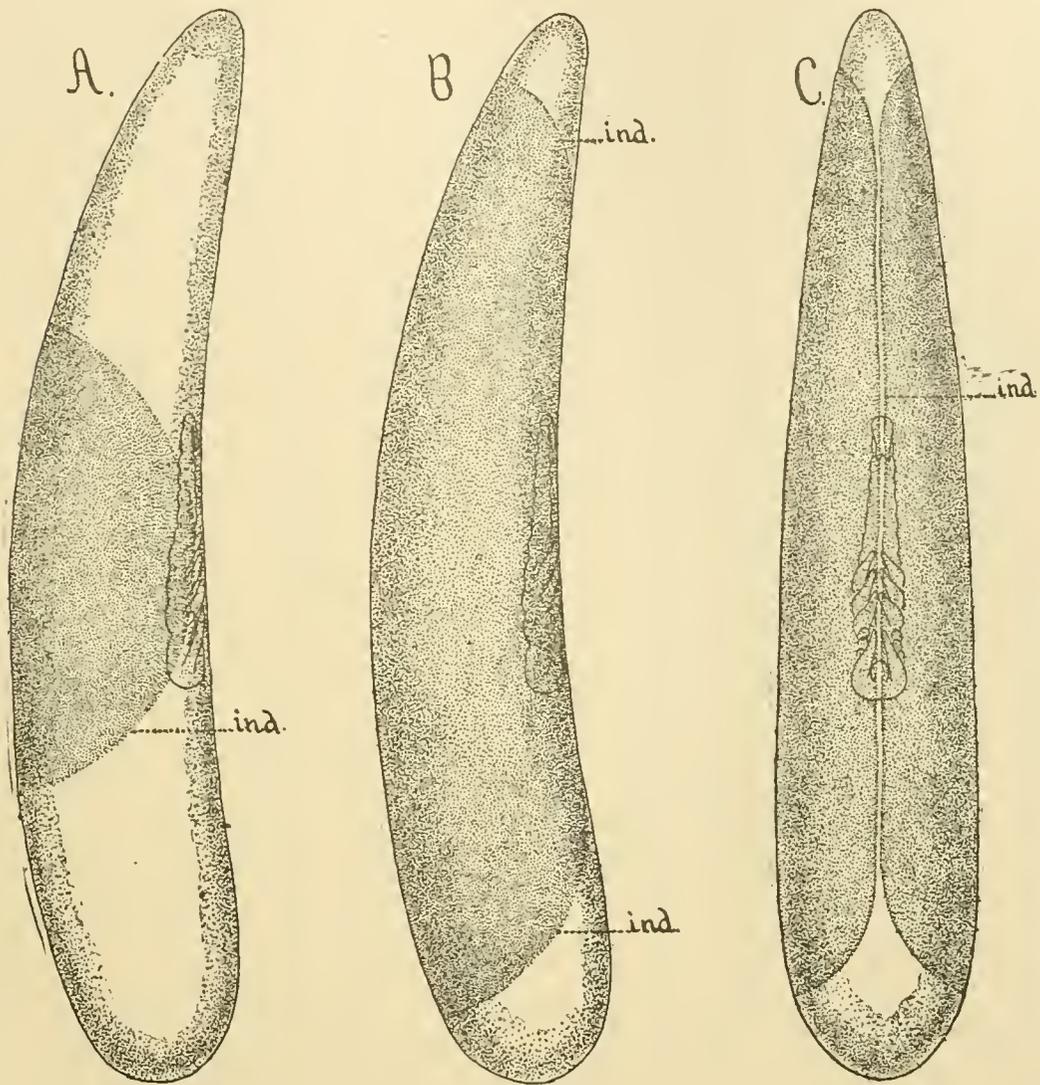


FIG. III.

Two stages in the spreading of the indusium. *A*, lateral view of egg just after the arrival of the embryo on the dorsal yolk; *B*, lateral view of the egg with the indusium nearly reaching the poles; *C*, same egg seen from the dorsal surface.

definitely excludes the outer indusium from any further share in the development of the embryo. Even in Stage E, this cell-layer was reduced to an exceedingly thin membrane. (Pl. III, Fig. 22, *am.*<sup>1</sup>) It seems to fuse with the serosa and to retain a connection with the inner indusium only at the ex-

treme anterior pole of the egg. I confess, however, that my observations on this envelope are rather unsatisfactory.

After the completion of the processes described in the preceding paragraphs we may distinguish several envelopes in a median transverse section of the egg. Passing from without inwards we have (1) the chorion, (2) the Blastodermhaut-like cuticle secreted by the serosa, (3) the serosa, (4) the outer indusium, (5) the layer of dark, granular secretion, (6) the cuticle secreted by the inner indusium, (7) the inner indusium and (8) the amnion. While envelopes 1-7 invest the whole egg, layer 8, the amnion, covers only the embryo.

The general development of the embryo has been traced to Stage F, when it lies as a straight and attenuated body on the dorsal yolk with its head directed towards the caudal and its tail towards the cephalic pole of the egg.

Like all other insects that have a stage during which the body is greatly elongated (Coleoptera, Diptera, Lepidoptera) *Xiphidium* passes into a series of stages during which the germ-band is gradually shortened. The shortening is accompanied by a broadening of all the segments, a growth of the appendages, and very important internal changes. The completion of this process is reached in Stage G (Fig. 7). Besides a greater development of the appendages seen in Stage F, Fig. 7 also shows that the abdominal appendages have appeared. Of these there are nine pairs, exclusive of the pleuropodia and cerci, so that in *Xiphidium*, just as in *Blatta* and many other insects, every segment of the abdomen bears a pair of appendages. Starting with the basal segment there are eight pairs of stigmata. These are not all seen in the figure. Just back of each pair of tracheal invaginations appears a second pair of ingrowths—the metastigmatic depressions—seen as small white spots just outside the appendages, near the posterior edges of their respective segments. They are in line (homostichous) with the tracheal invaginations which occupy corresponding positions near the anterior edges of their respective segments.

The ventral flexure of the abdomen constitutes another very important difference between Stages G and F. In *Xiphidium*

this flexure always takes place between the 7th and 8th segments and is brought about during the shortening of the embryo. It is essentially the same flexure which is found in *Blatta* and in Decapod Crustacea.

In Stage G the antennæ have increased to nearly one-third the length of the embryo. The procephalic lobes on which the segmentation of the brain is plainly visible, have developed greatly. The appendages, instead of projecting laterally, as they do in the younger embryo, are folded over the ventral surface of the germ-band. The nerve cord is distinctly marked out. (See abdominal region, Fig. 7.)

It is in this stage, or one but slightly more advanced, that the embryo passes the winter. Cleavage and the succeeding stages up to F are passed within a month after oviposition — during the warm days of August and September. But even should October and November be mild and sunny, development seems to have come to a temporary standstill on reaching Stage G. Among the hundreds of embryos which I collected during three succeeding autumns, I did not find one that had passed far beyond this stage. Nevertheless if kept in a warm, moist atmosphere during winter, a certain number of eggs will continue their development almost to hatching.

Before passing on to later stages in the development I will here give a brief account of some anomalies in the development of the indusium.

#### 4. *Variations in the Development of the Indusium.*

In the preceding pages I have described what I take to be the normal development of the indusium of *Xiphidium*. A considerable number of embryos (about 100), being nearly one half of the total number examined for the stages thus far described, deviated more or less widely in so far as the indusium was concerned from what I consider the normal type of development. Unfortunately I did not discover the organ till it was too late in the season to obtain a large supply of material in the requisite stages, so that the variations here briefly noticed probably represent only a small fraction of those

observable in a large number of eggs. The variations may be tabulated thus :—

1. Variations in size. Normally the indusium is of the same size as one of the procephalic lobes (.2 mm. in diameter) so that the head of the embryo resembles a clover leaf as long as the organ is attached to it. When the chorion is removed the organ may be distinctly seen with the unaided eye as a milk-white spot on the translucent yolk. Occasionally, however, embryos will be found in which it is less than .1 mm. in diameter, and all variations between this and the normal size may be observed.

2. Variations from the typical circular form. These variations are very numerous and may be regarded as belonging to two classes. In one class the indusium is rounded in outline, while in the other it is ragged and more or less irregular. To the first class may be assigned the oval, cordate and multilobulate varieties not infrequently observed; to the second belong a number of irregularly stellate and rhizopod-like forms. In one of my preparations, midway between the two classes, the indusium is evenly rounded anteriorly and ragged posteriorly along that portion of its periphery which has just broken away from the head of the embryo.

3. There is a variation in the time at which the organ is set free from the head. This cannot be proved directly by observation of the organ itself, for it usually does not begin to form the circular fold till after its isolation, but differences in the embryo, especially in the prominence of the segments and appendages, show that the organ remains attached to the head in some cases longer than in others.

4. Variations in the development of the circular fold. These variations, alluded to above, are characterized by a greater or less distinctness in the folds that give rise to the inner and outer layers. All shades in the process may be found between the distinct and comparatively rare method described and figured (Fig. 3), and the more frequent and obscurer method whereby the three layers are formed by a shifting of the individual cells.

5. Variations in number. I have twice observed two indusia

in the same egg. In the first case the embryo itself was in every way normal, and the first indusium of the normal size and shape, and in the usual position. The second, somewhat smaller, though regularly circular organ, was situated in front of the first and a little to the right of the median line. The distance between the two organs was about double the distance between the first organ and the head of the embryo. The outlines of the second or more anterior organ were less definite than those of the first. The amnion and serosa had formed over the embryo, but neither of the indusia showed as yet any tendency to form envelopes. Whether these two organs were derived from the division of one original præoral cluster of cells, or were originally established as two separate centres on the blastoderm, I am unable to decide. The latter method would seem to be the more probable.

The other case is somewhat singular. The first indusium was normal in size and position, but irregularly heptagonal in outline. The second, situated a short distance to the side of the right procephalic lobe, was not more than a third the size of the first organ and quite regularly quadrangular. The embryo itself was normal and covered with the amnion and serosa. The envelopes had also formed over the two organs, which in this case also probably originated from two discrete centres in the blastoderm. The smaller organ had probably never been attached to the head of the embryo.

##### 5. *The Revolution of the Embryo*

During the first warm days of spring the *Xiphidium* embryo resumes its development. This is characterized for some time by a growth of the germ-band in breadth and length and a lengthening of the appendages. The body of the embryo, which in Stages F and G was much narrower than the egg now becomes almost as broad so that its pleural edges embrace the yolk. This increase in size brings the head somewhat nearer the lower pole, and there soon sets in a decided movement of the whole body in this direction. When the head has almost reached the lower pole, the amnion covering the face

of the cephalic end fuses with the overlying inner indusium. A rent appears in this fused portion of the envelopes and through it the head is soon seen protruding. Gradually more of the body is pushed through the orifice, first the mouth parts, then the thoracic legs and finally the abdominal segments, till the whole embryo comes to lie free on the surface of the yolk in the space between the inner indusium and its cuticle. The amnion and inner indusium, which during the evagination of the embryo have remained united at the edges of the rent are folded over the pleural region of the embryo onto the yolk. The two envelopes now form but a single layer enclosing the yolk like a bag. The inner indusium is united to the edges of the amnion and these in turn are united to the pleural edges of the embryo, with the ectoderm of which the amniotic cells are continuous. The small size of the amniotic cells as compared with the huge flattened elements of the inner indusium enables one readily to distinguish the limits of the two envelopes.

During its evagination from the cavity of the amnion the embryo gradually passes around the lower pole of the egg head first and begins to ascend the convex ventral surface. An embryo freed from all its envelopes except the two that take part in revolution is represented in Fig. 8, in the very act of turning the lower pole. The amnion and inner indusium are folded back over the yolk, the former (*am*) characterized by its small rounded nuclei, the latter (*sr.*) by its large flat elements. The line of juncture of the amnion with the body of the embryo is marked by a denser aggregation of nuclei. The ventral flexure still persists on the dorsal surface.

The cavity of the amnion contains a quantity of serum-like liquid, which during the evagination of the embryo is poured into the space separating the inner indusium from its cuticle. This liquid collecting at the lower pole, may function as a lubricant and cushion, and thus facilitate the movements of the germ-band. In hardened specimens it is found as a granular magma enveloping the appendages. It is not shown in Fig. 8.

In many respects the embryo in Stage H has advanced considerably beyond that represented in Fig. 7. In the head, the

eye is distinctly marked out and its cells are arranging themselves to form the ommatidia, as is evident from the regular series of pale dots. The labrum, now considerably enlarged, is spade-shaped in ventral aspect. The antennæ have grown in length, and the saltatory legs (*p*3) are assuming their definitive characters. The large tapering pleuropodia stand out prominently on the first abdominal segment. Near the bases of the legs the thoracic stigmata are distinctly seen. They had made their appearance in Stage G, but for obvious reasons could not be shown in the figure.

The anterior end of the embryo continues to move up the ventral surface of the egg, straightening out as it rises. Finally the flexed terminal segments of the abdomen are again bent back to their original position in line with the rest of the body. Since their flexure these segments (the 8th–11th) have been the only portion of the body provided with a completed dorsal wall (*vide* Fig. 7). After the bending back of the abdominal tip its segments still retain a certain independence and make no attempt to embrace the yolk of the posterior pole as do the segments in front of them. It is for this reason that the abdomen presents a constriction just in front of the eighth segment. This constriction is especially noticeable in profile view.

The turning of the lower pole of the egg seems to take place very rapidly compared with other equally important processes of development, such as the passage of the embryo through the yolk. I infer this from the relative scarcity of embryos in the act of returning to the ventral surface. I have, however, succeeded in finding all the stages in the process of revolution, and feel quite as confident of having correctly interpreted my preparations as if I had studied the living egg.

#### 6. *The Stages Intervening between Revolution and Hatching.*

Fig. 9 represents an embryo that has just straightened out on the ventral surface of the yolk, which the reader may imagine as extending up beyond the head to nearly twice the

length of the embryo and terminating in the pointed anterior pole. A comparison of Figs. 8 and 9 shows that, although the former embryo has completed its revolution, it is nevertheless in an earlier stage so far as the development of its organs is concerned. This is particularly noticeable in the labrum, antennæ and mouth parts, the eyes and the saltatory legs. Hence we may infer that the time for turning the lower pole is subject to considerable variation.

In Fig. 9 it will be observed that many of the abdominal appendages have disappeared. Pairs are, however, retained on the 8th to 11th segments ( $ap^{8-cc}$ , ( $ap^{11}$ )). The pleuropodia are also still present though concealed behind the bases of the metathoracic legs. The disappearance of the appendages on the 2d-8th segments probably has its immediate mechanical cause in the lateral stretching which characterizes these segments in their attempts to embrace the yolk.

The embryo continues its growth as before in two directions—the body constantly lengthening and thus bringing the head nearer the pointed anterior pole, while its lateral walls, enveloping more and more of the yolk, gradually grow towards each other and finally unite in the median dorsal line. The union begins with the 7th abdominal segment, just in front of the segments which have for some time been provided with a dorsal wall, and continues headward. I am not certain as to what becomes of the amnion during this process. Its cells appear to take no part in the formation of the dorsal wall, but very probably degenerate and become supplanted by the cells of the advancing ectoderm. It must be remembered that a hard and fast line cannot be drawn between the amnion and the pleural ectoderm; the cells of both structures passing into one another by insensible gradations. My reasons for supposing that the amnion proper takes no part in building up the embryo are mainly of a theoretical nature and will be given in the latter part of this paper.

Concerning the fate of the inner indusium there can be little doubt. While the embryo is continually advancing towards the cephalic pole and enclosing more and more of the yolk—this envelope, which, as above stated, is characterized by

huge flat cells and nuclei, is being as gradually restricted to a more and more limited yolk surface. In consequence of this restriction its component cells become broader radially and narrower tangentially. In this stage the envelope functionally corresponds to the "dorsal organ" of other insects. It cannot, however, be thus designated without still further increasing the number of heterogeneous structures included under that unfortunate term, since the "dorsal organ" of other insects is a thickening of an envelope represented in *Xiphidium* by the serosa.

The thickened inner indusium is soon reduced to a cap of cells on the anterior pointed pole of the egg. As the head of the embryo advances to cover more of this pole, the envelope is pushed further forward and finally stripped from the yolk altogether. The anterior cranial walls then close over the pole and thus effectually separate the yolk from the inner indusium. The latter is reduced to a small conical mass, the cells of which soon show unmistakable signs of degeneration.

Soon after the embryo has thus rid itself of its envelopes and has taken into its mesenteron the whole mass of yolk not utilized in the processes of development hitherto undergone, a chitinous cuticle is shed from its entire surface. This may be designated as the first larval cuticle. It appears first on the ventral abdominal surface and spreads thence headward and dorsad. The progress of cuticularization is readily traceable by staining embryos in this stage, for the parts over which the cuticle is formed will not take the color; where it is being deposited the stain takes faintly and where it has not yet appeared, the stain, of course, penetrates easily. Ayers ('84) observed in *Æcanthus* that the secretion of the cuticle began on the ventral surface of the embryo and extended dorsad. This is just what we should expect from the fact that the dorsal hypodermis is ontogenetically a more recent formation than that of the ventral surface.

The first larval cuticle is about  $5\mu$  thick and consists of three layers. The innermost is apparently homogeneous and stains deeply in Orth's lithium carmine while the middle layer remains clear and vitreous. The outer layer is radially striated

and has the distinctly yellow tint of old chitin. Its outer surface is minutely papillate. On the appendages the cuticle is much thinner than it is on the trunk and though it stains it does not show a differentiation into three layers.

Before shedding the first cuticle the hypodermis secretes a second larval skin which persists till after hatching.

In Fig. IV, I have attempted to represent semi-diagrammatically the condition of the envelopes at a time when the eyes begin to acquire pigment. The chorion (*ch.*) is much distended and the egg larger and more resistant to the touch than it was during the autumn. Passing from without inward we first meet with the cuticle secreted by the serosa (*sr. c.*). Then follows the serosa itself (*sr.*) to the inner face of which the remains of the outer indusium (*ind.*<sup>1</sup>) are applied. At the extreme anterior end of the egg both these cellular envelopes appear to be much thickened and pass into a cylindrical pedicel of granular plasma which I shall call the columella (*cl.*). This in turn is continuous with a conical mass of cells (*ind.*<sup>2</sup>), the remains of the inner indusium which was stripped from the head in a preceding stage. Its cells, as shown in the figure, are in an advanced stage of disintegration. The cytoplasm of the different elements is reduced to a mass of granules and the chromosomes have become agglomerated into little spheres floating in the clear nuclear plasma. The process of degeneration is similar to that which I have described as occurring in the "dorsal organ" of *Blatta*. Between the mass of degenerating cells and the head of the embryo lies a granular coagulum (*am. s.*). This I take to be the amniotic serum which is forced up into the anterior pole by the enlarging of the embryo and the consequent decrease in the space between the body walls and the chorion. The columella and the remains of the inner indusium are held together and thus temporarily prevented from complete disintegration by the thick cuticle of the latter. This cuticle still envelops the embryo and extends forward to the anterior pole where it seems to be attached to the inner face of the outer indusium. Passing further inward we next meet with the first larval cuticle (*lv. c*<sup>1</sup>), which has been shed, and the second larval cuticle (*lv. c*<sup>2</sup>), which is still in organic

connection with the hypodermis. In a little later stage than the one here described the columella and the conical lump of inner indusial elements have disintegrated, and can no longer be distinguished from the granular amniotic serum.

The changes in the configuration of the embryo since its arrival on the ventral yolk, relate mostly to the appendages. At first the antennæ are of about the same thickness as the

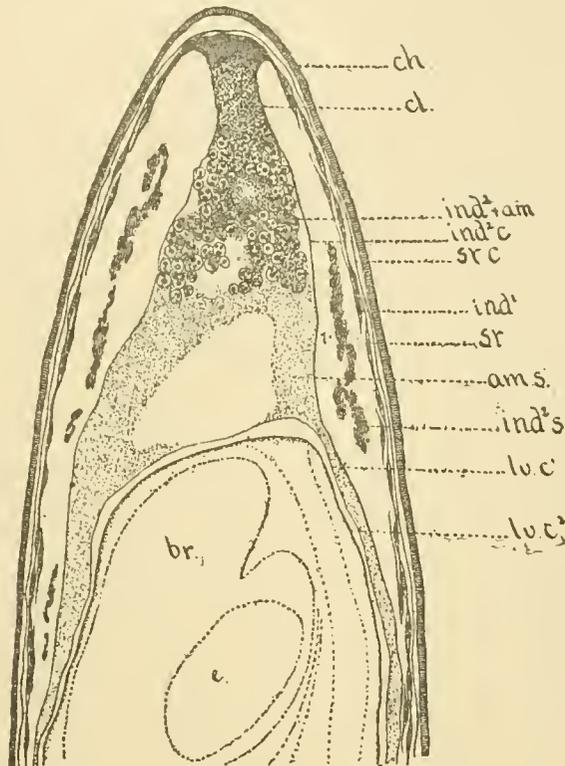


FIG. IV.

Sagittal section through the anterior pole of a *Xiphidium* embryo, with pigmented eyes. *ch.*, chorion; *cl.*, columella; *sr. c.*, Blastodermhaut; *sr.*, serosa; *ind<sup>2</sup> + am.*, remains of the inner indusium and amnion; *ind<sup>1</sup>*, outer indusium; *ind<sup>2</sup> s.*, secretion of the inner indusium; *am. s.*, amniotic secretion; *lv. c<sup>1</sup>*, first larval cuticle; *lv. c<sup>2</sup>*, second larval cuticle; *br.*, brain; *e.*, eye.

legs. The dark line running parallel with their inner edges, and distinctly marked in Fig. 9, is in section seen to be a mesodermal partition dividing the cavity of the appendage into two tubular sinuses. The antennæ grow directly tailward till their tips reach the femorotibial joint of the hind legs, when they diverge laterally, describe an arc, and then grow forward. When the tips have reached the head further progress is arrested

by the envelopes, but as the growth of the appendages does not cease, the arcs surrounding the hind legs gradually move tailward. This movement is arrested just before the time for hatching, when the antennæ have grown to nearly twice the length of the embryo.

The mouth-parts and thoracic appendages have been gradually assuming their adult characters in the meantime.

The pleuropodia, as described in a former paper ('90<sup>a</sup>), are shed during hatching and just previous to that event may be found attached to the pleural cuticle by means of very slender pedicels.

In the male the appendages of the 9th and 11th abdominal segments persist, the former as the stylets, the latter as the cerci. In the female the cerci also persist but together with them also the pairs on the 8th, 9th and 10th segments (Figs. 9 and 10—*op*<sup>1</sup> (*ap*<sup>8</sup>)—*op*<sup>3</sup> (*ap*<sup>10</sup>)). These are converted into the gonapophyses.

Apart from the eyes little pigment is developed in the hypodermis during embryonic life, unless we regard as such the brown granular secretion of the inner indusium.

A number of eggs kept in the house the greater part of the winter hatched May 15th–18th, but I am inclined to believe that out of doors the regular time for hatching is later, probably not till the end of May. *Xiphidium fasciatum* apparently does not hatch till early in June, since I found larvæ of this species on Naushon Island June 9, which could not have been more than a few days old. Inasmuch as the imagines of *Xiphidium cusiferum* oviposit on the average about Sept. 1st, the whole postembryonic development cannot occupy more than three months. As this Locustid is monogoneutic, nine months is therefore required for embryonic development. Even if we deduct the period of quiescence due to cold weather, it will still be apparent that the embryonic stages must succeed one another very slowly in *Xiphidium* as compared with those of other Ametabola (*e.g.* *Blatta*), not to mention the Metabola.

7. *The Development of Orchelimum vulgare.*

This Locustid oviposits like many of the smaller members of the family in the pith of dead plants. I found the eggs in Ohio during the last days of September in the stems of the wild lettuce (*Lactuca canadensis*), so common along the edges of fields and thickets, and in the petioles of the common elder (*Sambucus canadensis*). Oviposition probably takes place in the beginning or towards the middle of September. In the case of *Lactuca* and a few other plants which I did not identify, the insects had invariably selected for oviposition the main stem of the flower-panicles. From base to apex this portion of the stem was punctured at intervals, and a single egg thrust into the pith a short distance above each orifice. It is an easy matter to recognize the punctures by the little tufts which the insect evidently gnaws from the woody fibre, before inserting its scimeter-shaped ovipositor. Great care must be taken in splitting the stem, so as not to tear or cut the eggs which adhere very firmly to the pith.

The eggs are larger than those of *Xiphidium ensiferum*, being fully 6.-6.25 mm. long. In shape they are very similar to *Xiphidium* eggs except that the sides are compressed. In the fresh state they are smooth and opaque, and of a pale drab or bluish tint. In this respect, as also in the flattening of their lateral faces, they form a transition to the eggs of our larger Locustidæ, *e. g.* *Cyrtophyllus concavus*, *Amblycorypha uhlerii* and *Microcentrum retinervis*.<sup>1</sup> The chorion is not readily wetted with water, but like that of the *Xiphidium* egg, immediately becomes transparent when immersed in alcohol. The outer envelope is then seen to have a yellow tint, deepening into brown at the poles.

As would be expected from its close systematic affinity the embryonic development of *Orchelimum* does not differ much from that of *Xiphidium*. I have not seen all the stages, nor have I, as yet, sectioned any of my material, but the stages which I have examined are essentially the same as those

<sup>1</sup> For a description of the eggs of these species see an article on Orthoptera, by Prof. C. V. Riley, in the *Standard Natural History*, Vol. II. pp. 188-189.

described in *Xiphidium*. The embryo of *Orchelimum* passes through the yolk in the same manner as the *Xiphidium* embryo, shortens on the dorsal yolk, then grows apace, moves around the lower pole and finally begins the yolk-enveloping process on the ventral surface of the egg in the same way as the *Xiphidium* embryo. It also develops an indusium which is set free from the head and spreads over the yolk while the embryo is passing through it backwards. In *Orchelimum* the inner indusial layer also secretes a brownish pigment-like substance which enables one to follow its movements as it gradually covers more and more of the yolk. A clear slit is likewise left on the dorsal surface between the folds of the organ. But in the time of closure of this slit *Orchelimum* differs from *Xiphidium*. In the latter insect we found that the slit closed soon after the embryo had straightened on the dorsal yolk, before it had shortened very decidedly. In *Orchelimum* the closure is considerably delayed. The embryo shortens, then grows in length and breadth, passing beyond Stage G of *Xiphidium* and its head nearly reaches the lower pole before the two folds of the indusium meet and fuse. Frequently in this stage, when the embryo is about to revolve, the polar ends of the slit are still open, the membranes having fused over the embryo. In a little later stage, however, the indusium has completely enveloped the yolk.

## II. REMARKS ON GASTRULATION IN THE ORTHOPTERA.

Although many important observations have of late been contributed to the embryology of the Insecta, our knowledge of the formation of the germ-layers in the Orthoptera cannot be said to have made any signal advance. As late as 1889 so few forms of this order had been studied that I felt justified in expressing some doubt as to whether their mesentoderm was formed in the same manner as in the higher Metabola (Coleoptera, Diptera, Lepidoptera). My doubts were confirmed by a study of *Blatta*, when I failed to find the oral formative centre of the entoderm ('89<sup>b</sup>).<sup>1</sup>

<sup>1</sup> We need not go far to seek the reasons for this gap in our comparative studies. The eggs of the Orthoptera are almost without exception extremely

Bruce ('86) appears to have been the first to describe the origin of the mesentoderm from a median ingrowth of the germ-band in the Orthoptera. The species which he studied, is, I have every reason to believe, *Stagmomantis carolina*. His description is very meagre and his figures are unsatisfactory.

More convincing are Graber's figures and description of mesentoderm formation in *Stenobothrus variabilis* ('88, Pl. XIV, Fig. 11; Pl. XV, Fig. 13). His Fig. 11 shows that there is in the median line a distinct infolding of the ventral plate cells—a true invagination. In a more recent paper ('90), the account is briefly repeated without any important additions.

In his recent study of the embryogeny of *Blatta germanica*, Cholodkowsky ('91<sup>a</sup>) gives an account of the formation of the germ-layers more in harmony with what we know of the process in the Coleoptera than the account which I gave. But he has not come to any definite conclusion respecting the formation of the entoderm, and although he maintains that there is a distinct blastoporic groove running the length of the germ-band, he does not figure it in surface view, and most of his sections betray such an amount of distortion in his preparations that one may hesitate to regard the slight depressions in his figures (Figs. 7, 8, 10, etc.) as indicating invagination. Nevertheless I believe from renewed study of the Orthoptera, that Cholodkowsky is correct in deriving the mesoderm from a median proliferation of the primitively one-layered germ-band, and the entoderm from two formative centres—one in the oral and one in the anal region.

In *Xiphidium*, soon after its first appearance, the blastoporic depression, when seen from the surface (Fig. 1), is a straight refractory from a technical point of view. The cells of the embryo are often smaller and less distinct than they are in the Metabola. Moreover, the great quantity of yolk and its singular brittleness in hardened specimens renders paraffin sectioning most unsatisfactory, and rather than incur the great expenditure of time which working with celloidin involves, the student gladly selects some Coleopteran or Dipteran egg which is all that can be demanded from a purely technical point of view. Nevertheless the Orthoptera constitute, by common consent, one of the most primitive orders of the Insecta; their eggs are large and may be readily procured in great numbers; their development is so gradual that all the requisite stages may be obtained without the least difficulty.

groove extending nearly the entire length of the germ-band and dividing it into two symmetrical halves. Anteriorly the groove is rounded and seems to end rather abruptly, but posteriorly it bifurcates, each of the two grooves thus arising being continued for a short distance to either side till they gradually fade away. There can be no doubt, it seems to me, that the bifurcated termination of the blastopore is the homologue of the similar structure first figured by me in *Doryphora* ('89, Pl. XVIII, Fig. 71; Pl. XIX, Fig. 73) and subsequently seen by Graber ('90) in the corresponding stages of *Lina tremulæ* (Pl. II, Figs. 25 and 27). More recently Cholodkowsky has observed a similar widening of the blastopore in *Blatta*. He attempts to identify it with the posterior depressions of Graber's "lateral gastrulation."

In Stage B (Fig. 2) when the caudal annio-serosal fold has covered the ligulate portion of the germ-band, the blastopore presents a widening of its anterior end at a point which probably lies just in front of the definitive mouth. This widening was observed in several embryos, and I therefore take it to be a normal occurrence. It also has its homologue in the *Doryphora* embryo (see my Fig. 70, Pl. XVIII, '89). In the stage under consideration (Fig. 2) the anal bifurcation has grown more indistinct and is apparently about to disappear.

The closure of the blastopore proceeds simultaneously in two directions: from its anterior end backwards, and from its posterior end forwards, so that the last portion of the groove to disappear lies in that part of the germ-band which is to become the thoracic or baso-abdominal region.

In sections the groove is seen to be much shallower than it appears in surface view. Along its whole extent its floor is somewhat thickened and in this portion — destined to form the mesentoderm — the cells soon lose their columnar shapes and become more polygonal in outline and more irregular in their arrangement. The groove closes in such a way that no tubular cavity results as in the Coleoptera and Diptera; the cells at the edges of the depression appear to glide over the median elements, so that after the fusion of the edges in the median line the mesentoderm has the form of a solid cord

applied to the inner surface of the germ-band. The process whereby the inner layers are formed is, therefore, a slurred invagination. In this respect *Xiphidium* resembles *Blatta*.

The further differentiation of the mesentoderm is quite as difficult to follow in *Xiphidium* as in other Orthoptera. In these stages the embryo cannot be satisfactorily isolated from the yolk and sectioned by itself, and so friable is the yolk that it is almost impossible to obtain thin sections through the entire egg by the ordinary methods. After studying a few series of sections obtained by means of the celloidin method I can, however, affirm that the invaginated cells give rise to both entoderm and mesoderm. The former has a bipolar origin, as has been made out in the higher forms; in *Apis* by Grassi ('84); in *Hydrophilus* by Heider ('89); in *Doryphora* by myself ('89); in *Musca* by Voeltzkow ('89) and by Graber ('89); and in *Chalicodoma* by Carrière ('90). The anal is considerably larger than the oral formative centre and its elements seem to arise in part from the bifurcation and in part from the deeper portion of the blastopore just in front of the bifurcation.

In *Xiphidium*, just as in the higher Metabola, a pair of entoderm-bands grows towards the baso-abdominal region from either entoderm-pole. Each band, consisting of only one layer of much-flattened cells, meets that of its respective side and then begins to envelop the yolk by proliferation at its ventral and dorsal edges. Transverse sections show that at first the bands are only two or three cells in breadth and that these are closely applied to the dorsal faces of the mesomeres which are formed by this time.

I have made no observations on the relations of the proctodæum to the posterior end of the blastopore, but in regard to the anterior end and its relation to the stomodæum my results are more definite. Figs. 32-34 represent three successive sections through the head of an embryo in Stage D. The last section (Fig. 34) passes through the stomodæum (*st.*) which is just forming as a rounded depression in the cephalic ectoderm. Its large columnar cells are regularly arranged and have their nuclei in the inner ends. The next section (Fig. 33) passes just in front of the stomodæum and cuts two masses of cells in

the median line. The upper of these masses is a thickening of the ectoderm distinctly separated on either side from the elements of the same layer by the peculiar character of its cells. These are much smaller than those of the remaining ectoderm and stain more deeply, especially in the inner portions of the layer. The lower mass of cells is entirely cut off from the ectodermal thickening, though its elements are very similar in size and staining qualities. The ectodermal thickening marks the point where the paired labrum is about to appear (*cf.* Fig. 35). In the next section (Fig. 32), which also passes through the labral region, we again meet with the thickening of the ectoderm. Unlike its portion in the preceding section, it is not bounded below by a curved line, but juts in as a ragged mass of cells, in which it is possible to distinguish a pair of lateral wings and a median projection. The median portion thus proliferated beyond the limits of the ectoderm, is the anterior or oral entoderm centre—the lateral wings I regard as mesodermal. By combining Figs. 32 and 33 the flattened mass of cells underlying the ectoderm in the latter section is seen to be the backward continuation of the mesentoderm. Section Fig. 34 shows that this median unpaired mass splits into two masses, one on either side of the mouth. In this paired condition the bands run backwards through the trunk of the embryo.

Essentially the same condition of the germ-layers in front of the mouth persists till the labrum is definitely formed, as I have observed in a few series of sections. It is difficult to account for the late and intimate union of the mesentoderm with the ectoderm in the labral region, unless we suppose that the blastopore originally extended as far forward as this region and here closed in such a manner that the three layers were not at once separated into ectoderm on the one hand and mesentoderm on the other. It is mainly on this supposition that I take the labral region to coincide with the anterior widening of the blastopore seen in Fig. 2. This widening probably does not coincide with the stomodæum, but lies in front of it, and the definitive mouth is a later formation arising independently from the ectoderm alone.

I would here insert a few observations on gastrulation in *Stagmomantis carolina*, *Gryllus luctuosus*, and *Æcanthus niveus*.

In Fig. 12 the egg of *Stagmomantis* is represented in outline for the purpose of showing the relatively small size of the germ-band which arises as in other forms from a thickening of the blastoderm on the ventral face of the yolk. It is seen to lie somewhat nearer the broad posterior than the pointed anterior pole. It is but slightly longer than broad, and its wider anterior end, which is directed towards the upper pole of the egg, foreshadows the procephalic lobes. Fig. 11 shows that the germ-band of the Mantid, unlike that of *Xiphidium*, is raised above the niveau of the yolk and has its marginal cells sharply separated from the extra-embryonal blastoderm — or serosa — as it is now called. This much flattened layer is, nevertheless, encroaching on the edges of the germ-band to form the amnio-serosal fold (*ams.*). At the anterior edge lies a small cluster of cells (*p. o.*) but little larger than those of the germ-band. I believe that these may represent all that remains of an indusium in *Stagmomantis*.

The narrowly pear-shaped blastopore is very short. Sections show it to be a deep groove, which like the median infolding of other forms (*Doryphora*, *Hydrophilus*, *Musca*, etc.) is deepest posteriorly and grows shallower headward. As I failed to find any of the stages immediately following the one figured, I could not trace out the formation of the germ-layers.

According to Bruce ('86, p. 17), who studied the same species of *Stagmomantis*, "When the union of the folds (of the amnion and serosa) is effected and the embryo is separated from the surface and covered ventrally by the amnion, the under layer is formed, as in *Mcloë* and *Thyridopteryx* as an ingrowth from the median line of the embryo." This remark, together with his Figs. XLII–XLIV, Pl. IV, shows that he could not have observed the formation of the layers from a groove and that he must have based his inference on a stage later than the one I have figured.

In *Gryllus luctuosus* the blastopore is more like that of *Xiphidium*. The outline of the egg is shown in Fig. 14. The

germ-band is relatively much larger when compared with the yolk-mass than the germ-band of *Stagnomantis*. It arises on the ventral surface very near the lower pole. That such is the correct position of the embryo may be easily ascertained, since the mother-insect thrusts her eggs into the ground with their long axes perpendicular to the surface. In a glass jar containing a few inches of earth, many eggs were deposited between the surface of the glass and the earth, so that the exact position of the apical pole could be noted, and the egg removed and hardened with this pole constantly in sight. Thus it was possible to determine the exact topographical relations of the embryo to the yolk throughout the important stages of early development.

During gastrulation the germ-band of *Gryllus* (Fig. 13) is more elliptical and somewhat narrower than the germ-band of *Stagnomantis*. Its edges are also distinctly marked off from the blastoderm and here, too, the amnio-serosal fold (*ams.*) arises along the entire periphery. The blastopore (*bl.*) is much narrower than the corresponding depression in *Stagnomantis*. It is deepest posteriorly.

The discovery of an invaginate gastrula in *Gryllus* made it extremely probable that this stage had been overlooked in the other members of the same family which have been studied from an embryological standpoint. Neither Korotneff in his study of *Gryllotalpa* ('85), nor Ayers in his study of *Æcanthus* ('84), succeeded in finding an invagination. I was unable to secure the eggs of any of our native *Gryllotalpæ*, but I collected a great number of *Æcanthus* eggs in Ohio during the last days of September. An examination of these soon convinced me that Ayers had not seen the youngest stages in the development of the germ-band. The youngest germ-band that he figures (Figs. 1-18) lies near the posterior end of the egg with its tail pointing towards the micropylar pole. According to Ayers "A tract of the blastoderm along the median line of the ventral (concave) side, lying nearest the deep or primitively head-end of the egg, becomes thickened into a germinal band, which is the first trace of the *body* of the embryo." But this is *not* the first trace of the body of the embryo, nor does it

arise on the concave face of the egg. The germ-band of *Æcanthus*, like that of *Gryllus*, first makes its appearance as a thickening of the blastoderm on the *convex* surface near the lower pole of the egg. This convex surface is, therefore, the ventral surface and the micropyle marks the "primitively head-end" of the egg as is shown by the fact that the procephaleum is originally directed towards this and not towards the opposite pole, which Ayers incorrectly calls the "primitively head-end." The germ-band, however, soon leaves its position on the convex ventral surface and, moving around the lower pole tail first, comes to lie on the concave dorsal yolk. It is clear that Ayers could not have seen the stages preceding the arrival of the germ-band on the dorsal surface, and it is during these very stages that the blastopore forms and closes.

Before turning the lower pole the germ-band of *Æcanthus* resembles that of *Stagnomantis*. Its anterior is distinctly wider than its posterior end and represents the future procephalic region. A narrow, but distinct groove runs from the oral to the anal end as in the forms we have been considering. At the posterior end the groove bifurcates much as in *Xiphidium*. That this median groove gives rise to the mesentoderm admits of little doubt after what has been said of other Orthoptera. The amnio-serosal fold appears to arise simultaneously along the entire margin of the germ-band as in *Gryllus*.

It follows from the observations here recorded, fragmentary as they are in many respects, together with Graber's observations on *Stenobothrus*, that the Orthoptera can no longer be regarded as *hors de ligne* so far as the formation of their germ-layers is concerned. In all the families of the order, save the Phasmidæ, an invaginate gastrula has been found, and there can be little doubt that the investigator who is so fortunate as to study embryos of this family will find in them essentially the same process of germ-layer formation.

The view is now pretty generally held that in the Insecta both mesoderm and entoderm arise from a median longitudinal furrow — the former layer throughout nearly the entire length, the latter only in the oral and anal regions of the germ-band — and that the vitellophags, or cells left in the yolk at a time when the remaining cleavage products are traveling to the surface to form the blastoderm, take no part whatsoever in the formation of the mesenteron, but degenerate *in situ* and finally undergo dissolution. Discussions of the literature on this subject are to be found in the papers of Heider ('89) and Graber ('89, '90), and so few are the facts accumulated since these résumés were penned that I may dispense with an historical consideration of the insect germ-layers in the present paper.

In the interpretation of the insect gastrula the entoderm has always played an important rôle. The origin of the mesoderm has long been known and has been duly provided for in the various germ-layer hypotheses which have from time to time been advanced. But the true origin of the lining of the mid-gut has been ascertained only within the last few years, so that we cannot expect to find an adequate treatment of this layer in the older theories. Led astray by what had been observed in Crustacea and Arachnida, some writers chose to regard the vitellophags as forming the mesenteron and shaped their theories accordingly (Oscar and Richard Hertwig, '81). But now that it has been shown that the vitellophags take no part in forming the lining of the mid-gut, their morphological position is rendered even more obscure, and we are brought face to face with the question: Are the vitellophags a portion of the entoderm which has been set apart very early in development for the purpose of yolk-liquefaction or are they an entirely new segregation of cells belonging to none of the conventional germ-layers? Those who defend the former alternative maintain that the vitellophags of insects are entodermal in function inasmuch as they digest yolk and closely resemble the amœboid Crustacean yolk-cells which are actually converted into the lining of the mesenteron. On the other hand it is urged, that as the yolk-cells arise and function before the blastoderm is com-

pleted and hence some time before the germ-layers are formed, they cannot properly be assigned to the entoderm.<sup>1</sup>

It is probably best to await the results of further investigation before deciding on the phylogenetic relations of the vitellophags to the entoderm. Heider ('89) has also expressed himself to this effect and I fully endorse his opinion when he says: "Immerhin wird man vorläufig über vage Vermuthungen nach dieser Richtung nicht hinauskommen, und ist die Frage nach der Auffassung der Dotterzellen bei dem Nachweise, dass sie an dem Aufbau des Embryos keinen Antheil nehmen, meiner Ansicht nach von geringerer Wichtigkeit."

<sup>1</sup> Besides these vitellophags which with Cholodkowsky ('91<sup>a</sup>) we may call the primary yolk-cells—there are other cells which detach themselves from the blastoderm or embryo and enter the yolk. These Cholodkowsky calls secondary yolk-cells. While the origin of the primary yolk-cells has been quite satisfactorily demonstrated, this cannot be said of those of the second class. They appear to descend into the yolk at different times in different species. Thus, according to Patten ('84), all the cleavage products in *Neophylax* ascend to the surface, the yolk-cells subsequently descending from the blastoderm. I claimed a similar total migration of the cleavage products to the surface in *Blatta* ('89); Cholodkowsky, however, claims that some of the cells never reach the surface, but remain in the yolk. Be this as it may, in later stages I believe it can be shown that cells do migrate into the yolk from the embryo and especially from the entoderm-centres. This was shown by me to be the case in *Doryphora*, where many cells pass into the yolk from either entoderm pole (Pl. XIX, Fig. 82; Pl. XX, Fig. 88). I have since observed an exactly similar phenomenon in *Telea polyphemus* in a corresponding stage of development. Graber, ('89, p. 11) too, has made a similar observation on *Melolontha*, where he saw "vom invaginierten Blastodermwulst aus unter lebhaften Theilungserscheinungen ganze Ströme von Zellen in den Dotter hineinwandern, Zellen die freilich von den primären, gleichzeitig vorkommenden und auffallend grosskernigen Centroblastelementen ganz enorm verschieden sind, und die sich überhaupt durch ihre ganze Beschaffenheit als unzweideutige Abkömmlinge, man könnte sagen Auswürflinge eines wahren Keimblattes, erweisen." So far as the migrant cells described in *Doryphora* are concerned, I am sure they come from the entoderm. They occur only at or near the entodermal Anlagen and may be traced from this germ-layer into the yolk. These cells are not actively dividing like those described by Graber, but actively disintegrating. (May not Graber have mistaken disintegration-figures for caryokinetic figures?) In somewhat later stages no traces of these migrant cells are to be found. The yolk is segmented at the time of their leaving the entoderm and their invasion appears not to disturb in the least the activities of the vitellophags. Whether there is any relation between these evanescent entoderm cells and the "secondary mesoderm" of Reichenbach ('86), the "spores" of F. H. Herrick ('86), or the "chromatin nebulae" of Bumpus ('91) is a question which cannot be answered at present.

Among those who take a decided stand on the relations of the vitellophags to the definitive entoderm, Graber and Cholodkowsky may be mentioned. Graber ('89, p. 10), after introducing the superfluous and inapplicable term "centroblast,"<sup>1</sup> says: Dabei nehme ich zugleich, was indessen kaum misbilligt werden dürfte, stillschweigend auch an, dass dieses gegenwärtig, wie es scheint, von der Darm- and Gewebsbildung ausgeschlossene Zellenlager auch früher niemals eine dem echten Entoderm anderer Thiere entsprechende Rolle inne gehabt habe, sondern vielmehr dem letzteren gegenüber ein neues, wahrscheinlich mit der stärkeren Entwicklung des Dotters im Zusammenhang stehendes Differenzierungsproduct ist."

Cholodkowsky ('91<sup>a</sup>) does not dismiss the matter so briefly. Like Graber he draws a hard and fast line between primary and secondary yolk-cells, and admits no phylogenetic continuity between the vitellophags and the definitive entoderm. The vitellophags belong to none of the germ-layers. His reasons for not regarding them as a precociously segregated portion of the entoderm are neither new nor conclusive. Like other recent investigators he admits that the vitellophags are in part digested or discharged from the alimentary tract along with the remains of the yolk after hatching. But he is not satisfied that the yolk-cells should play a humble rôle in the insect economy. Some of them were predestined to a higher function than yolk-liquefaction — viz: to give rise to the blood, the fat-body and even to the germ-cells. He therefore supposes that the vitellophags are undifferentiated cells. But this supposition is not supported by the facts. That they are on the contrary, considerably specialized is shown by their limited function and mobility, their gradual and prolonged growth (especially in some Orthoptera), their inability to undergo caryokinesis or even akinesis, and their suspicious relations to the bacteria-like corpuscles of Blochmann. On *a priori* grounds we should not expect to derive whole sets of tissues from such specialized elements.

<sup>1</sup> Superfluous because we have enough names for these cells already, inapplicable because the termination "blast" is properly applied only to cells or tissues of a germinal character — not to decrepit elements like the yolk-cells.

But a more weighty objection may be adduced. It has been shown by Heider ('89) and Heymons ('90), not to mention many previous investigators, that the fat-body and sexual-cells arise from the mesoderm, and my own studies fully confirm this view. Concerning the origin of the blood there is some doubt, but it should be stated that Cholodkowsky has made no satisfactory observations of his own on this point and that, although some facts point to a derivation of the blood from the yolk-cells, others as definitely point to its origin in the mesodermal layer.

After taking for granted that the vitellophags are undifferentiated cells, that they have nothing and, what is more, never have had anything to do with the entoderm, and that they give rise to blood-corpuses, adipose-tissue and germ-cells, Cholodkowsky ushers in the parblast theory. It was to have been hoped that this theory might have been permitted to end its days in peace within the confines of vertebrate embryology where it originated. Fortunately, however, it has grown too old and decrepit, even under the skillful medical treatment which it has received from time to time, to be of any service in invertebrate morphology.

There is something almost ludicrous in Cholodkowsky's application of the parblast theory to the Insecta when we consider the methods which he employed in preparing the yolk of the *Blatta* egg. The capsules opened at both ends were subjected to the action of undiluted Perenyi's fluid for 12 hours and the eggs after treatment with the customary grades of alcohol, cleared in green cedar oil 24 hours. Thence they were transferred to paraffine (55–60° C.) and left 3–5 hours. The result of this heroic method is apparent enough in the distortion of the tissues, but its effect on the yolk is quite remarkable.

Both Blochmann ('87) and myself ('89) described the yolk of the *Blatta* egg as consisting of a mass of homogeneous and granular albuminoid bodies sharply polygonal from mutual pressure and interspersed with spherical oil-globules. We also described a peculiar distribution of the polygonal bodies; those of a homogeneous nature constituting an oval central core invested with the granular bodies. I further claimed that the

*Blatta* egg exhibited a yolk-segmentation which though faint and appearing late was, nevertheless, comparable to the yolk-segmentation in such forms as *Doryphora*.

Cholodkowsky ('91<sup>a</sup>) thus describes the yolk: "So kann ich, z. B., nicht bestätigen, dass der Dotter aus einzelnen polygonalen Dotterkörpern bestehe, wie derselbe von Blochmann (und Wheeler) beschrieben und abgebildet wird. Der ganze Dotter besteht aus einer continuirlichen plasmatischen Substanz, deren Vacuolen grössere und kleinere Fetttropfen enthalten. Die Continuirlichkeit der Dottermasse tritt nun um so deutlicher hervor, je besser die Objekte conservirt sind. Das Bild (ich möchte sagen, das Trugbild) der polygonalen Dotterkörper entsteht durch Bersten des Dotters nach der Bearbeitung mit nicht ganz passenden Reactiven." . . . "Auch kann ich die Blochmann'sche Unterscheidung des 'inneren' und 'äusseren' Dotters nicht annehmen; der ganze Unterschied in den Färbungsverhältnissen der beiden angeblichen Theile des Dotters lässt sich einfach dadurch erklären, dass die Farbe aus den peripherischen Theilen des Eies leichter als aus den inneren mit Säure ausgezogen wird." And at p. 58 he remarks: "Es ist bemerkenswerth, dass bei *Blatta germanica* eine Dotterzerklüftung vollkommen fehlt. Ich kann also mit Wheeler nicht übereinstimmen, wenn er sagt (p. 359), dass bei *Blatta* der Dotter, wenn auch sehr spät (nach Bildung der Extremitäten) sich furchen soll; höchst wahrscheinlich war Wheeler zu dieser irrigen Annahme durch die ausserordentliche Brüchigkeit des Dotters verleitet."

On reading these criticisms I re-examined my preparations and must emphatically re-assert what I claimed in my description of the yolk of the *Blatta* egg. Among my preparations I find several mature ovarian eggs hardened in Perenyi's fluid — not, however, treated with that vigorous reagent for 12 consecutive hours — and these show the yolk-bodies very distinctly as polygonal masses. There are no traces of a "Bersten des Dotters." Eggs killed in ordinary alcohol and mounted *in toto* show the polygonal yolk-bodies distinctly and in these same specimens the distribution of the different yolk-elements may be followed by carefully focusing. That Cholodkowsky should

be unable to detect the outlines of the segments in the yolk of eggs treated for half a day with Perenyi's fluid is not surprising, especially as this segmentation is of very short duration in *Blatta* as in other Orthoptera. It is present, however, as I have convinced myself from eggs mounted *in toto* and from sections.

If prolonged immersion in Perenyi's fluid can bring about a complete fusion of the yolk-bodies and an obliteration of their true structure, what must be its effect on the vitellophags scattered through the yolk? And how much importance are we to attach to Cholodkowsky's assertion that the fat-body, blood-corpuscles and sexual-cells arise from the vitellophags, and to the parablast theory as applied to the *Blatta*-ovum?

Let us return from this digression to the germ-layers. The curious fact that the definitive entoderm of the Insecta arises from two separate centres—one oral and the other anal—is too recent to have given rise to much speculation. Since the entoderm of other animals arises from a single centre it is tacitly assumed that such must originally have been the case with the Insecta, and that the present bipolar condition must be due to secondary modification. Starting with this postulate, there are, of course, many ways in which the bipartition of the original unipolar entoderm may be supposed to have taken place. Two of these possibilities are worked out in the hypotheses of Kowalevsky ('86) and Cholodkowsky ('91<sup>a</sup>).

Kowalevsky has expressed his views so clearly and concisely that I cannot do better than quote his own words: "Wenn wir jetzt versuchen, diese Bildung des Ento- und Mesoderms bei den Musciden mit der Bildung dieser Blätter bei anderen Thieren zu vergleichen, so sehen wir erstens, dass hier auch eine Art sehr in die Länge ausgezogener Gastrula entsteht, und dass aus dem eingestülpten Teil das Ento- und Mesoderm sich bildet. Also in diesen allgemeinen Zügen finden wir eine Uebereinstimmung. Es scheint mir aber, dass die Parallele noch weiter gezogen werden kann. Namentlich wenn wir der Bildung des Ento-Mesoderms bei *Sagitta* uns erinnern, so finden wir bei derselben dass der eingestülpte Teil des Blastoderms in drei parallele Säcke zerfällt, von

denen der mittlere das Entoderm liefert, die seitlichen aber das Mesoderm. Bei den Musciden entsteht auch eine solche Einstülpung wie bei *Sagitta*, und auch der mittlere Teil—allerdings nur an beiden Enden vorhanden—liefert das Entoderm, die seitlichen Teile liefern das Mesoderm: also ähnlich dem, was wir bei der *Sagitta* beobachten. Um die Aehnlichkeit weiter zu führen, kann vorausgesetzt werden, dass bei der so in die Länge gezogenen Gastrula der Insekten der mittlere, das Entoderm liefernde Sack so ausgezogen ist, dass er in der Mitte ganz verschwindet und nur an seinem vorderen und hinteren Ende bestehen bleibt. Bei dieser Auffassung wird es von selbst schon folgen, dass die sich schliessende Rinne fast auf ihrer ganzen Länge nur das Mesoderm liefert.

Jetzt bleibt noch die Frage übrig: wie verhalten sich die Flächen der Gastrula zu den Flächen des sich bildenden Entoderms. Bei der *Sagitta* wird die äussere Oberfläche der Blastula nach der Einstülpung zur inneren Oberfläche des Darmkanals, d. h. die Seiten der Zellen, welche bei der Blastula nach aussen gerichtet waren, werden im Darmkanal nach seinem Lumen gerichtet. Bei den Insekten kann dasselbe auch vorausgesetzt werden. Wenn wir uns die eingestülpte Rinne vorstellen, so sind deren Oberflächen ganz ähnlich gelagert wie bei der Gastrula; wenn wir weiter die Bildung der beiden Entodermanlagen dem mittleren Sacke der *Sagitta* vergleichen, so bleibt die Lagerung der Zellenflächen noch ganz dieselbe. Wenn wir dann voraussetzen, dass der mittlere Sack durch die weite Ausbreitung und durch das Eindringen der Masse des Dotters gewissermassen in seinen vordern und hintern Teil zersprengt ist, so kommt der Dotter ins innere des hypothetischen Sackes, und die Zellen, die den Dotter bedecken, werden zu dem Dotter in derselben Beziehung stehen, wie bei der *Sagitta* zu der eingestülpten Fläche."

A few years after these remarks were written Heider ('89) and myself ('89) at about the same time published observations on the Coleopteran germ-layers which seemed to support the hypothesis of the celebrated Russian embryologist. As further support to Kowalevsky's view I believe we may point to such gastrulas as that of *Stagmomantis* described above. In this

gastrula, which is so very short and broad, we may suppose that the oral and anal entoderm-centres are really continuous, covering the floor of the blastopore from end to end. In sections, it is true, I failed to detect any differentiation of the cells forming the walls of the furrow, into entodermal and mesodermal elements, but this would also be the case in the elongated gastrulas of other insects in a correspondingly early stage (*Xiphidium*, *Doryphora*). As favoring a purely mechanical separation of an originally single entoderm Anlage, it may be noted that the most rapid elongation of the germ-band occurs at a time when the entoderm is differentiating from the mesentoderm Anlage. There is probably more than an accidental correlation of these two processes. During this time some germ-bands (*Doryphora*, *Xiphidium*) double their length.<sup>1</sup> Inasmuch as the lengthening of the superficial layers of the embryo is much more rapid than the differentiation of the entoderm, this germ-layer must lag behind. In most insects the embryo is at the time of its greatest elongation much longer than the yolk-mass and must again shorten to the length of this mass, so that a rapid proliferation of the entoderm may be superfluous, since this layer would have to readjust itself to the yolk with the contraction of the embryo. It may, therefore, be an advantage for the entoderm to be somewhat retarded in its growth. In the Orthoptera, where the embryo lengthens rapidly, shortens, and then lengthens again to envelope the yolk we may suppose, for reasons to be given in the sequel, that yolk has been acquired. This seems also to be suggested by the histological structure of the embryonic entoderm; this layer consisting of large polygonal cells in the Coleoptera, which have only a medium amount of yolk, while in the Orthoptera the attenuate entoderm-bands consist of a very few flat cells.<sup>2</sup>

It is probable that when more forms have been carefully

<sup>1</sup> *Blatta* forms a very rare exception in this respect.

<sup>2</sup> A very similar condition may be observed in the case of the blastoderm. In the Coleoptera and Diptera, which have a medium or small amount of yolk, the newly formed blastoderm is a deeply columnar epithelium; in the Orthoptera it is a true pavement epithelium.

studied a method of entoderm formation midway between the unipolar and bipolar methods will be found to obtain in some insects. We must admit that a contribution of elements to the entoderm from the interpolar region of the furrow is not with certainty precluded in several of the species which have been studied. Thus Heider, ('89) while inclined to believe that such a contribution does not take place in the anterior portion of the germ-band, believes that it may take place in the posterior abdominal segments. Cholodkowsky ('91<sup>a</sup>) is inclined to accept a still more diffuse origin for the entoderm. "Untersuchungen zahlreicher Schnittserien machen es wahrscheinlich, dass an verschiedenen Stellen des Keimstreifens sich einzelne Zellen vom äusseren Blatte abspalten und an der Bildung des inneren Blattes betheiligen, so dass die Entstehungsart des letzteren sehr complicirt erscheint."

Starting with the same postulate as Kowalevsky, viz: that the bipolar is derivable from a unipolar condition of the entoderm — Cholodkowsky ('91<sup>a</sup>, '91<sup>b</sup>) proceeds to account for this phenomenon in a very different way from his compatriot. He takes the small, round blastopore of *Astacus*, stretches it till it equals the insect blastopore in length, introduces a number of modifications — such as the median groove and the pairs of lateral depressions and believes that he has found an explanation "sehr klar und ungezwungen" for all the different blastopores, not only in the Insecta, but also in the meroblastic eggs of vertebrates. It was Kleinenberg who said: "Gewagte Hypothesen, kühne Schlüsse nützen der Wissenschaft fast immer, die Schemata schaden ihr, wenn sie die vorhandenen Kenntnisse in eine leere und dazu noch schiefe Form bringen und beanspruchen tiefere Einsicht zu geben." The latter part of this aphorism seems to be particularly applicable to Cholodkowsky's exposition. As Graber ('91) has briefly pointed out, there are no grounds for comparing the *Astacus* blastopore with the entire insect blastopore. In the Decapods this orifice is confined to the anal region and if comparable at all to the median furrow in insects, must be compared with the caudal entoderm pole. This is all that is admissible, since the mesoderm of the Decapoda arises from the anterior lip of the blastopore and

proliferates headward. That such is its origin has been shown by Bobretzky and Reichenbach for *Astacus* ('86), by Paul Mayer for *Eupagurus* ('77), and by Bumpus for *Homarus* ('91). To Cholodkowsky both the extent and position of the blastopore are of little consequence as is abundantly evident from his reply to Graber's well-founded objection. It is this very neglect of what are generally and, I believe, rightly considered two of the most important matters in the discussion of the germ-layers, which stamps Cholodkowsky's hypothesis as superficial and inadequate.

There is, however, one redeeming suggestion in his hypothesis, viz: that the diverging grooves at the posterior end of the blastopore in insects may correspond to the "Sichelrinne" of vertebrates. Certainly the relations of the grooves to the median furrow in *Xiphidium* (see Fig. 1.) closely resemble in surface view the relations of the "Sichelrinne" to the primitive streak in the chick as figured by Koller ('81) and in the triton as figured by Oscar Hertwig ('90, p. 99).

While most investigators probably agree with Kowalevsky and Cholodkowsky in deriving the bipolar from a unipolar condition of the entoderm, Patten does not share this view ('90). In his opinion, which is based on Kleinenberg's interpretation of the gastrula, the blastopore is restricted to the oral region, and such depressions as occur at the posterior end of the germ-band, as well as the formation of teloblasts in that region, are supposed by him to have no connection with the blastopore, but to be merely the instruments of unipolar growth. "The Arthropod body represents an outgrowth from the trochosphere, but the trochosphere itself, the coelenterate stage, has disappeared. Hence there is no such thing as a gastrula in Arthropods and strictly speaking, no germ-layers." It is clear that this view must stand or fall with Kleinenberg's theoretical conclusions on which it is based, and we may venture to say that E. B. Wilson's recent work ('90) has rendered this foundation very insecure, notwithstanding Patten's rather confident assertion that "in *Lopadorhynchus* it is certain that the greater part of the mesoderm arises from the ectoderm at the growing tip of the tail, and has nothing to do with primitive mesoderm."

But it would be out of place to consider the widest bearings of Patten's hypothesis in this paper since I am concerned with it only in so far as it bears on the germ-layers of insects. Starting with the assumption that the blastopore is confined to the mouth, he attempts to show that the median furrow is a purely secondary structure. "That the median furrow of insects is merely an ontogenetic adaptation is sufficiently evident from the fact that it may be present or absent in closely related forms." This, however, is not the case. On the contrary the furrow or a slight modification of it is, we have every reason to suppose, universally present in the Insecta, at least in the Pterygota, and this wide occurrence of the structure is one of the surest indications of its high antiquity and phylogenetic importance.

In the latter part of his discussion Patten admits that there are "structures in Arthropods which may represent remnants of gastrulas. For example, if the mouth and œsophagus of Arthropods is primitive—and there is no reason to suppose it is secondarily acquired—then we must look for primitive entoderm at its inner end. I have figured in 'Eyes of *Acilius*,' at the very anterior end of the embryo, a great sac of entoderm cells which probably arise by invagination, although the process was not directly observed. The sac, which soon opens outward by the œsophagus, afterwards becomes solid, and finally is converted into two longitudinal bands, one on either side, extending backwards to the middle of the body, where they become continuous with similar bands extending forwards from the posterior end of the embryo." Patten admits that true entoderm is formed at two widely separated regions of the body, but he implies that only the anterior centre is comparable to the entoderm of other animals, the posterior centre being a new and purely adaptive formation. It is just here that his theory appears to me to fail, since it does not explain why the oral and anal centres should resemble each other so very closely in origin, method of growth and histological structure.

## III. THE INDUSIUM AND ITS HOMOLOGUES.

In none of the Pterygota hitherto studied has there been found any trace of a structure comparable to the indusium of *Xiphidium* and *Orchelimum*. The organ appears to have been retained by the Locustidæ and completely lost by the embryos of other winged insects. In some of the Apterygota, however, there is an embryonic organ which gives a clue to the possible homologues of the indusium. I allude to the so-called "micropyle" of the Poduridæ.

During the summer of '91 I was so fortunate as to secure the eggs of *Anurida maritima* in great numbers. They are much larger than any of the Poduran eggs hitherto studied — so large that they may be removed from their choria by means of dissecting-needles and partially stained for surface views. It is also an easy matter to obtain good sections.<sup>1</sup> When first deposited the eggs are provided with a thin transparent chorion and vitelline membrane, but after cleavage, which is total, is completed and the blastoderm formed, a yellow, peculiarly striated chitinous membrane is secreted from the surface. The egg then enlarges till the chorion and vitelline membrane are burst. The striated membrane was described by Ryder ('86), but he failed to observe that it is attached to a large circular ring — the "micropyle." In section (Fig. V) this organ is seen to be a very decided thickening of the blastoderm which at this time covers the whole yolk-mass as a single layer of minute columnar cells. In the "micropyle" the cells and nuclei are much enlarged and often considerably vacuolated. Surface views prepared according to the partial staining method show that the embryo is already faintly outlined on the yolk and that the ring-shaped organ lies just in front of its head (Fig. VI). The egg being spherical, the embryo is curled in a semicircle and the "micropyle" thus comes to lie on the dorsal surface nearer the head than the tail of the germ-band. In the figure a more advanced embryo is represented as spread out on a flat

<sup>1</sup> I mention this because the few fragmentary accounts that have been published on the development of the Poduridæ are based on the study of the embryo viewed through the chorion and other envelopes. This has given rise to some errors which I hope to point out in a future paper.

surface. The resemblance of the "micropyle" to the indusium is apparent at a glance (*cf.* Fig. 2, Pl. I). I have followed the organ in *Anurida* through the later stages by means of sections and find that it persists for some time as a simple thickening of the blastoderm, still connected with the peculiar striated membrane which stands away from the surface of the blastoderm at all other points. Finally, when the embryo has become flexed dorsoventrally and the body-walls are closed, it sinks into the yolk and is absorbed.

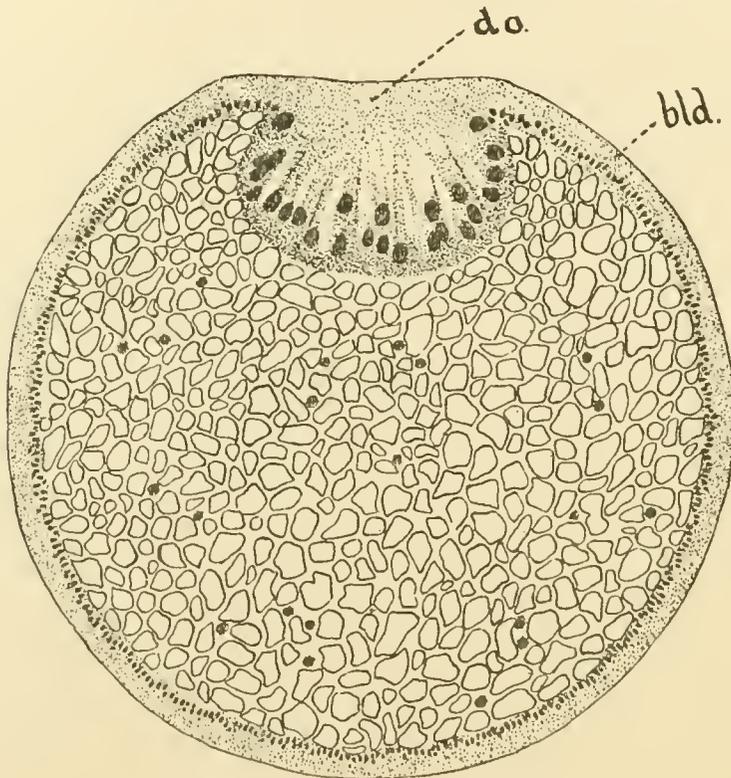


FIG. V.

Median section of the egg of *Anurida maritima*. *d.o.*, "micropyle"; *bld.*, blastoderm.

Although much simpler in its structure, I do not hesitate to homologize this "micropylar" organ in *Anurida* and the Poduridæ in general with the indusium of *Xiphidium*. A possible objection to this homology, on the ground that the indusium arises on the ventral face of the egg, while the Podurid "micropyle" is dorsal, has little weight, since the organ bears in either case the same relation to the head of the embryo. Provided, therefore, the egg of *Anurida* were to acquire yolk

and become greatly elongated, like the *Xiphidium* egg, the micropylar organ must come to lie on the same surface of the yolk as the germ-band.

It has been repeatedly suggested, and, I believe, on very good grounds, that the Podurid "micropyle" is the homologue

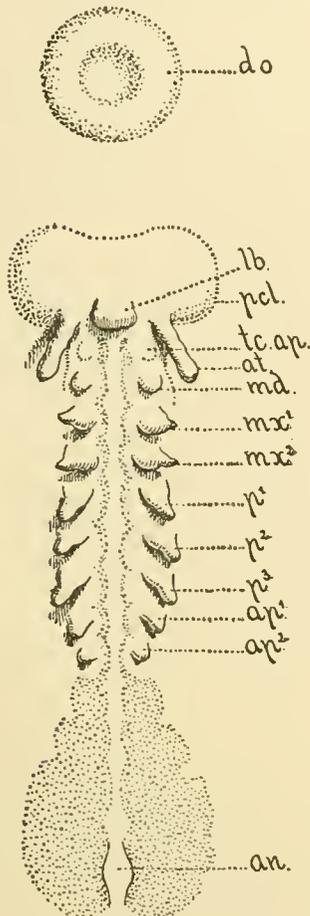


FIG. VI.

Embryo of *Anurida maritima* spread out on a flat surface. *d.o.*, "micropyle" *lb.*, labrum; *pcl.*, procephalic lobe; *at*, antenna; *tc.ap.*, minute appendage of the tritocerebral segment; *md.*, mandible; *mx*<sup>1</sup>, *mx*<sup>2</sup>, first and second maxillæ; *p*<sup>1</sup>-*p*<sup>3</sup>, first to third thoracic appendages; *ap*<sup>1</sup>, first abdominal appendage (= left half of colophore); *ap*<sup>2</sup>, second abdominal appendage; *an.*, anus.

of the crustacean "dorsal organ." In both groups the organ arises soon after the germ-band is mapped out on the yolk, and in both groups it is a circular or oval thickening of the blastoderm lying in the median dorsal line distinctly nearer the head than the telson. In the Crustacea its centre often shows a depression to the walls of which the Blastoderm-haut is attached, while standing away from the surface of the egg at other points. An exactly similar condition obtains in the Poduridæ; a slight depression marks the centre of the organ in *Anurida*, while in *Anurophorus* (Lemoine, '87) there appears to be a deep pit at the attachment of the chitinous envelope. This depression is comparable to the depression seen in Fig. 3 in *Xiphidium*, where the circular fold is encroaching on the disk.

Up to the stage represented in the figure just referred to, the indusium will bear close comparison with the crustacean "dorsal organ." In the first stages of its spreading it also resembles to some extent the saddle-shaped "dorsal organ" of *Oniscus*, *Porcellio*, and *Ligia*. But beyond this point it differs widely from its homologues, and it is difficult to see why it

should persist, and instead of sinking into the yolk, envelop the whole egg, secrete a granular and thereupon a chitinous layer, and finally, during revolution take on the function of a true serosa. That the organ is rudimental is shown by its tendency to vary, especially during the earlier stages of its development; that it still performs some function is indicated by its somewhat complicated later development and by its survival in but very few forms out of the vast group of Pterygotous insects. This seeming paradox may be explained, if we suppose that the indusium was on the verge of disappearing, being the last rudiment of some very ancient structure. As such a rudiment it no longer fell under the influence of natural selection, and for this reason began to vary considerably like other rudimental organs. Some of these fortuitous variations may have come to be advantageous to the embryo, and were perhaps again seized upon by natural selection; the nearly extinct organ being thus resuscitated and again forced to take an active part in the processes of development.

Pursuing the homologies of the indusium still further we come to the Arachnida, where we find in the primitive cumulus of spiders a structure comparable in many ways to the Podurid "micropyle," as v. Kennel ('85, '88) and Lemoine ('87) have suggested. There is, however, so much difference of opinion regarding the position and signification of the primitive cumulus that I should hardly be willing to agree with these authors, were it not for two of Claparède's figures of the *Pholcus* embryo ('62, Figs. 6 and 7, Pl. I). These show in the median dorsal line a thickening which forcibly recalls the "micropyle" of *Anurida*. Still it must be admitted that Claparède has failed to prove the identity of this thickening with the primitive cumulus.

In Pentastomids the "facette" or "cervical cross" described by Leuckart ('60) and Stiles ('91) is very probably the homologue of the crustacean dorsal organ and the insect indusium.

Although no homologous structure has yet been detected in the Myriopoda, the occurrence of a dorsal-organ-like structure in such widely separated groups as the Hexapoda, Araneina, Pentastomidæ, and Crustacea is sufficient reason for regarding

it as exceedingly ancient and as well-developed before the existing subdivisions of the Arthropoda were established. To seek a homologue of the "dorsal organ" among existing annelids may be regarded by some as a hopeless undertaking. Still I would call attention to Apathy's observation ('88) on *Clepsine bioculata*. The adult of this species has long been known to possess a chitinous plate in the median line between the head and the præclitellum. Apathy finds that this plate is the remnant of an embryonic sucking-disk, the glandular cells of which secrete a bundle of byssus-like threads that harden on contact with the water and serve to anchor the undeveloped young to the ventral concavity of the mother-leech. A similar organ is also found in the young of *Clepsine heteroclita*. It is certainly no great step from this embryonic sucking-disk of the Hirudinea to the Phyllopod "cervical gland" which is also used as a sucker, and which Fritz Müller ('64) and Grobben ('79) regard as homologous with the "dorsal organ" of the Amphipoda.

#### IV. THE ENVELOPES AND REVOLUTION OF THE INSECT EMBRYO.

##### 1. *The Amnion and Serosa.*

The formation of two cellular envelopes, the amnion and serosa, by a folding of the primitive extra-embryonal blastoderm, is rightly considered one of the most characteristic features of the Hexapod embryo. The envelopes are not, however, common to all insects. An amnion is completely lacking in the Poduridæ,<sup>1</sup> and consequently the extra-embryonal blastoderm in these forms is strictly comparable to the corresponding portion of the blastoderm in Crustacea, Myriopoda, and Arachnida. This is proved by the fact that it ultimately forms the definitive dorsal body-wall. So far as our present knowledge extends, the Apterygota may be regarded as Hexapoda Anamniota, and

<sup>1</sup> Lemoine ('87) describes a cellular "membrane amniotique" in *Anurophorus*, but he does not represent it in his figures and did not study it in section. I therefore incline to doubt the correctness of his observation, especially as I can find no traces of a cellular envelope in the *Anurida* egg, which on account of its size is a far more favorable egg for study than that of *Anurophorus*.

placed over against the Pterygota, which are characterized by the possession of an amnion (Hexapoda Amniota). There is a gap between these two groups of insects similar to the gap between the amniote and anamniote vertebrates. Whether it will be filled by the future study of such orthopteroid forms as *Machilis*, *Lepisma* and *Forficula* remains to be seen. For the present I am inclined to believe that the amnion first made its appearance in the ancestral Pterygota. Even if it be contended that the amnion was once present in the Apterygota and subsequently lost, its origin could not consistently be pushed further back than the Hexapoda, since this envelope is lacking in the Myriopoda, which, there is reason to believe, lie in the direct line of descent. The proof that the so-called amnions of *Pcripatus*, Scorpions and Pseudoscorpions are the homologues of the insect amnion is not forthcoming. Judging from the few descriptions of their formation, they appear to have arisen independently within their respective groups.

Just as many of the Pterygota develop only rudiments of wings or have altogether ceased to develop these organs in the adult state, so the embryos of the Pterygota in some cases develop only rudimental envelopes or none at all. It is reported that the amnion is lacking in the Proctotrupid Hymenoptera (Ayers, '84) and rudimental in Muscidæ (Kowalevsky, '86; Graber, '89) and viviparous Cecidomyidæ (Metschnikoff, '66). Certain ants of Madeira are incidentally mentioned by Metschnikoff as having the envelopes represented only by a small mass of cells in the dorsal region. The absence or abortion of the amnion is almost certainly a secondary condition. The Proctotrupidæ are egg-parasites and undergo an extremely aberrant embryonic and larval development. Both these and the other insects mentioned belong to groups characterized by high specialization. This is notably the case with the ants and with the Muscidæ which show considerable aberration in their embryonic and larval stages. The pædogenesis of the Cecidomyids studied by Metschnikoff stamps them also as aberrant. Moreover the embryos of other Orthorrhaphous Diptera (Simulidæ, Chironomidæ, Tabanidæ) have perfectly normal envelopes.

Many attempts have been made to explain the origin of the amnion in insects. It first appears abruptly and fully developed in the Orthoptera just as the vertebrate amnion appears abruptly in the Reptilia. One school, represented by Nusbaum ('87) and v. Kennel ('85, '88), regards the insect amnion as a structure of high phylogenetic value and would trace it to some organ in the lower Arthropods or in the worms. According to another view advocated by Will ('88) and myself ('89), the amnion has had no such remote phylogenetic history, but has arisen more recently in response to certain purely mechanical conditions of development.

Nusbaum advances the opinion that the cellular envelopes of the insect embryo are homologous with the "dorsal organ" of Crustacea. The saddle-shaped "dorsal organ" of *Ligia* and *Oniscus* is regarded as the key to this homology, the two flaps which clasp the sides of the Isopod embryo being equivalent to undeveloped amnioserosal folds. But I have shown in the present paper that the indusium of *Xiphidium* is very probably the homologue of the crustacean "dorsal organ," and as there is besides a well developed amnion and serosa in *Xiphidium*, Nusbaum's hypothesis must fall to the ground. His assertion was certainly premature that the "deux séries des organes aussi caractéristiques que le sont l'organe dorsal et les enveloppes embryonnaires, s'excluent réciproquement dans les deux groupes des Arthropodes, c'est-à-dire chez les Trachéates et les Crustacés."

So far as the insect envelopes are concerned v. Kennel's views do not differ essentially from Nusbaum's. He likewise homologizes the crustacean "dorsal organ" and the Poduran "micropyle" with the Hexapod amnion and serosa. But he goes further and includes under the same homology the amnion of *Peripatus*, Scorpions and *Chelifer* and the chitinous envelopes of Myriopods. He supposes all these structures to represent remnants of the annelid trochophore. I feel confident that he has jumbled together at least three categories of organs which cannot be regarded as homologous *inter se*, viz.: (1) the series of structures typically represented by the Crustacean "dorsal organ"; (2) the cellular envelopes of insects;

(3) the chitinous cuticles. As stated above, the amnions of *Peripatus* and Scorpions probably also represent structures of independent origin and no wise homologous with the envelopes of insects. It is perhaps unnecessary to add that the reduction of all these structures to the annelid trochophore is in the present state of our knowledge little more than a wild guess.

Graber ('90) has criticized the view advanced by Will and myself, that the insect amnion arose by an invagination of the germ-band like that of some Myriopods (*Geophilus*). His contention is certainly in great measure well-founded. Still I believe that it does not affect the essential point of the hypothesis which implies that the amnioserosal fold is the mechanical result of a local induplication of the blastoderm due to rapid proliferation in a single layer of cells.

Ryder ('86) has sought a mechanical explanation for the amnion, and although his paper treats mainly of the vertebrate amnion, he evidently implies that the homonymous envelope of the Insecta had a similar origin. According to him "the amnion in all forms has arisen in consequence of the forces of growth resident in the embryo, encountering peripheral and external resistance either in the form of a rigid outer egg-shell (zona radiata) or decidua reflexa, or even the walls of the uterine cavity itself, supposing of course that a large vesicular blastoderm containing yolk has been formed by epiboly."

This view applies with little alteration to the Insecta. There is the vesicular one-layered blastoderm filled with yolk and the germ-band arising by rapid proliferation at one point. The resistance of the yolk being less than the external resistance of the tightly fitting chorion and vitelline membrane on the one hand combined with the peripheral resistance of the extra-embryonal blastoderm on the other, the germ-band is forced to invaginate. This invaginative process is favored by the displacement of yolk during its liquefaction and absorption by the growing embryo. We may suppose that this invagination which results in the formation of the amnioserosal fold, assumed a definite and specific character in different groups of insects.

Conditions similar to those to which the insect germ-band is subjected during its younger stages are often present in the ova and young of other animals, and would be expected to lead to the formation of structures similar to the insect amnion. And this is found to be the case. A hasty glance through the animal kingdom at once suggests a number of parallel instances: the invagination from which the Cestode head develops in the *Cysticercus*; the similar invagination in the larval Gordiid; the origin of the Nemertine in the *Pilidium*; the formation of the definitive trunk in *Aulastoma*, according to Bergh ('85); the development of the trunk and Scheitelplatte in *Sipunculus*, according to Hatschek ('84); the formation of the young Spatangid in the Pluteus, according to Metschnikoff, and the somewhat similar conditions in the development of the *Antedon*, according to Barrois ('88); the formation of the trunk in the *Actinotrocha* of *Phoronis* (E. B. Wilson '81); the development of the Polyzoan within the statoblast (Oka '91; Davenport '91). I need hardly say that the development of the amnion and serosa in vertebrates is a strictly analogous case. A case still more to the point, because occurring in the Insecta, is the formation of the imaginal disks. In this process we have all gradations till we reach the extreme in *Musca*, where the hollow disks whose inner walls bud forth the imaginal appendages are almost completely abstricted from the original hypodermis. The resistance of the chitinous cuticle of the larva in causing the invagination of the disks admits of easy observation. It certainly cannot be claimed that in all the different forms here enumerated genetic relationship lies at the bottom of the mutual agreement in the methods of forming the trunk or certain organs. On the contrary, everything goes to show that these similar methods in widely separated groups have been independently acquired under the stress of similar developmental conditions.

Perhaps the most difficult point to explain in the view here advanced, is the complete abstriction of the amnion from the serosa in nearly all insects. It is more natural to suppose that the inner envelope would remain continuous with the outer, so that the embryo could the more readily be everted

during revolution. The only explanation I have to offer, will be given in connection with a discussion of the movements of the germ-band. In that connection the variations in the development and amputation of the envelopes in the different groups of insects may also be treated to greater advantage.

## 2 *The Yolk.*

To my knowledge, the quantity of yolk in the insect egg has not been made the subject of comparative study. It has long been vaguely stated (*vide* Brauer, '69 and '70) that the eggs of Ametabolous insects contain relatively more yolk than the eggs of the Metabola. In other groups of animals (Crustacea, Annelida, Mollusca, Vertebrata) it is often observed that absence of yolk is correlated with free larval development, while in eggs provided with an abundance of yolk the larval stages are either lacking or considerably modified. This same law obtains also in the Hexapoda, though it can hardly be formulated so concisely as in other groups of animals. And this is not surprising when we stop to consider that, as regards complexity of organization, the difference between the simplest insect larvæ, such as those of the Muscidæ and their highly specialized imagines, is far from being as great as the differences between the trochophore and the Annelid, or the Nauplius and the crustacean.

Beginning with the Orthoptera we find that the egg is provided with an abundance of yolk, — the germ-band when first formed in most cases covers only a very small portion of its surface, and when it reaches its maximum length before revolution is no longer than, and usually not so long as, the egg.<sup>1</sup> The period of embryonic development is greatly prolonged; most of the species are monogoneutic and oviposit in the fall, the larvæ not hatching till the following spring or summer. There is practically no metamorphosis.

In the most highly metabolic insects (Muscidæ) on the other hand, the quantity of yolk is comparatively limited. The germ-band before revolution is nearly double the length of the egg,

<sup>1</sup> To this rule *Gryllotalpa* seems to be a noteworthy exception.

so that the head and tail ends nearly meet. Embryonic development is completed in a day, and the larva must pass through a complex metamorphosis to reach the imaginal state.

The chasm between these two extremes is bridged by the less metabolic insects (Coleoptera, Neuroptera, Lepidoptera, Hymenoptera, etc.). The quantity of yolk is intermediate between that of the Orthopteran and Dipteran egg. The germ-band, like that of the Muscidae, is longer than the egg when it reaches its full length. But it is at this time much narrower than the yolk-mass, whereas in the Muscidae it embraces nearly half the circumference of the yolk. The larvæ usually hatch after a period of ten to thirty days in a relatively more advanced stage of organization than Dipteran larvæ.

It is probable that the quality of the yolk is also an important factor in development. The yolk of the Orthoptera and Rhynchota is dense and resembles that of the crustacean and Arachnid egg, while the yolk of the Metabola seems to have a much looser molecular structure. Hence, bulk alone is no criterion of the amount of yolk in an insect's egg.

The view here advocated, that the eggs of the Ametabola contain more yolk than those of the Metabola, admits of some exceptions. Thus the 17-year locust (*Cicada septendecim*) is a large insect with incomplete metamorphosis, but it nevertheless produces a great number of very small eggs. This is, however, seen to be a greater advantage to the insect than the production of a few large eggs, when we consider the extremely long period of larval life and the vicissitudes to which the larvæ may be subjected during all this time. Similarly, *Meloë angusticollis* produces a great number of very small eggs, while the eggs of the smaller beetles (*Doryphora, c.g.*) are much larger. But *Meloë* is a parasite form, and probably only a few of its many offspring ever succeed in gaining access to the eggs of the bee. The larvæ, as shown by their hypermetamorphosis, are subjected to very varied conditions, and this would still further tend to reduce the number of successful individuals. As in anemophilous plants many germs are produced, but very few are destined ever to prosper. Many other exceptions to the general rule, like these two, are probably due to habits

which necessitate the production of a great number of ova at the expense of their size. The opposite exception occurs in the parasitic Pupipara, where the nourishment of the single larva within the parent is equivalent to the production of a large yolk-laden egg.<sup>1</sup>

The question naturally arises: Were the eggs of the primitive Insecta poor or rich in yolk? As all the evidence of comparative anatomy, embryology and paleontology goes to show that the Metabola are the more recent, the Amctabola the more ancient forms, we are justified in maintaining that primitive insects, or at any rate the primitive Pterygota supplied their eggs with a considerable quantity of yolk. At first sight the Apteriygota, which have holoblastic eggs, would seem to constitute a serious obstacle to this view, but it must be remembered that total cleavage is not necessarily a criterion of paucity of yolk (witness Arachnida, Crustacea, and Myriopoda). Furthermore, the eggs of some Thysanura, *Anurida*, *e.g.* are provided with an abundance of yolk. Holoblastic cleavage in this group is probably a Myriopod trait, as was long ago suggested by Metschnikoff ('74). We might perhaps conclude that the superficial type of cleavage, like the embryonic en-

<sup>1</sup> The differences between the eggs of different insects with respect to the amount of yolk is systematically disregarded by Graber ('90). This is shown by his classification of germ-bands as microblastic and macroblastic, brachyblastic and tanyblastic. These distinctions are readily shown to be distinctions in the amount of yolk and not in the germ-band. Thus the just-established germ-bands of the Saltatory Orthoptera appear to be very small because the eggs contain an enormous quantity of yolk; while the germ-band of the Muscidæ appears correspondingly large on account of the small quantity of yolk. The amount of yolk fluctuates even within the limits of the single orders so that the newly-formed germ-bands appear to differ in length more than they really do. In the Orthoptera we have the following series in which the amount of yolk decreases, the germ-band in consequence appearing to increase: *Melanoplus*, *Mantis*, *Cecanthus*, *Gryllus*, *Xiphidium*, *Blatta*, (?) *Gryllotalpa*.

Graber's further classification of germ-bands as orthoblastic and ankyloblastic, or straight and curved, is equally artificial. In the great majority of cases the shape of the germ-band depends upon the yolk surface on which it arises, or over which it happens to grow. The uselessness of such a classification is also shown in the case of *Xiphidium* and *Orchelimum*, where the just-established germ-band is straight, but becomes curved in passing to the dorsal surface, and thereupon again becomes straight. To which of Graber's classes does this germ-band belong?

velopes and the wings, originated in the ancestral Pterygota. But Lemoine ('87) claims that the segmentation of the Poduran *Anurophorus laricis* approaches the superficial type, so that this latter may have had a still more remote origin. It is, however, hopeless to speculate on this subject till the eggs of many more Thysanura and Myriopoda, including the Symphyla, have been studied.

The relations of yolk-quantity to the movements of the embryo will be considered in the following paragraphs.

### 3. *Blastokinesis.*

According to Hallez ('85 and '86) "La cellule-oeuf possède la même orientation que l'organisme maternel qui l'a produite: elle a un pôle cephalique et un pôle caudal, un côté droit et un côté gauche, une face dorsale et une face ventrale; et ces différentes face de la cellule-oeuf coincident aux faces correspondantes de l'embryon." This law was founded on a study of the eggs of *Periplaneta*, *Hydrophilus* and *Locusta*, but it finds full support in the descriptions and figures of all investigators of insect development.<sup>1</sup> My own observations, based on some thirty different insects, accord perfectly with those of Hallez.

In most eggs the cephalic and caudal poles are readily distinguishable, the micropyle being usually located at or near the former. In exceptional cases, however, it is located at the caudal pole. There is frequently a slight flexure in the longitudinal axis of the egg, foreshadowing the dorsal and ventral, and consequently also the lateral regions of the mature embryo. The more nearly the egg approaches the spherical form, as in certain Lepidoptera and Coleoptera and in the Trichoptera, the more obscure become the relations of the egg-surfaces to the body-surfaces of the mature embryo. There is, however, every reason to suppose that these relations still exist.

The practical value of Hallez' law was shown in studying the *Xiphidium* egg; all the movements of the germ-band could

<sup>1</sup> The only exception is Ayers, who was undoubtedly mistaken in regard to the orientation of the young *Æcanthus* embryo.

at once be referred to the axis of the mature embryo. When the eggs of other insects are oriented in the same manner, it is seen that the germ-band invariably arises on the ventral surface of the yolk with its procephaleum directed towards the cephalic, and its tail towards the caudal pole. No matter what positions it may subsequently assume, it always returns to its original position before hatching. Frequently the germ-band, when newly formed, lies nearer the lower than the upper pole (*Calopteryx*, *Æcanthus*, *Stagmomantis*, *Hydrophilus*, etc.). The usual movements are very simple; from a position of rest on the ventral surface of the egg, the germ-band moves through an arc till its body is completely inverted. Then it rests and again passes back through the same arc to its original position on the ventral yolk. These movements may be compared to the single vibration of a pendulum. The ascending movement I shall designate as *anatrepsis*, the descending as *katatrepsis*, the intervening resting stage as the *diapause*. The general term *blastokinesis* may be used to include all the oscillatory movements of the germ-band.

Inasmuch as the germ-bands in other Arthropods (Crustacea, Myriopoda, Arachnida, and Thysanura) exhibit no movements comparable to those of the lower Pterygota, and since, moreover, the insect germ-band is formed in exactly the same manner as that of other Arthropods and ultimately returns to its original position, no matter what oscillations may intervene, it is safe to infer that blastokinesis has been acquired within the Hexapod and probably even within the Pterygote group. We may also infer from the intimate relations of envelope-formation to blastokinesis in most forms, that both of these processes arose at about the same time.

No attempt has been made to account for the origin of blastokinesis. It has occurred to me that it may be due to causes of a purely physiological nature. The eggs of the primitive Pterygota were, as I have attempted to show, provided with a considerable amount of food yolk. Like their modern descendants they were probably also invested with dense chitinous envelopes. These must render the respiration of the embryo difficult as compared with embryonic respiration in annelids, mollusks and

vertebrates, or even as compared with the Crustacea, which usually have much thinner envelopes than insect eggs. Special provision is also made in many of the Crustacea for aerating the eggs. Now the cells of the rapidly growing insect embryo not only absorb and metabolize the yolk but also give off a certain amount of waste matter. — That this is not wholly of a gaseous nature is seen in older embryos which have considerable accumulations of uric salts in the blood corpuscles and fat-body. Waste products are undoubtedly given off during the stages preceding anatrepsis, and probably permeate the yolk in the immediate neighborhood of the germ-band. As the oxidation of these waste products is very probably retarded by sluggish transpiration, and as growth under such conditions would be seriously impeded, we may suppose that the embryo has acquired the habit of moving to another part of the egg where the yolk is as yet unpolluted. Here it grows apace till the surrounding yolk is again charged with excreta. Growth is then temporarily suspended and the embryo moves back to the ventral surface. The embryo reaches a considerable size before katatrepsis, so that its rotation must cause a considerable circulation in the yolk bodies. This would also serve to aerate the yolk and to bring fresh pabulum in contact with the assimilating cells of the embryo. It may also be noted that in many insects the movements set in at critical periods of growth. Thus in *Xiphidium* anatrepsis occurs during the addition of new segments, and in many other forms it immediately precedes the formation of new segments. In the Orthoptera, katatrepsis usually occurs in the spring and is the signal for a decisive advance in the development of the heart, sexual organs, compound eyes, etc. During this period, also, the abortion of such rudimental structures as the pleuropodia, abdominal appendages and envelopes seems to be hastened. In short, the whole process of katatrepsis, at least in *Xiphidium*, has the aspect of rejuvenescence. It will be remembered that the amnion is formed just before or during anatrepsis. It is probable that the complete abstriction of this envelope from the serosa is a device for favoring the movements of the embryo. The germ-band is thereby set adrift on the yolk and enabled to

migrate to some other surface. This, of course, necessitates a secondary union of the envelopes previous to katatrepsis.

The hypothesis set forth in the preceding paragraphs is also supported indirectly by the fact that in the eggs of the Metabola which are less abundantly provided with yolk than the eggs of the Ametabola, blastokinesis is either faint or wanting. Aeration would be much less necessary in such small eggs. The lengthening and shortening movements seen in the embryos of the Metabola as well as in those of the Ametabola may suffice to keep the yolk circulating. The Lepidopteran germ-band, it is true, exhibits movements, but the eggs of these insects are laid in exposed situations and provided with unusually thick envelopes, so that the movements of the embryo, though differing widely from the typical blastokinesis of lower forms, have perhaps been independently acquired for a similar purpose.

I had intended to give a comparative description of blastokinesis in the different orders of insects but as the known facts have been recently summarized in a masterly manner by Korschelt and Heider ('92) I shall confine my remarks mainly to the Orthoptera. Although Graber, Ayers and others have studied representatives of this very important group, they have given but fragmentary and often inaccurate accounts of the relations of the embryo to the yolk-mass at different periods of development.

I may begin my account with the Saltatoria which comprise the three families Gryllidæ, Locustidæ and Acrididæ. As representatives of the first, *Gryllus luctuosus* and *Æcanthus niveus* were studied. In both of these insects as was pointed out at p. 42 the germ-band arises on the ventral surface of the yolk near the caudal pole. During the formation of the envelopes anatrepsis sets in and carries the germ-band to the dorsal surface where it rests through the winter in an inverted position with its head directed to the caudal and its tail to the cephalic pole. In the spring the envelopes over the head end first fuse and then rupture; the embryo is thereupon everted and during katatrepsis passes around the caudal pole to regain its upright position on the ventral yolk. The envelopes during

this process are stripped back over, and finally drawn into the yolk, where they undergo dissolution when the body walls have met in the median dorsal line. The defects in Ayers' description of *Æcanthus* ('84) were pointed out at p. 43.

*Gryllotalpa*, the only other Gryllid, which has been studied, seems to differ considerably from *Gryllus* and *Æcanthus*. Examination of Korotneff's figures ('85) shows that this difference is probably more apparent than real. In his surface views, there is a wide gap between his Fig. 2, representing the egg in a preblastodermic stage, and his Fig. 3, representing quite an advanced embryo. One is thus left without any guide to the exact relation of the just-established germ-band to the yolk-surfaces. Korotneff's defective account of the formation of the germ-layers would seem to show that he did not study these early stages closely. It is obvious that *Gryllotalpa* is blastokinetic both from Korotneff's statement that the embryo moves during revolution and from his figures 5, 7, and 8, but the exact nature of the process is not clear. The possibility of the embryo's passing to the opposite surface of the egg is not precluded by the conditions seen in Figs. 7 and 8. Judging from *Gryllus* and *Æcanthus* I am inclined to think that the embryo exhibits both ana- and katatrepsis, but that Korotneff has overlooked the former and misinterpreted the latter movement.

In the Locustidæ, as represented by *Xiphidium* and *Orchelimum*, we find a modification of the blastokinetic process observed in *Gryllus*. Instead, however, of arising near the caudal pole, the germ-band is formed on the middle of the ventral surface, and instead of passing around the caudal pole during anatrepsis it passes through the yolk as if to reach the dorsal surface by a shorter path. Katatrepsis is essentially the same as in the Gryllidæ, the embryo passing around the caudal pole. This lack of coincidence in the anatreptic and katatreptic paths is one of the most striking peculiarities of Locustid development; since it is known to occur in no other insect. It is probable that the anatreptic embryo originally passed around the lower pole, but that owing to the formation of the embryo higher up on the ventral surface, and perhaps also to an acqui-

sition of yolk at the lower pole, this movement has been deflected.

*Melanoplus femur-rubrum* was studied as a representative of the Acrididæ. The germ-band is formed very near the caudal pole of the egg, but still on the convex ventral surface. During the formation of the envelopes the posterior end of the body grows around the pole onto the dorsal surface, while its head remains fixed at the pole. It is not until the germ-band has reached a stage corresponding to Stage F. in *Xiphidium* that its head leaves the pole and the whole body moves upward on the dorsal surface. It soon comes to a standstill and passes the winter in this inverted position. In the spring it moves back around the lower pole and, like the Gryllid and Locustid embryo in a corresponding stage, proceeds to lengthen and envelop the yolk till its head reaches the cephalic pole.

Packard ('83) seems to have been the first to study the development of Acridians (*Melanoplus spretus* and *M. atlantis*). But he had no conception of the true relations of the embryo to the yolk, as is shown by his Fig. 1, Pl. XVII, where the egg is depicted with the micropylar end uppermost. Leuckart ('55) long ago showed that the Acridian micropyle is located at the caudal pole. If the egg figured by Packard be inverted, it will represent the embryo on the point of undergoing katatrepsis.

The same error is committed by Graber in his accounts of *Stenobothrus variabilis* ('88, '90). Misled, like Packard, by the position of the micropyle, he has mistaken the caudal for the cephalic pole. To mean anything his figures must be inverted. As I have not yet studied the later stages of *Melanoplus* in section, I will not attempt to describe the details of katatrepsis. Graber claims to have observed that the pleural ectoderm, where it passes into the amnion proliferates a thin cell-lamella to form the dorsal wall, while the amnion remains intact and still covers the ventral face of the embryo. This account is not substantiated by his figures (1 and 2, Pl. I). The two thin cell-lamellæ extending over the dorsal surface have every appearance of being the walls of the heart, and therefore mesodermal, although it is difficult to see how this organ could be so completely formed in so early a stage. As

Graber has paid no attention to the movements of the *Stenobothrus* embryo, and as he most assuredly has not demonstrated from a careful study of the later stages that the lamella in question is really converted into the dorsal wall, I cannot attribute much value to his observation.

The foregoing observations go to show that the blastokinetic processes are essentially the same throughout the suborder Saltatoria. Each family presents certain deviations from the type, which is probably most closely adhered to in the Gryllidæ. *Anatrepsis* is aberrant in the Locustidæ, while the Acrididæ are aberrant in the tardy separation of the procephaleum from the lower pole. Notwithstanding these deviations the Saltatoria form a clearly circumscribed group embryologically as well as anatomically, and were it not for *Gryllotalpa* would be separated by a wide gap from all other Orthoptera. *Gryllotalpa* is a generalized form, as Brauer has pointed out from a study of its anatomical peculiarities ('86), and his conclusions are to some extent substantiated by the large size of the germ-band as compared with the yolk mass.

In the Cursoria, as represented by *Blatta germanica*, movements of the embryo are far less apparent. The germ-band never leaves the ventral surface, on the middle of which it first appears. I have shown, nevertheless ('89, text-figures, p. 348), that it moves down the yolk after the rupture of the envelopes till its tail reaches the lower pole. The tail then remains stationary, while the head gradually rises to the cephalic pole as the body walls develop and invest more and more of the yolk. Slight as are these movements, they nevertheless recall the blastokinesis of the Saltatoria. I would regard the movement of the whole *Blatta* embryo towards the caudal pole as ana-treptic; katatrepsis is probably represented only by the upward growth of the embryo. The very late occurrence of the former movement may be due to its rudimental character, since it is too weak to carry the germ-band around the caudal pole.

Few observations have been published on the relations of the embryo to the yolk in the Gressoria. In *Mantis*, as I have shown, the germ-band when first formed lies somewhat nearer the posterior than the anterior pole. The embryo never leaves

the ventral surface of the egg, but whether or not it exhibits any traces of blastokinesis my limited material will not enable me to decide, and Graber ('77), Bruce ('86), and Viallanes ('90<sup>a</sup>, '90<sup>b</sup>), have contributed no observations bearing on this point. It is clear, nevertheless, that in its development, *Mantis* resembles *Blatta* more closely than either of these forms resemble the Saltatoria. This merely confirms the view which has long been held respecting the affinities of the Blattidæ and Mantidæ. From the structural similarity of the Phasmidæ and Mantidæ we may venture to infer a similarity of embryonic development.

It thus appears that the Orthoptera are clearly separable into two groups—the Saltatoria on the one hand and the Gressoria and Cursoria on the other. The Saltatoria are decidedly blastokinetic whereas the non-saltatory forms retain only faint reminiscences of blastokinesis (*Blatta*). I am inclined to believe that primitive embryological features have been preserved more faithfully in the Saltatoria than in other Orthoptera. That the habits of oviposition are more primitive in this group is shown by Brongniart's discovery of a fossil Blattid provided with an ovipositor ('89). Moreover, several features in the development of the Saltatoria show great conservatism, *c.g.* the retention of the indusium in the Locustidæ, the order in which the metameres arise, and the myriopod-like habitus of the *Xiphidium* embryo in Stage D.

Not only does a study of the Saltatoria throw light on the development of other Orthoptera, but it brings the order into closer union with the Odonata and Rhynchota. The blastokinesis of the Gryllidæ agrees closely with that of the Hydrocorisa among the Hemiptera—*c.g.* *Corixa*, as described by Metschnikoff ('66). *Ranatra* and *Zaitha* will bear even a closer comparison with the Gryllidæ. In the much elongated egg of the former, which has the cephalic pole marked by the pair of diverging pneumatic threads, the germ-band arises as usual on the ventral surface with its head directed upwards. As the envelopes develop it passes around the lower pole and finally assumes an inverted position on the dorsal surface. During katatrepsis it returns over the same path. The inclusion ob-

servable in *Corixa* and probably also in *Ranatra*, of a small quantity of yolk between the caudal amnion and the overlying serosa when the embryo first passes to the dorsal surface, is often observed in the Saltatoria. It is no great step to pass from the conditions seen in the Hydrocorisa to the "entoblastic" condition of other Hemiptera (*Pediculus*, *Aphis*, *Cicada*) and the Odonata (*Calopteryx*), where the germ-band instead of passing to the dorsal yolk during anatrepsis, comes to lie in the middle of the yolk, or even near the ventral surface (*Pyrrhocoris*). The Thysanoptera, as may be inferred from Jordan's brief statement ('88), the Corrodentia (Mallophaga) according to Melnikow ('69), and the Psocidæ, according to Packard ('84), are also referable to the "entoblastic" type. Concerning the embryonic development of the Plecoptera and Dermaptera nothing is known.

So far only the Homomorpha have been considered. The eggs of the Heteromorpha, as I have attempted to show, contain less yolk. Blastokinesis is nearly or quite lost in this more recent group, a fact that perhaps indirectly tells in favor of my view that the movements of the embryo have been acquired for the purpose of ventilating the yolk and supplying the growing embryo from time to time with fresh pabulum. The transition to the Heteromorpha is probably represented by the Ephemeridea. According to Burmeister's account of the development of *Palingenia horaria* (I quote from Zaddach, '54): "am dritten Tage, nachdem das Ei gelegt war, hatte sich der Keimstreif gebildet, der zungenförmig war, und sich über zwei drittel der Eilänge erstreckte, also in Form und Ausdehnung ganz dieselben Verhältnisse zeigte, wie im Phryganidenei." This may, perhaps, be taken to indicate that the Ephemeridea exhibit no blastokinesis; but the subject requires urgent investigation.

Among the Heteromorpha it is especially the Coleoptera which still show distinct though abortive movements of the germ-band. *Hydrophilus* may be taken as an example. As may be seen from Heider's figures, the germ-band forms on the lower ventral surface of the egg. As it grows in length, and the amnion is formed, the tail curls around the caudal pole on-

to the dorsal surface, but soon separates from the serosa so that a small amount of yolk is enclosed between the two envelopes. Later the yolk is expelled from this region and the envelopes become applied to each other. A true movement then sets in and carries the anterior portion of the germ-band forward up the ventral surface till the procephaleum overlaps the cephalic pole (*Cf.* Heider's figures, 4 *c*, 6 *a*, 7 *a* and 9, Pl. II. ('89). A certain similarity of these movements to those exhibited in *Blatta* leads me to believe that they represent a weakened blastokinesis.

Whether or not similar movements occur in the other so-called "ectoblastic" forms (Diptera, Hymenoptera, Siphonaptera, Neuroptera, Trichoptera) cannot be decided at present. If such movements occur at all they are probably exceedingly weak.

As stated above, the Lepidoptera have developed embryonic movements peculiar to themselves. In all the members of the order hitherto studied, the germ-band arises on the ventral surface of the egg, and its envelopes are formed while it is still in this position. As development proceeds the convex ventral surface of the germ-band, with its adherent amnion, moves back from the ventral serosa and soon comes to lie in the middle of the yolk. Hereupon the ventral surface of the embryo becomes concave, and its dorsal surface is applied to the dorsal serosa. I have already remarked that this movement of the embryo may have been independently developed for the same purely physiological purposes as blastokinesis in the Homomorpha. The fact that the movement is represented in the Trichoptera only by the change in flexure of the longitudinal embryonic axis, would seem to indicate that it has been acquired since the Lepidoptera diverged from the Trichopteroid ancestor.

Graber ('90) has recently made the interesting discovery that the Phytophagous Hymenoptera closely resemble the Lepidoptera in the movements of the embryo and in the amputation of the envelopes. This, taken together with the striking resemblance between the eruciform larvæ of the two groups, appears to point to a closer relationship than has usually been claimed.

While studying the movements of the embryo and the formation of the envelopes in the different orders and families of insects, with a view to testing the current classification, which is the outcome of a great amount of comparative anatomical and paleontological work, I have been especially impressed with two facts: First, the embryological data in no wise conflict with the generally accepted classification of Brauer. The developmental variations within limited groups are never greater than the post-embryonic differences in the members of the same groups. Usually there is great uniformity in embryological development between systematically allied insects of the same order; the wide gaps usually occur between the orders just where gaps have long been pointed out by comparative anatomy and paleontology. Second, developmental differences between members of different allied families of Orthoptera are greater than the differences between remotely related families in more recent orders. For example, the differences between a Locustid and an Acridian or a Locustid and a Gryllid embryo, or between any of the Saltatoria and the Blattidæ, or Mantidæ, are greater than the differences between an embryo Hydrophilid and a Chrysomelid, a Tabanid and a Chironomid, or a Bombycid and a Shingid. Frequently, it is true, the differences between the extremes in the higher orders are considerable, as between the Tenthredinidæ and the Proctotrupidæ among Hymenoptera, or the Chironomidæ and Muscidæ among Diptera. If any conclusions bearing on classification can be drawn from the few embryological data which I have collected, they refer to the ordinal value of the various Orthopteran families. It would appear that these groups have really more than family value. They are older than the families of more recent groups, and therefore exhibit greater divergence. The Rhynchota will probably be found to present conditions similar to the Orthoptera. There are certainly more considerable differences between the embryos of such forms as *Pyrrhocoris* and *Ranatra* than there are between the embryos of widely separated families among the Coleoptera.

#### 4. *The Elimination of the Embryonic Envelopes.*

Anatrepsis and katatrepsis in the lower insect orders, or the completion of the envelopes and their rupture in the higher orders, are separated by a distinct interval, during which the germ-band undergoes a considerable development. But during this interval, the diapause, no change is noticeable in the envelopes themselves beyond a thinning of the amnion with the increased growth of the embryo. The elimination of the envelopes is preceded by katatrepsis just as their formation was preceded or accompanied by anatrepsis. This elimination is immediately followed by the completion of the dorsal body-wall and may take place in a variety of ways. Korschelt and Heider ('92) distinguish the following types in this process:

1. The amnion and serosa become continuous and, after the eversion of the embryo, are drawn back over the yolk to form a single layer of cells. As the dorsal growth of the body-walls proceeds, both envelopes are drawn together and pushed into the yolk to form a sack or longitudinal tube which is ultimately enclosed by the walls of the mesenteron and absorbed. To this type belong the Odonata, Rhynchota, some Orthoptera (*Blatta*, *Ecanthus*, *Gryllotalpa*) and some Coleoptera (*Hydrophilus*).

2. The serosa is shed from the yolk and the amnion alone contracts on the dorsal surface preparatory to being drawn into the yolk and absorbed. (Certain Coleoptera, *e.g.* *Doryphora*.)

3. The serosa alone is agglomerated and drawn into the dorsal yolk, the amnion being cast off. (Certain Diptera [*Chironomus*] and Trichoptera.)

4. Both envelopes are shed. (Lepidoptera and certain Hymenoptera.)

In *Xiphidium* we may perhaps recognize a fifth type, in which as in the fourth, both amnion and serosa are shed. But while the serosa is in great part shed as a simple membrane, the indusium which is a modified portion of the serosa, together with the amnion is drawn together in a mass and cut off from the embryo. It is more than probable that other types of envelope elimination will be discovered when more forms have

been studied. *Musca* may perhaps be regarded as representing a distinct type, since in this highly modified form the rudimental amnion and the serosa are neither shed nor agglomerated and engulfed in the yolk, but are supposed to form the definitive body-wall. (Kowalewsky, '86; Graber, '89.)

It is clear that the revolution of the insect embryo includes three distinct processes: first, the eversion and katatrepsis of the germ-band; second, the formation of the dorsal walls; and third, the elimination of the envelopes. The mechanical cause of eversion and katatrepsis is probably a contraction on the part of the envelopes after their fusion and rupture over the ventral surface of the embryo. After the embryo is everted from the amniotic cavity, or exposed after the rupture of the amnion and serosa, these envelopes temporarily form the dorsal covering of the yolk. Do they ever form the definitive dorsal body-wall? For both envelopes this is claimed to be the case only in *Musca*. In all other insects the serosa, at least, takes no part in forming the permanent body-wall, as it is either shed or engulfed in the yolk. The question is, therefore, restricted to the fate of the amnion. In many insects (Lepidoptera, Hymenoptera, Phytophaga, some Diptera and Coleoptera), it has been shown that the amnion takes no part in the formation of the definitive body-wall, although a decision on this point is rendered difficult by the fact that no hard and fast line can be drawn between the ectoderm of the germ-band and the cells of the amnion. In other insects the decision is even more difficult. Still, I may say that I have seen nothing in the insects I have studied, to convince me that the amnion is converted into a portion of the permanent body-wall. Even in *Musca* it seems probable that the amnion and serosa only temporarily function as the body-wall, and that their cells are ultimately replaced by true ectodermal elements from the germ-band. In *Blatta* and *Xiphidium* I have seen appearances which lead me to believe that at least a part of the amnion may be eliminated by such a process of cell-substitution. I incline, therefore, to the views of Korschelt and Heider ('92), who hold that the envelopes are probably completely eliminated, and that the entire body-wall is derived from the ectoderm of the germ-band.

If this be the correct view, it follows that the dorsal body-wall is formed in essentially the same manner in all insects — by a growth and meeting of the germ-band edges. This process is, therefore, remarkably simple and uniform compared with the processes whereby the envelopes are eliminated. The great variability in the latter case has been dwelt on by Graber ('88) in a paper devoted to dorsal-wall formation in the Insecta. After reviewing all the literature on the subject and contributing many new facts, he proceeds to base a classification of the insects hitherto studied, on the "Keimhüllenzustände." He finds some fault with the current classification on the ground that insects which systematists regard as closely related often present great differences in their respective methods of dorsal-wall formation, whereas remotely related insects often agree very closely in this respect. Thus *Lina* and *Hydrophilus* differ more than *Hydrophilus* and *Æcanthus* in the processes whereby the dorsal-wall is formed. In considering Graber's views, I may pass over the awkward and kakophonous nomenclature which he has introduced, to what I regard as his main error, *vis.* the superficial analysis of his subject. Graber's term "Keimhüllenzustände," I take it, includes the formation of the envelopes as well as their condition preceding and during their elimination. Now I have attempted to show that there is nothing in the formation of the envelopes nor in the concomitant anatropsis of the germ-band in the different insect orders to conflict with the current classification. Nor is there anything in the closure of the dorsal-wall in different groups — restricting this term to the confluence of the pleural edges of the germ-band — to support Graber's conclusion. His statement must therefore be restricted to the elimination processes. That these are highly variable must be admitted, but they are probably of very little taxonomic value, as Graber would probably have observed, had he attempted to account for the wide differences in allied forms and the agreement of remotely related species. It is my opinion that this high degree of variability in the elimination process is to be traced to the same causes as the variability of the indusium, *vis.*, the rudimental character of the envelopes. Up to the close of the diapause

the envelopes subserve a distinct function, but as soon as the germ-band has invested the yolk with its own ectoderm, they have become functionless, or rudimental. Long before this time, in fact ever since their completion, the envelopes show no traces of cell-division. Moreover, their involution into the yolk or complete shedding shows conclusively that their morphological value is at this time reduced to *nil*. Whether both envelopes are shed instead of being drawn into the yolk, or whether one is shed and the other drawn into the yolk, may depend to some extent on the ease with which the pleural folds can close without their temporary assistance. But which of these processes shall occur in a given insect is probably a matter of no vital importance to the embryo, and has probably played no rôle in the struggle for existence. The involution of the envelopes, it is true, may add assimilable matter to the embryo, but enough energy to counterbalance this addition is probably consumed in metabolizing the dead cells. Hence the adoption of this process may be of no greater advantage to the embryo than the complete sloughing of the useless envelopes.

The insect envelopes, therefore, present only another case of an organ which has become specialized for a particular function at the expense of its formative power. This same phenomenon recurs in insect ontogeny. During cleavage certain cells are segregated for the express function of yolk-metabolization (vitellophags), while the remaining cells go to form the blastoderm. Later the cells of the blastoderm separate into those of the germ-band proper and those of the specialized envelopes. Still later, if the insect be metabolic, another splitting occurs, a portion of the hypodermis being set aside in the form of the imaginal disks to supplant the specialized primitive larval hypodermis. The formative material of the insect, like that of other organisms, thus undergoes a successive splitting into a specialized and a comparatively non-specialized portion. The former, being incapable of metamorphosis, is cast off or broken down, while the latter persists until a new segregation takes place. The analogy of this process to that occurring in rhizomatous plants, Polyzoa, etc., need not be pointed out in detail.

## V. NEUROGENESIS IN THE INSECTA.

I. *The Nerve-cord.*

The first traces of the central nervous system of *Xiphidium* make their appearance at a very early stage, before the blastopore is closed and while the envelopes are still incomplete. In this stage (Fig. 2) surface preparations made according to the methods given in the latter part of this paper, show a number of pale spots scattered over the procephalic lobes. They frequently occur also in the maxillary region, and, were it possible to remove the amnio-serosal fold without injuring the surface of the germ-band, would probably be found to extend still further caudad. The meaning of these spots is apparent when sections of embryos in Stages B–D are examined. In a transverse section (Fig. 25) through the middle of the abdomen of an embryo in Stage D, the ectoderm, which bulges out somewhat on either side of the median line, is seen to consist of two kinds of elements. First, there are a few large, clear, polygonal cells with spherical nuclei (*nb.*), lying in the deeper portion of the layer; and second, a much greater number of small and more deeply stainable cells (*db.*), differing in no essential respect from the cells forming the remainder of the ectodermal layer, such as the appendages. The latter cells have smaller, oval or cuneate nuclei, which appear to contain more chromatin than the large inner cells. While the small cells form a continuous layer, the large elements make their appearance singly or in small clusters, as seen in the figure. It is these pale clusters underlying the darker cells which produce the pale spots seen from the surface.

The large pale cells may be called neuroblasts — since it is they that give rise to the purely nervous elements of the cord.<sup>1</sup>

<sup>1</sup> The term “neuroblast” was originally used by Whitman ('78 and '87) to designate the two offspring of the large posterior macromere of the *Clepsine* egg, which give rise by a process of budding to two rows of cells — the “neural rows.” From these rows the nerve cord arises. His ('89) subsequently employed the same term “neuroblast” to designate such of the offspring of the “Keimzellen” as give rise directly by differentiation and not by further divisions to the ganglion-cells, or, to use Waldeyer's term, to the neurons of the vertebrate central nervous system. More properly the term would have been applied to the “Keimzellen” themselves, and by mistake it has been thus used by at least one recent writer (C. L. Herrick, '92, p. 430, Fig. 10). Haeckel (*Anthropogenie*, 4th ed. p. 268, '91) uses ‘neuroblast’ in the sense of ectoderm in general.

The remaining cells which cover the neuroblasts and extend down between them in the median line, give rise to purely integumental structures and may therefore be called dermatoblasts. The two thickenings of the ectoderm are to become the lateral cords (*Seitenstränge*). They extend from the anterior edge of the eleventh abdominal segment, just in front of the anus, to the mouth, where they diverge and pass without interruption into the brain. The groove which separates the lateral cords and which is very faint in Fig. 25, is the neural furrow (*Primitivrinne*). It appears soon after the closure of the blastopore and takes the place of this depression. It is deepest anteriorly.

All the neural structures develop in an anteroposterior direction, beginning with the brain; hence different stages in the development of the lateral cords may best be studied in the same embryo. Fig. 26 shows a section passing through the first abdominal segment of the embryo from which the section in Fig. 25 was taken. Here we see a distinct advance in structure. The neural furrow (*n.g.*) is more clearly marked and the neuroblasts (*nb.*), four in either lateral cord, have arranged themselves side by side in a regular layer in the deepest portion of the ectoderm. Over them the dermatoblasts (*db.*) also form a single regular layer, while the cells lying in the median line on either side of the neural furrow have grown more elongate. Sections further forward show essentially the same conditions — the neuroblasts which were at first differentiated as small clusters or as isolated cells, have arranged themselves throughout the anterior portion of the embryo as an even layer entad to the dermatoblasts.

The further changes in the development of the nerve-cord, are brought about — first, by a proliferation of the neuroblasts; second, by a proliferation of the dermatoblasts and a deepening of the neural furrow; third, by the development of the median cord; fourth, by the formation of the connectives and commissures, and fifth, by the development of the neurilemmata. These changes which occur simultaneously may be described singly for the sake of convenience.

As cross-sections show, the neuroblasts are arranged in from

3-5 longitudinal rows in either lateral cord. In surface view these rows may often be followed through one or two segments as continuous strings of cells. I assume that there were originally four of these rows, but that owing to the pressure exerted by the developing appendages on the lateral edges of the cords and to a more rapid growth of the neuroblasts than of the germ-band, the primitive regular arrangement has been considerably obscured. The neuroblasts are polygonal in outline from mutual pressure. When they divide, as they very soon do, their spindle axes are directed at right-angles to the surface of the body. As soon as one cell has been given off, the nucleus rests for a short time and then again divides in the same direction. This process continuing, a column of cells is budded off from each neuroblast and stands at right angles to the surface of the germ-band. The divisions do not take place simultaneously in all the cells although corresponding neuroblasts in either cord will frequently be found in the same phase of caryokinesis, especially in the earlier stages of their proliferation. A section (Fig. 27) through the first maxillary segment of an embryo in Stage F shows that each of the eight neuroblasts has produced a row of daughter-cells. The large succulent mother-cells are evenly rounded on their outer surfaces which are overlaid by the dermatoblasts. Their inner faces are flat or concave and in every case closely applied to the latest daughter-cell. The nuclei of the mother-cells are spheroidal and take no deeper stain than the pale succulent cytoplasm which surrounds them. The neuroblasts are in all essential respects typical proliferating cells like the terminal cells in plant-shoots and the teloblasts of annelids. The daughter-cells ( $g^1$ ) are at first characterized by their small size, cuneate outline and deep stain. Their nuclei are considerably flattened, probably from mutual pressure. These characters are retained by the daughter-cells till they have been pushed some distance from the neuroblast by later offspring, when they become larger and considerably paler and assume the appearance of definitive ganglion cells ( $g^2$ ).

Turning now to a somewhat older embryo (Stage G, Fig. 28) we see that the columns of daughter-cells have greatly increased

in length, while the neuroblasts remain to all appearances unaltered. The increase in the number of daughter-cells is so great that they are forced to arrange themselves in several rows. In the figure this is best shown in the progeny of the innermost neuroblasts, and the tapering columns there formed may be regarded as typical.

In my preliminary note ('91<sup>c</sup>) I held that the daughter-cells themselves divide to form the multiple rows in each pillar. I incline to think that I was mistaken on this point. The daughter-cells probably never divide but are directly converted into ganglion cells. All reproductive powers seem to be confined to the neuroblasts. Some of the nuclei of the daughter-cells exhibit peculiar chromatic structures which I may have mistaken for caryokinetic figures; this being an easy error to make in the case of small cells killed by means of heat, since the achromatic portions of the spindles are obliterated by this method.

The last stages in the proliferation of the neuroblasts are shown in Fig. 31, which is taken from an advanced embryo (Stage J). The columnar arrangement is no longer visible since the individual cells are now converted into the definitive ganglionic elements. On the outer periphery of the ganglia, however, neuroblasts are still to be found, and extending from them short series of small flattened cells ( $g''$ ), their latest progeny, still distinguishable from the ganglion cells by their deeper stain. It will be noted that these cells, which like their precursors will become ganglion cells, are no longer budded off at right angles to the surface of the nerve-cord but parallel to it, a condition undoubtedly due to a lack of space. Finally the neuroblasts stop proliferating and shrink to the size of their progeny. Their chromatin then shows signs of senility. Beyond this point I have been unable to trace them satisfactorily. They are probably broken down and absorbed by the growing ganglion cells. Some of them may persist as ganglion cells of a particular character and function, though I deem this improbable.

The dermatoblasts play an important part in the development of the ventral nerve-cord, as will be seen by returning to the younger stages. We left these cells as a layer covering

the neuroblasts and continuous laterally with the general ectoderm. In the median line they extend to the deepest portion of that germ-layer in the form of a few compressed cells (Fig. 26, *db.*). These compressed cells form the walls and bottom of the neural furrow. The proliferation of the neuroblasts has caused the lateral cords to bulge out enormously (Fig. 27), so that the dermatoblastic layer becomes stretched and attenuated. Such divisions as occur in the cells of this layer seem to be confined to the outer surface and do not extend into the furrow. The spindle axes lie parallel to the surface, as shown at *nb.* The bulging of the lateral cords naturally brings about a deepening of the neural furrow, since the cells at its bottom have a fixed attachment. At this point in Fig. 27 there is seen a triangular cell-mass, capped by a single large element (*mn<sup>b</sup>.*), a true neuroblast which resembles in nearly all respects the neuroblasts of the lateral cords. Its more pyramidal outline is obviously the result of its position between the converging walls of the furrow. To the same mechanical cause is due the shape of the cell-mass, which consists of the heaped up daughter-cells of the neuroblast. Inasmuch as the proliferating cell occurs in the median line, and together with its offspring and the dermatoblastic cells of the median furrow, is equivalent to the "Mittelstrang" of authors, I shall call it the median-cord neuroblast. Its exact origin I have not been able to determine. To judge from the number of cells which it has given off it must have begun to proliferate at about the same time as the lateral-cord neuroblasts. There can be no doubt, it seems to me, that it originated as a polygonal ectoderm cell like the lateral cells seen in Figs. 25 and 26, but whether it was originally median in position or arose unilaterally I am unable to decide. The pale surface spots of embryos in Stage B show that neuroblasts are arising at a time when the blastopore occupies the position of the neural furrow and hence, if the median cells are median in position from the first, they must arise somewhat later than their sister neuroblasts.

There is one important difference in the arrangement of the mother-cells of the lateral and median cord. Whereas the former, as has been stated, form continuous though irregular rows

from mouth to anus, the latter constitute an interrupted series between the same two points. They are single, isolated cells, which occur only intersegmentally. That such is their distribution may be distinctly seen in frontal sections like the one represented in Fig. 30. This section passes through the first to fifth abdominal segments at the level of the median cord neuroblasts (*mnb.*), which are seen to lie distinctly between the segments, where the walls of the neural furrow dilate at intervals for their accommodation. At first the daughter-cells are given off in the same direction as those of the lateral cords, but soon the triangular space to which they are confined will no longer contain the older cells of the series and these are pushed along the floor of the neural furrow. This produces an angular flexure in the cell-column, but later the whole mass, including the neuroblast, assumes a horizontal position. This change in the position of the median cell-mass is seen to have taken place in the median sagittal section from an embryo in Stage G (Fig. 29). The neuroblast (*mnb*) is in each segment directed caudad, while the mass of small and deeply stainable daughter-cells (*mg*) is wedged in under the commissures. The section passes through the sub-oesophageal ganglion, which consists of the fused ganglia of the mandibular and both maxillary segments (*md. g*; *mx. g*<sup>1</sup>; *mx. g*<sup>2</sup>), and through the pro- and mesothoracic ganglia (*p. g*<sup>1</sup>; *p. g*<sup>2</sup>). Transverse furrows (*i. g*<sup>1</sup>; *i. g*<sup>2</sup>), which I shall consider later, separate the unfused ganglia from one another, and as the median cord cells lie in front of these furrows, they must be regarded as belonging not to the intersegmental region of the ectoderm, but to the posterior portions of the separate ganglia. Each ganglion possesses a median cord neuroblast, so that, beginning with the mandibular, which is the first ganglion in the nerve-cord proper, and ending with the tenth abdominal, there are in all sixteen median mother-cells. Each of these, after producing its quota of ganglionic elements, deteriorates in the same way as the mother-cells of the lateral cords.

The development of the Punksubstanz may be readily followed in *Xiphidium*. It arises in each ganglion as two separate masses. Each of the daughter-cells of the lateral neuroblasts

sends out a cytoplasmic process which soon ramifies. The mass of fibres thus formed increases in size very probably by the addition of further ramifications till the Punktsubstanz is definitely established as a scarcely stainable body, lying on either side of the median line in the deepest portion of the lateral cord (Fig. 27, *p.s.*) In its earliest stages the formation of the substance is easily followed, but very soon the felted fibres become too dense for analysis by ordinary methods of investigation. It is only after a distinct mass is formed in either half of a ganglion that the longitudinal commissures, or connectives as they are best called, make their appearance, and unite the hitherto isolated centres in two longitudinal series. Very soon the transverse commissures, or commissures proper, of which *Xiphidium*, like all other insects, has two in each segment, make their appearance. The daughter-cells of the median cord neuroblasts take no part in the formation of the anterior commissure. Whether they contribute fibres to the posterior commissure or not, I must for the present leave undecided. I have seen no evidence in the median cord of a distinct and isolated Punktsubstanz centre, such as is described and figured by Graber for some Coleoptera ((90) Pl. V, Fig. 66). I deem it more probable that in *Xiphidium* the commissures arise wholly from the Punktsubstanz masses of the lateral cords. Both commissures are distinctly seen in cross section in Fig. 29.

The connectives and commissures incompletely divide the cellular portion of each ganglion into five parts,—two lying laterad to the connectives and a median series of three smaller portions separated from one another by the two commissures. The former may be called lateral gangliomeres, and the three median portions respectively the anterior, central, and posterior gangliomere.<sup>1</sup> Of the median divisions the posterior is distinctly the largest from the first. This is due to its being formed in great part by the progeny of the median neuroblast, whereas the anterior and central gangliomeres consist of a comparatively small number of cells, contributed by the lateral neuroblasts.

<sup>1</sup> These are equivalent to Graber's laterale Zellenlager, vorderes, centrales, and hinteres Medianlager.

It is not till after the commissures and connectives are formed that the inter-ganglionic regions become clearly marked out. Throughout the early stages, in fact till the embryo reaches the ventral surface of the egg (Stage J), the ganglia are as long as their respective segments and are separated from one another only by the intersegmental constrictions. These have grown very deep in Stage G, especially in the thoracic and abdominal regions. In the median line, as shown in sagittal section (Fig. 29, *i.g*<sup>1</sup>, *i.g*<sup>2</sup>.), they form deep tubular ingrowths which may be called furcal pits. Since these pits are median in position they are to be regarded as differentiated interganglionic portions of the neural furrow. They therefore belong to the median cord. They are not found between the mandibular and first maxillary, nor between this and the second maxillary ganglion, and are also wanting between the eighth and ninth, and ninth and tenth abdominal ganglia. Evidently their absence in these cases is due to early fusion to form the infracesophageal and last abdominal ganglion. In the thoracic segments the furcal pits are converted into chitinous apodematous structures which give attachment to some of the leg-muscles. It is interesting to note that in the abdomen also furcal pits are distinctly developed as late as Stage K. Here, too, they serve for the attachment of a few weak muscle-like structures, which run from their tips to points in the adjacent abdominal wall, perhaps corresponding to the insertions of the rudimental appendages. Later both muscle-like cords and abdominal furcæ disappear, — the latter by a very simple process. It will be remembered that at this time the embryo is growing in length and continually covering more and more of the yolk. The tail end is practically fixed at the lower pole of the egg, while the head slowly moves upwards. The body-wall is thus stretched in both a longitudinal and lateral direction. Hence the intersegmental constrictions, so deep in Stage J, must gradually become shallower, and the furcal pits, which are nothing but portions of these constrictions, are drawn out from between the connectives to form part of the sternal integument. The stretching not only draws out the folds in the embryonic body-wall, but also reduces it to a much thinner layer

of cells. The length of the individual segments is thereby greatly increased and the nerve cord, which is firmly attached in the infraœsophageal region and more loosely in the terminal abdominal segments, is compelled to lengthen. The separate ganglia, besides assuming a somewhat fusiform outline, are scarcely affected by this traction, whereas the connectives are drawn out into thin threads denuded of all ganglionic cells and covered only by the neurilemma.

The presence in the abdomen of temporary furcal pits corresponding to the persistent furcæ of the thorax admits of an easy explanation, if we take these structures to be correlated with the development of ambulatory appendages. The temporary abdominal appendages have usually been regarded as the rudiments of once functional walking-legs, and they are still so well preserved in the Orthoptera that it need not surprise us to find traces of correlated structures which served for the attachment of some of their muscles.

The progeny of the median neuroblast together with the interganglionic portion of the neural furrow have been accounted for; the former becoming the posterior gangliomere, the latter a portion of the sternal integument; but I have not yet accounted for the remaining portion of the median cord—*vis.* the intraganglionic walls of the neural furrow. This portion of the groove is crossed by the two commissures and separates those portions of the lateral cord which will ultimately constitute the anterior and central gangliomeres. Its cells are of an epithelial nature. Those of the opposite walls of the furrow become applied to one another by the swelling of the lateral cords. The lumen is thereby obliterated though its walls are still continuous on the outer surface of the ganglion with the integumental ectoderm. The two lips of the furrow finally fuse and the ganglion together with the portion of the furrow included between its two halves is liberated from the ectoderm. It is these epithelial walls thus set free from the integument which appear to give rise to the outer and inner neurilemmata. Both these neural envelopes are ectodermal; there are no traces of mesodermal structures taking any part in their forma-

tion and it seems to me that they can have only two possible sources—they either arise from some of the progeny of the neuroblasts or from the intraganglionic portion of the median cord. I deem it highly improbable that they should arise from the former source, since the daughter-cells of the neuroblasts have every appearance of being early specialized as ganglion cells. Furthermore, the cells of the neurilemmata when they definitely appear, closely resemble the cells of the neural furrow both in size and in the great avidity with which they take the stain. The outer neurilemma covers first the inner surface of the ganglion—then the outer or neuroblastic surface;—the thin cellular membrane apparently progressing laterad in either case and meeting near the origin of the nerve-trunks. The inner neurilemma, which envelops the Punktsubstanz is completed before the outer envelope. Histologically both envelopes resemble each other in every respect.

The fusions of ganglia in the nerve-cord take place gradually and may be easily followed in *Xiphidium*. Several stages of these fusions are represented in Fig. VII, A–D. In Fig. A, the nerve-cord is shown much as it appears in Stage F. The ganglia form an unbroken series from mouth to anus. The connectives are very short and not as yet distinguishable from the surface. Fig. B, is taken from an embryo just turning the lower pole (Stage H). Here the mandibular and two maxillary ganglia, and also the three terminal abdominal ganglia still remain as in the preceding stage, while the other ganglia are being drawn apart by the stretching of the embryo, so as to show their short connectives. In Fig. C, the subœsophageal and last abdominal ganglia are established as two fused masses. The number of ganglia comprising each of these masses may still be easily determined by counting the commissures. It will be noticed that in this stage the first abdominal is closely approximated to the metathoracic ganglion and that the second and third abdominal also lie close together. Between the other ganglia the connectives have lengthened. In the later stage represented in Fig. D, the connectives are still longer; the first abdominal ganglion has fused with the metathoracic, and the second and third abdominal form a single mass.

These fusions become more intimate as the time for hatching approaches, so that the ventral cord finally consists of only ten ganglia instead of sixteen, the original number.

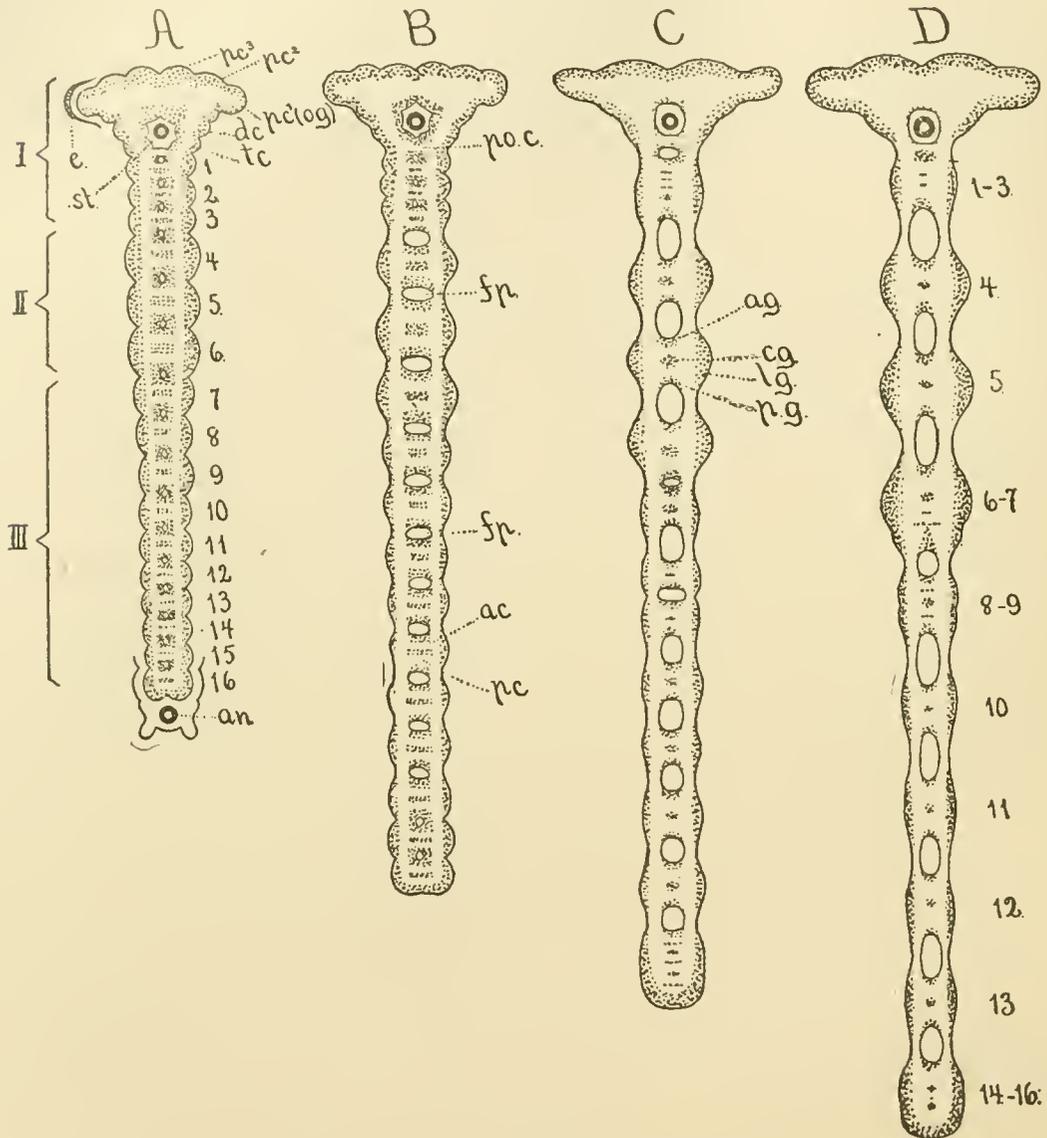


FIG. VII.

A-D. Diagrams of four consecutive stages in the development of the brain and nerve-chain of the *Xiphidium* embryo. I, cephalic; II, thoracic; III, abdominal region; *st.*, stomodæum; *an.*, anus; *e.*, optic plate; *pc*<sup>1</sup>(*o.g.*), first protocerebral lobe, or optic ganglion; *pc*<sup>2</sup>, *pc*<sup>3</sup>, second and third protocerebral lobes; *dc.*, deutocerebrum; *tc.*, tritocerebrum; 1-16, the sixteen postoral ganglia; *p.o.c.*, postoral commissure; *fp.*, furcal pit; *ac.*, anterior; *pc.*, posterior ganglionic commissure; *ag.*, anterior; *pg.*, posterior; *cg.*, central; *lg.*, lateral gangliomeres.

The description of the ventral nerve-cord of *Xiphidium* here given applies equally well to the other Orthoptera which I have studied (*Blatta germanica*, *Melanoplus femur-rubrum*). The

points in which the Blattid and Acridian nerve-cord differ from that of the Locustid are so insignificant that I need not burden the reader with their enumeration. I will stop to mention only two peculiarities in *Blatta*. Here I fail to detect the pale spots in the "slipper" stage of the germ-band, and sections show that the neuroblasts do not differentiate as early as they do in *Xiphidium*. They are, however, readily detected in late stages, when they stand out with even greater distinctness than in the Locustid. The median cord neuroblasts, though present and occupying positions corresponding to their homologues in *Xiphidium*, are more difficult to trace, probably on account of the smaller size of the embryo.

Neuroblasts, or cells of a similar character have been described and figured by a number of investigators of Arthropod development. Perhaps the earliest mention of these cells is to be found in Reichenbach's beautiful *Astacus* monograph ('86), where the nerve-cord is described as consisting in an early stage of two kinds of cells—a few large pale elements arranged in a single layer and confined to the periphery, and a much greater number of small and more deeply stainable cells forming the bulk of the ganglia. The developing ganglia of the cray-fish resemble the ganglia of the Orthoptera in many particulars. The number of large cells in the lateral cords in Reichenbach's figures (notably his figures 114-133) is 3-6, the average being 4 or 5, the same as in *Xiphidium*, *Blatta*, etc. Furthermore the ganglia of *Astacus* show a foliated arrangement of the smaller cells, which is not unlike the condition seen in the older ganglia of the Orthoptera. Some of the figures (188 and 189 for example) show a single neuroblast-like cell surmounting the median cord cell-mass. There are, however, two points in Reichenbach's work, which throw some doubt on the homology of his large cells with the neuroblasts of the Orthoptera. First, Reichenbach neither figures nor describes these cells as dividing to form ganglion cells. This negative observation, however, loses much of its force when we consider that caryokinetic figures are singularly absent from all of Reichenbach's figures, excepting his surface views of young embryos, and when we recall the fact that amitotic

division is a very general phenomenon in the Crustacea (according to Carnoy, '85). A more serious objection to the homology under consideration is Reichenbach's statement that the large cells 'gehen schliesslich in die von Leydig, Dietl, Krieger und anderen beschriebenen, grossen Ganglienzellen über.' This, too, is an objection only if the neuroblasts really degenerate, a point on which I am still doubtful.

Nusbaum found huge succulent cells in the young nerve cord of the embryo *Mysis* ('87). He compares them with the large cells of Reichenbach and believes that they have a similar fate. Similar cells were also observed in the brain of *Oniscus murarius* "pendant les stades relativement jeunes." On one point only does he add to Reichenbach's observations: he depicts (Fig. 78) a caryokinetic figure, which from its size and position must be referred to one of the large cells. Its spindle axis is directed at right angles to the surface of the body. This observation, small and incidental as it is, would tend to show that the large cells proliferate as in the Orthoptera. I am inclined to think that a renewed study of the Crustacean nerve cord will show that the ganglion cells are budded forth from the large cells and that these are equivalent to the insect neuroblasts.

Korotneff ('85) was the first to find gangliogenic cells in the Insecta. At p. 589 in his description of the *Gryllotalpa* embryo he says: "Einige der Ektodermzellen, welche die Nervenauftreibung bedecken, fangen an zu wachsen, ihre Kerne vergrössern sich bedeutend und zeigen dabei eine karyokineticische Figur. Grösstentheils sind diese Zellen (Ganglien) so angeordnet, dass einer einfachen platten Ektodermzelle eine wachsende Neuroektodermzelle folgt. Hat sie eine bestimmte Grösse erreicht, so sinkt jede wachsende Zelle in die Tiefe des Ektoderms und wird von den benachbarten, unveränderten Zellen bedeckt. Jede Ganglienzelle theilt sich dabei, eine ganze Folge von neuentstandenen Zellen bildend, nur an der Fig. 60 ist leicht zu unterscheiden, welche Gruppe von Zellen der oben gelegenen Ganglienzelle entspricht. Durch eine solche Vermehrung von Zellen wird der Nervenstrang mehr und mehr in die Höhe getrieben." The Fig. 60 referred

to in this description is taken from a stage corresponding to my Fig. 28. Both in this figure and in Fig. 61 he represents four neuroblasts in one of the lateral cords. Korotneff seems not to have seen the early stages of proliferation.

In the developing nerve-cord of *Doryphora* I observed ('89, p. 366) that "the outer layer of cells continuous with the hypodermis stands off somewhat from the ganglionic thickenings, leaving a space which is in early stages occupied by several large, clear, oval cells which divide rapidly by caryokinesis, and might be called *ganglioblasts*, as the products of their divisions reinforce the mass of ganglion cells." In my figures the polar-axes of the neuroblast spindles lie parallel to the surface of the ganglia. Re-examination of my preparations has convinced me that this observation is essentially correct. I find also that the newly-formed daughter-cells of the neuroblasts occasionally divide caryokinetically and thus give rise to further generations of daughter-cells. The daughter-cells are not budded forth in regular rows, but very irregularly. I am not sure that I can distinguish the median cord neuroblasts in *Doryphora*, though I believe that I have detected homologous structures. In my figure 72 I represented circular intersegmental patches in the median line between the lateral cords. Closer examination shows these to be clusters of cells of the same appearance and dimensions as the lateral-cord neuroblasts. They are very clearly brought out by Graber in his figures of *Hydrophilus* ('89, Figs. 40, 41, and 43, Pl. III) and are described as taking part in the formation of the posterior gangliomere of each ganglion. I doubt whether the large cells constituting the posterior gangliomere of *Periplaneta* in Miall and Denny's Fig. 43 ('86) to which Graber refers, are to be regarded as the equivalents of the median-cord clusters in *Doryphora* and *Hydrophilus*. *Periplaneta* very probably has in each segment only one median-cord neuroblast, which atrophies before the close of embryonic life, and the large cells in Miall and Denny's figure probably arise from the daughter-cells and are therefore merely large ganglionic elements.

Graber figures and describes ('89, p. 47, Pl. X, Fig. 130) a cross-section through an abdominal ganglion of a *Mccloulontha*

embryo in which he finds a neuroblast in either lateral cord and three symmetrically arranged cells of the same character in the median cord. Similar median cells were seen in *Lucilia*. He refers to Korotneff's observations on *Gryllotalpa* and states that he has found the lateral "ganglionäre Grosszellen" in *Lina*. He is inclined to regard them as a widely occurring differentiation of the ectoderm.

In a subsequent paper ('90) Graber describes and figures a foliated condition of the ganglia in the nerve-cord of *Stenobothrus*. In Fig. 52 the neuroblasts are distinctly seen, and in one lateral cord five, in the other four pillars of cells may be distinguished. So far as the neuroblasts are concerned, he cannot be said to have added anything to Korotneff's account.

Nusbaum ('91), in a recent Polish paper on the development of *Meloë*, figures neuroblasts in the lateral cords. They are frequently represented in mitosis—the spindle-axes being in some cases perpendicular to the surface of the ganglia (Figs. 94, 95) while in others (Fig. 107) they are parallel to the surface, as in *Doryphora*. Such portions of the text as were translated for me contained nothing new on these structures.

Viallanes, in two recent papers ('90<sup>a</sup>, '90<sup>b</sup>) on the structure of the nervous system in the embryo *Mantis*, comes to conclusions agreeing with my own, which were arrived at independently. His observations on the neuroblasts may be briefly summarized in his own words ('90<sup>b</sup>, p. 293): "A l'origine le bourrelet primitif n'est qu'un simple épaississement de l'exoderme, c'est-à-dire une région de ce feuillet dont les cellules sont devenues colonnaires et ont augmenté de volume. Bientôt ces cellules se multiplient et se divisent en deux couches, l'une superficielle (*dermatogène*), l'autre profonde (*gangliogène*). A une période plus ou moins tardive, suivant la région considérée, la couche des cellules dermatogènes se sépare de la couche des cellules gangliogènes et devient l'hypoderme. Les cellules gangliogènes en se multipliant donnent naissance aux cellules *ganglionnaires*."

Viallanes' figures do not show a regular arrangement of the cells budded forth from the neuroblasts, and he has not described the neuroblasts of the median cord, probably because

his attention was concentrated on the structure of the brain. He has observed the degeneration of the gangliogenic cells, or neuroblasts. In a late stage ('90<sup>b</sup>, p. 301), he says "Ils montrent des signes évidents de décrépitude; beaucoup des cellules gangliogènes ont déjà disparu, les autres sont en voie d'atrophie."

Our knowledge of the median cord cannot be said to have made much advance since this structure was first described by Hatschek ('77). While all writers agree that it originally extends as an uninterrupted structure from the mouth to the anus, there is wide difference of opinion respecting the ultimate fate of its inter- and intraganglionic portions. Hatschek ('77), Tichomiroff ('82), and Korotneff ('85) maintain that the interganglionic portions remain attached to the integument when the nerve-cord is liberated and that they ultimately disappear. Ayers ('84) on the other hand holds that the whole median cord is liberated from the ectoderm, but does not affirm that the interganglionic portions form a constituent part of the ganglia.

Graber ('90) has very recently come to a conclusion which differs from the views hitherto advanced. With Ayers he holds that the interganglionic portions of the median cord are delaminated from the ectoderm along with the intraganglionic portions, but he goes further and claims (p. 103) that "das Zellenmaterial des interganglionalen Mittelstranges, zum Theil wenigstens mit den Ganglien vereinigt wird, oder mit anderen Worten, dass eine Vergrösserung des ganglionalen Mitteltheiles auf Kosten des interganglionalen erfolgt."

As will be inferred from the above descriptive paragraphs, I hold to Hatschek's view that the interganglionic portions of the median cord take no part in the formation of the ganglia but are drawn out from between the connections and constitute a portion of the sternal integument. Graber's researches on this portion of the nerve cord are limited to the Coleoptera and as the insects of this order certainly differ to some extent from the Orthoptera in the formation of the nervous system, I have no grounds for doubting the correctness of his observations. I believe, however, that Ayers' account of the median cord in *Æcanthus* is open to criticism. After

clearly implying that the median cord is set free from the ectoderm along its whole extent he remarks (p. 252): "Between the successive pairs of ganglia the median ingrowth atrophies, and at the time of the closure of the dorsal wall of the body there is seen between the connecting cords of two adjacent pairs of ganglia, a small triangular or cylindrical mass of cells, concerning the fate of which I am not absolutely certain. I believe, however, that they go to form a part of the internal skeleton. The chitinous rods in the thoracic region to which the muscles of the legs and wings are attached probably arise from the remnants of the median invagination, but in the abdominal region they may disappear entirely without giving rise to such structures." If I understand this passage correctly, Ayers implies that the chitinous rods are originally interganglionic portions of the median cord. But if this is the case, how can the median cord separate completely from the ectoderm unless we are to suppose that there is a reunion of the interganglionic portions with the integument to form the endoskeletal structures? The chitinous rods are directly continuous with the chitin of the integument so that until observations are forthcoming to show that portions of the integument can loosen and pass into the body-cavity and subsequently reunite with the integument, I must regard Ayers' account as inadequate.

I am still in some doubt as to the exact origin of the commissures. Grassi ('84), Ayers ('84), Heider ('89), and Graber ('90), all maintain that the commissural fibres arise from the median cord cells. *A priori*, there are no reasons why the daughter-cells of the median neuroblast should not send out processes to form Punktsubstanz and thus form a commissure. From the position of these cells, however, I regard it as highly improbable that anything but the posterior commissure could be formed in this way. The isolated Punktsubstanz masses in the Coleopteran median cord in Graber's and Heider's figures may arise from cells equivalent to the daughter-cells of the median neuroblasts of the Orthoptera. It is very improbable that the dermatoblastic cells which form the walls of the median cord in the region of the anterior commissure, and which I regard as

non-nervous, should take part in forming the fibres of that structure.

Regarding the origin of the neurilemmata in insects, there is still considerable doubt. The inadequacy and inconsistency of Nusbaum's observations on *Blatta* ('83) have been sufficiently pointed out by Eisig ('87) and Korotneff ('85). Nusbaum derived the median cord (which, by the way, he did not recognize as the median cord) from the entoderm, and compared it with the vertebrate chorda. So far his observations and conclusions were erroneous, but he derived the inner and outer neurilemma from the cells of this "chorda"—an observation which agrees essentially with my own.

Korotneff's view that the neurilemmata arise from migrant mesoderm cells has not been confirmed by recent writers, who are inclined to derive these envelopes from the ectoderm (Heider, '89 ; Graber, '90). Though I venture to say that my own observations are somewhat more definite than those hitherto published, I cannot regard them as in any way final.

## 2. *The Brain.*

In the following account of the *Xiphidium* brain, I shall use the nomenclature employed by Viallanes in his recent papers ('90<sup>a</sup>, '90<sup>b</sup>), since his studies on the brain-development of *Mantis religiosa* agree very closely with my own. Before passing to a description of my sections I would refer the reader to the diagrammatic figure (VII) which represents the main points in the structure of the embryonic brain. Here it is seen that the ventral nerve-cord bifurcates just in front of the mandibular segment and passes on either side of the mouth, where it forms two successive pairs of ganglia. The posterior of these (*tc.*) is the *tritocerebrum*, or third brain segment. Its two halves are united by the *infraesophageal commissure*, shown in the figure as a broad white band connecting the Punktsubstanz-masses of the ganglia. The anterior pair of swellings (*dc.*) constitute the *deutocerebrum*, or second brain segment. From this portion the antennæ are innervated. Further forward the deutocerebrum passes into a large paired

supraoesophageal mass, the *protocerebrum*, or first brain segment, which constitutes the greater portion of the brain. Each of its halves may be separated into three lobes; the first, or outermost lobe ( $pc^1$  [*o.g.*]) forms the optic ganglion of the larva and imago, while the second and third lobes ( $pc^2$ ,  $pc^3$ ) ultimately form the bulk of the brain proper. The third lobe is united with the contralateral lobe by the broad *supraoesophageal commissure*. Such is the structure of the Orthopteran brain reduced to its simplest terms. It may now be considered a little more in detail.

Like the nerve-cord, of which it is simply a modified portion, the brain arises from neuroblastic cells. These first make their appearance in clusters (the spots seen on the procephalic lobes in Fig. 2). Later they form a single layer of proliferating centres continuous with and in every way comparable to the neuroblasts of the ventral nerve-cord. Like the latter they are covered externally by a layer of dermatoblastic cells.

That the deuto- and tritocerebral ganglia are strictly homodynamous with the ganglia of the nerve-cord is clearly shown in *Xiphidium*. In the first place these brain segments are directly continuous with the segments of the cord; second, they have at first the same size and shape as the latter, and third, they present on the average four neuroblasts in cross-section on either side. The suppression of the median cord in the deutocerebrum (if it be not drawn forward into the protocerebrum), is perhaps sufficiently explained by the presence of the stomodæal invagination. A partial suppression of the median cord in the tritocerebrum may be due to the same cause. The infraoesophageal commissure is perhaps the morphological equivalent of both the commissures of a ventral ganglion.

The early clustered condition of the neuroblasts is seen in Figs. 32-34 at *nb*. At the edges of these cross-sections a rounded mass of pale cells ( $pc^1$  [*o.g.*]) is distinctly marked off from a more deeply stainable layer which encloses it on nearly all sides. This mass, the future optic ganglion (first protocerebral lobe), is delaminated from the ectoderm at a very early stage. The cells of the mass agree with the neuroblasts

in their slight affinity for stains; they differ in the more elongate shape of their nuclei and cytoplasm. The dark layer enclosing the optic ganglion on all except its innermost face is the *optic plate* and will give rise to the compound eye. Passing towards the median line in these sections (especially in Fig. 33) two other thickenings may be distinguished (*pc*<sup>2</sup>, *pc*<sup>3</sup>) — the second and third protocerebral lobes.

The cross-section, Fig. 36, runs through the labrum of an older embryo (Stage F) and shows a considerable advance in the structure of the brain. The three protocerebral lobes are distinctly marked out. In the second and third, the neuroblasts have arranged themselves in a row and have budded forth strings of ganglion cells. In the first lobe (*pc*<sup>1</sup> [*o.g.*]) no teloblastic arrangement is ever present. The cells are small and narrow and early assume a radial arrangement around the Punktsubstanz core at the base of the mass. The cells of the optic plate, which stand away from the surface of the ganglion, already show a tendency to differentiate in that they have become smaller and narrower than the dermatoblasts covering the two other lobes of the protocerebrum. At the juncture of the second with the third lobe, several large dermatoblastic cells are intercalated (*igl.*). They are continuous with the dermatoblasts covering the second protocerebral lobe. This intercalated mass is called by Viallanes the *bourrelet ectodermique intraganglionnaire*. I shall call it the intraganglionic thickening.

A still more advanced stage in the development of the brain is seen in Fig. 37 (Stage G). This section passes above the base of the labrum. Owing to the active proliferation of the neuroblasts, the mass of the protocerebrum is greatly augmented. The Punktsubstanz has made its appearance as a confluent mass.<sup>1</sup> The optic plate is much thickened and its small cells are about to arrange themselves to form the ommatidia. The intraganglionic thickening (*igl.*) presents an interesting appearance. The edge of the optic plate is united with the inner edge of the optic ganglia, but between it

<sup>1</sup> I have seen nothing to corroborate Cholodkowsky's view ('91) that there are at first three distinct and separate pairs of Punktsubstanz masses in the brain.

and the second protocerebral lobe there is a fissure ( $\omega$ ) which extends in some distance. Examination of a number of successive sections and stages has convinced me that this fissure is not the result of artificial rupture during sectioning but that it is brought about by an infolding of the intraganglionic thickening. The shape and position of the involuted mass may be clearly seen from the surface in Stages H and I (see Figs. 8 and 9, *igl.*).

In the frontal section (Fig. 39) are shown the relations of the protocerebrum to the outer brain-segments and to the ventral cord. Only a small portion of the optic plate ( $c$ ) is cut. Beneath it lies the optic ganglion ( $pc^1$  [*o.g.*]), the small cells of which contrast with the large cells of the brain proper. The second protocerebral lobe ( $pc^2$ ) still contains many neuroblasts which are budding forth their last progeny. The older daughter-cells have already assumed an irregular arrangement. The brain is separated from the attenuated dermatoblastic, or integumental layer ( $db.$ ) beneath which the outer neurilemma ( $cnl.$ ) is forming. The inner neurilemma ( $inl.$ ) envelops the Punksubstanz portions of the brain. The broad supraoesophageal commissure ( $s.cm$ ) connects the third protocerebral lobes of the two sides. As shown in the figure, the deutocerebrum is distinctly præoral. At  $an$  is shown the point where the antennary nerve leaves the fibrous portion of this brain segment. Caudad to the deutocerebrum lies the tritocerebrum, a pair of somewhat smaller ganglia united by the infraoesophageal commissure. It is this segment which according to Viallanes innervates the labrum and the frontal ganglion. Besides the supra- and infraoesophageal commissures and the connectives which arise in the third protocerebral lobes and traverse the deuto- and tritocerebrum to pass into the mandibular ganglion and thence through the nerve-cord, I may call attention to two other masses of Punksubstanz which lie in front of the supraoesophageal commissure. These are shown at  $p.$  in the figure. They appear to be connected by a small band running parallel to the robust supraoesophageal commissure. I did not succeed in finding these connected Punksubstanz masses in all embryos of this stage, and as they were not

seen in later stages, their morphological value as indicating the presence of another segment in front of the protocerebrum is somewhat doubtful.

The three divisions of the protocerebrum may still be recognized in the transverse section (Fig. 40) of an embryo which has passed beyond Stage J. In the median line at *mc* lies a rounded and compact mass of cells which I regard as the præ-oral representative of the median-cord cell-masses of the ventral cord. A large cell, which I take to be a neuroblast, lies at the outer periphery of this median cell-mass at a younger stage. Structures at a corresponding position in the brains of other insects (*Hydrophilus*, *Musca*) and likewise comparable to the median-cord have been described by Heider ('89) and Graber ('89, p. 49). It is not improbable that the brain neurilemmata may have their origin near this median cerebral mass, just as the neurilemmata of the ventral cord probably arise from the non-ganglionic portions of the neural furrow.

Two other interesting structures are shown in Fig. 40: the intraganglionic thickening (*igl.*), now completely separated from the integument and lying as a deeply stainable mass wedged in between the optic ganglion and the second protocerebral lobe, and a peculiar pale thickening at the edge of the optic plate (*th*).

In later stages it is very difficult to locate the intraganglionic mass, so that I am unable to decide whether it atrophies or persists in a modified form as a portion of the brain. The researches of Viallanes on *Mantis* would seem to lend great probability to the former alternative.

The thickening at the lateral edge of the optic plate is constant in Stages G to J and somewhat later. It soon entirely disappears, without taking any part in the formation of the eye, so far as I have been able to observe. Does it represent an abortive ocellus?

The fully established optic nerve is shown in Fig. 41 (*o. n.*). In a much earlier stage it may be found as a delicate band of cells connecting the posterior edge of the optic ganglion with the optic plate (Fig. 38, *o. n.*). I agree with Viallanes, that it seems to arise from the ganglion and to grow outwards into the ommatidial layer; for there is from the first a sharply defined

intercepting membrane between the nerve and the plate, whereas the nerve passes without interruption into the ganglion. But I am led to lay little stress on these appearances by the researches of Watase ('90) and Parker ('90) on the adult ommatidia of a number of Arthropods. They have shown in a very convincing manner that each retinula-cell is the termination of an optic nerve fibre. The retinulæ are undoubtedly modified optic plate cells; and judging from recent observations on percipient cells in other forms (vertebrate eye, ear, olfactory nerves, v. Lenhossék's observations on *Lumbricus* ('92), etc.), we must suppose that the nerve fibres grow out from the retinula-cells, pass through the optic nerve and enter the ganglion. Such prolongations from all the retinulæ would be amply sufficient to form the optic nerve, although it is probable that some of its fibres are centrifugal prolongations from optic ganglion cells. It has been suggested that the optic nerve may be established at a very early stage, when the optic plate and optic ganglion are still closely applied to each other (*vide* Figs. 32-34), and that the nerve may not become visible till the two Anlagen separate with further development. But I do not think this is the case in *Xiphidium*. Sections like Fig. 36 show a distinct separation of the optic plate and ganglion in the region of the future optic nerve; and Viallanes has made an exactly similar observation on *Mantis*.

The sympathetic nervous system arises in part at least from the dorsal median wall of the œsophagus. At three separate points (Fig. 61) the ectoderm becomes thickened and its outer cells enlarge and assume the character of ganglion cells. The most anterior of these thickenings (*f. g.*) is the frontal ganglion. It arises just behind the base of the labrum. The two other thickenings which are placed further back (*rg<sup>1</sup>*, *rg<sup>2</sup>*) are the second and third visceral ganglia. I have not followed the development of the nerves which unite these ganglia and ramify from them.

Concerning the origin of the peripheral nervous system I have no positive data. In a few cases I have seen appearances which led me to believe that they arise as outgrowths from their respective ganglia.

The development of the brain of *Blatta germanica* and *Melanoplus femur-rubrum* agrees in all essential respects with the development of the *Xiphidium* brain. Certain Hemiptera, e.g. *Ranatra fusca*, conform very closely to the type of brain structure seen in these Orthoptera. I may mention in this connection that the brain of *Anurida maritima* shows the typical division into proto-, deuto- and tritocerebral segments with great distinctness. The last segment especially is remarkably distinct.

Until very recently the detailed study of the embryonic Hexapod brain has been limited to the Coleoptera and the results obtained have been naturally enough extended to include not only other insects but other Arthropods as well. The Coleoptera, however, are far from being primitive forms and the rôle which they play in contemporary embryological literature is largely attributable to the unusual technical advantages presented by their eggs. As far as development is concerned, the simpler brain of the Orthoptera and Ametabola in general offers many points of resemblance to the Crustacea and Myriopoda,<sup>1</sup> whereas the brain of the Metabola, like so many other points in their organization bears witness to a considerable amount of modification. It is therefore more consistent with our general views of phylogeny to reduce the Coleopteran brain to the Orthopteran type than to proceed *vice versa*.

We owe the most important contributions to the subject of Orthopteran brain development to Viallanes. After a decade of study devoted to the histological structure of the adult Arthropod brain he has selected *Mantis* as a subject for embryological investigation. His previous careful study of the adult brain of other Orthoptera (*Ædipoda coerulea* and *Caloptenus italicus*, '87<sup>b</sup>) has enabled him to avoid the confusion with which the inexperienced investigator is overwhelmed when attempting to follow the rapidly increasing complication of neural structures. With his usual skill and patience he has traced the development not only of the main structural features but of many details, so that we have a well-established point

<sup>1</sup> See the papers of St. Remy ('90) and Viallanes ('87<sup>a</sup>, '87<sup>b</sup>).

of departure for further comparative studies. So far as my own observations are concerned I am able to corroborate Viallanes' results on nearly all important points. I must state, however, that I have not followed the development into such detail.

In the light of these researches a reconsideration of the Coleopteran brain must be undertaken. Patten's description of the *Acilius* brain ('88) and my description of the brain of *Doryphora* ('89) need revision and alteration. We described the organ (see my Fig. 72, Pl. XIX) as consisting of three segments, each of which was subdivided into a brain portion, continuous with the ganglia of the ventral cord, an optic ganglion portion and an optic plate portion. Between the third brain and the mandibular segment, a segment was found which I designated as intercalary. This segment is also clearly seen in some of Patten's figures (Figs. 2 and 2<sup>a</sup> s4 Pl. VII). Thus according to our account there were four premandibular segments or seven segments in the entire head. Our results were obtained almost exclusively from surface views — by itself a defective method. But greater error was incurred, it seems to me, in ascribing segmental values to the various prominences of the optic ganglion and optic plate.

In order to bring our observations into harmony with the results obtained from a study of the Orthopteran brain, our figures must be interpreted in a very different way from that in which we chose to interpret them. Our first brain-segment is probably no segment at all, but merely a slight elevation often seen near the median line at the extreme anterior end of the germ-band. Our second, third and intercalary segments are equivalent to Viallanes' third protocerebral lobe, deutocerebrum and tritocerebrum. The three divisions of the optic ganglion are not parts of three segments, but the whole structure belongs to the protocerebrum, of which it forms the first lobe. In the same way we cannot regard the optic plate as trisegmental since it has no connection with the deuto- and tritocerebrum but only with the optic ganglion. It follows that the ocelli of Coleoptera are not originally formed on different segments as Patten would have us believe, but

belong to one segment—the protocerebrum. That such is the case I am convinced from a study of the eyes in embryos of *Dytiscus verticalis*, a form closely related to *Acilius*.

As will be seen in the profile view Fig. 8, the optic ganglion and optic plate of *Xiphidium* are at first folded back so as to lie along side the deuto- and tritocerebrum. The antennal furrow runs forward, separating the optic ganglion from the brain but stops when it reaches the protocerebrum. The value of this furrow as completely separating the second and third brain-segments from the optic ganglion was overlooked by Patten and myself: hence our false interpretation of the structures lying laterad to it.

I believe that I am justified in putting this new interpretation on the Coleopteran brain, because it harmonizes with Heider's careful study of *Hydrophilus* ('89). He has failed to find indications of segmental constrictions in the optic plate and optic ganglion and his figure 4 *A. B.* at p. 37 agrees closely with Viallanes' description of the *Mantis* brain. It should be observed that the embryos of *Hydrophilus* are much larger than those of *Acilius* and *Doryphora* and therefore much more favorable for surface study. On the other hand it may be urged that Heider evidently did not employ so good a method of surface preparation as Patten.

The distinct invagination associated with the formation of the optic ganglion in Coleoptera and described by Patten, Heider and myself, is probably homologous, as Viallanes suggests, with the intraganglionic thickening of the Orthopteran embryo. This structure in *Mantis*, and probably also in *Xiphidium*, takes no part in the formation of the optic ganglion, which arises—at least in great part—by delamination as in the Crustacea (see Parker ('90)). Only the outer or lateral portion of the optic plate becomes the compound eye, so that in a later stage the intraganglionic thickening is separated from the edge of the eye by a considerable space. The thickening then lies just laterad to the antennal furrow as shown in Fig. 8. Whether or not the invagination in the Coleoptera really plays any part in forming ganglionic tissue as has been claimed, must be decided by renewed investigations.

Concerning the researches of Cholodkowsky on the brain of *Blatta germanica*, I must say a few words, since I have described the brain and nerve-cord of this form as agreeing in all essential respects with those of *Xiphidium*. Cholodkowsky lays great stress on the existence of three distinct pairs of Punktsubstanz masses in the supracæsophageal ganglion as indicating the presence of three segments. When we come to examine his figures we find that he takes a very unusual view of brain-segmentation, for the three pairs of Punktsubstanz masses are seen to belong (Fig. 46 Pl. IV; Fig. 67 Pl. VI) to the protocerebrum and correspond to the centres of its three lobes. He did not distinguish the deuto- and tritocerebral segments! Such of his remarks on the development of the brain and ventral nerve-cord as are at all comprehensible show similar glaring defects in observation. Thus he has failed to detect the small dermatoblastic cells which from the first cover the brain and nerve-cord. He asserts that these organs are at first naked and are only subsequently covered by an overgrowth of the integument from the sides of the body. The antennal and neural furrows do not play the part in development that he ascribes to them. The last abdominal ganglion of the mature embryo does not consist of four but of three fused ganglia; the fusion of the second and third abdominal ganglia was completely overlooked.

### 3. *General Remarks on the Nervous System.*

The nervous system of Arthropods is by common consent derived from the nervous system of annelid-like forms, and it is to this group that we naturally turn in seeking an explanation for certain structures in the Hexapod brain and nerve-cord.

In a brief preliminary paper Patten ('88) made the statement, that "the ventral cord and brain of Arthropods is at first composed entirely of minute sense-organs, which in scorpions have the same structure as the segmental ones at the base of the legs." This would seem to indicate that the Arthropod nervous system can be traced back to the condition seen in Polychæta — *Lopadorhynchus*, according to Kleinenberg ('86) — where both brain and nerve-cord arise in connection with and

ultimately supplant certain larval sense-organs. So far, however, as the Hexapoda are concerned, Patten's statement is, to say the least, inapposite, since, as I have pointed out, both brain and nerve-cord arise from peculiar ectodermal cells—the neuroblasts—which under no circumstances can be regarded as primitively sensory. They are simply generalized cells, like the teloblasts of worms and the meristem of plants.

The development of the nerve-cord in the Hirudinea and Oligochæta agrees more closely with the conditions seen in insects. As Whitman has shown for *Clcpsine* ('87), and E. B. Wilson for *Lumbricus* ('89), the nerve-cord is proliferated forward from a pair of neuroteloblasts situated at the posterior end of the germ-band. Hence, in these worms, the whole of the nerve-cord is condensed, as it were, into two huge mother-cells, whereas in the Insecta it is condensed into a single layer of huge cells. There are reasons, however, for believing that this layer is, in part at least, derived from a few large cells situated just in front of the anus, and therefore corresponding to the Annelid neuroteloblasts.<sup>1</sup> That there are only two rows of these cells in Annelids, while there are eight in insects, can form no very serious objection to their homology, as I pointed out in my preliminary note ('90<sup>e</sup>).

Certain conditions in the Crustacea also lend probability to the view that the Hexapod neuroblasts may be budded forth from a præ-anal row of teloblasts.<sup>2</sup> Patten ('90) has pointed out in *Cymothoa* a row of proliferating cells which form ectoderm, and Nusbaum ('91) has described a very similar condition in *Ligia*. I have observed the same phenomenon in *Porcellio*, and believe it to be of general occurrence throughout the Isopoda. The cells are budded forth so as to form regular transverse and longitudinal rows. Reichenbach describes and

<sup>1</sup> What I have called the neuroblasts in insects therefore correspond to the "neural cell-rows" in Annelids (the cells *np. c.* in E. B. Wilson's Fig. 59, Pl. XIX, and the cells *nc.* in Whitman's Figs. 9 and 11, Pl. V).

<sup>2</sup> The neuroblasts of the last row (*tb.*, Fig. 56, Pl. VI) in the nerve-cord of *Xiphidium* are always distinctly larger and clearer than the neuroblasts of the remainder of the cord. They may be true neuroteloblasts and give rise to the neuroblasts, but as I have never found unmistakable caryokinetic figures in them, I am still in doubt as to their homology with the neuroteloblasts of worms.

figures a very similar budding-zone of ectoderm cells in the Decapod *Astacus* ('86). That a portion of the ectoderm cells thus budded forth in these forms goes to form the nervous system, admits, it seems to me, of very little doubt. The clustered condition of the neuroblasts in the young germ-band of *Xiphidium* may be due to the rapidity with which the germ-band grows in length and breadth; the original regular arrangement of the cells being thereby disturbed and not re-established till a somewhat later stage.

Although I have maintained a phylogenetic connection of the insect neuroblasts with the neural cell-rows of Annelids, I admit that they may be regarded from an entirely different standpoint, *viz.* as having arisen independently in insects by a process of precocious segregation; but it should be noted in this connection that it is just the oldest Pterygota, the Orthoptera, which show this segregation most clearly, while in the more recent forms (Coleoptera, *etc.*) the process is more obscure.

A comparison of the histogenesis of the insectean with the histogenesis of the vertebrate nervous system brings out some interesting analogies. The neuroblasts may be compared with His' Keimzellen which divide by caryokinesis to form his neuroblasts. These are directly converted into ganglion-cells by sending out axis-cylinder processes. They correspond, therefore, to the daughter-cells of the insect neuroblasts, which are likewise converted into ganglion-cells. The "Keimzellen" of vertebrates differentiate close to the central canal, which is, of course, morphologically the outer surface of the cord, just as the Arthropod neuroblasts lie at the surface of the cord. In both groups the daughter-cells appear to be budded off in the same direction morphologically; though in vertebrates the ganglion-cells migrate, while in insects they are pushed inwards by their newly proliferated sister-cells. The early separation of the neural ectoderm in vertebrates into Keimzellen and sustentacular tissue (spongioblasts of His) is paralleled in insects by the precocious splitting of the same germ-layer into neuroblasts and dermatoblasts, the latter giving rise to supporting tissue in the form of neurilemmata.

The majority of authors hold that the Arthropod brain is either wholly or in part homologous with the Annelid brain. Patten ('90) alone takes the view that the Annelid prostomium is absent in Arthropods, and that the brain of the latter is formed by the moving forward of three segments which are postoral in the Annelids. Apart from the fact that we have as yet no means of deciding whether what we call the first segment of the Arthropod head (protocerebrum) is really a single segment or a complex of several, it is extremely improbable that so highly important a structure as the Annelid brain should have completely disappeared in the Arthropods. So great is the resemblance between the Arthropods and Annelids in all the more important morphological features and even in the detailed structure of the ventral nerve-cord, that the complete elimination of the brain certainly makes strong demands on one's credulity.

Will ('88) goes to the opposite extreme and regards the præoral portion of the Arthropod brain as the exact homologue of the Annelid brain. He goes so far as to call the procephalic lobes of *Aphis* the "Scheitelplatte." He finds that they lie at the pole of the egg opposite the blastopore, or rather what he takes to be the blastopore, and that they arise independently of the nerve-cord. Now the "Scheitelplatte" of *Aphis* must include at least the proto- and deutocerebral segments—probably also the tritocerebrum. The deutocerebrum in all the Orthoptera which I have examined is provided with a pair of true mesodermic somites and with a pair of appendages, the antennæ. Each mesodermal somite sends a hollow diverticulum into an antenna, which is thus shown to be homodynamous with the other appendages of the embryo. The tritocerebral segment also contains a pair of abortive somites and in *Anurida maritima*, as I have lately ascertained, bears a pair of minute but distinct appendages (see Fig. VI, *tc. ap.*). Viallanes ('87<sup>a</sup>) and St. Remy ('90) have found that the second pair of antennæ in Crustacea belong to the tritocerebral segment. These facts go to show that the deuto- and tritocerebral segments are homodynamous with the postoral segments and, as the "Scheitelplatte" of *Aphis* must extend at least as far back as the

tritocerebral segment, it cannot be homologized with the Annelid Scheitelplatte, a structure which is not segmented.

A view midway between Will's and Patten's probably accords best with the facts at our disposal. The Arthropod protocerebrum probably represents the Annelid supraoesophageal ganglion, while the deuto- and tritocerebral segments, originally postoral, have moved forward to join the primitive brain. This is essentially Lankester's view ('81), according to which in Arthropods "the præoesophageal ganglion is a syncerebrum consisting of the archicerebrum and of the ganglion masses appropriate to the first and second pair of appendages which were originally postoral, but have assumed a præoral position whilst carrying their ganglion-masses up to the archicerebrum to fuse with it."

In comparing the Arthropod with the Annelid brain much stress has been laid on the fact so clearly brought out by Kleinenberg ('86) — that the Annelid supraoesophageal ganglion originates independently of the ventral nerve-cord. Several investigators — Balfour ('80), Schimkewitch ('87), Will ('88) and others — have fancied that they could detect a similar ontogenetic discontinuity of the brain and nerve-cord in Arthropods. But more recent observations all tend to prove that there is a direct continuity of the central nervous system from the time when the ganglia first make their appearance. So far as the insects are concerned I may note that Will's conclusions were based on the defective surface observation of a form (*Aphis*) ill adapted to the study of the central nervous system.<sup>1</sup>

Even granting that the Annelid brain arises independently of the nerve-cord — and this is not yet settled — at least so far as the Oligochæta are concerned (see E. B. Wilson, '89) — Lankester's view of the Arthropod brain is in no way invalidated. The line of separation corresponding to the Annelid prototroch must fall in front of the deutocerebral segment, since it has been shown that this segment in some insects contains a pair of well-

<sup>1</sup> Little value can be attached to Cholodkowsky's assertion that in *Blatta* the supraoesophageal ganglion originates independently of the nerve-cord, since he has failed to see the deuto- and tritocerebral segments which are quite as well developed in *Blatta* as in other Orthoptera.

developed mesodermic somites. When we stop to consider the intimate union of the proto- and deutocerebral ganglia from the time of their first appearance, we need entertain little hope of finding traces of a separation which existed, if indeed it existed at all, in a very remotely ancestral period.

## VI. THE DEVELOPMENT OF THE REPRODUCTIVE ORGANS IN THE INSECTA.

### 1. *The Gonads.*

The following account of the development of the sexual organs is based almost exclusively on *Xiphidium*. Some attention was devoted to the study of *Blatta*, but this form proved to be so much less satisfactory and to depart so little from the *Xiphidium* type, that it was abandoned.

Before passing to a description of the sexual organs and their ducts, it will be necessary briefly to consider the mesodermal somites, since the history of the organs under consideration is intimately bound up with the history of the middle germ-layer. The mesoderm of *Xiphidium*, like that of other insects, is coextensive with the blastopore and hence reaches from the region of the definitive mouth to the region of the definitive anus. At first a continuous cell-layer, it soon splits up into segmental masses as metamerism sets in. These are further divided in the median ventral line so that each segment has a pair of mesoderm blocks. Each of these acquires a cavity and the somites are established.

The appendages are from the time of their first appearance intimately connected with the somites, since each of the latter sends a hollow diverticulum into the appendage of the corresponding half of the body. All the somites are fully formed when the embryo has reached Stage F. There are then eighteen pairs in all. The most anterior pair occupies the deutocerebral segment and sends hollow diverticula into the antennæ. The walls of these somites are much thinner than those of succeeding pairs and, curiously enough, persist much longer. The pair in the tritocerebral segment is very small

and indistinct and disappears very early. Each of the succeeding segments, with the exception of the eleventh abdominal has a distinct pair of somites. In the last abdominal, mesoderm is present, but I have been unable to find a trace of a true coelomic cavity.<sup>1</sup>

The youngest embryo in which I was able to detect reproductive cells had almost reached Stage F. In still earlier stages careful scrutiny failed to reveal any differentiation of the mesoderm cells. These cells, it is true, vary considerably in size and appearance but I have found it impossible to fix on any one set of elements which might be brought into connection with the germ-cells of older embryos. It is not, therefore, till the somites are established as distinct sacs that unmistakable primitive germ-cells make their appearance. In frontal sections of embryos in Stage F (Fig. 52 *gd*<sup>1</sup>, *gd*<sup>3</sup>), the primitive germ-cells are seen to lie in the inner wall of the somite. They are considerably larger than any of the surrounding mesoderm cells, and much paler. The chromatin of their nuclei is arranged in a more delicate skein. Like the neuroblasts they stain very deeply in picric acid. Normally, they occur only in the first to the sixth abdominal segments, each cluster being confined to the inner wall of a somite. The reproductive organs of *Xiphidium* are therefore truly metameric in their origin. There is nothing to show that they arise from vitellophags which have migrated into the somitic wall; nor can they arise from the entoderm, since they are differentiated before the entoderm-bands have reached the basal abdominal segments in their growth backward from the oral and forward from the anal formative centre. I conclude, therefore, that the primitive germ-cells are enlarged and modified mesoderm-cells. In explanation of Fig. 52 it may be noted that the plane of section is somewhat oblique so that it

<sup>1</sup> I mention this because Graber ('90) has recently described a coelomic cavity in the anal segment of *Hydrophilus* (Fig. 29, p. 62). Cholodkowsky also describes and figures ('91, Fig. 49 Pl. IV) such a cavity in the eleventh abdominal segment of *Blatta*. Every little slit in the mesoderm is not a coelomic cavity, and the figures referred to show only small spaces between the mesoderm cells of the telson. This may have been produced artificially, for aught the figures show to the contrary.

strikes only the lowermost germ-cell of the cluster in the third abdominal segment (*gd*<sup>3</sup>) and in the fourth segment passes completely under the cluster. Even at this time certain mesoderm-cells, the future epithelial elements (*ep.*) begin to flatten out and apply themselves to the surfaces of the germ-cells.

The exact relations of the primitive germ-cells to the walls of the somite are readily seen in transverse section (Fig. 53). The inner face of the triangular somite is applied to the surface of the yolk and besides giving rise to the germ-cells will ultimately form the splanchnic, or visceral layer. The remainder of the cœlomic wall is somatic, or parietal, and is converted into fat-body and musculature. The heart arises where the outer edge of the splanchnic passes into the somatic layer. In the section figured the entoderm is still wanting on the left side, whereas on the right side a single cell (*en*) of the right posterior band has already reached the segment. Similar inequalities in the rate of growth of the entoderm-bands are by no means infrequent.

In this stage some of the primitive germ-cells show a tendency to leave the wall of the somite and to drop into the cœlomic cavity. This is distinctly seen in Fig. 53. These cells sometimes enlarge considerably, become vacuolated and take on the appearance of young ova. A cell of this kind, nearly filling the cœlomic cavity, is shown in Fig. 55. I do not believe that the cells are loosened from the cœlomic wall during the process of sectioning.

Although the clusters of germ-cells normally occur only in the first to the sixth abdominal segments, in one somewhat older embryo (Stage G) a well developed pair of clusters was found in the tenth segment. One of these is shown in sagittal section in Fig. 56 (*gd*<sup>10</sup>). It resembles the normal clusters in every particular. The same section shows the diverticulum of the tenth somite (*m. d.*). In the next section laterad to the one figured, the hollow tip of the diverticulum is seen to terminate in the right appendage of the segment. A similar relation of the cœlomic diverticula to the appendages obtains in all the abdominal segments in front of the tenth.

The primitive germ-cells, which at first occupy only a limited portion of the splanchnic wall, increase in number during a stage midway between F and G. Beautiful caryokinetic figures may then be found in some specimens—showing that the primitive germ-cells themselves proliferate. New germ-cells may be added to the clusters by a differentiation of elements in the splanchnic wall but I have seen nothing to convince me that this occurs. The epithelial cells become much flattened and stain more deeply so that they stand out distinctly among the pale rounded germ-cells which they invest. The inner wall of the somite soon becomes too small to contain all the rapidly accumulating cells and is forced to send out a solid diverticulum. This is directed anteriorly, and in a little later stage fuses with the wall of the antecedent somite. This fusion is probably preceded by the shortening of the embryo which takes place during a stage immediately succeeding Stage F. The result of the fusion is the formation of a continuous strand of germ-cells with their accompanying epithelial cells. For some time the typical hexametameric arrangement is still visible in the strand, but later the whole mass shortens very decidedly to form the definitive ovary and testis and all traces of metamerism are lost. In the present paper I shall not follow the development of these organs further, but will pass on to a description of the sexual ducts. This will enable me to supplement the recent work of Heymons ('91) who has given us an extended and valuable account of the development of the sexual organs in *Blatta*, but has contributed only a few observations on the development of the ducts.

## 2. *The Male Ducts.*

The sexual ducts like the germ-cells are modified portions of the mesodermal somites. While considering the exceptional embryo in Fig. 56, attention was directed to the diverticulum (*m. d.*) of the tenth abdominal somite. This diverticulum, which is quite normal, is destined to form the terminal portion of the deferent duct (spermaduct) and the seminal vesicle of the adult insect. At the base of the divert-

iculum a constriction is formed which converts the proximal portion into a thin cord but leaves the distal end expanded as a hollow sac, which I shall call the terminal ampulla. The remainder of the deferent duct — *viz.* the portion extending from the sexual gland in the sixth to the anterior end of the tenth segment is formed by a cord-like thickening in the splanchnic wall of the three intervening somites. Anteriorly the cells of the duct pass into the epithelium enveloping the germ-cells. There is no lumen in the duct proper except towards its end where it widens into the terminal ampulla. Thus only the appendage diverticula of the tenth segment go to form the ends of the ducts ; in all the other abdominal segments the diverticula break down and disappear, together with their respective appendages, before the embryo reaches Stage H. In the thoracic, oral and antennary segments, however, the diverticula are converted into the muscles of the persistent appendages.

The further history of the male ducts is readily followed in partially stained embryos mounted *in toto*. Sex is determined, so far as I have been able to make out, during or soon after kataropsis, at which time the appendages of the second to the seventh abdominal segments disappear. Fig. 42 represents the caudal end of an embryo just after kataropsis (Stage J). Appendages still persist on the eighth to eleventh segments while the pleuropodia, not seen in the figure, have begun to degenerate. The testes (*ts.*) and the spermaducts (*m. d.*) are represented in blue. The former have shortened considerably and moved caudad so that they now lie in the sixth and seventh segments. The long terminal threads run forwards from the anterior ends of the testes, while the spermaducts run backwards and end in the terminal ampullæ (*ta. m.*) which still fit into the cavities of the tenth pair of abdominal appendages (*ap.* 10). A section through these appendages is seen in Fig. 57, showing very clearly the connection of the ampullæ (*ta. m.*) with the ducts (*m. d.*). In front of this section the ducts have no distinct lumen.

In Fig. 43, taken from a slightly older embryo, the appendages of the eighth segment have completely disappeared,

while those of the tenth have grown smaller and approached the median ventral line. They have, in fact, grown too small to contain the ampullæ which are drawn away from them and lie between the ninth and tenth segments a little in front of the abortive appendages. At the same time the inner faces of the ampullæ have become flattened and applied to each other in the median line. Each of these sacs has a pointed tip directed caudad. The more arcuate course described by the ducts in this stage is undoubtedly due to the mutual approximation of their terminal ampullæ. The appendages of the eleventh segment, the cerci (*cc.* [*ap*<sup>11</sup>]), which in the preceding stage were rounded like the other abdominal appendages, have become oval.

A more advanced embryo is represented in Fig. 44 (somewhat younger than Stage K). The appendages of the tenth segment (*ap*<sup>10</sup>) have almost completely disappeared. Those of the ninth segment, the future styli (*ap*<sup>9</sup>) have lengthened and now point outwards and forwards. The cerci have grown more pointed. The terminal ampullæ lie completely in the ninth segment, having shifted their position headwards. The movement takes place in such a way that what were the posterior faces of the ampullæ in the younger stage (Fig. 43) are applied to each other, while the pointed tips are directed forwards. The ducts are thereby rendered still more arcuate towards their terminations. An intermediate stage in this singular movement is shown in Fig. 45, where only small portions of the posterior faces of the ampullæ are as yet applied to each other. Comparison of Figs. 43, 44 and 45 shows that the pointed tips remain united and move forward while the surfaces of mutual contact are being shifted. Finally in Fig. 46, which represents the abdominal end of an embryo ready to hatch, we see that the terminal ampullæ have increased considerably in size at the expense of the thickness of their walls. They have also lengthened, and brought still more of their surfaces in contact in the median line. The pointed tips of the ampullæ extend into the eighth segment. It may also be noted that the points where the spermaducts meet the ampullæ have moved forward. The appendages of the tenth segment

have long since disappeared and the pleuropodia have lost their organic connection with the embryo, so that only two pairs of abdominal appendages persist, the stylets (*st.* [*ap*<sup>9</sup>]) and the cerci (*cc.* [*ap*<sup>11</sup>]), both provided with setæ. The pointed fusiform cerci are now folded back so as to bring their insertions on the anal segment into view.

Beyond this stage the development of the male ducts was not followed in *Xiphidium ensiferum*, but several larval stages of an allied species, *X. fasciatum*, were studied for the purpose of connecting the embryonic with the adult condition.

It will be noticed that in *Xiphidium ensiferum* there exists at the time of hatching no external opening to the spermaducts; the ampullæ are completely closed sacs applied to the ventral hypodermis of the ninth abdominal segment, and the ducts connecting them with the testes have no lumen. In the *X. fasciatum* larva 10 mm. long the ejaculatory duct has made its appearance as an unpaired invagination of the hypodermis in the median line between the ninth and tenth segments. Fig. 47 shows the sexual organs of such a larva seen from within, the ventral scutes of the tenth and anal segments having been entirely removed. The prominent terminal ampullæ, which become the seminal vesicles of the adult, are considerably enlarged and their walls have increased in thickness. The short spermaducts, now provided with a small lumen, run from the under surface of the sacs to the prominent testes. Only the outer opening of the invagination which is to form the ejaculatory duct is seen at *m.o.* It runs forward as a flattened chitin-lined depression beneath the seminal vesicles. Sagittal sections show that there is as yet no communication between the lumina of the mesodermal and ectodermal portions; it is not till a later stage that such a communication is established.

### 3. *The Female Ducts.*

The oviducts, like the vasa deferentia, are derived from a pair of cœlomic appendage-diverticula, but in the female the diverticula belong to the seventh abdominal segment. The diverticula of the female embryo also become constricted

proximally and end in terminal ampullæ, which are from the first somewhat smaller and more elongate than the homodynamous structures of the male. The appendages to which the ampullæ belong are also less prominent than the tenth pair of appendages in the male. Examination of several series of cross-sections from embryos in Stage J—this being the stage in which the sexes differentiate—reveals the curious fact that in the female, besides the pair of ampullæ in the seventh, a pair is also retained in the tenth segment. Figs. 58 and 59 represent two sections taken from such an embryo—the former passing through the tenth, the latter through the seventh abdominal segment. In Fig. 58, the two terminal ampullæ (*ta. m.*), and small portions of the ducts leading to them, are still preserved, but the cells and nuclei, especially in the ducts, are being broken down. The ampullæ soon share the same fate. In the seventh segment (Fig. 59, *f.d.*) the cavity of the diverticulum still opens into the cœlomic cavity of the same segment (*coe*). Its distal ampullar end is applied to the ectoderm where it bulges out to form the small seventh abdominal appendage (*ap7*). The condition of the diverticulum after the constriction of its proximal portion is shown in Fig. 60, taken from a somewhat more advanced embryo. In this figure, the connection of the oviduct with the posterior end of the young ovary (*ov.*) is distinctly seen. The cells of the duct pass over into the epithelial cells of the ovary, just as the cells of the spermaducts become continuous with the testicular epithelium.

We may now turn to surface views of the female reproductive organs. The specimen represented in Fig. 48 is in the same stage as the male embryo represented in Fig. 42. Five consecutive pairs of abdominal appendages are still present (*ap7*, *ap11*). Of these, the ninth and eleventh pairs are very prominent, while the tenth pair has grown very small. The ovary (*ov.*) extends back to the seventh segment where it joins the oviduct. This ends in the terminal ampulla, which lies near the posterior edge of the segment in the seventh abdominal appendage. The terminal ampullæ of the tenth segment have not yet disappeared. They are represented in blue because I regard them as the homologues of the persistent male ampullæ.

In a more advanced embryo (Fig. 49, Stage K) the male ampullæ have disappeared completely, and the tenth pair of appendages, while growing smaller, have moved up to the inner posterior insertions of the ninth pair. The ampullæ have increased in size and have come to lie at right angles to the longitudinal axis of the embryo. This causes the oviducts to describe an arc. It is thus seen that the movement of the female ampullæ is essentially the same as that of the male, but considerably weaker. Traces of appendages on the seventh segment are still apparent.

From this stage we may pass to a brief consideration of the female embryo ready to hatch (Fig. 50). The ovaries (*ov.*) have now assumed their definitive characters. Although the pointed and flattened ampullæ have approached the median ventral line, they are still separated by a wide space. Even in this advanced stage slight thickenings of the integument over the posterior edges of the ampullæ may be taken to represent the remains of the seventh pair of abdominal appendages. The appendages of the tenth segment appear to have joined the inner bases of the ninth pair. I must say, however, that my observations on this pair of appendages are unsatisfactory, notwithstanding I have taken considerable pains to follow their history. The appendages of the eighth and ninth segments undoubtedly form the two anterior pairs of gonapophyses. The third pair has been described by Dewitz ('75) and others as arising from the inner bases of the second pair and is therefore supposed to belong to the ninth segment. I believe, however, that the tenth pair of embryonic appendages persists and moves forward to join the ninth pair, whence they grow out during early larval life as the third pair of gonapophyses. In the embryo the line separating the ninth and tenth segments is certainly very vague, especially on the ventral surface, so that the possibility of a fusion of the two pairs of appendages is by no means precluded. That this fusion should occur is certainly no more remarkable than the migration of the male ampullæ from the tenth to the ninth segment. Both of these forward movements may be in some way connected with the forward migration and fusion of the ganglia belonging to the eighth, ninth, and tenth segments (*cf.* Figs. 42-46, *ag*<sup>1-3</sup>).

The female larva, like the male, has no external orifice to the sexual organs at the time of hatching. It is even more backward than the male, inasmuch as the terminal ampullæ of the oviducts have not yet met in the median ventral line. The first traces of a vagina were found in *Xiphidium fasciatum* larvæ about 10 mm. long (Fig. 51). Here the terminal ampullæ meet, but the surfaces of mutual contact are limited to the pointed tips. The vagina (*vg*) is a short and broad invagination of the hypodermis between the seventh and eighth segments. Its tip extends to the juncture of the terminal ampullæ. In a somewhat later stage the ampullæ open into each other and into the vagina. The three pairs of gonapophyses (*op*<sup>1</sup>–*op*<sup>3</sup>) are already assuming their definitive characters.

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For an excellent résumé of the little work that has been done on the embryonic development of the sexual organs in insects I would refer the reader to Heymons' recent paper ('91). Here I shall consider only three contributions, —two of Heymons' ('90 and '91) which treat mainly of the sexual glands, and Nusbaum's paper on the development of the ducts ('84).

Although the results of my study of *Xiphidium*, so far as they go, agree in many respects with Heymons' account of *Blatta*, several not unimportant differences must be pointed out. The first difference relates to the stage in which the germ-cells make their appearance. Heymons ('91) claims that he can detect them before the somites are established, at a time, in fact, when the abdominal region of the embryo is still unsegmented. This would correspond to a stage in *Xiphidium* midway between *B* and *C*. In *Blatta* certain mesoderm-cells at this time enlarge and assume a clear and succulent appearance. There is apparently no definite relation between the position of these modified cells and the future segments, and even in a later stage when they become integral portions of the somite-wall, they are quite irregular in their distribution. Heymons regards them as largely dissepimental in position. In *Xiphidium*, which is, on the whole, a far more favorable form for the study of the sexual organs than *Blatta*, I was

unable to detect the presence of germ-cells till the somites were established (Stage a little younger than F). At this time they formed strictly metameric cell-clusters each of which was confined to the median portion of the splanchnic wall of its respective somite. These cells rarely, if ever, strayed into the dissepimental region during this stage. It is, of course, conceivable, that *Xiphidium* and *Blatta* may differ very considerably in respect to the point under consideration, but I suspect, nevertheless, that Heymons has mistaken the young vitellophags for sexual-cells, notwithstanding his assertion to the contrary. At any rate, to be complete, his figures should show the vitellophags, which are undoubtedly present in the stages he studied and which occupy the very location of his "sexual-cells" in his Figs. 2 and 3.

Another point on which we differ is the distribution of the germ-cells. According to Heymons they occur in *Blatta* in the second to seventh abdominal segments, whereas I find them in *Xiphidium* in the first to sixth. Heymons says emphatically: "Im ersten Abdominalsegment treten niemals Genitalzellen auf." But this is certainly an error, for in several *Blatta* embryos I find unmistakable germ-cells forming a pair of isolated clusters in the first abdominal segment. Here they also persist till a comparatively late stage when they are drawn into the second segment during the contraction of the sexual Anlage. The peculiarly modified pleuropodia in *Blatta* form so efficient a means for determining the exact position of the first abdominal segment and its somite in series of sections both longitudinal and transverse, that I feel confident of not being mistaken in this matter. I admit, however, that fewer germ-cells occur in the first than in the succeeding abdominal segments. According to Heymons comparatively few germ-cells occur in the seventh pair of abdominal somites. In these I have never seen traces of germ-cells in *Xiphidium* but I cannot, of course, assert that they never occur, especially as I have shown that germ-cells may be found even as far back as the tenth segment. It is interesting to note that Heymons, too, found germ-cells in some of the posterior abdominal segments in *Blatta*.

In his first paper ('90) Heymons made the following statement in regard to the genital ducts: "Von besonderer Wichtigkeit scheint mir nun die Thatsache zu sein, dass beim Männchen der ursprünglich angelegte Ausführungsgang nicht in seiner ganzen Länge zum Vas deferens wird, sondern dass sich sein distaler Abschnitt später wieder zurückbildet, ohne je functioniert zu haben. Der wirklich als Ausführungsgang dienende Endtheil des Vas deferens, welcher sich mit dem ectodermalen Ductus ejaculatorius verbindet, entsteht erst nachträglich an dem ursprünglich angelegten Ausführungsgang. Beim Weibchen dagegen bildet sich der ganze primitive Ausführungsgang zum Oviduct aus."

This is the very opposite of what I have found: in *Xiphidium* it is the male duct which at first occurs in both sexes in the tenth abdominal segment—whereas in the female the oviducts are an independent formation, the original male duct being soon broken down. In the female both pairs of ducts are established simultaneously since they are both cœlomic diverticula.<sup>1</sup>

In his more recent paper Heymons ('91) describes the genital ducts as terminating at the posterior edge of the seventh abdominal segment. As he mentions this fact before he comes to a description of the embryo with determinate sex, I assume that he regards these ducts as common to both sexes. What he saw was without doubt the pair of oviducts, not the deferent ducts. From personal observation I can state that the male ducts of *Blatta* end at first in terminal ampullæ enclosed by the appendages of the tenth abdominal segment just as in *Xiphidium*, whereas the female ducts terminate in much flattened ampullæ in the seventh segment. Whether or not a rudimental male duct is present in the tenth segment of the female *Blatta* embryo I have been unable to decide. Perhaps Heymons found something of this kind and while confounding the sex of the embryos he studied, was led to make the above quoted remark.

<sup>1</sup> For the sake of greater exactness, I may state that the anterior pair is, perhaps, formed a little sooner than the posterior pair, since the somites develop from before backwards.

Of the few observations which have been made on the development of the genital ducts in insects, Nusbaum's ('84) are the most important. Their agreement with Palmén's anatomical researches on Ephemeroidea ('84) has been regarded as sufficient warrant of their accuracy. Nusbaum studied the developing ducts in Mallophaga, Pediculidæ, Blattidæ, and Culicidæ and came to the conclusion that the testes and deferent ducts in the male and the ovaries and oviducts in the female are mesodermal, while the seminal vesicles, ejaculatory duct and accessory glands in the male, and the uterus, vagina and accessory glands in the female are ectodermal. He therefore draws the line between mesodermal and ectodermal portions at the juncture of the seminal vesicles and deferent ducts in the male and at the juncture of the oviducts and uterus in the female. This is at variance with my results, since I have found that the seminal vesicles and uterus are mesodermal. These structures are described by Nusbaum as if he had traced their derivation from the ectoderm step by step. Yet he seems not to have studied embryos but only larvæ, and it is during embryonic life that the uterus and seminal vesicles are formed.<sup>1</sup> Furthermore his investigations were carried on without the aid of sections. The differentiation of the uterus and seminal vesicles from the ectoderm is far from satisfactorily shown in his figures. I cannot therefore regard Nusbaum's work as contributing any evidence in favor of Palmén's view. Palmén concluded from a careful study of the Ephemeroidea that the genital ducts of insects originally

<sup>1</sup> This is Nusbaum's description ('84, p. 40): "Auf der Bauchseite des vierten, von hinten an, Abdominalsegmentes entstehen zwei paarige Hautepithelverdickungen die sich einander nähern um sich dann zu einem hufeisenförmigen unpaaren Körper zu vereinigen. Bevor aber noch die Vereinigung zu Stande kommt, lösen sich diese Keime von der Haut ab und verwachsen, wie gesagt, mit den Enden der noch soliden Vasa deferentia. . . . In dem vorderen Theile des soliden hufeisenförmigen Keimes entstehen zwei vordere geschlossene Höhlen; der mittlere Theil bleibt noch weiter solid, der hintere verlängert sich in zwei seitliche solide Auswüchse. Die zwei vorderen Höhlen verlängern sich nach vorn und differenzieren sich in die zwei Vesiculæ seminales (innere Schläuche) des definitiven birnförmigen Körpers. Mit denselben communiciren die sich aushöhlenden Vasa deferentia." An essentially similar description is given by Nusbaum for the female (p. 41).

had paired openings on the surface of the body. This view, which I fully endorse, has a good basis of anatomical data ; but Nusbaum has not shown that there is a double opening to the sexual organs or even a distinctly paired Anlage to the ectodermal portion of the sexual apparatus. According to his own figures the vagina and ejaculatory duct are unpaired from the first ; the structures on which he laid stress as being paired ectodermal portions were nothing more nor less than the unmodified terminations of the mesodermal ducts — the terminal ampullæ.

#### 4. *General Considerations.*

The foregoing account of the development of the sexual organs differs sufficiently from the accounts of other authors to justify a brief consideration of some general questions.

First in regard to the germ-cells. These arise as six metameric pairs of clusters in the splanchnic walls of the mesodermal somites. Since the single layer of cells forming the walls of each somite corresponds to the peritoneal epithelium of Annelids, Heymons' conclusion that the germ-cells of insects arise in essentially the same manner as the germ-cells of Annelids, is certainly well-founded. In both groups the germ-cells are local proliferations of the epithelium lining the body cavity. In Annelids the germ-cells lose their connection with the peritoneum and drop into the body cavity where they undergo maturation. I have called attention to a similar process in the *Xiphidium* embryo. Whether these germ-cells disintegrate or again attach themselves to the wall and become invested with epithelial cells, I must leave undecided. I am inclined to adopt the latter alternative, since I have found no traces of germ-cells in the cœlomic cavities in stages but little older than the one figured. (Fig. 53.) Heymons has observed in *Blatta* a similar migration of the germ-cells into the cœlomic cavity.

I have alluded to the fact that *Xiphidium* exhibits more pronounced metamerism in the early arrangement of the germ-cells than *Blatta*. Strictly speaking the germ-cells in the form studied by Heymons are not at all metameric since

they arise, if his account is correct, before metameres are established. It is only on the supposition that the germ-cells of *Blatta* are precociously segregated that their method of origin can be satisfactorily compared with the conditions seen in Annelids, for in this group the germ-cells are not differentiated—so far as I am aware—until after the somites have reached a considerable degree of development. Providing, therefore, that I have not overlooked the germ-cells in precœlomic stages, *Xiphidium* must be regarded as presenting more primitive conditions than *Blatta*.

In *Xiphidium* and *Blatta* six, and therefore more than half the total number of abdominal segments, produce germ-cells.<sup>1</sup> In one case I found well-developed clusters in the tenth segment, so that if we omit the eleventh or telson-segment, which is rudimental and hence cannot be expected to produce germ-cells, and if, moreover, Heymons is correct in stating that reproductive elements occur in the seventh, only two abdominal segments fail to produce germ-cells! This consideration lends support to Heymons' suggestion that "ursprünglich die Sexualzellen auch in den hinteren Segmenten des Abdomens noch in derselben typischen Weise auftraten." The resemblance of the insect-embryo to Annelids in which a great number of consecutive segments produce ova and spermatozoa, is very obvious. The high development of the appendages and their musculature in the thoracic and oral segments of insects perhaps sufficiently accounts for the complete elimination of the germ-cell clusters in these regions. It may also be noted that they are normally absent in the abdomen in the very segments which longest retain traces of *quondam* ambulatory appendages—viz. the eighth to the eleventh.

The indications of metamerism which are so transitory in the sexual Anlage of the Orthoptera would appear to be retained throughout life in some of the Thysanura. In *Iapyx*, according to Grassi ('89), the arrangement of the egg-tubes is "nettement métamérique," and his Fig. 44, Pl. IV, represents in either ovary seven egg-tubes, occurring in consecutive abdominal seg-

<sup>1</sup> In a single instance (Fig. 55, Pl. VI) what I took to be a sexual cell was found in one of the cœlomic cavities of the metathoracic segment!

ments, beginning with the first. The solid testes show no traces of metamerism. In the young *Lepisma*, according to the same authority, the egg-tubes are also segmental, there being five in either ovary, a pair in each of the five basal abdominal segments. In the adult this character is no longer noticeable. In certain species of *Lepismina*, there are six sacs in either testicle, united in pairs on either side. These also lie in the basal abdominal segments. "L'organisation segmentaire des ovaires chez *Iapyx* se répète chez *Machilis*. Cette répétition dans des formes très éloignés l'une de l'autre comme le sont précisément *Iapyx* et *Machilis*, tend à donner au fait une sérieuse valeur morphologique. Les tubes ovariens de *Machilis* sont au nombre de sept de chaque côté." In the latter form Oudemans ('87) also figures seven egg-tubes strung along the oviduct, but without a clearly marked metameric arrangement. In the male there are three pairs of testicular sacs.

In all these Thysanura the female, as might be expected, adheres more tenaciously than the male to the metameric scheme. It will also be observed that the number of egg-tubes (five to seven pairs) is about the same as the number of germ-cell clusters in embryo Orthoptera. The position of the organs is also identical, viz. in the first to seventh abdominal segments. We might conclude, therefore, that the sexual organs of the higher Thysanura represented an embryonic or arrested condition.

A difficulty is encountered, however, when we stop to ask the question: Is the individual egg-tube in such a form as *Machilis* homologous with an individual egg-tube in *Blatta* or any other Pterygote? So far as structure is concerned, this would appear to be the case. We should also say that each egg-tube of *Iapyx* or *Machilis* was a metameric unit. But the lowest number of egg-tubes in the *Blatta* ovary is sixteen, and as this is more than double the number of metameres which contribute germ-cells in the embryo, the egg-tube in this form cannot be regarded as a metameric unit. We must conclude, therefore, either that the individual egg-tubes are not homodynamous in the Pterygota and Apterygota, or that the ovaries in *Iapyx*, *Machilis*, etc., are not primitively metameric. The possibility of there being an acquired metamerism, or pseudometamerism

in these cases is suggested by Grassi: "Cette disposition qui est nettement métamérique préserve l'ovaire du danger d'être détérioré de quelque manière que ce soit. Le danger existe particulièrement quand les oeufs sont près d'arriver à maturité et provient de ce que, chez *Iapyx*, la différentiation segmentaire de la musculature et de la cuticule est avancée au point que les métamères ont acquis une grande indépendance de mouvement. Cette indépendance est beaucoup moindre chez *Campodea* et c'est pour cela que cet insecte n'offre pas la disposition indiquée plus haut." If this be an adequate explanation, the resemblance of the sexual organs in the Thysanura to those in the Orthoptera is due to secondary causes. At all events, this question must remain open till Thysanuran embryos can be studied.

The metameric mesodermal origin of the germ-cells in embryo Orthoptera is too much like the origin of the germ-cells in Annelids to be considered as secondary and I fully agree with Heymons ('91), and Korschelt and Heider ('92) in regarding the sexual organs of such forms as the Rhynchota (Aphidæ, Cicadidæ) and Diptera (*Chironomus*, *Cecidomyia*) as derived by a process of precocious segregation from metameric gonads like those of the Orthoptera. These exceptional forms frequently exhibit peculiar and aberrant features (parthenogenesis, pædogogenesis) like the Crustacea which have a similar precocious segregation of germ-cells (*e.g.* *Moina*, according to Grobben, '79).

The genital ducts of the insect embryo are not so readily reduced to the Annelid type. Many authorities, it is true, have regarded them as modified nephridia but apart from their paired mesodermal origin and tubular structure there was very little to support such a view.<sup>1</sup> But now the prevailing view receives fresh support from the fact that the ducts in both sexes arise as hollow diverticula of the coelom. Though temporarily obliterated the lumen of the duct is very probably a persisting remnant of the coelomic cavity. This is certainly

<sup>1</sup> My statement in a former paper ('89) that the genital ducts might arise from tracheal involutions is erroneous. What I saw and figured (Fig. 80, Pl. XIX) was a section through the terminal ampullæ of the deferent ducts, and not as I supposed, through their orifices.

the case with the cavities of the terminal ampullæ which are never obliterated.

In seeking for some clue to the true nature of the cœlomic diverticula one naturally turns to *Peripatus*. Unfortunately the two accounts of the development of the nephridia and genital ducts in this curious Arthropod—the one by v. Kennel ('85 and '88), the other by Sedgwick ('85 and '88)—contradict each other in many particulars. Both authors, however, agree in deriving the mesodermal portion of the nephridium from hollow diverticula of the somites (similar to those seen in the Orthoptera, in that they extend into the appendages!), and both agree in regarding the sexual ducts as modified nephridia. But Sedgwick derives the nephridium from the portion of the diverticulum located in the appendage, while Kennel derives it from the inner lower angle at the base of the somite. According to Kennel only the funnel-portion arises in this way, the long duct being formed by a tubular invagination of the ectoderm. On the other hand, Sedgwick derives the funnel and the greater portion of the duct from the mesoderm and believes that only a very small portion of the duct arises by invagination from the ectoderm. These differences apply, of course, to the sexual ducts as well. According to v. Kennel's account not only their unpaired terminal portion (opening in his form on the antepenultimate segment) but also the deferent ducts and uteri are ectodermal; only a short piece, corresponding to the nephridial funnel, and uniting the uteri to the ovaries, and the deferent ducts to the testes, has a mesodermal origin. According to Sedgwick the dorsomedian portion of the cœlom persists in the segments caudad to the fifteenth and is constricted off from the remainder of the somite. The dissepiments are broken down between the adjacent abstricted portions of the somites, so that a hollow tube is formed on either side. These tubes receive the germ-cells from the entoderm and form the sexual glands.<sup>1</sup> In the segment bearing the anal papillæ

<sup>1</sup> Sedgwick claims that the germ-cells originate in the entoderm and later on migrate into the cœlomic wall. In this particular I prefer to adopt v. Kennel's account, according to which the germ-cells have a mesodermal origin, since it accords better with the facts of Annelid development and with my own observations.

(which in all probability are reduced ambulatory appendages) a complete separation of the cœlom into a lateral (diverticular) and a dorsomedian (genital) portion does not take place, so that the two cavities remain confluent. The portion of the cœlomic wall surrounding the proximal cavity joins the sexual-gland while the diverticular (nephridial) portion acquires an external opening, "which, however, is much nearer the middle line than in the case of the anterior somites, and, indeed, may be described as being common with that of the opposite side. However this may be, the two openings soon become definitely united to form a single opening, while the tubes themselves persist as the generative ducts. Whether any large portion of the latter are ectodermal in origin, that is to say, derived from a growth of the lips of the opening at its first appearance, it is impossible to say."

If we accept Sedgwick's account it is easy to reduce the genital ducts of insects to the type seen in *Peripatus* and consequently to Annelid nephridia. In the first place, everything goes to show that the appendage diverticula of the cœlom are homologous both in *Peripatus* and Orthoptera. In both cases the oviducts and deferent ducts arise from these diverticula by partial constriction. Just as the ducts of *Peripatus* run into the cavities of the anal papillæ, so the sexual ducts of *Blatta* and *Xiphidium* run into the rudimental abdominal appendages. In both cases there are terminal ampullæ, for as such I venture to regard the slight distal widening of the cœlomic diverticula in Sedgwick's Figs. 42 and 44. A comparison of these figures with my Figs. 56 and 59 will show the close resemblance between insects and *Peripatus* better than paragraphs of description.

As the exact limits of the ectodermal portions of the ducts of *Peripatus* have not been clearly ascertained, further comparison with the Insecta cannot at present be undertaken. In the Insecta only the vagina and ejaculatory ducts with their respective accessory glands arise from the ectoderm. These structures are median and unpaired in all insects except the Ephemeridea, one of the oldest and most primitive groups. In this group, as Palmén has shown ('84), the ducts of both sexes

have independent openings. The oviducts open at the posterior edge of the seventh, the deferent ducts, which are continued into a pair of penes, at the posterior edge of the ninth segment. There is no ductus ejaculatorius proper since, according to Palmén, the chitinous cuticula covering the surface of the body does not extend in beyond the lips of the orifices.<sup>1</sup> In the females of *Heptagenia* the oviducts open at the bottom of an infolding of the hypodermis between the seventh and eighth segments. This infolding, the ovivalvula, accommodates the mature eggs till the time for oviposition, and may be regarded as a structure on the way to becoming a vagina. Morphologically it is simply an intersegmental depression differing from those which separate the sternites of other segments only in being somewhat exaggerated. Palmén observed that the male genital ostia are not opened till the last nymphal ecdysis.

A comparison of the nymphal Ephemeropterid with the Orthopteran embryo is very instructive. In *Xiphidium* and *Blatta* the female ampullæ lie at the hind end of the seventh, the male at the hind end of the ninth abdominal segment. Just as the deferent ducts of Ephemeropterids extend into the penes and open to the exterior, so the terminal ampullæ originally extend into a pair of appendages, albeit on the tenth segment and not opening to the exterior. If the penes of Ephemeropterids are really modified ambulatory appendages they would be homologous with the styli of Orthoptera. The curious persistence of these appendages in existing Orthoptera may be due to their having once functioned as penes, long after the other abdominal ambulatory appendages had disappeared. It would be necessary to suppose, if this view were adopted, that the terminations of the male ducts had moved backwards. But this whole matter is very

<sup>1</sup> Palmén claims ('84, p. 82) to have found no chitinous lining in the terminal portion of the ejaculatory ducts and oviducts of Ephemeropterids—an observation from which he naturally infers that these ducts are mesodermal throughout their entire length. I have found, however, that there is in the nymph of a species of *Blasturus* very common in the ponds of Worcester, Mass., a distinct chitinous lining to the ejaculatory ducts for some distance inward from the orifice of either penis. My attention was attracted to this lining during the ecdysis of the insect, when I saw the membrane withdrawn from the ducts along with the cuticle covering the external surfaces of the penes and terminal abdominal segments.

obscure, for why should the ampullæ in *Xiphidium* move from the tenth into the ninth segment? The answer to this enigma depends on further comparative embryological research. The long persisting closure of the ostia of the male ducts in Ephemeroidea is probably an embryonic trait. That the vagina and ejaculatory duct of higher insects may have arisen from a simple intersegmental depression like the ovivalvula receives some support from the fact that the ectodermal portions of the sexual apparatus make their appearance so late ontogenetically. To obtain in *Xiphidium* a condition essentially like that in Ephemeroidea it would only be necessary to have each terminal ampulla in both sexes open to the exterior.

The original termination of the sexual ducts in modified ambulatory appendages—which is so clearly seen in both sexes in embryonic Orthoptera—is very probably a primitive feature. In the Malacostraca among Crustacea and in Diplopod Myriopoda the sexual ducts terminate on more or less modified ambulatory limbs; in both sexes in the former group, only in the males in the latter. In the insect embryo the male genital appendages are larger than those of the female; hence, perhaps the larger size of the ampullæ filling their cavities. The ampullæ are probably very important structures from a phylogenetic standpoint. They may perhaps represent the nephridial Endblasen of *Pteropoda* and *Annelids*, providing these latter structures are mesodermal. In Annelids the Endblasen occasionally function as temporary receptacles for the sexual products, a function which seems to have been retained in the male insect, where they become the vesiculæ seminales.

Within the group Eutracheata<sup>1</sup> the position of the sexual openings is subject to great variation. Thus in Diplopods and Pauropods the ducts open behind the second pair of legs, usually between the second and third segments. In Chilopoda, on the other hand, they open on the penultimate segment. In the Symphyla the unpaired genital orifice is situated on the fourth segment, which probably corresponds to the first abdominal segment in insects. Even within the division Apterygota great variation is observable. In the Collembola in

<sup>1</sup> Under this heading I would include the Myriopoda and Hexapoda.

both sexes the orifice lies at the posterior edge of the fifth abdominal segment. In the Thysanura the female opening usually lies on the eighth, and that of the male on the ninth abdominal segment. In female Orthoptera and Ephemeroidea the sexual organs open behind the seventh, in the male behind the ninth abdominal segment, while in the Plecoptera and many other insects the female orifice is said to lie at the posterior edge of the eighth segment. Although these facts of adult anatomy point to great instability in the segmental termination of the sexual ducts, the evidence from embryology is more conclusive. The female *Xiphidium* embryo has at first two pairs of ducts, and in the male the single pair shift their position from the tenth to the ninth segment. The former fact proves conclusively that the male and female ducts are not homologous but homodynamous structures, and the latter that ducts may shift their insertions from one segment to another during ontogeny. The inference is, that sexual ducts may arise in any nephridium-bearing segment from the pair of nephridia which best subserve the sexual function and at the same time interfere least with the development and function of other organs, and that a phylogenetic shifting of the ducts has probably taken place repeatedly. The position of the genital ostia on a particular segment cannot therefore be regarded as a character of high morphological value, at least for the larger groups.

Two conflicting views have long been entertained respecting the morphological significance of the gonapophyses. Under this term, introduced by Huxley ('77), we may include the appendages of the eighth to the tenth abdominal segments in the female and such of their homologues as persist in the male. In the female, these appendages go to form the ovipositor. According to Lacaze-Duthiers ('49-'53) they are not true appendages, *i. e.* homodynamous with the legs, mouth-parts, etc., but simply modified ventral sclerites. Haase ('89), too, believes that the gonapophyses are not true appendages but "Integumentbildungen von etwas höherer Werthigkeit als die Griffel," or styloid processes which are found inserted at the bases of the legs in some Myriopods and Thysanura. A similar view ap-

pears to be held by Grassi ('89). All these authors base their conclusions solely on comparative anatomical data.

Other observers, including Weismann ('66), Huxley ('77), Uljanin, Kowalevsky ('73), Kraepelin ('72), Dewitz ('75) and Cholodkovsky ('91<sup>a</sup>) regard the gonapophyses as homodynamous with the true ambulatory appendages. Most of these authors adduce support for their views from the origin of the ovipositor during the larval and pupal stages. The ovipositor and sting have been traced in Orthoptera and Hymenoptera to two pairs of imaginal disks—one situated on the eighth, the other on the ninth abdominal segment. On the latter segment the pair of disks gives rise to a bifurcate or double pair of appendages. (Dewitz, Kraepelin, etc.) But the mere fact that these appendages arise from imaginal disks is not sufficient evidence of their homodynamy with ambulatory appendages, since the wings of the Metabola also arise from imaginal disks, yet cannot belong to the same category as the ambulatory appendages. The imaginal disks of the gonapophyses must be traced into the embryo and a connection clearly established between them and the embryonic appendages, before the view advocated by Huxley, Uljanin and others can be said to rest on a secure foundation. *Xiphidium* supplies this hitherto missing evidence. In this form there can be no doubt concerning the direct continuity of the embryonic appendages with the gonapophyses. One embryo which had just completed katatrepsis still showed traces of all the abdominal appendages. The pairs on the eighth, ninth and tenth segments were somewhat enlarged. In immediately succeeding stages the appendages of the second to sixth segments disappear; the pair on the seventh disappear somewhat later. Up to the time of hatching the gonapophyses could be continuously traced, since in *Xiphidium* there is no flexure of the abdomen as in other forms to obscure the ventral view of the terminal segments. From the time of hatching Dewitz ('75) has traced the development of the ovipositor in another Locustid (*Locusta viridissima*) so that now we have the complete history of the organ.

While there can be no doubt about the appendages of the eighth and ninth segments, which go to form the two outer

sheaths of the ovipositor or sting, the development of the innermost pair of blades is by no means so satisfactory. But whether this pair is only a portion of the ninth pair of appendages, as most authors claim, or represents the tenth pair of appendages, as I maintain, the main question at issue is in no wise affected; for it still remains true that the ovipositor consists of two or three pairs of modified ambulatory limbs.

In the male *Xiphidium* embryo it was claimed that the pair of appendages on the ninth segment persists to form the definitive styli; those of the eighth and tenth segments disappearing very early. The continuity of the styli with the embryonic appendages was quite as satisfactorily observed as the continuity of the ovipositor blades. Cholodkowsky has made an exactly similar observation on *Blatta* ('91<sup>a</sup>). The styli are, therefore, the homologues of the second pair of gonapophyses. Haase must therefore have gone astray in seeking to homologize the styli with the styloid processes, or "Griffel," for the styli are modified ambulatory appendages. Moreover, if my interpretation is correct, he cannot have found, as he claims, the evanescent rudiments of styli in young female Blattids, since the second pair of "anal palps" are the homologues of the styli (*vide* Huxley, '77).

#### VII. THE SUBCÆSOPHAGEAL BODY IN XIPHIDIUM AND BLATTA.

This structure, of which I have elsewhere ('92) given a brief preliminary account, makes its appearance in the *Xiphidium* embryo, in a stage a little earlier than F. The somites in the oral and thoracic segments are then established as closed sacs. The stomodæum is still a relatively shallow depression, and the entoderm-bands starting from its inner end have made but little progress. Sagittal (Fig. 61) and frontal sections (Fig. 62), through the heads of embryos in Stage F, show several interesting details. A pair of somites (*coe*) lie in the mandibular segment, and previous to this stage there was also a pair of small somites with indistinct cavities in the tritocerebral segment (*tc*). The planes of section in the two figures are such that the deutocerebral somites are not shown. A mass

of cells, the subœsophageal body, colored pink in the figure, extends between the œsophagus and the mandibular somites. The origin of this mass is obscure. It may arise from the ectoderm of the œsophagus, to the inner end of which it is attached (Fig. 61), or it may come from the entoderm (*en*). I believe, however, that it arises from neither of these sources, but from the mesoderm, which in a preceding stage formed the abortive somites of the tritocerebral segment. In frontal section (Fig. 62) the mass of cells is  $\Lambda$ -shaped, with the juncture of its two arms attached to the lower surface of the œsophagus. The distal ends of the arms are applied to the anterior walls of the mandibular somites. The separate cells are often sharply wedge-shaped and appear to be separated by clear spaces. They grow somewhat, lose their triangular outline and become more rounded. At the same time they tend to fuse in curved strings, with their broad edges applied to one another. This condition is seen in Fig. 63, which is taken from a section through the organ of an embryo in Stage G. The cytoplasm is now very granular, and has a distinctly yellow tint even in unstained sections; like the neuroblasts and germ-cells it absorbs picric acid with avidity. Vacuoles have begun to make their appearance, and the walls between adjacent cells are disappearing. The volume of the nuclei remains constant, but the cytoplasm enlarges up to the time of hatching. Fig. 64 is a part of a section through the subœsophageal body of a 7 mm. larva of *Xiphidium fasciatum*. The condition of the organ is essentially the same as at the time of hatching. The increase in volume of the cytoplasm is clearly shown. Instead of being granular, as in the younger stages, the protoplasm is now so filled with small vacuoles that it is reduced to a coarse reticulum.

In the subœsophageal body of a larva 9 mm. long, signs of degeneration have begun to appear. The small vacuoles fuse in the centres of the cells, leaving only the cell-walls as ragged envelopes. The nuclei become somewhat polygonal in outline. At this time the organ is found attached to the anterior ends of the salivary glands and to the large trunks which run forward into the head from the first thoracic tracheæ.

A section from a very young nymph (10 mm. long), is shown in Fig. 65. The cytoplasm of the fused cells is reduced to a ragged mass in which the irregular nuclei are suspended. Their chromatin is aggregated in rounded masses—a sign of advanced degeneration. In this stage the organ is much shrunken in size so that one is led to conclude that part of it has already been absorbed. In a little later stage the last traces of the organ have disappeared.

A subœsophageal body essentially like the one here described occurs also in *Blatta*. It, too, has the characteristic yellow tint. In his study of the development of *Blatta* Cholodkowsky appears to have seen this peculiar structure, though he regarded it as a portion of the fat-body. At page 52 ('91<sup>a</sup>), he says: "Die Entodermlamelle umwächst den Nahrungsdotter dorsalwärts und von allen Seiten; der Vorder- und Hinterdarm liegen nun ausserhalb des Nahrungsdotters und werden vom homogenen Dotter umspült, in welchem (besonders neben dem *Œsophagus*) kleine blasse Zellen liegen, die sich in Fettkörper zu verwandeln scheinen."<sup>1</sup> The organ is shown in Cholodkowsky's Fig. 68, Pl. VI. In other writers on insect embryology I find no mention of this interesting structure.

In the Rhynchota, to judge from a few observations on the embryos of *Zaitha fluminea*, the subœsophageal body occurs in a slightly modified form. Here it consists of a number of loose spherical cells lying on either side and a little below the œsophagus. The nuclei are large and spherical and the compact and finely granular cytoplasm has a distinct yellow cast. Though these cells vary in size (11–15 $\mu$ ) they are always larger than the cells of the surrounding tissues (6.3 $\mu$ ). Beyond this stage I could not trace the organ in *Zaitha* on account of lack of material.

The subœsophageal body may always be readily distinguished from the fat-body of the oral and more posterior segments by the peculiar structure and arrangement of its cells and by its yellow tint. I therefore regard it as an organ *sui generis*. It belongs to the category of embryonic or early larval organs,

<sup>1</sup> The italics are mine.

and this alone would suffice to distinguish it from the fat-body which persists throughout life.

It is perhaps premature to advance any hypothesis as to the function and morphological significance of the subœsophageal body, but I may call attention to its possible homology with an organ in the Crustacea. The researches of Viallanes and St. Remy go to show that the tritocerebral segment of insects is homologous with the second antennary segment of Crustacea. In the latter group of Arthropods this segment is provided with the green-gland, a structure which develops from the mesoderm and is generally regarded as a modified nephridium. The subœsophageal body, providing it arises from the mesoderm of the tritocerebral segment, may be all that remains of this same pair of nephridia in the cephalic region of insects.

#### VIII. TECHNIQUE.

*Xiphidium* eggs, like those of other Orthoptera are not easily sectioned in the younger stages, because their yolk bodies are rendered so brittle by the hardening fluids and are cemented together with so little protoplasm that they disintegrate during the process of cutting. After the appearance of the appendages the embryo may be readily dissected away from the yolk either in the fresh or hardened egg and mounted or sectioned by itself. In the study of the envelopes, where it is necessary to section the whole egg, the following method gives fairly good results :—

The eggs are taken from the galls and killed by being placed for about a minute in water heated to 80° C.<sup>1</sup> They are then transferred for preservation to 70 per cent alcohol in which they should remain for several weeks, if not months, in order to allow the yolk to harden and to shrink away from the chorion. The neglect of this simple precaution has led many to exaggerate the difficulty of studying insect eggs or to abandon them altogether. After remaining in the alcohol for some time, the chorion may be removed by tearing it

<sup>1</sup> Alcoholic picrosulphuric acid also proved to be an excellent killing reagent.

open at the broad pole and gently pushing against the narrow pole of the yolk with one needle, while holding on with the other to the chorion at the same pole. In the earliest and latest stages the chitinous blastodermic membrane comes off with the chorion, in other stages it adheres firmly to the yolk and prevents satisfactory staining. If aqueous stains like Orth's lithium carmine or Grenacher's alum carmine are used, the eggs should be left in them but a short time and carefully watched as the yolk-bodies have a peculiar tendency to absorb water till they lose the polygonal shapes they acquired by mutual pressure, finally swell and fall asunder. This is especially liable to occur in the younger stages when the blastodermic membrane is removed. I have as yet found no other insect egg with yolk capable of imbibing so much water. In Grenacher's borax carmine there is no swelling, a reason which has induced me to use this stain in preference to the aqueous solutions; though the two stains mentioned give excellent results if used with due precautions. After dehydrating and clearing with cedar oil, the eggs are kept from two to three hours in melted paraffine ( $55^{\circ}\text{C}$ ). Older embryos in which most of the yolk has been metabolized need not remain in paraffine more than an hour.

Embryos isolated from the yolk in the anatreptic stages, as well as later embryos used in sectioning, were stained in Czokor's alum cochineal. The bluish color of this stain is preferable to the borax carmine in serial sections, as it is less wearisome to the eye.

In the study of the entire embryo three different methods may be followed with advantage.

METHOD I. — The isolated embryo is stained with borax carmine, all excess of the stain is removed by prolonged immersion in acid alcohol, and the preparation mounted in clove oil or balsam. In such preparations many of the details of internal structure, such as the arrangement of the cœlomic sacs, may be very clearly distinguished. This method was very extensively used by Graber; in fact it seems to have been the only method which he employed for surface study. In this respect it is decidedly inferior to

METHOD II.—The hardened eggs or embryos, freed from their envelopes, are transferred from seventy per cent alcohol to Delafield's or Ehrlich's hæmatoxylin, in which they are left not longer than thirty or forty seconds. Then they are suddenly returned to seventy per cent alcohol, and a drop of twenty per cent HCl is allowed to fall through the alcohol onto the embryos, which almost instantly change color. As soon as they pass from a red to a salmon tint the fluid must be hastily removed and replaced by fresh seventy per cent alcohol, to which a trace of ammonia has been added. The nuclei gradually turn blue and throw the embryo out in bold contrast to the pale yellow yolk. In older isolated embryos, the stain faintly tinges the surface protoplasm, accentuates the shadows, and leaves all the sharp depressions unstained. When embryos thus treated are mounted in glycerin or balsam and examined with widely opened diaphragm and Abbé condensor under a moderately low power (about sixty diameters), the surface relief is exquisitely sharp and clear. The exact delimitation of the appendages, both permanent and evanescent, the tracheal orifices, cœnocytic invaginations, segments of the brain and nerve-cord, etc., may be traced with great precision, as the figures on Plate I will testify.

The method here given with several modifications of my own, was taught me by Dr. Wm. Patten, who has used it with great success in his studies of Arthropod development, more especially in his work on the brain and eye of *Acilius*. A very similar method seems to have been used by other investigators (*vide* Foster and Balfour, *Elements of Embryology*, 1883). Unfortunately, surface preparations with hæmatoxylin are not permanent, probably on account of the acid used to extract the stain. The color gradually fades, often disappearing completely in the course of a few weeks. I therefore prefer Czokor's alum cochineal, washing in water instead of acidulated alcohol. These preparations are nearly or quite as clear as the hæmatoxylin preparations and keep indefinitely.

METHOD III.—This is really only a compromise between Methods I and II. Embryos in the katatreptic stages are allowed to remain in Czokor's alum cochineal till the stain has

penetrated as far as but not into the yolk. They are then washed in water, dehydrated and mounted in balsam. The sexual ducts together with their ampullæ may be distinctly traced on the yellow background of the yolk and structures which lie just beneath the integument, like the œnocyte clusters and the nerve-cord, may be more readily studied than in specimens prepared by Methods I and II. The figures on Plate V and Fig. 10, Plate I were drawn from such partially stained embryos.

The methods here described give good results, not only with *Xiphidium* and *Blatta*, but also with all the other insects and crustaceans which I have examined.

The outlines of the figures in the plates were drawn with an Abbé camera.

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WORCESTER, MASS., May 10th, 1892.

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## EXPLANATION OF PLATE I.

*(Xiphidium ensiferum, Scud.)*

FIG. 1 (A). Surface view of embryo during gastrulation. *p.o.*, indusium; *pc.l.*, procephalic lobe; *bl.*, blastopore; *a.*, anal bifurcation of the blastopore; *ams.*, amnioserosal fold.

FIG. 2 (B). Surface view of embryo with amnioserosal fold closed over trunk-region. *pcl.n.*, neuroblast-centres on the procephalic lobes. *o.*, anterior widening of the blastopore. Remaining letters as in Fig. 1.

FIG. 3 (C). Surface view of embryo with the amnioserosal fold encroaching on the indusial thickening; *po.am.*, amnioserosal fold of the indusium; *z.*, pedicel temporarily uniting the indusium with the head; *at.*, antenna; *md.s.*, mandibular segment; *mx.s.*<sup>1</sup> first maxillary segment; *mx.s.*<sup>2</sup> second maxillary segment; *p.s.*<sup>1</sup>-*p.s.*<sup>3</sup> first to third thoracic segments; *a.s.*<sup>1</sup> first abdominal segment. Remaining letters as in Fig. 1.

FIG. 4 (D). Surface view of embryo just after the separation of the indusium from the head. Letters as in Figs. 3 and 1.

FIG. 5 (E). Indusium spreading over the yolk. View of embryo nearly completely submerged in the yolk, on its way to the dorsal surface. *tc.s.*, tritocerebral segment. Other letters as in preceding figures.

FIG. 6 (F). Surface view of elongate embryo on dorsal surface of yolk. *lb.*, labrum; *md.*, mandible; *mx.*<sup>1</sup> first maxilla; *mx.*<sup>2</sup> second maxilla; *p.*<sup>1</sup>-*p.*<sup>3</sup> the three thoracic appendages (legs); *coe.*, cœlomic sac of first abdominal segment showing through the body wall; *pl.* (*ap.*<sup>1</sup>), pleuropodium (appendage of the first abdominal segment); *v.*, yolk; *envl.*, cellular envelopes torn away from the ventral face of the embryo; *cc.* (*ap.*<sup>11</sup>), cerci (appendages of the eleventh abdominal segment).

FIG. 7 (G). Surface view of shortened embryo on dorsal yolk. *pc.*<sup>2</sup> second protocerebral lobe; *pc.*<sup>3</sup> third protocerebral lobe; *dc.*, deutocerebrum; *tc.*, tritocerebrum; *e.*, eye; *x.*, metastigmatic, or œnocyctic invagination; *ap.*<sup>4</sup> fourth abdominal appendage. Remaining letters as in Fig. 6 (F).

FIG. 8 (H). Surface view of embryo turning the lower pole of the egg. *sr.*, inner indusium functioning as the serosa; *am.*, amnion reflected back over the yolk and continuous with the membrane *sr.*; *at.*, antenna; *pl.*, right pleuropodium; *igl.*, intraganglionic thickening.

FIG. 9 (J). Surface view of embryo just after returning to the ventral face of the egg. *e.*, eye; other letters as in Fig. 8 (H).

FIG. 10 (K). Surface view of advanced ♀ embryo just before the secretion of the larval cuticle. *d.o.*, "dorsal organ"; *f.d.*, oviduct; *ta.*, terminal ampulla of oviduct; *op.*<sup>1</sup> (*op.*<sup>8</sup>), *op.*<sup>2</sup> (*ap.*<sup>9</sup>), first and second pairs of gonapophyses (appendages of the 8th and 9th abdominal segments). *cc.* (*ap.*<sup>11</sup>), cercus.

1 (A)



po  
pel  
bl  
a  
ams

2 (B)



po  
pelu  
o  
am  
bl

3 (C)



po am  
pel  
o  
al  
mds  
mx<sup>1</sup>  
mx<sup>2</sup>  
ps<sup>1</sup>  
ps<sup>2</sup>  
a<sup>1</sup>

4 (D)



po  
pel  
o  
mds  
mx<sup>1</sup>  
mx<sup>2</sup>  
ps<sup>1</sup>  
ps<sup>2</sup>  
a<sup>1</sup>

5 (E)



po  
pel  
d  
mds  
ps<sup>1</sup>  
ps<sup>2</sup>

6 (F)



pel  
lb  
at  
md  
mx<sup>1</sup>  
mx<sup>2</sup>  
p<sup>1</sup>  
p<sup>2</sup>  
p<sup>3</sup>  
coi  
pl (ap<sup>1</sup>)  
v  
cm<sup>1</sup>  
cc (ap<sup>2</sup>)

7 (G)



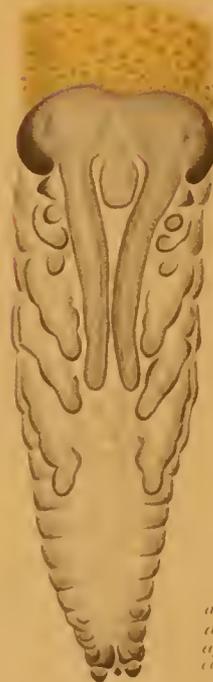
ps<sup>1</sup>  
ps<sup>2</sup>  
dc  
c  
lc  
lb  
md  
at  
mx<sup>1</sup>  
mx<sup>2</sup>  
p<sup>1</sup>  
p<sup>2</sup>  
pl (ap<sup>1</sup>)  
x  
ap<sup>3</sup>  
cc (ap<sup>3</sup>)

8 (H)



sr  
am  
tgi  
al  
p<sup>3</sup>

9 (I)



sr  
am  
tgi  
c  
ap<sup>8</sup>  
ap<sup>9</sup>  
ap<sup>10</sup>  
cc (ap<sup>10</sup>)

10 (K)



d  
tbl  
to  
ap<sup>11</sup>  
ap<sup>12</sup>  
cc (ap<sup>12</sup>)





## EXPLANATION OF PLATE II.

(Figs. 11 and 12, *Stagmomantis carolina*; Figs. 13 and 14, *Gryllus luctuosus*; Figs. 15-20, *Xiphidium ensiferum*.)

FIG. 11. Surface view of gastrula of *Stagmomantis*.  $\times 150$ . *p.o.*, rudiment of indusium?; *bl.*, blastopore; *ams.*, amnioserosal fold extending just over the edge of the oval germ-band.

FIG. 12. Outline of egg of *Stagmomantis* showing the position and relative size of the germ-band during gastrulation.

FIG. 13. Surface view of gastrula of *Gryllus*.  $\times 150$ . *ams.*, amnioserosal fold extending just over the edge of the germ-band; *bl.*, deeper posterior end of blastopore.

FIG. 14. Outline of egg of *Gryllus* showing the relative size and position of the germ-band during gastrulation.

FIG. 15. Surface view of a *Xiphidium* embryo during the closure of the amnioserosal fold over the mouth. Eight segments in the trunk. *p.o.*, indusium; *y.*, pale area between the indusium and the head; *pc.l.*, procephalic lobe; *ams.*, edge of amnioserosal fold. *o.*, stomodæum; *md.s.*, mandibular segment; *mx.s.*<sup>1</sup>, first maxillary segment; *n.g.*, neural groove; *s.*, serosal nuclei; *a.s.*<sup>1</sup>, first abdominal segment.

FIG. 16. Surface view of head during the separation of the indusium. Stage of Fig. 3 (C), Plate I more highly magnified. *p.o.*, indusium; *nn.*, shrunken nuclei of the indusium; *s.*, serosal nucleus; *z.*, pedicel connecting the indusium with the head; *pc.l.*, procephalic lobe; *lb.*, labrum; *o.*, stomodæum; *at.*, antenna; *tc.s.*, tritocerebral segment; *md.s.*, mandibular segment; *mx.s.*<sup>1</sup>, first maxillary segment.

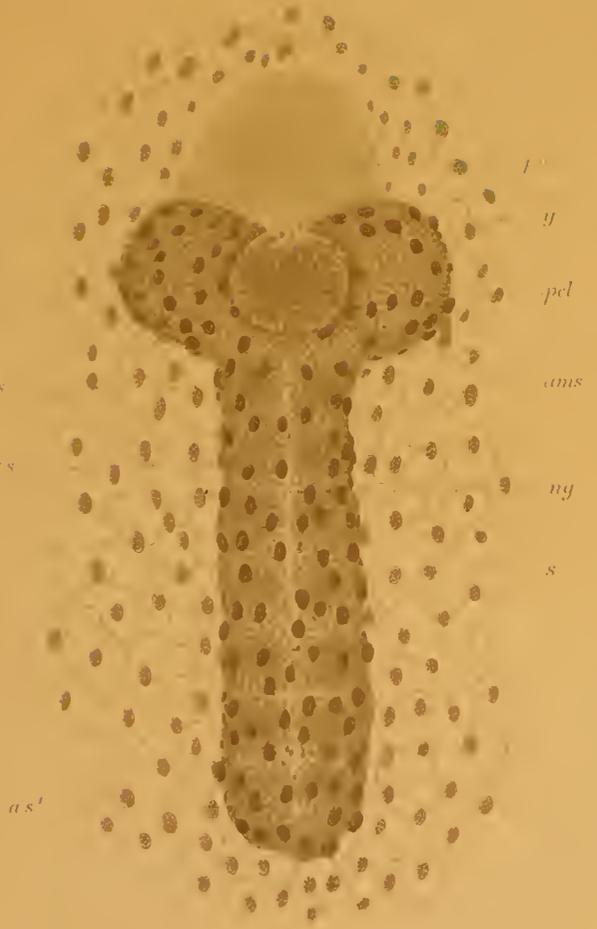
FIG. 17. Median transverse section through the indusium while still a simple thickening of the blastoderm (serosa).  $\times 230$ . *s.*, serosa; *p.o.*, portion of indusium with normal nuclei; *nn.*<sup>1</sup>, shrunken nuclei; *nn.*<sup>2</sup>, less shrunken nuclei; *d.*, thickened periphery of the organ; *v.*, yolk.

FIG. 18. Median transverse section of the indusium while the amnioserosal fold is closing over the disk.  $\times 230$ . Letters as in Fig. 17.

FIG. 19. Median transverse section through the indusium after the complete closure of the amnioserosal folds.  $\times 230$ . *am.*<sup>1</sup>, outer indusial layer; *p.o.*, inner indusial layer; *nn.*<sup>1</sup>, shrunken nuclei.

FIG. 20. Median transverse section through the indusium just after it has begun to spread.  $\times 230$ . Letters as in the preceding figures.

15.



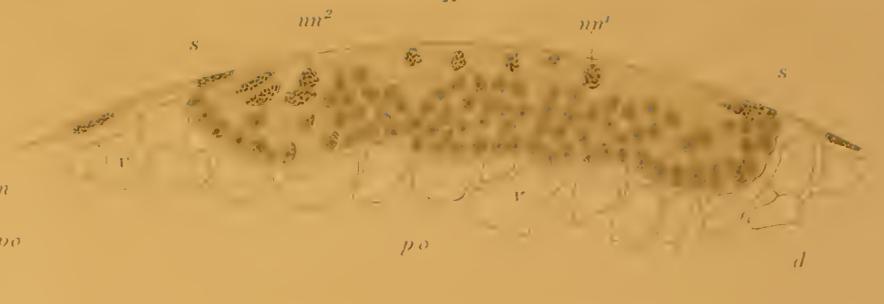
y  
 pel  
 ans  
 ng  
 s  
 as'

16.



nm  
 po  
 s  
 pel  
 o  
 at  
 tes  
 mds  
 mxs'

17.



s  
 m<sup>2</sup>  
 nm'  
 s  
 v  
 po  
 d

18.



s  
 s  
 v  
 po  
 v

20.



po  
 am  
 s  
 nm'  
 v

11.



po  
 bl  
 ans

12.



ans  
 bl

15.

14.

19.



po  
 am'  
 s  
 v





## EXPLANATION OF PLATE III.

*(Xiphidium ensiferum.)*

FIG. 21. Median longitudinal section through the head of an embryo over which the envelopes have just closed.  $\times 230$ . *o.*, stomodæum; *am.*, amnion; *s.*, serosa; *p.o.*, indusium; *z.*, pedicel uniting the indusium to the head of the embryo; *ec.*, ectoderm; *ms.*, mesoderm; *vph.*, vitellophag; *v.*, yolk.

FIG. 22. Median tranverse section of the indusium when it has reached half way round the egg.  $\times 230$ . *p.o.*, inner indusial layer; *am.*,<sup>1</sup> outer indusial layer; *s.*, serosa.

FIG. 23. Three normal cells from the indusium.  $\times 700$ .

FIG. 24. Three cells with shrivelled nuclei from the indusium.  $\times 700$ .

FIG. 25. Transverse section through basal abdominal region of an embryo passing to the dorsal surface of the yolk.  $\times 230$ . *nb.*, neuroblasts; *db.*, dermatoblasts; *am.*, amnion; *ms.*, mesoderm; *ec.*, ectoderm.

FIG. 26. Transverse section through first maxillary segment of an embryo passing to the dorsal surface of the yolk.  $\times 230$ . *ng.*, neural groove; *en.*, entoderm. Other letters as in Fig. 25.

FIG. 27. Transverse section through the second maxillary segment of an embryo in the stage of Fig. 6, (F) Plate I.  $\times 230$ . *mx.*,<sup>2</sup> second maxilla (trilobed); *mn.*, median-cord neuroblast; *ps.*, Punktsubstanz; *vph.*, vitellophag. Other letters as in Figs. 25 and 26.

FIG. 28. Transverse section through the mesothoracic ganglion of an embryo in a stage somewhat younger than G (Fig. 7, Pl. I).  $\times 230$ . *g.*,<sup>1</sup> younger offspring of the neuroblasts; *g.*,<sup>2</sup> older offspring of the neuroblasts (ganglion cells); *ecd.*, ectoderm; *mc.*, median cord ("mittelstrang"). Remaining letters as in preceding figures.

FIG. 29. Sagittal section through nerve-cord a little to one side of the median line. Embryo in Stage G (Fig. 7, Pl. I).  $\times 175$ . *md.g.*, mandibular ganglion; *mx.g.*,<sup>1</sup> first maxillary ganglion; *mx.g.*,<sup>2</sup> second maxillary ganglion; *ig.*,<sup>1</sup> *ig.*,<sup>2</sup> first and second interganglionic depressions; *p.g.*,<sup>1</sup> first thoracic ganglion; *p.g.*,<sup>2</sup> second thoracic ganglion; *mn.*, median cord neuroblasts; *mg.*, their progeny; *inl.*, inner neurilemma.

FIG. 30. Frontal section through the base of the abdomen of an embryo somewhat older than Fig. 6, (F), Pl. I.  $\times 175$ . *nb.*, neuroblasts; *mn.*, median cord neuroblasts in the intersegmental regions; *p.*,<sup>3</sup> metathoracic leg; *pl.*, pleuropodium; *ap.*,<sup>2</sup>-*ap.*,<sup>5</sup> second to fifth abdominal appendages grazed by the knife.

FIG. 31. Transverse section through the mesothoracic ganglion of an embryo in a stage between Fig. 9 (J) and 10 (K) Pl. I.  $\times 230$ . *hy.*, hypodermis (product of dermatoblasts); *nb.*, neuroblasts; *g.*,<sup>n</sup> latest progeny of the neuroblasts; *g.*, ganglion-cells; *inl.*, inner neurilemma; *enl.*, outer neurilemma; *ps.*, Punktsubstanz.







## EXPLANATION OF PLATE IV.

*(Xiphidium ensiferum.)*

FIG. 32. Transverse section through the head of an embryo in the stage of Fig. 5 (E) Pl. I.  $\times 230$ . *lb.*, labrum; *pc.*<sup>1</sup> (*o.g.*), *pc.*<sup>2</sup> *pc.*<sup>3</sup> first, second and third protocerebral lobes; *op.*, optic plate; *cn. ms.*, mesentoderm.

FIG. 33. Next following section to that represented in Fig. 32.  $\times 230$ . *nb.*, neuroblast; *am.*, amnion. Remaining letters same as in Fig. 32.

FIG. 34. Next following section to that represented in Fig. 33.  $\times 230$ . *st.*, stomodæum. Remaining figures the same as in Figs. 32 and 33.

FIG. 35. Transverse section through the labrum, in a stage intermediate between E and F (Figs. 5 and 6, Pl. I.)  $\times 230$ . *pc.*<sup>3</sup> third protocerebral lobe; *lb.*, labrum; *cn. ms.*, mesentoderm.

FIG. 36. Transverse section through labrum and brain of an embryo in Stage F (Fig. 6, Pl. I.)  $\times 230$ . *lb.*, labrum; *pc.*<sup>1</sup> *pc.*<sup>2</sup> *pc.*<sup>3</sup> first, second and third protocerebral lobes; *op.*, optic plate; *nb.*, neuroblast; *db.*, dermatoblasts; *coc.*, head-cælom; *ms.*, mesoderm cells; *igl.*, intraganglionic thickening; *st.*, stomodæum; *am.*, amnion.

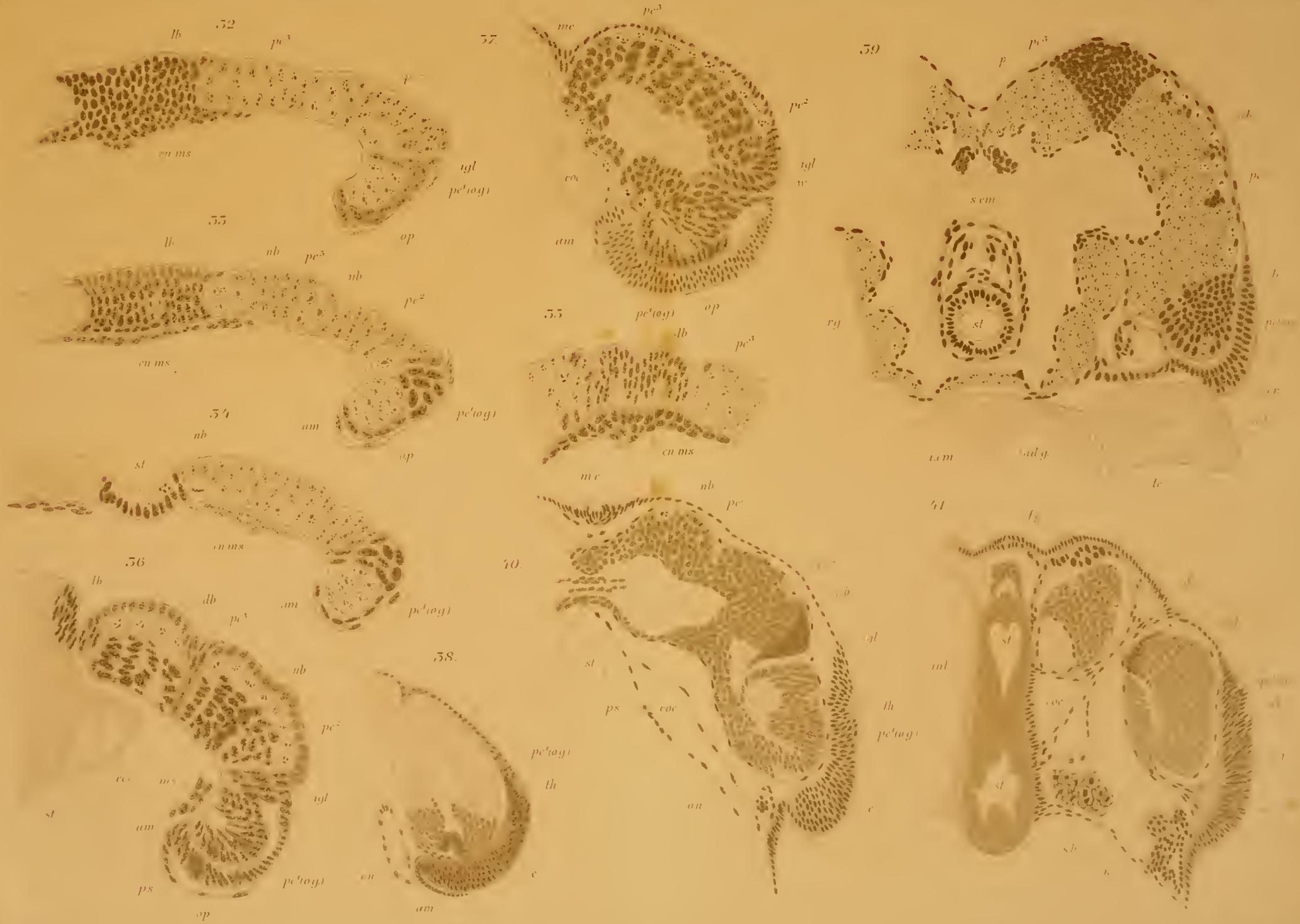
FIG. 37. Transverse section through prælabral region of an embryo in stage somewhat later than F (Fig. 6, Pl. I.)  $\times 145$ . *mc.*, median cord; *pc.*<sup>1</sup> (*o.g.*), first protocerebral lobe (optic ganglion); *w.*, orifice of involution of the intraganglionic thickening. Remaining letters as in Fig. 36.

FIG. 38. Transverse section through optic ganglion and optic plate of an embryo in Stage G (Fig. 7, Pl. I.)  $\times 145$ . *th.*, clear thickening in the optic plate; *e.*, eye; *on.*, optic nerve; *pc.*<sup>1</sup> (*o.g.*), optic ganglion; *am.*, amnion.

FIG. 39. Frontal section through the brain of an embryo somewhat older than I (Fig. 9, Pl. I.)  $\times 175$ . *p.*, problematical brain segment; *pc.*<sup>1</sup> (*o.g.*), optic ganglion; *pc.*<sup>2</sup> *pc.*<sup>3</sup> second and third protocerebral lobes; *dc.*, deutocerebrum; *tc.*, tritocerebrum; *e.*, eye; *md.*, mandible; *md.g.*, mandibular ganglion; *r.g.*, recurrent ganglion; *st.*, stomodæum.

FIG. 40. Transverse section of brain through the supraœsophageal commissure of an embryo in Stage K (Fig. 10, Pl. I.)  $\times 175$ . *nb.*, neuroblasts; *igl.*, interganglionic thickening; *th.*, clear thickening in the optic plate; *coe.*, head-cælom; *ps.*, Punktsubstanz; *on.*, optic nerve. Remaining letters as in Fig. 39.

FIG. 41. Transverse section from same series as that represented in Fig. 40, but passing through the frontal ganglion.  $\times 175$ . *dc.*, deutocerebrum; *f.g.*, frontal ganglion; *sb.*, subœsophageal body. Remaining letters as in Figs. 39 and 40.







## EXPLANATION OF PLATE V.

(*Xiphidium cusiferum*, Figs. 42-46, 48-50. *X. fasciatum*, Figs. 47 and 51.)

FIG. 42. Tip of abdomen in surface view from a ♂ embryo which has just passed the lower pole. (Stage J, Fig. 9, Pl. I.)  $t^5-t^8$ , fifth to eighth abdominal stigmata; *ts.*, testis; *m.d.*, vas deferens; *ta.m.*, terminal ampulla; *ap.*,<sup>8</sup> appendages of eighth abdominal segment; *st.* (*ap.*<sup>9</sup>), stylets (appendages of ninth abd. seg.; *ap.*,<sup>10</sup> appendages of tenth abdominal segment, in the cavities of which the ampullæ lie in this stage; *cc.* (*ap.*,<sup>11</sup>) cerci; *prd.*, proctodæum; *an.*, anus.

FIG. 43. Tip of abdomen in surface view from a ♂ embryo somewhat older than the one shown in Fig. 42. Letters same as in Fig. 42.

FIG. 44. Tip of abdomen in surface view from a ♂ embryo somewhat older than the one shown in Fig. 43. *v.*, yolk; *ag.*, last abdominal ganglion. Remaining letters as in Fig. 42.

FIG. 45. Vasa deferentia (*m.d.*), with their terminal ampullæ (*ta.m.*), from an embryo just before the development of the larval cuticle.

FIG. 46. Tip of abdomen of a ♂ embryo ready to hatch. Letters same as in Figs. 42-44.

FIG. 47. Tip of abdomen opened and seen from within from a ♂ larva 1 cm. long. *m.o.*, sexual orifice, *c.n.*, connectives; remaining letters as in Figs. 42-44.

FIG. 48. Tip of abdomen of ♀ embryo in a stage corresponding to that represented in Fig. 42. *ov.*, ovary; *f.d.*, oviduct; *ta.f.*, terminal ampulla; *m.d.*, vas deferens and terminal ampulla of the male type, still persisting;  $t^6-t^8$ , sixth to eighth abdominal stigmata; *ap.*,<sup>7</sup> persisting appendage of the seventh abdominal segment; *op.*<sup>1</sup> (*ap.*<sup>8</sup>), *op.*<sup>2</sup> (*ap.*<sup>9</sup>), *op.*<sup>3</sup> (*ap.*<sup>10</sup>), three pairs of abdominal appendages which become the gonapophyses (ovipositor); *an.*, anus; *cc.* (*ap.*<sup>11</sup>), cerci; *v.*, yolk.

FIG. 49. Tip of abdomen of ♀ embryo seen in full surface view in Fig. 10. (*K*), Pl. I. *mnb.*, location of median cord neuroblast; *cm.*, posterior commissure; *cn.*, connective; *ag.*, last abdominal ganglion. Other letters as in Fig. 48.

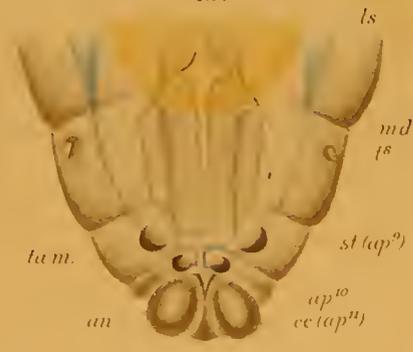
FIG. 50. Tip of abdomen of ♀ embryo ready to hatch. Letters same as in Figs. 48 and 49.

FIG. 51. Tip of abdomen of ♀ larva 1 cm. long, opened and seen from within; all the parts being dissected away except the reproductive organs. *vg.*, vagina; *op.*<sup>1</sup> (*ap.*<sup>8</sup>), *op.*<sup>2</sup> (*ap.*<sup>9</sup>), *op.*<sup>3</sup> (*ap.*<sup>10</sup>), three pairs of gonapophyses. Other letters as in Fig. 48.

42.



43.



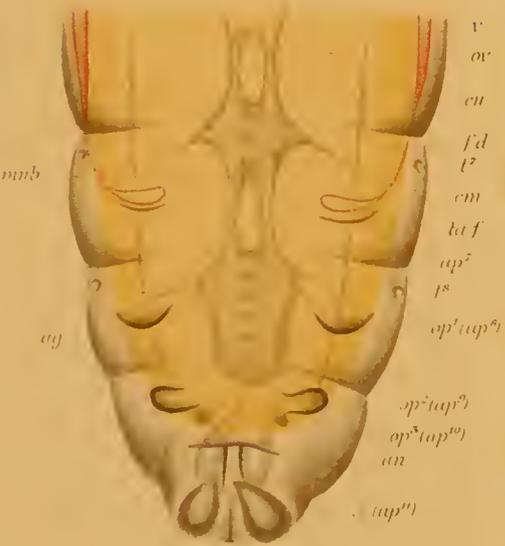
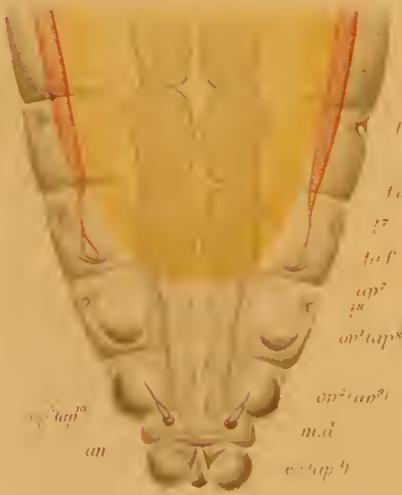
44.



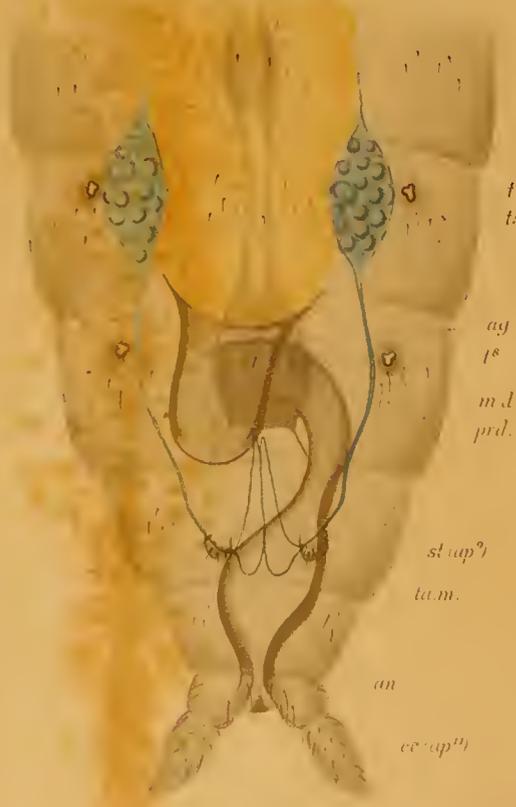
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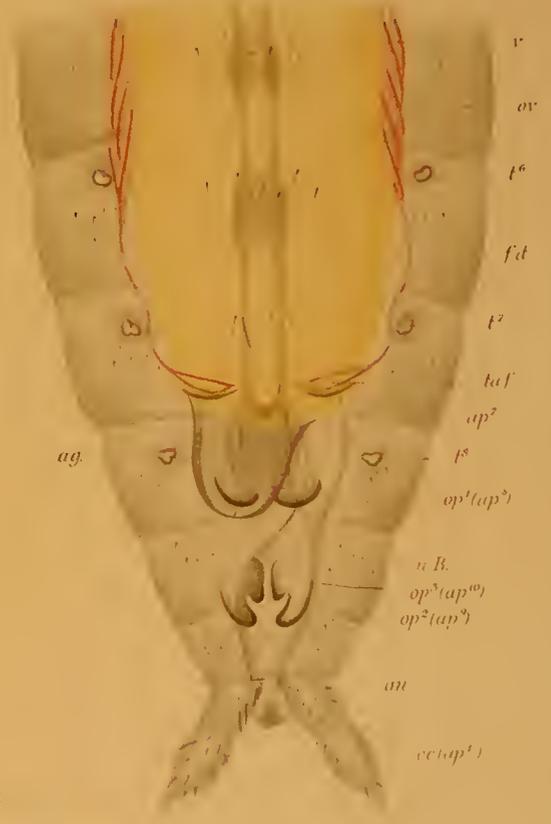
48.



46.



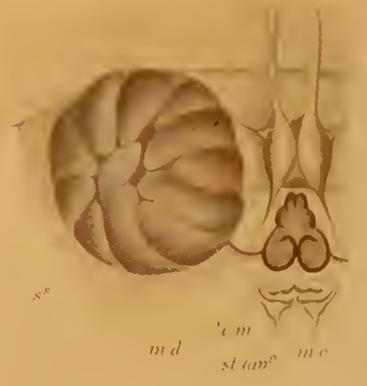
50.



51.



47.





## EXPLANATION OF PLATE VI.

(*Xiphidium ensiferum*, Figs. 52-63; *X. fasciatum*, Figs. 64 and 65).

FIG. 52. Frontal section through first to fourth abdominal segments, showing segmental arrangement of the gonads. Embryo in Stage F (Fig. 6, Pl. I).  $\times 230$ . *ml.*, longitudinal ventral muscle; *gd.*<sup>1</sup>*gd.*<sup>2</sup>*gd.*<sup>3</sup> gonads of first, second and third abdominal segments; *coe.*, cœlomic cavity; *ecd.*, ectoderm; *ep.*, epithelium.

FIG. 53. Transverse section through the third abdominal segment of an embryo in Stage F, (Fig. 6, Pl. I).  $\times 230$ . *nc.*, nerve cord; *am.*, amnion, *sms.*, somatic wall of somite; *spms.*, splanchnic wall of somite; *v.*, yolk; *en.*, entoderm. Remaining letters as in Fig. 52.

FIG. 54. Frontal section through the fourth abdominal segment of an embryo in Stage F (Fig. 6, Pl. I).  $\times 230$ . The diverticula point towards the head. *v.*, yolk; *gd.*<sup>4</sup> gonad of fourth abdominal segment; *coe.*, cœlomic cavity; *ep.*, epithelium.

FIG. 55. Section through a somite from the third thoracic segment showing a single enlarged germ-cell protruding into the cœlomic cavity.  $\times 230$ .

FIG. 56. Sagittal section through the end of the abdomen of an embryo in Stage G (Fig. 7, Pl. I). *tb.*, neuroteloblast?; *nb.*, neuroblasts; *coe.*<sup>7</sup>-*coe.*<sup>10</sup> cœlomic cavities of the seventh to tenth abdominal segments; *m.d.*, diverticulum of the tenth abdominal somite which becomes the vas deferens and its terminal ampulla; *gd.*<sup>10</sup> gonad in tenth abdominal segment (abnormal and atavistic); *nc.*, nerve cord in unflexed portion of abdomen.

FIG. 57. Transverse section through ninth abdominal segment of embryo represented in Fig. 42, Pl. V, cutting the tenth pair of appendages.  $\times 175$ . *msc.*, muscular tissue; *prd.*, proctodæum; *nc.*, nerve cord; *h.*, heart; *ecd.*, ectoderm; *m.d.*, vas deferens; *ta.m.*, terminal ampulla; *ap.*<sup>10</sup> appendage of the tenth abdominal segment.

FIG. 58. Transverse section of the abdomen of an embryo in the stage represented in Fig. 48, Pl. V. The greater portion of the section passes through the ninth, its anterior portion through the tenth abdominal segment.  $\times 175$ . *coe.*<sup>10</sup> cœlom of tenth abdominal segment; *bl.*, blood corpuscle; *ta.m.*, terminal ampulla of vas deferens; *m.d.*, vas deferens disintegrating; *op.*<sup>3</sup> (*ap.*<sup>10</sup>), third pair of gonapophyses; *nc.*, nerve cord; *prd.*, proctodæum; *ec.*, ectoderm; *h.*, heart.

FIG. 59. Transverse section through the seventh abdominal segment, taken from the same embryo as the section in Fig. 58.  $\times 175$ . *v.*, yolk; *en.*, entoderm; *bl.*, blood-corpuscle dividing; *h.*, heart; *coe.*<sup>7</sup> cœlom of the seventh abdominal segment; *f.d.*, oviduct; *ta.*, terminal ampulla; *oe.*, oenocytes; *ec.*, ectoderm; *nc.*, nerve-cord; *ap.*<sup>7</sup> appendage of seventh abdominal segment.

FIG. 60. Transverse section through the seventh abdominal segment of an embryo somewhat older than that in Fig. 48, Pl. V.  $\times 175$ . *ov.*, ovary; *mc.*, median cord; *ad.*, fat-body; *spms.*, splanchnic mesoderm. Other letters as in Fig. 59.

FIG. 61. Sagittal section through the head of an embryo in Stage F (Fig. 6, Pl. I).  $\times 175$ . *f.g.*, frontal ganglion; *r.g.*<sup>1</sup> first recurrent ganglion; *r.g.*<sup>2</sup> second recurrent ganglion; *v.*, yolk; *pc.*<sup>3</sup> third protocerebral lobe; *ms.*, mesoderm; *lb.*,

labrum; *st.*, stomodæum; *tc.*, tritocerebrum; *md.g.*, mandibular ganglion; *en.*, entoderm; *s.b.*, subœsophageal body; *coe.*,<sup>3</sup> cœlom of mandibular segment.

FIG. 62. Frontal section through the head of an embryo in Stage F (Fig. 6, Pl. I). × 175. *pc.*<sup>1</sup> (*o.g.*), optic ganglion; *md.*, mandible. Remaining letters as in Fig. 61.

FIG. 63. Transverse section through the subœsophageal body from an embryo in Stage G (Fig. 7, Pl. I.). × 500.

FIG. 64. Section through subœsophageal body of larva 7 mm. long. × 500.

FIG. 65. Section through the subœsophageal body of a nymph 1 cm. long. × 500.





# A CONTRIBUTION TO THE PHYSIOLOGY OF COLORATION IN ANIMALS.

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1. It is the purpose of the following pages to communicate some observations concerning one of the physiological causes of coloration in animals, the term coloration meaning the relative distribution and shape of the colored parts on the surface of an animal. The reader knows that we have to discriminate between two kinds of coloration in animals, (1) coloration determined merely by the structure of the superficial tissues, and (2) coloration produced by specific pigments. In the second case the pigment may or may not be exclusively contained in specific pigment cells, the chromatophores. It is my intention in this paper to deal with coloration produced exclusively by chromatophores.

For the physiologist the question arises which circumstances determine such an arrangement of pigment cells as to produce a fixed and typical design. There are two possibilities; the pigment cells are formed in the same place where they are found, or are produced irregularly all over the surface, and by secondary causes are forced to migrate to certain places and to gather there.

While studying the physiology of development in fish, I was struck by the tiger-like coloration of the yolk sac of *Fundulus*. The observation of the development of this coloration showed me that in this case the coloration is produced by a migration of the chromatophores from the place where they first appear to the blood vessels, and this migration seems to be the result of an attraction which the thin-walled blood vessels, or what is more probable their contents, exert upon the amœba-like chromatophores.

2. When the eggs of *Fundulus* are fertilized artificially, at a temperature of about 20°, as a rule in three days the circulation at the surface of the yolk begins, and at the same time

scattered chromatophores make their appearance. These chromatophores, the number of which increases steadily during the next days, do not show any special arrangement except that possibly their number is a little greater in those places where the vitelline arteries leave the body, and at a little distance from where the vitelline veins join the heart. There are two kinds of chromatophores, one containing a red and the other a black pigment, both sending out processes.

The processes of the black cells are comparatively few in number, whilst the red cells send out a much greater number of small processes. At first one finds these cells scattered in the lacunes between the yolk capillary vessels as well as spreading out partly over a blood vessel. About the fourth or fifth day, however, one can observe that many of the chromatophores which are near a blood vessel send out one or more processes on its surface. These processes follow the surface of the blood vessel very closely, and by no means spread out over its other side. This attachment to the surface of the blood vessel is most striking in those places where the blood vessel undergoes a bifurcation. Here the process of the chromatophore, rather than leave the surface of the blood vessel, undergoes the same bifurcation. This is at first more obvious in the black than in the red chromatophores, the former on the whole appearing more irritable. During the next days the number of chromatophores which send out processes to the blood vessels increases and the whole mass of those which had already stretched out their processes to a blood vessel creeps upon the surface of the latter. The striking feature in the chromatophores after having reached the blood vessel is the change in their dimensions. As a rule the diameter of a chromatophore before it comes in contact with a blood vessel is greater in every direction than the diameter of a capillary blood vessel. But as soon as it is on the blood vessel it stretches out in the longitudinal direction of the same in order to accommodate its whole mass on its surface. At the point of bifurcation of a blood vessel the chromatophore, as a rule, undergoes the same bifurcation. By the ninth day practically every chromatophore has crept upon a blood vessel; hardly a single chromatophore can longer be found in the gaps between them.

The blood vessels now have a covering of pigment which, however, is not uninterrupted, but shows many shorter or longer open spaces where the vessel is entirely free from pigment. It seems that the chromatophore remains at that point of the blood vessel to which it originally crept.

3. Therefore the history of coloration of the yolk sac in the *Fundulus* embryo seems to be as follows: The production of chromatophores takes place with the exception of a few spots in almost every part of the yolk sac. Whether the production of pigment and the origin of chromatophores is in some way dependent upon the formation of the blood and the blood vessels I cannot say; but as a matter of fact the chromatophores make their appearance at almost every point of the surface regardless of the blood vessels. As soon as a process of the chromatophores reaches the blood vessel the whole mass of the cell creeps upon it and remains there. This migration of the chromatophores reminds of the migrations of the white blood corpuscles. It is known that these blood corpuscles show a chemical irritability, a chemotropism. It may be that the chromatophores are also chemotropic, and that some of the contents of the blood vessels attract the chromatophores, or rather cause the protoplasm of the chromatophore to move toward and upon the blood vessel.

4. In order to find out whether the contents of the blood vessels attract the chromatophores I made the following experiment which is, perhaps, more interesting in itself than decisive for our question. I made the embryo develop without the heart-beat, to see if the migration of the chromatophores to the blood vessels would take place even without the blood circulating in the vessels. The embryo was put into sea water which contained a specific heart-poison, namely, potassium chloride. Five gr. of this substance were added to 100 ccm. normal sea water, after I had convinced myself that an addition of 3 gr. to 100 ccm. sea water is already sufficient to make the heart of a *Fundulus* embryo stop beating in a few minutes. The eggs segmented normally in a so strong solution of KCl, and quite a number developed into a normal embryo. I found it more favorable, however, to let the embryo first develop in normal sea water, and not put it in the poisoned sea water for

twenty-four hours after the impregnation. A small percentage of these embryos formed a heart, a circulatory system, arteries, veins, capillaries and blood corpuscles. But I was never able to find any sign of a heart-beat or circulation, although I watched the eggs continually. Nevertheless, these embryos kept on developing until the fourth day after the impregnation. A great number of chromatophores were formed, but not the slightest relation between these and the blood vessels was visible. In normal sea water by this time the first traces of migration were visible. It is my intention to give these experiments more detailed in another paper. As far as the causes of the orientation and migration of the chromatophores are concerned, they rather favor the idea that the stimulus for this orientation and migration is to be sought in the circulating blood.

5. From the above we conclude *that the typical coloration of the yolk sac of the Fundulus embryo is due to a specific, probably, chemical irritability of the chromatophores which are forced by this irritability to migrate to the blood vessels and gather on their surface.* If that be true we may expect that this same irritability also plays some rôle in the coloration of the embryo itself. I intend to study this question more in detail next summer, and will give here only a few facts which possibly answer the question affirmatively. It seems as if there were formed in the embryo two rows of pigment on either side corresponding to the cardinal veins. It may be, moreover, that the two dark spots which are formed, one near the tail, the other nearer the head of the embryo, correspond to the places where the vitelline arteries leave the body of the fish, and where indeed plenty of chromatophores are found. But be this as it may, it is important to remember that our explanation of coloration only can be applied in cases in which coloration is determined by chromatophores. And even in such cases we must not forget that chemical irritability is not necessarily the only kind of irritability of chromatophores. In certain animals and organs for instance, they are irritable by light. It is possible that in cases where blood vessels determine the orientation of pigment cells this only takes place as long as the walls of the vessels are sufficiently thin, as in the embryo.

## THE STRUCTURE OF THE LUNG.

W. S. MILLER.

THE difficulty in unraveling the various subdivisions of the bronchus of higher animals has made it impossible for us to gain a clear idea of the structure of the lung tissue. From a comparative standpoint it has also been impossible to gain light regarding the structure of the mammalian lung, mainly on account of the many variations in the different groups of animals. With these facts in view, I have attempted to rework the whole subject in question, guided by Dr. Mall and aided by a Fellowship granted by Clark University. I am also under obligations to Dr. Baur for most of the specimens of reptilian lungs.

Microscopic anatomy has told us that organs are built up of like component parts, and that when the structure of one of these parts is known, the structure of the whole organ is clear. Yet it is necessary to homologize these parts in different animals, and this has been one of my problems. In order to do this we naturally inquire into the evolution as well as into the development of the different lungs. When studied from these standpoints, the structure of the lungs becomes quite a simple affair, provided we employ methods to unravel the more complex lungs. This is made possible by the employment of the more recent methods of reconstruction, which were introduced by His, by Born and many others in embryology, and by Mall,<sup>1</sup> Spalteholtz<sup>2</sup> and myself<sup>3</sup> in histology.

The following methods were found to be of greatest value :

1. Injecting the lung with air or illuminating gas and then allowing it to dry.
2. Corrosion preparation in wax or in Wood's metal.

<sup>1</sup> Mall, Blut- u. Lymphwege, etc., *Abhandl. d. k. säch. Gesellschaft d. Wiss.*, Bd. XIV, 1887, and *The Blood-vessels of the Stomach, Johns Hopkins Hospital Reports*, Vol. I.

<sup>2</sup> Spalteholtz, His's *Archiv*, 1892.

<sup>3</sup> Miller, *Anatom. Anz.*, 1892.

3. Digestion in pepsin after the air-passages and blood-vessels had been injected with colored celloidin.

4. Reconstruction as well as free-hand modeling.

The first method is of greatest service in the study of the lungs of Amphibia and Reptilia. In these animals the large air-sacs permit a very careful study of the interior of the lung after it has been blown up, dried and cut open. The mammalian lung, however, is hard to dry, and the best specimens are of little value in the study of the finer structure.

Injections with wax and with Wood's metal proved of great value in the study of the blood-vessels, but are of little value in the study of the air-passages within the lobule. It is of great service in studying the bronchial tree of embryos which are sufficiently advanced to permit a cannula being tied in the trachea. But all that can be obtained with Wood's metal or with wax can be obtained equally well or better with celloidin. It is by all odds the most desirable method. The celloidin is to be colored with vermilion, Prussian blue or chrome yellow. After the vessels have been injected the organ may be placed in water for some time, and then the tissues are digested with pepsin and one half per cent. hydrochloric acid. For this latter procedure I am under obligations to Dr. Mixer of Boston. The digestion, of course, takes place best in the thermostat; and when the tissues are thoroughly softened, which often takes days, they are to be washed off in flowing water. In all cases I found that the corrosions are best preserved in a mixture of equal parts of glycerine, alcohol and water.

Direct modeling I found of great service in the study of small corrosions of the tips of the bronchi of embryos. For the more complex lungs the reconstruction method of Born was at last resorted to, by which, after devoting nearly two years to a single specimen, the desired light was obtained. The lung was first prepared by making a triple injection, by which the capillaries were filled with Prussian blue, the arteries with vermilion, and the veins with ultramarine-blue gelatine. A single lobule was then removed, imbedded in paraffine, and cut into sections 20  $\mu$  thick. The magnifying

power employed in reconstruction was one hundred, and each section was drawn on a wax plate two millimeters thick. As long as the terminal bronchus was in the section it was quite easy to locate all the air-cells from section to section. Beyond the terminal bronchus the location of the blood-vessels and the shape of the air-sacs served as guides. In this way all the air-cells communicating with one bronchus were drawn on wax plates, and the portions representing the air-spaces carefully cut out. The frame-work left, when piled up, gave an exact model of the air-sacs, and the pieces piled gave a "corrosion" of the same. The models were now cut in various directions in order to study the relation of the air-sacs to the terminal bronchus.

Throughout my study the relation of the blood-vessels to one another as well as to the air-passages has been constantly kept in mind, and it was found that they are distributed according to rule throughout the lung, and that the terminations of the bronchi are marked by sacs which have an arterial and a venous side. These I have termed the air-sacs. They are in turn studded with the air-cells, some of which are covered with arterial and some with venous capillaries. That the blood-vessels are related to the component parts of complex glands in this manner is by no means peculiar to the lungs, for it has been shown that the same exists in all organs in which the blood-vessels have been studied carefully.<sup>1</sup>

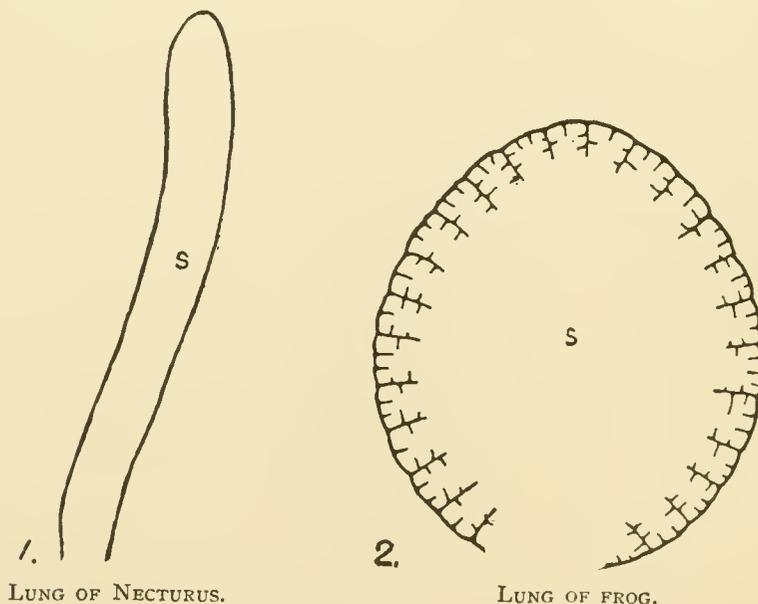
When lungs of different animals are placed side by side the various microscopic subdivisions in the more complex lungs must be homologized with the macroscopic subdivisions of the simpler lungs. Due to the many names which have been already employed in the description of the various portions of the lungs in different animals, the way in which I use the term "air-sac" may be confusing to the reader. It seems to me, however, that it would be more confusing if many new terms were introduced. The plan has been to call the terminal subdivision the air-cell; the first cluster of these the air-sac;

<sup>1</sup> I have already given a full history of the structure of the lung in the article "Lung" in *Buck's Reference Handbook of the Medical Sciences*—supplement volume. Wm. Wood & Co., New York, 1893.

and when a group of the sacs open into a common cavity with no distinct walls, I term it the atrium. From the stand-points of evolution and of development this may not be quite correct, for what is originally an air-sac in a simple lung is converted only in part into the air-sac of the complex lung; if the terminology is reversed, about the same holds true.

#### THE AIR PASSAGES.

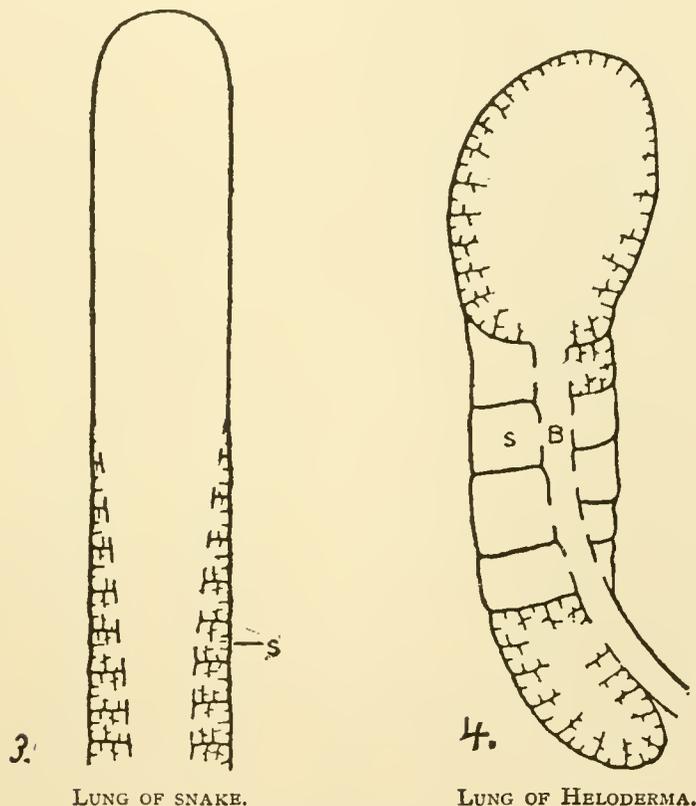
*Amphibian lung.*—Of the amphibians I have chosen for description the lungs of *Necturus maculosus*, the common mud puppy of the Mississippi Valley and the great lakes of the the West, and those of the common frog (*R. catesbiana*). In



*Necturus* the lungs consist of two elongated cylindrical-shaped bodies, the outer and inner surfaces of which are perfectly smooth (Fig. 1). Along the mesial side of the lung is seen the vein and on the opposite side the artery. Lateral branches are given off from each, which give rise to the capillaries. The distribution of the vessels is such, that we find an arterial twig placed nearly equidistant between two venous twigs.

In the frog (Fig. 2) the lungs are ellipsoid in shape and bluntly pointed behind. The exterior is smooth, but the inner surface is crossed by large bands which extend some depth into the central cavity. These form a coarse network, within

which smaller septa are found, to form a second and more delicate network. In this manner the inner surface is given a honeycombed appearance. We have in these two animals the extremes of the lungs of the amphibians; one smooth and the other honeycombed, but between them we find all degrees of division of the inner surface. The accompanying cuts give the general arrangement of the interior of the lungs of *Necturus* and of the frog. In the first the walls are perfectly smooth, while in the frog the septa, which are destined to break the



*S*, air-sac; *B*, bronchus.

lung into compartments in the higher animals, are already beginning to form.

*Reptilian lung.*—In the snake we find a single, much elongated lung (Fig. 3), perfectly smooth externally, and internally for the posterior two thirds of its length. The inner surface of the anterior third becomes more and more divided until we reach the anterior sixth, where we have the inner surface divided into many small sacs which communicate with the central cavity by nearly circular openings (Fig. 4).

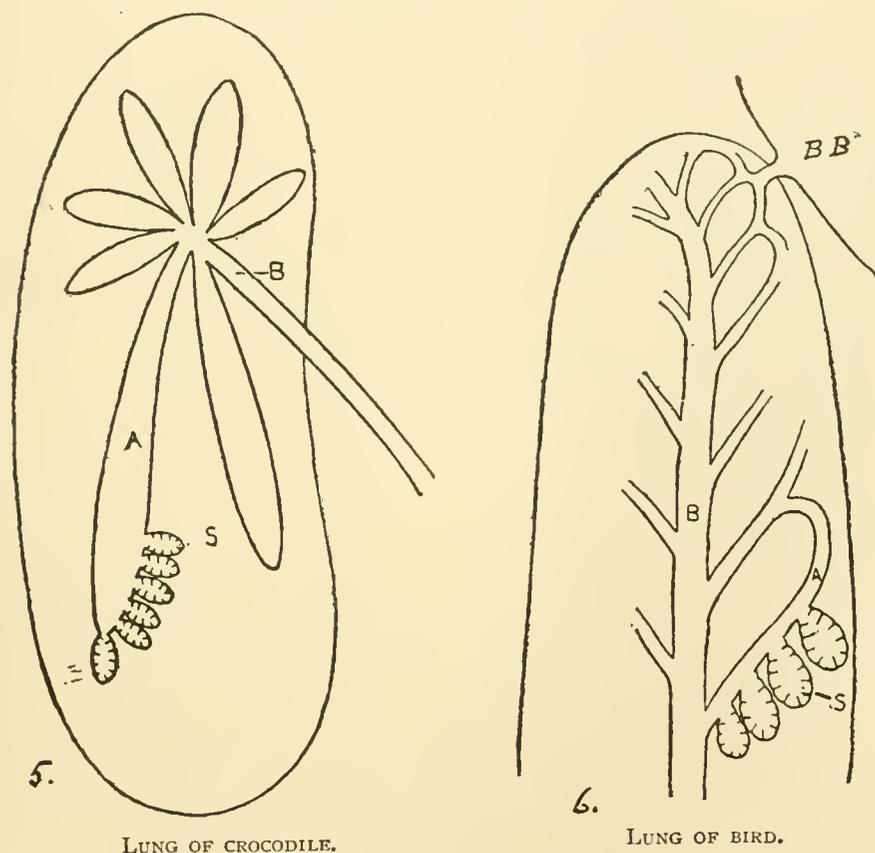
All the lungs examined thus far have a large central cavity, the inner surface of which is smooth or more or less subdivided; the bronchus opens into the anterior end, but is not continued into the interior. The central cavity is not yet divided into special cavities by partitions extending across it. The diagram in cut 3 shows the relation of this lung to that of lower animals. In many respects it is a continuation of the lung of *Necturus* and that of the frog. Even if we do view the snake's lung as degenerated, we can not fail to see that in that portion which is more complex it is slightly in advance of that of the frog. In the former the air-cells are already grouped in bunches forming the air-sacs. This grouping is more marked in other reptiles.

In some of the lizards (*Heloderma suspectum*) we find that this central cavity is divided into several smaller ones by means of partitions (Fig. 5). These smaller cavities or air-sacs are subdivided into air-cells, just as we have seen in the case of the frog's lung. The bronchus is continued into the lung (which it enters at its inner and anterior sixth) until it reaches the wall which divides the posterior sac from those lying in front of it; here it ends as a circular opening. This posterior sac is simpler in its structure than those lying anterior to it, just as the posterior end of the snake's lung was found to be simpler than the anterior. The subdivision of the inner surface of the lung begins at the anterior end and gradually proceeds backward. In general the whole lung can be viewed as somewhat more advanced than that of the snake. The air-sacs have encroached upon the central cavity until nothing but a small tube or semi-bronchus is left. I am not inclined to call this a bronchus, because it is perforated all along its walls with the openings of the air-sacs. It might be considered as a single atrium or third air-cavity which communicates with the bronchus. This third cavity is best seen in the crocodile.

The lungs of the turtles furnish beautiful gradations in their subdivisions into sacs, and in the complex division of these sacs. The posterior sac may be smooth like that of the *Necturus* lung, subdivided as in the frog's lung (Fig. 6), or it may attain the same degree of subdivision as the other sacs, a condition

which is found in Macrochelys. In Heloderma and the turtles each air-sac communicates with the bronchus by an oval or circular opening in its walls; this opening is the only one connected with the sac.

In the crocodile (Fig. 7) and alligator the bronchus enters the lung near its center, and passes somewhat obliquely into the lung until it reaches the junction of the lower and middle third; here it breaks up into eight to fifteen tubular passages. These tubular passages are studded with a great many air-sacs,



LUNG OF CROCODILE.

LUNG OF BIRD.

S, air-sac; A, atrium; B, bronchus; BB, air-bag.

as shown in cut 5. In these animals the lung for the first time gives the structure as it is found in mammals. There are many air-sacs which communicate with a common cavity, or atrium, all of which in turn communicate with a single terminal bronchus. A single lobule of the mammalian lung is simply enlarged to form the lung of the crocodile; the lung of the former is only a conglomerate of that of the latter.

*Avian lung.*—In many respects the bird's lung varies con-

siderably from that of the reptile or from that of the mammal. This difference seems to be so great that in the past it has been very difficult to compare them satisfactorily. As soon as the bronchus enters the lung it breaks up into a number of tubes, one of which is shown in Fig. 9. Each of these tubes rapidly breaks up into a number of pipes which lie in great part immediately below the surface of the lung and clasp it as tongs from all sides. These tubes are not real bronchi, because their structure is very different and because they do not end as do the terminal bronchi of mammals. In comparative anatomy they are known as *canaliculi aëriferi*, or air pipes. The pipes just below the surface of the lung anastomose freely, as shown in Fig. 9, and also send blind tubes into the interior of the lung, which meet but do not communicate with those from the opposite side. At points where two or more of the pipes unite there are large openings which communicate with the air-sacs or bags of the abdominal cavity, bones, etc. In order to avoid confusion, I shall speak of them as air-bags to differentiate them from the air-sacs of other lungs. The bags must be considered as an integral portion of the lung, for they are continuous with the air-pipes and are also developed directly from them.<sup>1</sup>

From each air-pipe, all along its course, the alveoli, or true lung structure, arise.<sup>2</sup> Now how shall this be brought into connection with the reptilian lung? It is very simple. The air-pipes are nothing more than the atria of the crocodile's lung, only that in the bird they are more turned upon one another and anastomose. From the air-pipes or atria, as I shall now call them, the air-sacs or proper lung substance arise. The whole is clear when cuts 5 and 6 are compared.

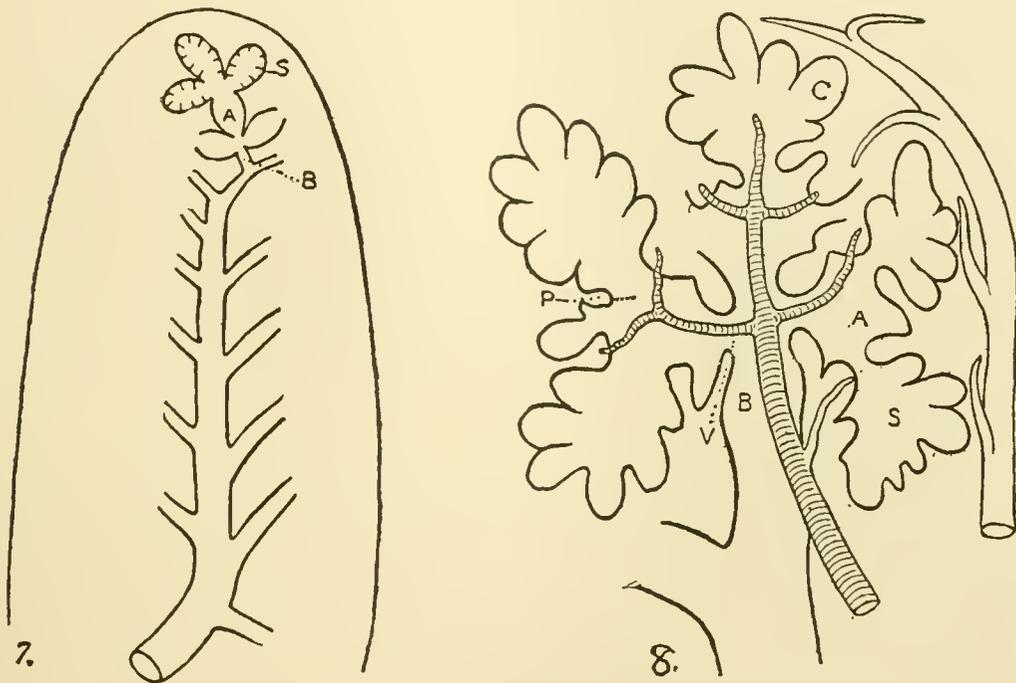
In the bird we have the important intermediate links between the reptile and the mammal (compare cuts 4, 5, 6, and 7). It is the first beginning of a branching bronchus. Up to the present we have had only a single terminal bronchus or a bronchus perforated along its sides. Up to the present we

<sup>1</sup> Weldon, *Proceedings of the Zoöl. Society*, 1883. Butler, *ibid*, 1889. Mall, *Journal of Morphology*, Vol. 5.

<sup>2</sup> F. E. Schultze, *Stricker's Handbuch*, 1871.

have seen that the lung becomes more complex by a continuous formation of septa, and the process of budding forms only a secondary rôle (Fig. 4). Now the budding begins to become more important and the septum-formation is secondary. This we must keep clearly in mind when we compare the development of the various lungs.

*Mammalian lung.*—In the study of the mammalian lung I have made use of specimens taken from the rat, rabbit, cat, dog, sheep and man; not only during adult life but also during the growing period and in foetal life.



MAMMALIAN LUNG.

TERMINAL BRONCHUS OF MAMMALIAN LUNG.

S, air-sac; C, air-cell; A, atrium; B, terminal bronchus; V, vestibule; P, air-sac passage. The artery is shaded, and the vein is in outline.

The last division of the bronchus, or terminal bronchus, before breaking up into the parenchyma of the lung, bears on its distal extremity a club-shaped expansion as shown in Fig. 11. From this expansion arise a number of passages which widen out into secondary expansions, and from the latter other passages lead out which open into central cavities set about with small irregular cells (Figs. 19 to 25). A good idea of this arrangement may be obtained if we compare it to a Pompeian house. The passage leading off from the terminal

bronchus is the "vestibulum" which opens into the "atrium." From this arises the "faux" or air-sac passage which leads into the "peristylum" or air-sac. This air-sac is set about with "cubacula" or air-cells. Cuts 7 and 8 fully elucidate these points.

From this description it will be seen that the air-sacs do not communicate directly with the terminal bronchus, as is usually described, but between each air-sac and terminal bronchus there is a cavity constant in all parts of the lung (Figs. 17, 18 and 19, pink color) which I have already termed the atrium.<sup>1</sup> The communication between the atrium and bronchus I have called vestibulum; that between the atrium and air-sac, air-sac passage, or, simply sac passage.

The terminal bronchus does not have a smooth cylindrical surface, but we find projecting from all portions small cells, the air-cells of the bronchus. The opening by which these cells communicate with the bronchus is surrounded by the smooth muscle fibres which line the bronchus. In sections of the lung we may recognize the terminal bronchi by their diameter, the thickness of their walls and the presence of smooth muscle fibres.

Leading out of the distal extremity of the terminal bronchus we find from three to six openings or vestibules. These are circular in outline, or nearly so; and the smooth muscle fibres lining the bronchus surround them, forming a sort of sphincter muscle. They have an average diameter of 0.2 mm. These openings do not all take the same direction (Fig. 19); usually one of them appears as though it were a continuation of the bronchus, while the others open out at various angles or may take a course nearly recurrent to that of the bronchus, as shown in Fig. 18. In sections which lie in such a plane that we look directly into the vestibules, the presence of smooth muscle fibres about the openings make them seem to be small terminal bronchi. They may, however, be distinguished from a bronchus by their diameters.

That the smooth muscle fibers found about the vestibules do not extend from the bronchus into the atrium, is shown in

<sup>1</sup> Miller, *Anatom. Anz.*, 1892.

sections which are cut in such a plane that a vestibule is divided at right angles. In such sections we find the bundles of muscle fibers, cut transversely, lying on either side of the divided vestibule, but they do not in the least enter into the formation of the walls of the cavity of the atrium.

The air-sac passages connecting an atrium with its air-sacs are somewhat smaller than the vestibules, having an average diameter of 0.143 mm. (Fig. 21). In sections the air-sac passages can always be distinguished from the vestibules by the absence of smooth muscle fibres.

The air-sacs present a great diversity of forms; they are very irregular and adapt themselves to the space they have to occupy. As seen in sections of the lung, they vary in diameter from 0.313 mm. to 0.511 mm. In Fig. 21 a corrosion model of an atrium with a single air-sac attached is shown. The irregularity of form is well shown in Fig. 22, which is a single air-sac removed from its atrium; it also shows a deep cleft nearly dividing it in half. The irregular contour of one air-sac fits into corresponding irregularities of the adjoining air-sacs. The walls of the air-sacs are quite thin and are made up of the capillary network of the blood-vessels, elastic tissue and reticulum. In sections lying parallel with, or perpendicular to, the pleura, the large irregular-shaped openings bounded by thin walls are the air-sacs. The small openings grouped about them are the air-cells.

The air-cells are about one fourth the size of the air-sacs; their walls are thin, and have the same structure as those of the air-sacs. There are three varieties of air-cells: those arising from the bronchus, those from the atrium, and those from the air-sac. Those arising from the air-sac are the more numerous, and are the most pronounced on its distal end. The air-cells arising from the atria and air-sacs have the same average diameter of 0.113 mm., while those air-cells which are found on the bronchus are smaller, having an average diameter of 0.047 mm. It is not uncommon to find in sections made perpendicular to the pleura that the plane of the section is such that it divides longitudinally a vestibule opening into an atrium. In rare instances it may pass through the vestibule, atrium,

sac-passage and air-sac. Usually it is quite difficult to determine the atria and air-sacs unless we have a complete series of sections to examine; I have already mentioned several points of distinction, but for general purposes attention to the following points will aid one to decide. The bronchi may be recognized by their containing smooth muscle fibres and by their diameter. Lying in more or less of a circle about the bronchus may be seen three, four or five openings nearly circular in outline and separated from the bronchus by a number of smaller openings; the first are the atria and the second are air-cells. On the outside of the circle of atria may be seen large irregular openings, the air-sacs, and grouped around each air-sac a number of smaller openings, their air-cells.

The above description shows that the lung of mammals is constructed after the plan of that of the birds, reptiles and amphibia. In general it may be viewed as a conglomeration of crocodile's lungs. In crocodiles, as in birds, these atria (they are not bronchi) give rise to many air-sacs. In the crocodile there is but one terminal bronchus; in birds several; and in mammals there are many. In both crocodiles and birds each atrium gives rise to many air-sacs; in mammals each atrium gives rise to but few air-sacs (see the various cuts). In reptiles the lung often shows a double structure; one or more air-bags and many air-sacs and cells. In birds this same combination is retained, only there are a great many air-bags extending far beyond the thorax, and in addition there is a communication of the atria with one another. In mammals the specialization is only single, *i.e.* atria with their air-sacs, but no air-bags.

From time to time it has been asserted that there is communication between the air-cells of adjoining lobules. This question I have already discussed in other papers. I think it sufficient to state that in careful reconstructions it is impossible to find any communication, and my corrosion preparations suggest the same. Moreover, it is impossible to inject a fluid from one bronchiole to a neighboring bronchus, which certainly could be done easily were there any communication between the air-

cells. As far as I have studied the subject, there is no secondary communication between adjacent air passages in any animal, excepting the bird.

#### THE BLOOD-VESSELS.

Besides the pulmonary artery and vein, we have to consider the bronchial vessels. I shall not consider the grosser vessels, for they have been described so often and can be demonstrated so easily by means of corrosion. The pulmonary artery follows the bronchus throughout its entire length, and when it reaches the last forked division of the bronchus it penetrates the lobule until it reaches a point beyond the terminal bronchus. Fig. 13 shows the relation of the artery to the bronchus just before the lobule is reached, while Fig. 15 includes also the vein. In all sections of the lung the bronchus and artery are side by side, while the vein is at all times as far away as possible from them.

After the terminal bronchus is passed, the artery divides quite abruptly into as many branches as there are atria, as shown in Fig. 25. From the atrial arteries the terminal arteries arise and supply the air-sacs. It is extremely difficult to demonstrate the real relation from the figures, as the relation is at once obscured by the many air-sacs. Cut 8, however, is an exact diagram from the corrosion specimen, reduced to a single plane. Each air-sac receives a single artery on its central side, *i.e.* the side over the center of the lobule, which at once breaks up into a rich capillary network, as shown in Fig. 16.

We have already seen that there are three kinds of air-cells, those arising from the terminal bronchus, those from the atria, and those from the air-sacs. Corresponding with this subdivision we also have three kinds of terminal arteries; those for the air-sacs, those for the atria and those for the air-cells arising from the terminal bronchus. This arrangement is of course what would be expected, for no doubt all the above subdivisions function alike and all must have an equal arterial supply. It does not seem as if any portion of the lobule is especially favored, because the small branches to the atria are smaller

than those to the air-sacs, and those to the air-cells of the terminal bronchus are smaller than those to the atria. None of the arteries seem to pass directly to the surface of the pleura, and it is very seldom that an artery passes to the periphery of the lobule. A cast of the terminal artery with all of its branches is given in Fig. 14. It was made by injecting with celloidin and then digesting the tissues in artificial gastric juice. The lobular artery, *L*, breaks up into the atrial arteries, *A*, which in turn give rise to the branches of the air-sac, *A. S.*

On reaching the air-sac the artery breaks up into small radicals which pass to the central side of the sac in the sulci between the air-cells, and are finally lost in the rich system of capillaries to which they give rise (Fig. 16). This net-work surrounds the whole air-sac and communicates very freely with that of the surrounding sacs. When two sacs adjoin each other a common net-work supplies both. In fact this is the rule throughout the lung. Not only are the air-sacs covered with this rich net-work of capillaries, but they also cover the atria and also those air-cells which arise directly from the terminal bronchus. The diameter of the capillaries is about  $7\mu$ , being slightly larger just beneath the pleura, and somewhat smaller deeper in the lung. In length they are slightly larger than their diameter, and the meshes of the net-work exceed, to a slight extent, the diameter of the vessels. After the net-work has extended over the whole air-sac or atrium to the peripheral side of the air-sac they begin to form larger vessels which are the beginnings of the veins.

The vein radicels collect the blood from the air-sacs rather than from the air-cells. They do, however, lie upon the cells of a sac rather than in the sulci between them. These radicels soon flow together, as shown in Fig. 16, and unite to form the air-sac vein. The veins lying on the peripheral side of the air-sac naturally lie between the lobules, and of course collect blood from the sacs of three distinct lobules. Were it not for the fact that the artery and bronchus lie in the center of the lobule and that there is more connective tissue between the lobules, it would be as easy to consider the vein in the center

and the artery on the periphery. This same statement may be applied to the liver. The veins keep on the periphery of the lobule (Figs. 17, 18 and 19), except in a few instances, when they send branches between the air-sacs to collect the blood from the terminal bronchus and the capillaries of the atria. All the larger veins run in the interlobular spaces, and are situated as far from the bronchus and artery as possible.

Considering the termination of the air passages and the arteries and veins, we have as histological unit of the lung, the *air-sac*. In all cases it has one artery and one vein which represent the termination of each system. The artery is on the central side of the sac, the vein on the peripheral, and between the two a rich capillary net-work, thus giving an arterial and a venous side to each air-sac.

*Bronchial artery.* — The distribution and termination of this vessel has caused much discussion on account of the difficulty in isolating it from the surrounding vessels. The coarser vessels are best separated by injecting them with gelatine in which there is suspended ultramarine blue. The granules do not pass into the capillaries. If, however, the capillaries which arise from this artery are to be injected, it is best to use Prussian-blue gelatine in which there are suspended granules of cinnabar. The blue passes through the capillaries and the cinnabar lodges in them, thus marking very definitely the direct path of the vessels.

The bronchial artery is distributed to the bronchus throughout its entire length, the walls of the blood and lymph vessels, and their connective tissue sheaths. There are two or more bronchial arteries for each bronchus and at no point do they anastomose with the pulmonary artery. Free injections into the bronchial arteries always flow into the pulmonary veins, and at no time into the pulmonary arteries unless the former are clamped when the backward pressure forces the fluid through the capillary into the pulmonary artery. The bronchial artery breaks up into capillaries which communicate quite freely with the pulmonary capillaries, but in general form quite large but short veins, which empty directly into the pulmonary veins. These small bronchial veins extend throughout the

length of the bronchus into the lobule. At this last point, the terminal bronchus, two small veins arise which are formed by the capillaries arising from the bronchial artery. These are on the opposite sides of the terminal bronchus and receive in addition a few small branches from the capillaries of the neighboring air-sacs, and in this way a short but quite large vein is formed which empties itself into the nearest pulmonary vein.

#### MEASUREMENTS.

In surveying the whole lung it was necessary to make many measurements and counts of the different parts in order to gain a clear idea of the ultimate histological unit. The measurements are all taken from dogs of about the same size, each weighing about seven kilograms. The measurements are in millimeters.

#### *Air Passages.*

	NUMBER.	DIAMETER.	AREA OF SECTION.
Trachea . . . . .	1	16. millimeters	201 sq. mm.
R. and L. Bronchi . . .	2	12.5 "	245 "
Lobar Bronchi . . . . .	6	7.6 "	272 "
1st Order " . . . . .	24	4.12 "	320 "
2nd " " . . . . .	164	2.44 "	766 "
3rd " " . . . . .	1,021	1.12 "	1,000 "
Lobular " . . . . .	16,000	.4 "	2,000 "
Atria . . . . .	64,000	.285 "	4,032 "
Air-Sacs . . . . .	192,000	.412 "	25,536 "
Air-Cells of Bronchi . .	320,000	.047 "	544 "
" " Atria . . . . .	320,000	.113 "	3,200 "
" " Air-Sac . . . . .	1,920,000	.113 "	17,280 "
Total of Air Cells . . .	2,560,000		21,024 "

*Blood-Vessels.*

	NUMBER.	DIAMETER.	AREA OF SECTION.
Pulmonary Artery . . .	1	15.5 millimeters	181 sq. mm.
R, and L. Branches . .	2	11.5 "	208 "
Lobar Arteries . . . .	8	5.96 "	223 "
1st Order " . . . .	24	3.96 "	293 "
2nd " " . . . .	164	2.26 "	656 "
3rd " " . . . .	1,021	1. "	801 "
Lobular " . . . .	16,000	.3 "	1,120 "
Atrial " . . . .	64,000	.165 "	1,344 "
Sac " . . . .	128,000	.165 "	2,688 "
Capillaries . . . .	600,000,000	.007 "	23,000 "
Sac Veins . . . .	192,000	.23 "	7,680 "
Atrial Veins . . . .	32,000	.45 "	6,098 "
Lobular Veins . . . .	16,000	.4 "	2,000 "
3rd Order " . . . .	1,021	1.22 "	1,194 "
2nd " " . . . .	164	2.44 "	765 "
1st " " . . . .	24	4.18 "	340 "
Lobar " . . . .	8	6.12 "	299 "
Venous Trunks . . . .	4	13.75 "	756 "

UNIVERSITY OF WISCONSIN, January, 1893.

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## DESCRIPTION OF PLATE VII.

FIG. 1. Lung of *Necturus lateralis*, inflated and dried. Natural size. The left lung is cut open; the right is in outline. The blood-vessels were injected, and the relation between the artery and vein is well shown.

FIG. 2. Lung of *Rana sylvatica*. Medium sized animal, prepared as Fig. 1. One half of the left lung is represented. The honey-combed appearance is well shown.

FIG. 3. Lung of *Bascanion constrictor*. Natural size; the posterior six centimeters are not shown. Note the smooth surface of the posterior portion; the gradual thickening of the wall in anterior portion, due to the gradual deepening of the air-sacs.

FIG. 4. A portion of Fig. 3.  $\times 5$  times. The appearance of the air-sacs as one looks into them, is clearly shown.

FIG. 5. Lung of *Heloderma suspectum*. Natural size. The position of the bronchus as continued in the interior of the lung is indicated by the red markings.

FIG. 6. Lung of *Graptemys lesneuri*. Natural size. The course of the bronchus within the lung is indicated by the blue thread. Note the simpler character of the posterior sac.

FIG. 7. Lung of *Crocodylia*; 87 cm. long. Natural size. A little below the center the breaking up of the bronchus into atria is shown. The two arrows at the upper part of the figure pass through air-sac passages and indicate the communication of two air-sacs with the elongated atrium. The finer openings are air-cells.

FIG. 8. A transverse section through the opposite lung of the same specimen as shown in Fig. 7. The plane of the section is through the terminal bronchus; the vestibules and a few of the atria are shown; also the air-sacs and air-cells.

Fig. 1.



Fig. 6.



Fig. 5.



Fig. 7.



Fig. 2.



Fig. 8.



Fig. 4.



Fig. 3.







## DESCRIPTION OF PLATE VIII.

FIG. 9. Corrosion specimen of the lung of the domestic fowl.

FIG. 10. Free hand model of the tip of the bronchus of the lower lobe of a fœtal cat just before birth.  $\times 18$  times.

FIG. 11. Wax corrosion of three terminal bronchi. The upper one shows the club shaped expansion ; the two lateral show the atria injected.  $\times 6$  times.

FIG. 12. Wood's metal corrosion of dog's lung. Shows terminal bronchi, atria and air-sacs.  $\times 10$  times.

FIG. 13. Celloidin corrosion of the artery and bronchus of a dog's lung. *A.* Artery ; *B.* Bronchus.  $\times 10$  times.

FIG. 14. Celloidin corrosion of artery of dog's lung. *L.* Lobular artery ; *A.<sup>1</sup>* *A.<sup>2</sup>* *A.<sup>3</sup>* *A.<sup>4</sup>* the branches within the lobule corresponding to the atria ; *A. S.* branches to air-sac.  $\times 10$  times.

FIG. 15. Triple injection with celloidin, digested in pepsin solution. The injection of the bronchus is not quite complete. *A.* Artery ; *B.* Bronchus ; *V.* Vein.  $\times 10$  times.

FIG. 16. Reconstruction from sections of an air-sac and its blood vessels.  $\times 50$  times. *A.* Artery ; *V.* Vein ; *X* cut surface from which another air-sac has been removed.

Fig. 13.



Fig. 10.

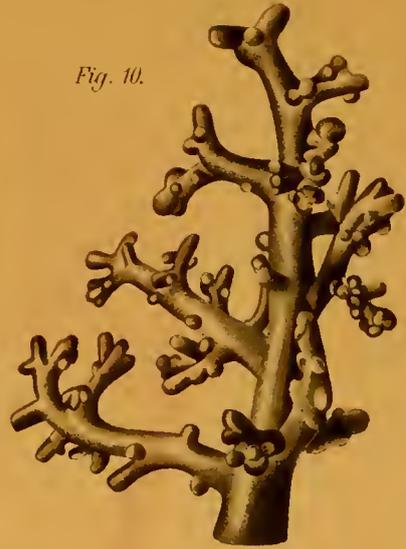


Fig. 11.



Fig. 16.



Fig. 12.



Fig. 15.

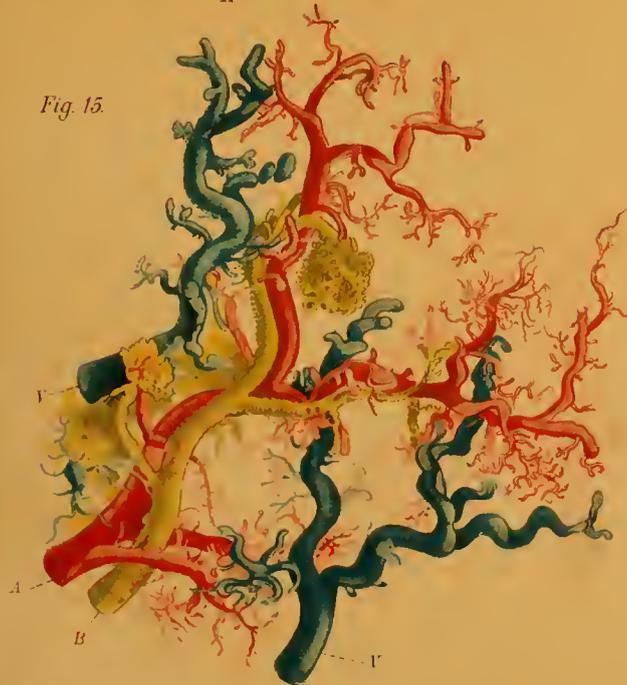
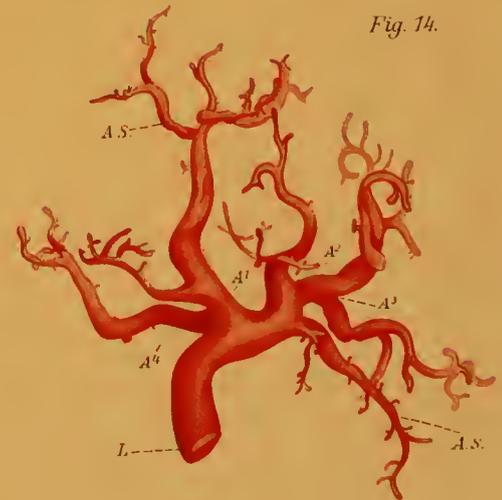


Fig. 9.



Fig. 14.







## DESCRIPTION OF PLATE IX.

FIG. 17. Reconstruction from sections of a lung lobule.  $\times 50$  times. The model is divided through its centre, and is viewed towards the pleura. The tip of the terminal bronchus is the colored yellow; the atria are colored pink; the air-sacs and air-cells are dark. The upper arrow indicates an air-sac passage leading from an atrium into an air-sac. The lower arrow passes through a vestibule into an atrium and its point may be seen in an air-sac.

FIG. 18. Reconstruction from sections of the lobule of the lung.  $\times 50$  times. Fig. 17 and 18 are the two halves of the same model which has been cut in half and its two cut surfaces exposed. The letters  $a$  and  $a$ , and  $b$  and  $b$  show how the two figures can be placed over each other. The lobule is divided in the centre and is the view towards the deeper portion of the lung. The colors indicate the same as in Fig. 17. The dotted line separates two lobules from each other, and just beneath it is the point where the bronchus bifurcates into the two terminal bronchi.

FIG. 19. The same as figure 17, showing only the terminal bronchus and the four vestibules which arise from it. Yellow, terminal bronchus; pink, atria.  $\times 33$  times.

FIG. 20. A portion of figure 17, in which only those air-sacs and air-cells just below the pleura are shown.  $\times 33$  times.

FIG. 21. A reconstruction corrosion of an atrium with a single air-sac attached.  $\times 50$  times.  $V$ . vestibule;  $A$ . atrium;  $P$ . cut air-sac passage;  $S$ . air-sac. A cast of an air-sac.  $\times 50$  times.

FIG. 22. A cast of an air-sac.  $\times 50$  times. The figure shows the irregular form of the air-sacs; also a deep cleft nearly dividing it in half. This form is quite common.  $P$ . air-sac passage;  $S$ . air-sac.

FIG. 23. Reconstruction corrosion of the lobule of the lung.  $\times 33$  times. At  $X$  a divided vestibule is seen. An atrium and its air-sacs have been removed.  $A$ . artery;  $V$ . vein.

FIG. 24. The atrium and its air-sacs which were removed from Fig. 23.  $\times 33$  times.  $X$  the divided vestibule.

FIG. 25. Reconstruction corrosion of the lobule of the lung.  $\times 50$  times. All the air-sacs of one side removed.  $B$ . terminal bronchus;  $A^1 A^2 A^3$  atria;  $P$ . air-sac passages;  $S$ . air-sac (the same as in Fig. 21);  $Ay$ . artery;  $V$ . vein.

Fig. 19.



Fig. 20.



Fig. 23.

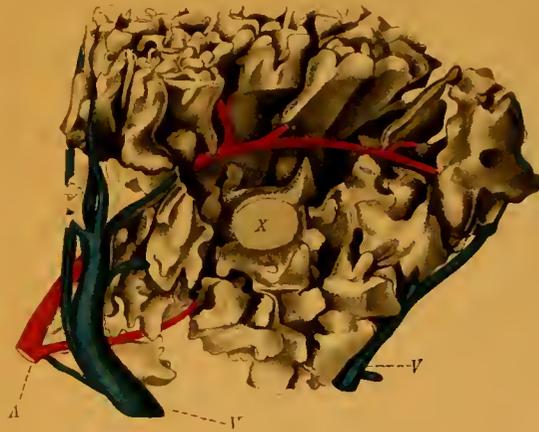


Fig. 24.



Fig. 17.



Fig. 22.



Fig. 18.



Fig. 21.

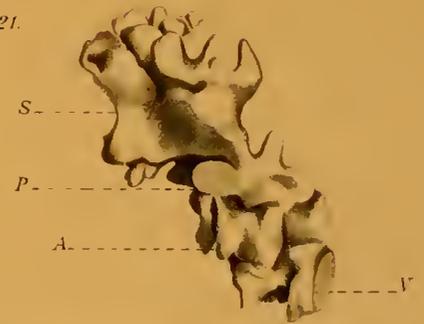
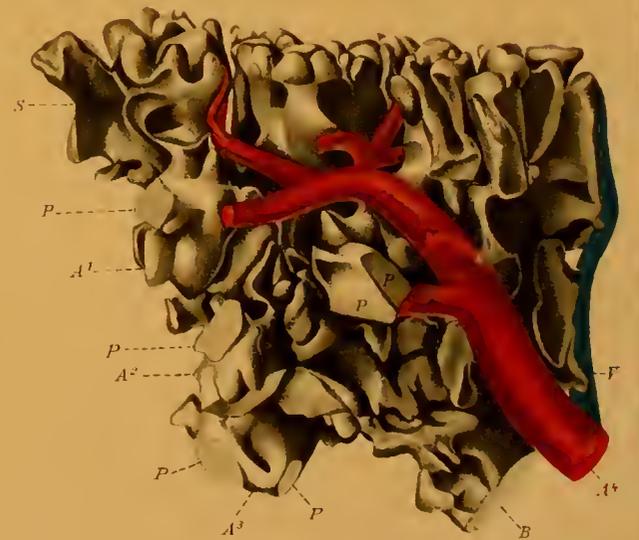


Fig. 25.





## THE DEVELOPMENT OF THE OPTIC VESICLES IN AMPHIBIA.

ALBERT C. EYCLESYMER.

NOTWITHSTANDING the searching observations made during the past score of years, but little has been found which would essentially modify the statements of the pioneer embryologists: that the eyes first appear as a pair of diverticula budding out from the sides of the anterior cerebral vesicle.

It should not be said, however, that we are wholly without observations pointing toward an earlier differentiation. Bischoff, Kölliker, His, Van Beneden and others have noted that in mammals the optic vesicles appear extremely early. Heape<sup>1</sup> finds in an early stage of the Mole, where the neural folds are closed along the center of the embryo, that "at the anterior end the floor of the neural groove, on either side, is swollen, and on the outer and anterior edge of the two masses a deep narrow groove indicates the commencement of the formation of the optic organs." Keibel<sup>2</sup> describes a like condition in the embryo of the guinea-pig. This apparently precocious development of the eyes in mammalia, showing no differentiation beyond the fact that depressions are present, is probably due to the retarded closure of the cephalic portion of the neural groove, and can scarcely be considered as a fact of phylogenetic significance. Whitman<sup>3</sup> discovered that in *Necturus* there is a very early appearance of the eye, "its basis being discernible as a circular area—after treatment with osmic acid, followed by Merkel's fluid—long before the closure of the neural folds of the brain."

Through the kindness of Professor Whitman I have been able to study the development of the eyes in *Necturus*, and

<sup>1</sup> *Quart. Journ. Micr. Sci.*, 1883, p. 106.

<sup>2</sup> *Arch. f. Anat. u. Physiol.*, 1889, p. 372.

<sup>3</sup> *Journal of Morphology*, 1889, p. 593.

find that at a stage (Fig. I) corresponding closely to that described, there is considerable differentiation, in that mitoses are more numerous in these regions, as well as a marked migration of the nuclei toward the periphery. This is the earliest indication I have been able to make out satisfactorily. In a few cases paired depressions have been found in earlier stages, even before the neural ridges have become prominent; but sections through these depressions show nothing in the way of histological differentiation, and one might justly consider them as nothing more than artifacts. A like condition occurs in *Amblystoma*, yet the cases are by far too rare to be of any special significance.

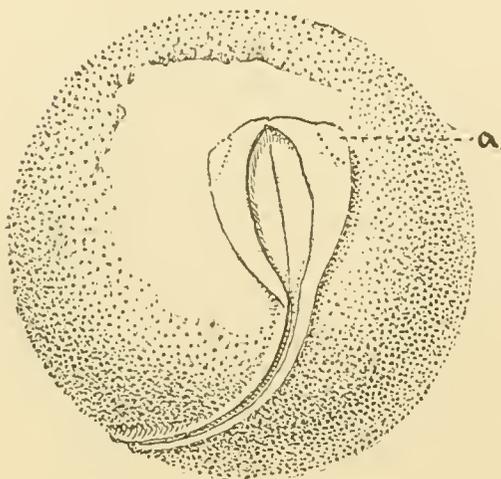


FIG. I.

Surface view of an embryo of *Necturus*.  
a. optic vesicles.

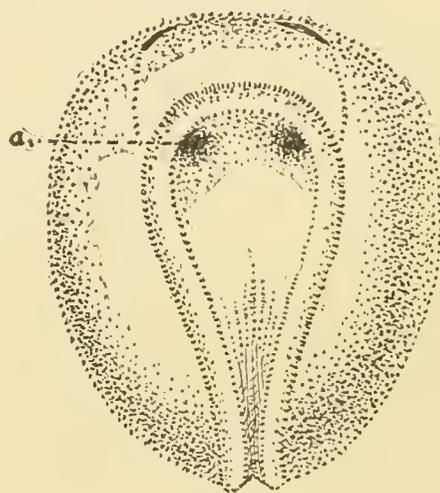


FIG. II.

Surface view of an early embryo of *Rana palustris*. a. optic pits.

The only thing which would dispel all doubt as to the meaning of these areas would be to find some form in which they are so well marked that they may be traced through the phases of involution of the neural plate to the future optic vesicles; this condition is perfectly fulfilled by *Rana palustris* (?) a form hitherto unstudied. For an abundant supply of material I am indebted to Dr. William M. Wheeler.

In an early embryonic stage (Fig. II) when the neural ridges are just forming and are widely separated, there appear in the anterior portion of the neural plate, on either side of the median

line and just within the cephalic fold, paired depressions (*a*) which are sharply contrasted with the surrounding parts by a much deeper pigmentation. A transverse section through these areas shows the condition represented in Fig. III. The hypoblast (*h*) is a single layer forming the roof of the mesenteron; in the median line a fold represents the anterior end of the chorda. The mesoblast (*m*) consists of a single layer of irregular scattered cells, which pass insensibly into the axial region, where all three layers are fused. The deeper layer of the epiblast has become much thickened, resulting in the formation of the broad epiblastic plates, which are united by the thinner median portion. In *Amblystoma* and *Necturus*

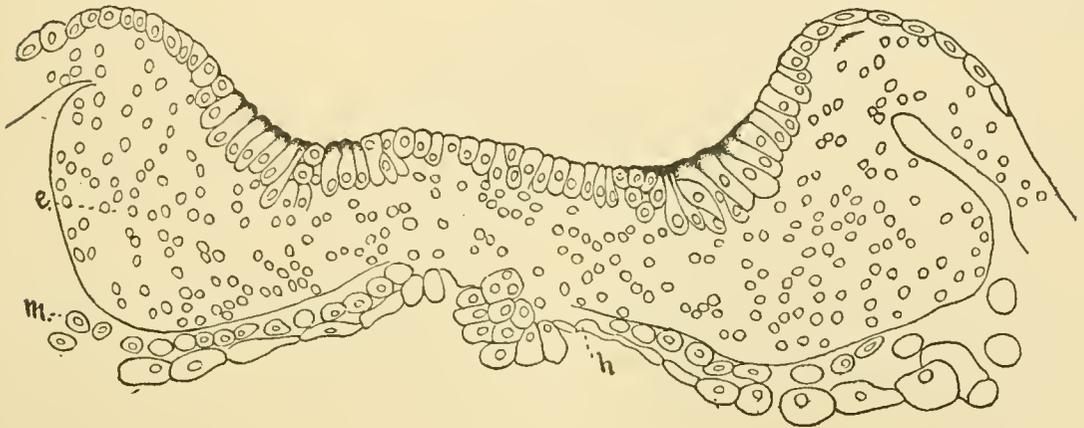


FIG. III.

Transverse section through optic pits. *h*. hypoblast. *m*. mesoblast. *e*. epiblast.

the superficial layer of the epiblast cannot be distinguished beyond the region of the neural ridge, while in *Rana* the two are very distinct, as shown in the figure, and it is in this layer that the optic pits are formed. In addition to the fact that these areas are sharply defined by the presence of pigment, to which more or less importance may be attached, they are further remarkable in that the elongation of the cells and the position of the nuclei are indicative of a considerable degree of histological differentiation. Between these areas the cells are undifferentiated, and resemble those of the superficial epiblast in other parts of the embryo. Sections posterior show the cells of this layer to be likewise uniform.

Fig. IV represents a section through a later stage when the folds are still widely separated. There is but little change in the histological character of the cells, except that their boundaries are less distinct.

Owing to division and loss of yolk, the cells of the embryo have gradually become smaller and more compact. The originally broad space enclosed between the neural folds has undergone a constant reduction in size, a rapid closing-in of the anterior portion has taken place, so that along the entire length of the embryo the folds are approximated. A section at this stage shows the condition represented in Fig. V. The canal is elliptical owing to the slight evagination of the optic areas which are the forerunners of the optic vesicles. Their

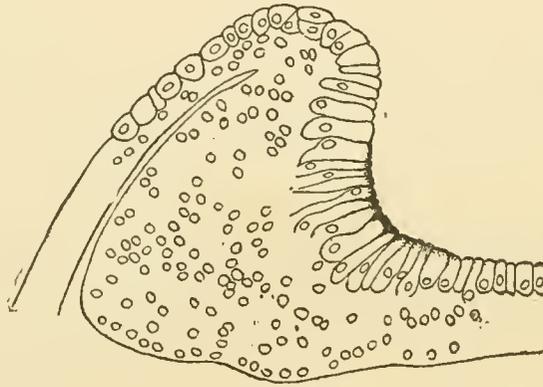


FIG. IV.

Transverse section through optic pits.

bilaterality is indicated only by the distribution of the pigment and one might justly consider them as derived from a common anlage. A marked migration of the pigment has taken place: instead of being located at the ends of the cells as in the earlier stages, it is found between them and nearer the periphery. The nuclei have likewise undergone a further migration toward the surface, so that the cells of the superficial layer have completely lost their identity.

It is of interest to note that in *Petromyzon* at a stage corresponding closely to the one above described, Kupffer<sup>4</sup> has observed that an unpaired basis for the eyes is present, as the

<sup>4</sup> Arch. f. Mikr. Anat. 1890, p. 510.

following quotation shows: "In der Mittelebene zeigt sich gar kein Merkzeichen, welches auf Duplicität der Anlage deutete, dieselbe ist vielmehr zunächst eine unpaarige. . . . Die später paarige Erscheinung des Organs wird nur darin angedeutet, dass in den lateralen Regionen der Erweiterung beiderseits Mitosen auftreten, die am Boden fehlen."

During the later stages of development the walls of the vesicles become thinner so that just before they invaginate

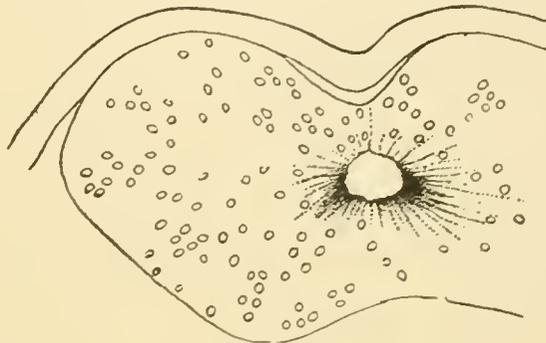


FIG. V.

Transverse section through optic vesicles.

to form the optic cups they consist of but a single layer of elongated cells with their nuclei located in their peripheral ends. A continued dispersion and migration of the pigment has also taken place, and is now more abundant in the stalk than in the portion which will later form the optic cup.

A more extended and detailed account, together with a discussion of the theoretical bearing of these facts, is reserved for a later paper.

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THE EMBRYOLOGY OF LIMULUS.—PART II.

J. S. KINGSLEY.

CONTENTS.

	PAGE
INTRODUCTION .....	196
THE MESODERM AND ITS DERIVATIVES .....	196
CÆLOM.....	197
COMPARISONS .....	200
NEPHRIDIA .....	203
COMPARISONS .....	206
MUSCLES.....	208
ENTOSTERNITE .....	209
ORGANS OF CIRCULATION .....	209
COMPARISONS .....	212
THE ALIMENTARY CANAL.....	214
MESENTERON .....	214
STOMODÆUM.....	217
PROCTODÆUM .....	219
COMPARISONS .....	219
THE NERVOUS SYSTEM .....	222
THE RESPIRATORY ORGANS .....	222
COMPARISONS .....	225
THE RELATIONSHIPS OF LIMULUS .....	227
THE CLASSIFICATION OF THE ARTHROPODA .....	247
BIBLIOGRAPHY .....	255
EXPLANATION OF PLATES .....	260

## INTRODUCTION.

THE preceding portion of this paper (this Journal, Vol. VII, p. 33) dealt with the habits, ovigenesis, the origin of the germ layers, and the development of the external form of *Limulus polyphemus*, together with some more general questions connected with the matters under discussion. In the present part the development of some of the organs is followed out, and in conclusion I have considered the bearings of the facts upon the systematic affinities of the Xiphosura and upon the classification of the Arthropods.

I would, before beginning, call attention to the fact that, disregarding the neuromeres, I have numbered the somites and the appendages, beginning with the first appendage. This may account for some apparent discrepancies later, and it also brings my article into harmony with those of other writers. At present we are in a transition condition, and no other course seems advisable. The matter of the neuromeres will be discussed in its proper place.

## THE MESODERM AND ITS DERIVATIVES.

As previously described the mesoderm arises by cell proliferation from a median longitudinal line on what will eventually form the ventral surface of the embryo. For this line of mesodermal formation the name primitive streak, borrowed from vertebrate embryology, seems especially appropriate. From this streak (*cf.* Figs. 42, 43) the middle layer grows outward on either side between ectoderm and entoderm (yolk), the process of differentiation beginning in front and gradually extending backwards, where the process continues for some time after it has ceased anteriorly and after the coelomic pouches have formed in the first somites of the body. This fact gives confirmation of the correctness of interpretation of anterior and posterior in the earliest embryos (*vide* '92, p. 47), a point upon which absolute certainty was impossible, since the features there described only become visible upon the employment of methods which coagulate the albumen and consequently kill the egg.

At first the mesoderm forms a continuous sheet across the egg, but one or two cells in thickness, and united to the ectoderm in the line of the primitive streak (Figs. 42, 43); and frequently there is also apparently a marginal connection between the two layers, the line of junction being indicated by a groove<sup>1</sup> (Figs. 42, 44). This line of union is apparently secondary, and I interpret it as a precocious differentiation of dorsal and ventral surfaces. I have seen no evidence of an augmentation of mesoderm by cell proliferation in this region.

With the appearance of metamerism the connection between mesoderm and ectoderm is lost, except in the stomodæal region (Fig. 43) and in the abdominal portion of the embryo where new cells continue to be added to the mesoderm for some little time. At first, after the separation of the two layers (Fig. 44), the mesoderm extends as an unbroken sheet, one or two cells in thickness, across the germinal area, the ectoderm outside this area losing its columnar character and becoming more flattened. As yet there is no appearance of coelomic cavities.

So far as my observations show metamerism obtains its first expression in the ectoderm. Thus Fig. 40 represents a longitudinal section to one side of the median line (where the coelom first appears) of an embryo, which in surface view showed differentiated cephalic and caudal areas, separated by a single somite. The boundaries of these regions are recognizable in the ectoderm of the section marked by arrows, while the mesoderm shows no corresponding metamerism.

CŒLOM. — Owing to difficulties of manipulation I have not been able to correlate surface views and sections, and so cannot say exactly how many somites are outlined when the coelom first appears. It is, however, preceded by a splitting of the mesodermal sheet into right and left halves,<sup>2</sup> a condition which is maintained (*cf.* Fig. 48) until after the appearance of the limbs. By this splitting are produced two mesodermal bands, the inner margins of which are undulating. As shown

<sup>1</sup> This has already been noticed and figured by Patten ('90, p. 375) who suggests a comparison with similar appearances in vertebrates.

<sup>2</sup> Except in oral and caudal regions.

by Fig. 45, which is a sagittal section through an embryo with seven differentiated somites, the mesoderm extends farther toward the middle line in a segmental than in an intersegmental area. Had the section passed further from the middle line the mesoderm would have formed a continuous sheet from head to caudal region.

The cœlom arises (Fig. 43) by a splitting of the lateral halves of the mesoderm, a pair of cavities being thus formed for each somite. These cœlomic cavities long remain distinct from each other, and the dissepimental walls persist until a later stage of development. The later history of the cœlom is, however, very difficult to follow on account of the subsequent appearance of numerous lacunæ in the mesoderm, which, so far as my observations go, have no connection with the primitive cavities which I would homologize with the cœlom of other forms. The cœlomic pouches appear in the six thoracic segments before they do in those of the abdomen (Fig. 46). An especially interesting fact is that there is no preoral cœlomic pouch, but the cavities of the first postoral somite send prolongations (Fig. 47) into the region in front of the mouth.

The walls of the cœlom differ in thickness. The somatoplure is usually several cells thick, while the splanchnoplure is only one cell in thickness and rapidly takes the form of a thin layer of pavement epithelium closely applied to the underlying yolk. A partial exception to this general statement occurs in the abdominal region where (Fig. 49) the somatoplure at first may also be a single cell thick. With the appearance of the limbs, as I pointed out in my earlier paper ('85, p. 532), the cœlom extends into these members, but it is soon excluded by the rapid growth of mesoderm in their interior. Of the fates of the cœlomic pouches I cannot speak with absolute certainty. The following is what appears to me the probable history. In all of the thoracic segments a portion (if not all) of the cœlom is gradually carried, with the advancing mesoderm, from the ventral on to the dorsal surface of the embryo. That a portion is thus carried is certain; but the rapid formation of lacunar spaces in the somatoplure renders

it impossible to say whether a portion is left in the ventral region of each segment. That a portion does so persist in the fifth segment will appear later.

Until after the splitting of the chorion only eight pairs of schizocœlia are produced, there being a pair for each<sup>1</sup> (Fig. 46) cephalothoracic and for the two anterior abdominal somites. At first these cavities are flat, and broader than long; the first pair, however, rapidly elongates and sends a diverticulum forward beneath the brain on either side of the œsophagus, into the preoral region. At first all of the cavities are distinct, and their walls epithelial in character, but soon it becomes difficult to follow their fate with certainty since a secondary splitting of the mesoderm soon produces a large number of anastomosing lacunar cavities (*e.g.* Figs. 53 *bs.*, 59 *lac.*, etc.) connected later with the vascular system and developing into the so-called "body-cavity" of the Arthropod, the existence of which much confuses the sections.

With the growth at first laterally, then dorsally and medially, of the two halves of the mesoderm, a portion (if not all) of the cœlom in somites II, III, IV, VI and VII, and a part of that in somite V, is carried towards the dorsal median line of the embryo, where, in the latest stages I have studied, the cavities, now run together, persist as a longitudinal tube (Figs. 63–67) beneath the pericardial sinus, on either side of the heart and its anterior arterial prolongation. Posteriorly this paired cavity does not at any time extend further back than somite VIII or IX. Whether in any of the somites all of the cœlom is thus carried to the dorsal surface I am unable to say, while I have not followed the fate of the cœlom of somite I. According to Kishinouye the anterior cœlomic pouches are pushed inward with the advancing stomodæum, and hence give rise to the splanchnopleure of the œsophagus and proventriculus. In somites I–IV, and also in somite VI, I am unable to recognize any ventral cavity as distinctively cœlom much later than the time when motion is seen in the appendages. In the posterior abdominal somites the cœlom persists

<sup>1</sup> According to Kishinouye there is no cœlom in somites II, III, IV of the Japanese *Limulus*.

on the ventral side until a later date, and is then apparently obliterated by a flattening of the cavities and a growing together of their walls. In the fifth somite, however, I can speak with more confidence,—for here the cœlomic cavities divide into two moieties, a dorsal and a ventral, and the latter remains upon the ventral surface and gives rise, in a way soon to be described, to the nephridia.

Concerning the fate of the dorsal portion of the cœlom I am uncertain. It persists, as has been said, as a perfectly distinct cavity with epithelial walls on either side of the central circulatory organ in the latest stages which I have studied. From its position and from its posterior termination I am inclined to think that this portion of the cœlomic epithelium is finally converted into the reproductive organs. In young *Limuli* an inch and a half in length I have found no traces of the cavity except as it might be represented in the gonads. I regret also that I have not been successful in tracing the history of the reproductive ducts. There are, however, so many lacunæ developed in the genital somite that I have not been able to follow the fate of the lower portion of the cœlom in that somite.

From this point the subsequent history of the mesoderm is best followed under the headings of the different organs, but before taking them up it is well to consider the facts already described.

*Comparisons.*—The only previous papers<sup>1</sup> dealing with the mesoderm of *Limulus* are those by Patten ('90), Kishinouye ('91, '92) and myself ('85), and Patten's remarks are only incidental to the discussion of an entirely different question. According to him there is a short slit-like invagination at the posterior end of the embryo, and from the walls of this inpushing much of the entoderm and mesoderm is produced, essentially as described above for mesoderm alone. Again

<sup>1</sup> Possibly an exception should be made made in favor of Dr. Packard's last paper, where ('85, p. 269) a few statements are made concerning this layer. He recognizes (Fig. 3) two cœlomic pouches in the region in front of the first pair of appendages; but his figures clearly show that he has not seen the true cœlom; the cavities described being either lacunar or artifacts. The cœlom does not exist in the plane of his section in the stage he has studied.

Patten describes an immigration of ectoderm cells from different portions of the germinal area ; and lastly, the middle layer receives further accessions from the marginal groove already referred to above. The cells of this marginal area are described as containing an extremely long, coiled, brilliantly refractile filament ; and some of these cells become elongated to form the dorsal muscles, the filament forming the longitudinal striation.

I have not seen, either in surface views or in sections, the slit-like invaginations described by Professor Patten ; I have seen no additions to the mesoderm from various points of the blastoderm, outside of the limits of the primitive streak ; I have not seen any cells, much less great masses of them, separate from the primitive streak and wander into and become scattered through the yolk ; and I have yet to see the peculiar origin of the dorsal muscle-cells which he describes. He figures (p. 374 Fig. 18 D, *pstr.*) a cylindrical rod of invaginative tissue, an appearance lacking in my sections, and refers to masses of entoderm cells at the inner end of the œsophagus which are quickly absorbed and which I am confident do not exist.

Kishinouye ('92) has the mesoderm in *Limulus longispina* arising from three sources :—from the cells forming the lower part of the blastoderm thickening ; from the primitive streak ; and from the yolk cells. The first portion forms the mesoderm of the cephalothorax ; the second the mesoderm of the abdomen ; the last probably the blood corpuscles. I must be permitted to express my scepticism upon this account of the origin and fate of the different portions of the middle layer. In the subsequent history however we are in close accord and only the points of difference need be noted. The order of the appearance of the mesoderm somites is the same and in them the coelom appears as schizocoelia. According to Kishinouye no coelomic cavities appear in somites II, III, or IV, and in the others they do not extend into the appendages. In both points the Atlantic *Limulus* differs ; cavities occurring in every postoral somite (Fig. 46) and at first extending into the limbs. In both species the coelom (or at least a part of each

cavity) migrates to the dorsal surface where it comes to lie at either side of the central circulatory organ. Kishinouye says it disappears before hatching. I do not find that it does so but find it persisting (*cf.* Fig. 87) after the assumption of the adult characters, for a portion of the length of the body. My belief that it gives rise to the gonads was referred to above.

Our knowledge of mesoderm formation in the Arachnida is deficient, but as far as it goes it agrees well with that of *Limulus*. Thus all accounts agree in the following: The mesoderm arises by a proliferation from a median ventral primitive streak, the proliferation continuing later in the posterior region than in front; next the mesoderm (+ entoderm in *Euscorpius*) separates from the ectoderm and forms at first a broad sheet across the ventral surface of the embryo; and, next, this sheet becomes divided into right and left bands connected behind; and into distinct somites in either half of the body. Later in each half of each somite a coelomic cavity is formed by splitting and these cavities extend temporarily into the corresponding cephalothoracic appendages. This will apply equally well to *Limulus*, but farther, differences in details are to be noted. Thus Balfour ('80, p. 174, Pl. XX, Fig. 13) describes cells from the yolk migrating into and adding to the mesoderm, while in *Euscorpius* (Laurie '90) the mes-entoderm is first differentiated from the ectoderm and then later it is divided into mesoderm and entoderm. From what we now know of Arachnid development I think it safe to regard the view of Balfour that the mesoderm was increased by migrations from the yolk as erroneous, certainly his figure ('80, Pl. XX, Fig. 13) does not require such interpretation, while the differences in scorpions is readily understood in connection with the peculiar features of the formation of the entoderm already discussed (Part I, p. 53 ff.)

Both Laurie (Scorpion) and Balfour (*Agalæna*) describe a prestomial coelom but without stating whether it actually belongs to the prestomial area or migrates to that region as in *Limulus*. Kowalevsky and Schulgin, on the other hand ('86) describe in the scorpion a preoral somite with a distinct cavity. Laurie has also studied the genital duct which according to him develops from the coelom of somite VII. Owing, however,

to its late appearance he is not certain as to its nephridial nature.

NEPHRIDIA (so-called *Coxal Glands*). — As has been described above, a portion of the cœlom of the fifth somite remains upon the ventral side of that somite, while the other cœlomic cavities are migrating toward the dorsal surface (Fig. 52). For a considerable time this cavity shows no changes worthy of remark, and first in about Stage G, (Fig. 32) is any modification noticeable. The cavity now begins to elongate and to become bent upon itself like the letter U, the rounded portion being directed anteriorly. The U now grows in length and, the posterior end being fixed, soon extends into somite IV. With this change in shape a differentiation in the epithelial cells becomes noticeable, most of them becoming cubical or columnar, while those upon the inner side of the inner end of the U retain their pavement character.

These changes, just before Stage H, produce the following results (Figs. 56–59): The cœlom (of somite V) is now divided into two portions, (1) a true cœlomic portion (the “end sac” of authors, Fig. 58 *nst.*) bounded by pavement epithelium on its inner side, and passing directly into the nephrostomial portion with columnar cells; and (2) the nephridial duct which passes forward (Fig. 57), as the proximal limb of the organ, to the loop (Fig. 56) and then backward, as the other limb (Figs. 57, 58, 59 *nd.*), to the posterior limits of somite V. As yet it has no connection with the exterior, but in the last section (Fig. 59) can be seen an inpushing of ectoderm (*no.*), which is to form its external aperture. In the various sections in the neighborhood of the coxal gland at this stage may be seen numerous lacunæ (*lac.*) in the mesoderm, which is rapidly assuming the trabecular condition characteristic of the later stages. I have never been able to certainly trace any connection between these lacunæ and the cœlomic cavity and am strongly of the opinion that none exists.

In Stage H (Fig. 54 *a-i*; Fig. 55, reconstruction) the same conditions are seen, except that the duct now opens to the exterior. We have here the end sac (*c* 5) which passes directly into the nephrostome without any sharp line of demarcation

(except that of change in the character of the cells) and this in turn into the two limbs of the duct. In this series of sections a peculiarity is seen, which in most cases does not make an appearance until a much later stage, *viz.* the formation of trabeculæ of mesodermal tissue which invade the cavity, and passing from wall to wall of the proximal portion of the organ tend to subdivide it and give it an anastomosing character.

The changes which have occurred up to the Stage I are represented in the horizontal sections (Figs. 61 *a-c*) and the sagittal section (Fig. 62). Figs. 61 *f* and *g* are reconstructions by projection of the whole series of which a few sections are represented in 61 *a-c*. The tube has now become more elongate, extending in front nearly to the anterior limits of somite IV, while the two limbs are relatively much more closely applied to each other than before. With this growth the regions are much more differentiated. The end sac (Fig. 61 *d*) is separated from the, now numerous, lacunæ of the mesoderm by a layer of pavement epithelium which in my series nowhere shows a break.<sup>1</sup> This cœlomic sac passes directly, as before, into the nephrostome (*nst.*) and this again into the duct, which shows no change, except increase in length, until in the distal limb, when about at the level of the end sac it becomes enlarged into an excretory vesicle or bladder, *cv.* (Figs. 60 and 61 *f, g*).

Later than Stage I, I have comparatively few notes which add to the information contained in my earlier paper ('85) and in the simultaneous one by Mr. Gulland ('85). One reconstruction, however, demands attention; that represented in Fig. 62. This is the nephridium in Stage K just before the molt in which the telson appears in the adult form. One error however is noticeable in it; the lateral amplification is too great and consequently the diameter of the tubes and the extent of the outgrowths are exaggerated. In this the same parts are recognizable as before but they have undergone some modifications. Thus in the region of the end sac a fenestration is

<sup>1</sup> In my earlier paper I was in doubt upon this point and thought that possibly ('85, p. 535) I had found such an opening, which led to the reconstruction of the tube with an internal funnel. Still I was not positive. I now feel confident that the opening of that paper was an artifact.

observable, the beginning of the anastomosing condition of the adult (*cf.* Fig. 55); while the proximal (internal) limb is thrown into a series of four, outwardly directed diverticula which are segmentally arranged and which occupy somites two to five. The external (distal) limb shows fewer modifications, the chief being that the excretory vesicle is relatively smaller than before; the external opening still persists and I have not found out when it closes. This figure corresponds quite closely to Gulland's ('85) Fig. 3, except that in his reconstruction the outgrowths from the proximal limb are represented as taking a sagittal direction and the anastomosing character of the inner end is carried still farther, a condition doubtless due to the older stage with which he worked.

Although I have not followed it out the appearance at this stage gives countenance to the view that the whole organ of the adult is derived from the cœlom of somite V and that the apparently metameric lobes figured by Packard ('80, Pl. III, Fig. 7, copied by Lankester '84 p. 153 Fig. 3) are not the remnants of the nephridia of the corresponding somites but are rather the derivatives of the diverticula shown in my reconstruction. Besides an increase in the size of the lobes all that is necessary to convert my reconstruction into the "coxal gland" of the adult are closure of the external opening, more or less complete fusion of the two limbs of the duct, accompanied by an increase in the anastomoses, the result being to convert cœlom and duct into the spongy tissue of the adult, the whole organ being a series of anastomosing tubes, the "cæca" of Lankester. This view of the morphology of the organ receives confirmation from the fact that I have seen no fusion of cœlomic spaces two to five while Kishinouye, as already stated, finds no cœlom at all, in the anterior somites which are later invaded by the coxal gland.

A word as to the external opening of the nephridium. Gulland ('85, p. 513) states that it is "at the base of the coxa of the fifth limb on the side next the fourth appendage and on the dorsal surface." I find upon repeated examination that it is rather upon the posterior side of the coxa of the fifth pair of legs, as I stated it in my earlier paper ('85).

*Comparisons.* — The foregoing account differs in some points from that which I gave in 1885. I then failed to recognize the genetic connection between the nephridium and the cœlom and also failed to recognize the closed condition of these organs. The history of our knowledge of the nephridia in *Limulus* can be briefly summarized.

These organs were first noticed by Packard ('75<sup>a</sup>) who from their histology and by exclusion, concluded that they were renal in function and homologous with the green gland of Crustacea. Five years later ('80) he redescribed and figured their adult structure and suggested a comparison in their position with the shell gland of the Entomostraca. Lankester in 1882 described these organs and compared them with the coxal glands of the scorpion and later ('84) gave the histology with some detail. In both papers he was inclined to compare them with the green gland of the Crustacea but still admitted the possibility or even probability of their being ectodermal or entodermal with a frame work of "skeletotrophic tissue." In the next year Gulland ('85) described the organ in young specimens while Kingsley at the same time gave an account of the early history of the organ, comparing it exactly with the shell gland of the Entomostraca and claimed that it and the genital ducts of both Crustacea and Arachnida should be regarded as Nephridia. In 1890 Kingsley stated that the coxal gland of *Limulus* was derived from the cœlom of somite V, that it terminated cæcally and gave in outline the above account of the origin of the segmentally arranged lobes. Kishinouye ('91<sup>a</sup>) stated that in *Limulus longispinus* the coxal gland is formed from the ventral part of the cœlom of somite V and later ('91<sup>b</sup>) describes the method more at length. His account agrees well with the foregoing excepting that the outgrowths of the metameric lobes occur at an earlier stage and the end sac (his "funnel") is in the mesoblastic dissepiment between the fifth and sixth appendage bearing segments.

All others who have had occasion to refer to the nephridia of *Limulus* have used the material included in the papers enumerated above. This is true of Eisig ('88) Loman ('88) and Sturanay ('91).

Of the coxal glands of the Arachnids we know considerable concerning the adult structure and little about the development. They have been found in Scorpions, Phalangids, Solpugids, Acarina and Araneina. They occur in either somite III or V or they may co-exist in the same individual in two somites at the same time. Usually the external duct becomes closed, as in *Limulus*, at an early date but in Phalangids (Loman '88) it remains open through life.

Laurie ('90) describes the development of the coxal gland in *Euscorpius italicus*. In the youngest stage studied it is a simple straight tube opening distally to the exterior and proximally by a funnel to the cœlom of somite V, which is a much larger space than in *Limulus*.<sup>1</sup> In the next stage the duct becomes bent on itself so that it appears in sections cut in three places. Its connection with the cœlom is still evident. Later it becomes more complicated and the whole gland becomes enveloped in a thin capsule of mesoderm cells, but the process is not further described. The external opening persists until after hatching.

Kishinouye ('90) has studied the development of the gland in *Agalæna*, *Lycosa*, and other spiders. In these it occurs in somite III and is described as consisting of a duct of ectodermal origin which breaks through to the cœlom. The schematic figures do not prove this origin of the duct.

Lebedinsky's account ('92) of the development in Phalangids is most complete. The first appearance of the nephridium is a weak outgrowth of the wall of the cœlom of somite III. The cells of this outgrowth become columnar while its external end grows into connection with the ectoderm of the first ambulatory appendage. This thickened portion, which is to form the duct of the organ, now grows inward, carrying the wall of the cœlom with it, so that its internal end is surrounded by a ridge. This inner ingrowth forms the nephrostome. The ectoderm is resorbed later, giving an opening to the exterior, and the tube becomes convoluted.

<sup>1</sup> Kowalevsky and Schulgin ('86) saw the organ when it was but little complicated; they describe its duct as ectodermal.

That these organs in *Limulus* and Arachnids are homologous admits of little doubt. It is scarcely more doubtful that they belong to the same category as the antennal glands and shell glands of the Crustacea. The correspondence, as I earlier pointed out, is exact between the coxal gland of *Limulus* and the shell gland of the Entomostraca. Their closure in *Limulus* and certain arachnids is paralleled for instance in the case of *Argulus* (teste Leydig, '89), where the shell gland is functional only in early life. On the other hand, as stated above, the coxal gland in Phalangids is functional in the adult.

In the light of recent investigations these organs must be regarded as nephridia, and the arguments to the contrary advanced by Eisig ('88) are without foundation. Two recent studies are of interest here: That of Sedgwick on the development of the nephridia in *Peripatus*, and those of Weldor ('89 and '91) on the relations of the antennal gland to the coelom in the Decapods. In *Peripatus* the development is strikingly like that in *Limulus*, except there are numerous pairs of nephridia in the former. In both there is the formation of small coelomatic spaces; in both (*cf.* Kishinouye, '91<sup>b</sup>) a division of the coelom into dorsal and ventral portions, and in both the conversion of the ventral coelom into end sac, nephrostome, duct, and bladder. It is interesting in this connection to note that according to Loman ('87) in *Phalangium*, where the nephridial opening does not close and the organ remains functional through life the Malpighian tubes—the other urinary organs—are lacking.

I am inclined to believe that the genital ducts of *Limulus* are also to be regarded as nephridia, but I have searched in vain for any trace of their development. Laurie's account of the origin of the ducts in the scorpion is, as he says, intelligible upon the ground that they are nephridia; their somewhat tardy appearance and lateness in opening to the exterior not seriously militating against such a view.

MUSCLES. — I have not attempted to trace the history of the muscles except to a slight extent. The muscles, which move the feet and which extend from the dorsal surface of the body down to the appendages, are developed along the interseg-

mental lines. The tissue from which they arise is the boundaries of the somites which extend inward toward the median line and which by their encroachment into the yolk outline the liver lobes. The early history of this portion is traced in connection with the alimentary canal. In the abdominal region the differentiation of the muscles of the gill appendages is accomplished in the same way, and it is interesting to note (see Fig. 79) that the anterior wall of the somite develops the extensor and the posterior the flexor of the corresponding appendage.

ENTOSTERNITE. — Passing between the alimentary canal and the nervous system in the cephalothoracic region, and serving at the same time to connect the pedal muscles of the right and left sides is a layer of tissue which serves as a tendon, or rather as a series of tendons, and which by its later chondrification or chitinization (vide Lankester, '84, p. 133) gives rise to the entosternite. It is to be noticed that in its development the entosternite (Figs. 74, 84, 86, 87 *cs*) is always fibrous and it arises from the fibrous tissue of the region. The other "cartilages" occurring as axial tendons in the gills and operculum (Fig. 79) present in their early stages a distinctly chondroid appearance.

ORGANS OF CIRCULATION. — The early history of the central circulatory organ — the heart — of *Limulus* was outlined in my paper of 1885. Later, Kishinouye has added to the account given there and has corrected some points in my description. So this early history need not be detailed here. The heart arises as a result of the extension of the mesoderm over the yolk towards the dorsal median line. Its walls are formed by the edges of the advancing tissue, and, according to Kishinouye, as the walls of the tube thus formed are interrupted in the intersomitic region, a series of segmentally arranged openings into the cavity — the ostia — are produced. The differentiation of the heart begins at first behind and gradually extends forward. In its early formation the heart of *Limulus* affords support to the theory of Bütschli ('82) in regard to the relations of the circulatory system to the segmentation cavity.

My present description begins with Stage H (Fig. 64). In this the heart may be seen with walls in which no definite arrangement of cells is visible and with two blood corpuscles in its interior. It is connected in this section with the dorsal ectoderm by a cord of cells, while on either side are two cavities, a dorsal and a ventral. The ventral is clearly the coelom of the somite, and its walls, somatopleure and splanchnopleure, are perfectly distinct. The upper cavity, the pericardium, is plainly lacunar, and is produced by a splitting of the mesoderm, and at this early stage is limited distally by the trabecular tissue so characteristic of the embryo of *Limulus*. Farther forward (Fig. 66) the heart is larger, and the cells of its walls are arranged in a single layer.

In the next stage (I, Fig. 66) the somatopleuric mesoderm has given rise to the alary muscles which are best developed in the anterior portion. In the abdominal region (Fig. 67), the heart is larger, but in all parts it as yet consists of the single layer of cells which were found in the preceding stage. Fig. 67, which passes through the plane of the genital operculum, shows on either side the posterior extension of the dorsal coelom. A few sections further back (Fig. 68) this cavity disappears from the sections. In longitudinal section (Fig. 82) the heart is seen to extend back to about the middle of the abdomen and forward to the anterior end of the yolk, following this down toward its junction with the stomodæum. At its anterior end the heart divides into two aortic arches (the "crosses aortiques" of Alph. Milne Edwards, '73) which I have called the sternal arteries. These arteries (Fig. 76) pass down one on either side of the stomodæum to dispose themselves at first as two tubes upon the upper surface of the ventral nerve chain. I have not satisfied myself of the way in which these sternal arteries arise but the observations which I have made are not incompatible with the view that dorsally at least they are interseptal. This point is however difficult to settle on account of the numerous lacunae, which, as already mentioned, early appear in the mesoderm and confuse the observer. At this stage (I) no other arteries arise from the heart.

In Stage K the conditions are much the same, the relations of the anterior end of the heart and the sternal arteries being shown in Fig. 77, drawn from a wax reconstruction.

In Stage L, the heart (Figs. 72, 73, 82) has nearly attained its adult condition so far as segmentation into chambers is concerned. In Fig. 73—representing a horizontal section—the anterior end of the heart is shown, enclosed in the pericardial space and supported by the alary muscles. In front, on either side are the roots of the sternal arteries but I have not seen at this or any earlier stage the frontal arteries of Milne Edwards. Fig. 72, taken at a lower level, shows the section of the sternal arteries on either side of the narrow duct connecting the proventriculus with the mesenteron.

It is not until late Stage H that the sternal arteries reach the nervous system. At first they extend themselves as two separate tubes along the dorsal surface of the nervous cords and extend backwards but a slight distance upon them forming the rudiments of the neural artery. There is at this time no trace of any tube beneath the nerves. It is especially interesting that this condition which is transitory in *Limulus* should be permanent in the Scorpion.

In Stage I the neural artery extends back behind the middle of the cephalothorax but its termination is indistinct. In somite VII (Fig. 67) no traces of it are to be found. The partition between the two arteries still persists (Figs. 70–71) but on either side the artery is extending itself around the nervous system and appearing beneath it, thus giving rise to the peculiar condition so well known as characteristic of the adult horseshoe crab. This condition is brought about, at least in part, by outgrowths from the dorsal tubes but whether there be cavities formed independently beneath the cord which are later taken into the neural artery I cannot say. Most of it is accomplished by the downward growth and the wrapping of these portions around the cord, there being as Milne Edwards has suggested a soldering of the two edges of the vessel and a subsequent resorption of the resulting lamella. As will be seen, by this process of formation the nerve does not float freely in the blood but is surrounded by a neurilemma which is part

of the arterial wall. In other words the artery is not morphologically inside the artery.

In Stage K, as shown by the reconstruction Fig. 77, this process has been completed at the anterior end of the ventral cord. Below, this figure shows the neural artery (*av.*) represented as filled with a solid mass, while the omission of the nerve cord leaves a central cavity from which proceed the openings for the nerves on the sides. In front (to the right) below, the forwardly directed process shows the nature of the outgrowths by which the ventral artery is extending itself beneath the brain.

In Stage L the dorsal portion of the neural artery has reached the abdomen, while in the somite of the fifth appendage the conditions are advanced as far as shown in Fig. 74. In the oldest embryos I have sectioned the arteries surrounding the nerves have extended themselves into the appendages (Fig. 89).

I have not attempted to follow the development of the blood sinuses, *etc.* They occur most abundantly in the abdominal region (Figs. 65, 68, 73, *etc.*) and that they are produced by a splitting of the mesoderm is easily seen in the development of the gills. The pericardial sinus belongs to the category of these blood spaces, the cœlom taking no part in its formation.

*Comparisons.*—I have above referred to Kishinouye's account of the formation of the heart in *Limulus* with which my recent observations are in fair accord. In my first account I described the heart as arising from a solid cord of cells but this was a mistake. A re-examination of the slide showed that at the stage described the heart was already formed and its cavity was filled with blood corpuscles.

In the Arachnida most observers have described the heart as arising from the coalescing edges of the somites, meeting in the dorsal median line, there being slight differences in details between the Scorpions (Kowalevsky and Schulgin, Laurie) and the Araneina (Schimkewitsch, Locy, Morin, Kishinouye). In the other groups, as far as I am aware, no detailed observations have been made.

The pericardium of the spiders, according to Schimkewitsch ('87) arises as a layer of mesoderm split off from the splanch-

noplure, while the somatoplure gives rise to the alary muscles. If this be so it is an important point of difference between *Limulus* and the Arachnids. Schimkewitsch further describes the pulmonary veins arising as outgrowths from the pericardium, the lateral arteries as outgrowths (*Ausstülpungen*) from the heart itself.

#### ALIMENTARY TRACT.

The alimentary tract of *Limulus*, like that of all Arthropods, consists of three divisions ; stomodæum and proctodæum, of ectodermal origin, and mesenteron (including the "intestine" and "liver"), derived from the entoderm. These parts are easiest considered in connection with each other.

MESENTERON.—The separation of the entoderm from the ecto-mesoderm by delamination was described (p. 46) in the first part of the present article. As will be remembered, I regard the whole of the nucleated yolk after that separation as the true entoderm. From the time of differentiation of this layer until the first molt there is but slight histological change in the region of the midgut and its diverticula aside from a slight increase in number and consequent decrease in size of the yolk (= entoderm) cells. There is, however, a very considerable change in the shape of the entoderm which may be summarized as follows :—

When the entoderm is first separated from the rest of the egg, it is, like the egg, spherical in outline. It then becomes gradually flattened (*cf.* Fig. 82) and more and more ovoid in outline, viewed from above, corresponding in this with the changes in shape of the embryo. As a result there may soon be distinguished a large semicircular mass of entoderm in the cephalothorax and a smaller, more cylindrical portion in the abdomen. Coincidentally with this change in outline the beginning of the differentiation of midgut and "liver" occurs. As the mesoderm extends itself peripherally from the median ventral line of the embryo, it gives rise to slight intersegmental ridges, the septa of Balfour. Until this centrifugal growth reaches its extreme these septa are slight in extent, but as it attains the margin of the carapax and, turning

on to the dorsal surface, begins to grow back to the dorsal median line, there begins a rapid centripetal growth of these septa, resulting in broad sheets of tissue which divide the peripheral portion of the yolk into a corresponding number of lobes, those of the first division being of course segmentally arranged. Thus there are in the cephalothoracic region six pairs of these lobes, while in the abdomen they are less distinct and less extensive and are only temporary, disappearing totally at an early stage. The fact of their temporary appearance in this region is, however, of considerable interest.

A similar process of lobulation of the yolk has been described by several authors for various Arachnids, and it may be regarded as characteristic of the large-yolked eggs of the group. It however occurs to a greater or less extent in other forms. Thus, in the Crustacea the lobulation of the midgut gland ("liver") is of the same character, while in the leeches, as described by Dr. Whitman ('73), the differentiation of the intestine and its diverticula is exactly the same. Were this process of differentiation of liver, lobes and intestine to go on regularly, it would result in the production of a *Limulus* with a paired liver in each somite, each half emptying by its own duct directly into the intestine. This, however, does not occur. With the development of the extensive muscular system of the gill-bearing appendages and the large blood sinuses in that region the abdominal midgut diverticula disappear. In the cephalothorax the primitive regularity shows the following modifications. The septa do not all grow at the same rate, and (Fig. 83) some are interrupted at points in their growth, so that two or more lobes remain in direct connection with each other. This occurs between lobes 1, 2 and 3, and also between lobes 4, 5 and 6. At the same time the inner ends of the septa become expanded by the development of the muscles of the feet so that they in places run together, cutting off lobes 1 and 2, and 5 and 6 from direct connection with the central mass. In this way the six primary liver lobes and the two hepatic ducts (*hep.*,<sup>1</sup> *hep.*<sup>2</sup>) of either side of the adult are differentiated. A later peripheral ingrowth of mesoderm still farther divides up the primary

lobes into lobules (Figs. 34, 35) resulting in the adult condition.

At the anterior end of the body an ingrowth<sup>1</sup> similar to the septa carries back the anterior end of the intestine, and intervenes to separate the first pair of lobes from each other. With this ingrowth this pair of lobes, which at first were at right angles, come to lie parallel to the principal axis of the body.

The central unsegmented part of the yolk which remains after the differentiation of the "liver" forms the "intestine" of the adult. It extends from the point of the first appearance of the stomodæum back to the posterior end of the body.

Until after the first molt after hatching the entoderm retains the same histological characters which it had at its first differentiation. It is a mass of yolk without lumen and is divided into a number of polygonal cells with clearly marked cell walls and central nuclei.<sup>2</sup> Excepting in a slight difference in size, it is impossible to distinguish histologically between the entoderm cells of Stages C and I. That some change does occur in the interval, of a chemical rather than of a histological character, is shown by the fact that while in the earlier stages the yolk is very difficult to section, in the later it cuts as readily as any other tissue of the body.

After the molt which produces the adult form (Stage L) the histogenesis of the epithelium of the midgut and its diverticula begins. It appears first in the intestine and later in the liver; and in the intestine it is first seen at the anterior end (Fig. 81). From the study of numerous sections (*cf.* Figs. 81, 85, 88) the process is clearly seen to be a direct conversion of the yolk-cells into the epithelial lining of the mesenteron. In Fig. 85—a sagittal section through the junction of stomodæum and mesenteron at early stage L—the entoderm cells, *en*, near the

<sup>1</sup> For clearness this and the lateral septa are considered as ingrowths, but they are to a large extent outgrowths as well, since the margin of the carapax is farther removed from the median line in the later than in the earlier stages, and it is coincidentally with this change in the relative position of the margin of the body that the septa are developed. This is even more marked in front than at the sides of the body.

<sup>2</sup> In my figures the yolk is represented as solid, neither cell walls or nuclei being shown. They are, however, very distinct in all of my preparations.

middle line, are seen to have assumed a columnar character and to be nearly free from yolk, while on either side they pass into a tissue crowded with yolk spherules (*ys.*) in which the cell boundaries cannot be followed and in which the nuclei are irregularly arranged. In Fig. 84—a transverse section of a slightly older embryo—the same conditions are shown upon a smaller scale. On the upper left side of the intestine (*mes.*) the cells have a well-marked epithelial character, while on either hand they pass directly into the normal yolk cells of the earlier condition. In a slightly older individual (Fig. 81) the whole anterior end of the intestine is free from yolk, and its lining cells (represented diagrammatically) have the character of a columnar epithelium, while at the posterior end they pass directly into the yolk cells which still fill the whole cavity in this region.

This rearrangement of the epithelium is well advanced in the intestine before it begins in the liver, and it advances more rapidly in the central than in the peripheral parts of the latter. It thus forms first the epithelium of the hepatic ducts (Figs. 72, 86) and then the secretory epithelium. In the latter I have failed to recognize an early differentiation of purely epithelial and excretory cells such as has been described in some spiders.

As will be seen, I regard the yolk in *Limulus* from the time of its delamination as true entoderm. I fail to recognize, at least here, the existence of “vitellophags” whose purpose is merely the metabolization of the yolk and which then degenerate. I look upon the yolk cells from the beginning as morphologically a true epithelium, the cells of which, being gorged with yolk, are crowded from their proper position, thus obliterating the lumen and obscuring their true nature. In the later stages, when there is a rapid development of tissues, there is a corresponding call upon the entodermal structures for nourishment. Then it is that the yolk cells act temporarily as “vitellophags” and, metabolizing the yolk, pass the products on to the other tissues. It is only then that, the yolk being out of the way, they are able to rearrange themselves as a true epithelium.

While this view is in full accord with the observations of most students of Arachnid development, it is at variance with some of the commonly received ideas of Arthropod embryology,

which are to the effect that the yolk cells are "vitellophags," degenerating and not contributing to the formation of the entodermal epithelium which arises from cells derived from some other source. I feel confident that this is not the case in *Limulus*. I have yet to see any evidence of degeneration of the yolk cells, and further, I have seen no cells other than those of the yolk which could supply the epithelium of intestine and liver. From the very time when the yolk is first included in a mesodermal envelope this layer is to be clearly traced (*sp.*) as a splanchnopleure closely enveloping the yolk, and in the later stages the same layer is seen (Figs. 84, 85) in exactly the same place and presenting the same conditions. In some sections which I have made of Hexapod eggs I have seen appearances which lead me to think that possibly in these forms the entoderm of authors is in reality splanchnopleure. The fact that most observers have closed their investigations before the development of a well-differentiated entodermal epithelium leaves a gap which renders their interpretations not conclusive on this point.

STOMODÆUM. — As already described (Vol. VII. p. 52) the anterior end of the primitive streak is marked by a spot where the cells are deeper and more columnar (Figs. 43, 45, *mo.*) than elsewhere in the median line, and this spot is usually, in Arthropods, regarded as marking the position of the future mouth, and all discussions of the transfer of the mouth from a preappendicular position to one behind the first pair of appendages are concerned with the connection of this spot with the mouth of the adult. In reality this spot marks the junction of stomodæum and mesenteron and forms the inner end of the foregut. Hence to call it the mouth is to introduce an element of confusion.

At the time of the first outlining of the limbs the invagination of the stomodæum begins, and the process is one which finds its closest parallel in the invagination of the neural canal in the vertebrates.<sup>1</sup> At first it is a shallow pit with small

<sup>1</sup> It is needless to say that this affords no foundation for the curious vagaries of Gaskell, as to the origin of the vertebrate nervous system from the alimentary tract of the Arthropod.

lumen and with an external opening pear-shaped in outline, narrower in front and wider behind. It is in fact enclosed by two ridges, one on either side, while posteriorly it is without distinct boundaries and passes directly into the ventral ectoderm. The lateral walls gradually unite in front (*cf.* Vol. VII, Figs. 30, 31), and are at the same time added to behind. It is by the continuation of this process that the stomodæum is invaginated and the mouth comes to occupy a position behind the first pair of appendages. Thus it will readily be seen that with regard to *Limulus*, at least, Claus is wrong when he says ('87, p. 129) that the preoral condition of the appendages is "nicht ein Lagenwechsel des Mundes . . . sondern eine im Laufe der Entwicklung vollzogene Aufwärtsbewegung der Gliedmassen mit entsprechenden Verschiebung der Ursprungsstelle des zugehörigen Nerven." It would seem probable that the same conditions obtain in the Crustacea, but detailed observations are as yet lacking.

At first the cells of the stomodæum invaginated in this manner form a low cubical epithelium, but they soon elongate and assume the columnar character which is found in this region in all the later stages. At an early date they also begin the secretion of the chitinous cuticle. At the outset all of the stomodæum is apparently formed by this invagination, and the tube is straight between mouth and the inner end. Later it begins to elongate by interstitial growth, and in this way a flexure in the sagittal plane is produced (*cf.* Figs. 81, 82). This flexure is also increased by the flattening of the embryo.

With the introduction of the flexure there begins a differentiation of the stomodæum, at first into an internal proventriculus (stomach of most authors) and an outer tube. Later, the latter in turn is subdivided into buccal cavity and œsophagus proper (Fig. 81). In the proventriculus there soon develop longitudinal folds, and before the connection of stomodæum with mesenteron is effected, the proventriculus has, except in size, the characters which it has in the adult.

After the epithelium of the midgut has been formed at the anterior end, the connection between fore- and midgut is

effected by a breaking down of the wall between them. At first the proventriculus empties directly into the mesenteron, but later (Fig. 72) the inner end of the former becomes drawn out in a slender tube which projects slightly as the "cone" into the intestines. The limits of the two regions, ectodermal and entodermal, at this stage are clearly distinguished by the chitinous cuticle upon the former.

PROCTODÆUM.— In striking contrast to its development in the Crustacea the proctodæum in *Limulus* is late in its appearance and small in extent. As late as Stage I (Fig. 82) it appears merely as a slight inpushing of the ectoderm upon the ventral surface. In Stage L it is wider but scarcely deeper than before (Fig. 81). In still older specimens (Fig. 88) the boundary between mesenteron and proctodæum has broken through and the now more elongate proctodæum has become thrown into inner folds. At the point of juncture between ectoderm and entoderm there appears, above and below in the section, an enlargement of the lumen of the tube and a second similar enlargement occurs within the entodermal portion of this tract. As to the meanings of these enlargements I have nothing to offer aside from the fact that in connection with them the Malpighian tubules of the Hexapods and the analogous structures of the Arachnids naturally suggest themselves.

*Comparisons.*— Almost nothing was previously known of the development of the alimentary tract of *Limulus*. In 1885 I gave essentially the same account of the formation of the stomodæum and the differentiation of the liver-lobes, illustrating both with diagrammatic figures. In the same year Brooks and Bruce in their preliminary paper ('85) describe the entodermal epithelium as arising from the yolk cells in the same way as I have done and they further say, though without any details, that the stomodæum arises as an ingrowth which at first goes upward and forward and then bends upon itself. Though not specifically the same in words the account given by Kishinouye ('91) of the development of the stomodæum is easily brought into harmony with the foregoing, and especially interesting is his statement (pp. 79–80) that "As the upper lip grows posteriorly, the mouth opening which was at first pre-appendicular

gradually shifts its position backward." Kishinouye has nothing to offer concerning mesenteron or proctodæum.

In the Arachnida almost all observers describe conditions closely similar to those obtaining in *Limulus*. Since the time of Balfour's paper ('80) the liver-lobes have been recognized as differentiated by the ingrowth of mesodermal septa into the yolk in the same manner as in *Limulus*, and the hepatic ducts (of course different in number) as formed in substantially the same way. Balfour, while thinking that certain observations possibly pointed to the origin of the hepatic epithelium from the cells of the thickened ends of the septa, still recognized the entoderm in the yolk. Balfour did not trace the formation of the entodermal epithelium but later authors agree that it arises in the spiders from the yolk cells, the differentiation beginning at first at the posterior end. So far as their observations go Locy, Morin (the text of his preliminary paper, the copies of the figures of his later article in Russian), and Schimkewitsch ('87) agree in the recognition of the yolk nuclei of spiders as the nuclei of the future entoderm. Later, Schimkewitsch ('90) changes his views; he now recognizes as entoderm in the "Tracheates," the smaller cells which lie on the ventral surface of the yolk, while the mass of the yolk cells in the Arachnids are the Anlage of the blood corpuscles. Such a view is impossible with *Limulus*, since before the yolk cells undergo any change they are entirely enclosed in the splanchnopleural layer of the mesoderm, through which migration to the blood vessels is impossible.

Faussek, in his account of the development of the Phalangids ('91) says that at the close of the embryonic period the yolk cells (derived as in *Limulus* by delamination) divide rapidly, and, with a small amount of protoplasm, begin to throw themselves down upon the mesodermal envelope of the midgut and its diverticula. At first these patches of entodermal epithelium are irregularly scattered but they soon begin at the anterior end, to arrange themselves in the cylindrical epithelium of this region.

In the scorpions the conditions are quite different. As already pointed out (Vol. VII, pp. 55 ff.) Kowalevsky and

Schulgin ('86) and Laurie ('90) show that in the early stages the entoderm is a distinct differentiation from the germinal area; it remains for a long time as a solid mass at the posterior end of the embryo and only later spreads itself out as a definite layer enclosing the yolk and forming the epithelium of the midgut and its diverticula. Of the details of the formation of the liver-lobes but little is said, but apparently it occurs through the ingrowth of the mesodermal septa as in *Araneina* and in *Limulus*. The different type of formation of the entoderm in the scorpions and *Limulus* is possibly the strongest objection which can be raised to the close association of the two forms, for while the two types can be reconciled, it implies an extremely long separation of the forms. Still it must be borne in mind that if this be advanced as an objection it must be equally valid in proving that the *Araneina* and the scorpions are but remotely connected, a thesis which I hardly care to defend, while on the other hand it would show closer relationship between *Limulus* and the spiders than between the former and the scorpions, a view which is negated by numerous other facts of structure and ontogeny.

*Limulus* also agrees with the *Arachnida* in the early appearance and elongation of the stomodæum and the shortness and lateness of appearance of the proctodæum. As yet, no observations are recorded as to the manner of invagination of the stomodæum, but as figured by Morin (in Korschelt and Heider '92) and Locy ('86) it bears close resemblance to that of *Limulus* in its bending and in the early differentiation of a terminal pouch clearly comparable to the proventriculus of *Limulus*. In the only sagittal section given by Laurie of the later stage of the stomodæum in the scorpion ('90, Pl. XVII, Fig. 48) the proventricular enlargement is not very apparent but the buccal cavity is well marked. Concerning the proctodeal region the comparisons reveal little of importance. In the spiders we have in this region the formation of the stercoral pocket<sup>1</sup> which is without parallel in *Limulus* (*vide*, however, *supra* p. 219 and Fig. 88).

<sup>1</sup> Kishinouye's observation ('90, p. 68) that the stercoral pocket is developed from the unpaired cœlom of the caudal region certainly needs confirmation.

## NERVOUS SYSTEM.

I leave all questions concerning the nervous system and sense organs untouched, except in so far as is necessary to explain some points which will appear later. I do this the more willingly since my friend, Dr. Wm. Patten, has for several years been devoting especial attention to this system. I find in *Limulus* that at an early stage the nervous system, viewed from the surface, presents the appearance of numerous circular pits (Fig. 29). These, which are shown in section in Figs. 49 and 65, I suppose to be what Patten ('89 p. 602) refers to in his statement that "the central cord and brain of Arthropods, is at first composed entirely of minute sense organs, which in the scorpion have the same structure as the segmental ones at the base of the legs." Kishinouye has also seen the same structures and compares them to the ommatidia of the eye. There is, however, no such differentiation of the nuclei as are shown in the figures of the latter. Unlike Patten, I interpret these in-pushings of the nuclei as centres for the rapid proliferation of nerve cells, and not as sense organs. I also am inclined to withdraw my original account of the formation of segmental sense organs ('90), as I am now inclined to believe that the structures which I described as sensory in structure (various figures "ss") are more probably glandular. The brain arises from three pairs of ganglia in front of the pair which innervates the first pair of appendages. I believe these three ganglia to represent: the first, the primitively preoral nerve centre, the homologue of the "brain" of the annelids; the other two to belong, like the deuto- and tritocerebrum of the Hexapod, to ganglia which have left the postoral and have wandered into the preoral region. Upon the real state of affairs I have, however, almost no actual observations, and base my opinions largely upon the conditions in other groups.

## RESPIRATORY ORGANS.

As is well known the respiratory organs of *Limulus* are borne upon five pairs of appendages situated on somites VIII—

XII. Each of these appendages has upon its posterior surface a large number of rounded quadrilateral lamellæ (the number varying with age), the "gill-leaves," and the whole organ is known as the "gill-book." The first of these appendages to appear is the most anterior one (VIII). It grows out from the body, not as a cylindrical process like appendages I–VI, but as a broad lobe with an oblique insertion upon the ventral surface of the body (Figs. 28, 32 *g*). At first this lobe consists merely of a fold of ectoderm (Fig. 78) marked off from the ventral surface by an impushing behind, and containing in its interior scattered branching mesoderm cells between which are numerous large lacunæ. At stage H this appendage shows the first appearance of the gill lamellæ in the form of a single fold of the ectoderm of the posterior surface at about midway of the length of the member. Very soon a second and smaller outgrowth appears between the first leaf and the body, and so on in regular succession (Fig. 79) the new leaves continuing to be added at the base of the appendage, the outer leaves being the larger and older.

When the first gill-leaf appears on appendage VIII, appendage IX is budded, and on this the first gill-leaf appears (Fig. 79) when on appendage VIII there are four or five lamellæ. The subsequent growth of these appendages and the new gill-leaves is but a repetition of that already given. At the molt with which the telson appears and with which my studies end, appendage X has appeared, but is as yet without lamellæ, while appendages VII and IX are well provided in this respect (Fig. 80).

At stage I (Fig. 79) the gills and gill appendages present the following appearances in their finer structure. The ectoderm has become columnar and has secreted on its free surface a thin cuticula. In the gill-leaves the two walls are united here and there by fine mesodermal filaments which extend between the two walls like the tie rods of an architectural structure. Between these trabeculæ are lacunæ without definite walls, and in these, scattered blood corpuscles are to be seen, showing that these spaces are in connection with the general circulatory system. These lamellæ are shown in cross section

in Fig. 68, where also may be seen, at either side of the gill-leaf larger lacunæ, forming the afferent and efferent blood channels of the lamella.

In the gill appendage as in the operculum (appendage VII) is a central rod of compact tissue somewhat resembling cartilage, but whose fate I have not traced. In my former paper ('85, Pl. XXXIX, Fig. 38) the line from the abbreviation for muscle, was by mistake of the lithographer, run to this structure. On either side of this rod are developed the muscles of the appendages. They have their origin in a narrow line on either side of the back (Fig. 67) and are inserted in the wall of the appendage at the lower end of the rod just referred to. In transverse section these muscles (Fig. 67) are seen to be fan-shaped, the line of insertion embracing nearly the entire width of the appendage. From its origin and insertion the muscle in front of the rod is seen to be antagonistic to that behind and we may consider the two as respectively flexor and extensor in function.<sup>1</sup> The flexors of one somite and the extensors of the next have their origins closely approximate, and their traction soon results in the drawing inwards a small patch of the dorsal ectoderm, thus producing in the adult the line of depressions on either side of the middle of the tack, and the corresponding chitinous ingrowths in the interior of the same region. One of these ingrowths of chitin secreting ectoderm is cut across in Fig. 68 *ent.*

In the last stage studied (Fig. 80) the conditions are essentially the same as before, except that the gill-leaves are larger and more numerous. The section, however, does not pass in the right plane to show the muscles and internal rod. For a description of this section I cannot refrain from quoting MacLeod's ('87, p. 4) description of the lung-book of a scorpion:—  
 “Nous trouvons en [la figure] la coupe d'un certain nombre de fines lamelles, les lamelles pulmonaires, placées horizontalement, libres à leur extrémité postérieure ou caudale, c'est-à-dire la plus rapprochée de l'extrémité caudal de l'animal, vers la droite

<sup>1</sup> I have been unable to identify with certainty these muscles with those of the adult as described by Benham ('83). At this early age they are not differentiated as they are later.

de la figure, et attachées en avant. Entre ces lamelles se trouvent des cavités en forme de fentes qui communiquent en arrière avec une cavité générale laquelle débouche à son tour à l'extérieure par une fente stigmatique."

*Comparisons.*—The only previous accounts of the gill development are by myself ('85) and Kishinouye ('91, p. 72), and the foregoing differs from them in being somewhat fuller in some details (Kishinouye calls the metastoma an appendage<sup>1</sup> hence his appendage IX is my VIII, etc.). The process is extremely simple—the outgrowth of lamellate processes from the posterior surfaces of the corresponding appendages, the newer lamellæ being formed proximally—and yet my early account does not seem to have satisfied Laurie, who says ('91, p. 137) "a detailed account of the development of these appendages *Limulus* may throw more light on the matter" of the homologies of the respiratory organs of the scorpion and the king crab. Had not Laurie been confused by some strange<sup>2</sup> ideas of the homologies I think that my previous account would have proved detailed enough for his purposes, for the two organs,—the gill of *Limulus* and the lung of *Scorpio*,—can be compared in detail in the simplest manner, without the invocation of any inversion, of any "parabranial stigmata," of any conversion of air space into blood space, or the like.

Several workers have described the development of the respiratory organs of the Arachnida, and from the papers of Metschnikoff ('71), Kowalevsky and Schulgin ('86), Locy ('86), Bruce ('87), Kishinouye ('90), and Laurie ('90 and '92), we may gain the following summary of the development of the lung books in these forms.

In these forms the lungs develop in connection with the abdominal appendages. These appendages grow out for a short

<sup>1</sup> See upon this Kingsley ('85, p. 541, and '92, p. 60).

<sup>2</sup> Thus Laurie says (*l.c.* p. 136): "The additional appendages of *Limulus* are directed towards the tail as one would expect abdominal appendages to be. Now if the appendage had sunk without invagination, one would expect it to be still directed towards the tail, unless there were some very good reason for its having changed its direction. If, on the contrary, it had become invaginated it would naturally be directed in the opposite direction towards the head, and this is what we find in the scorpion. The inpushing is from the beginning towards the head, and the aperture opens toward the tail."

distance and then an inpushing takes place just behind the appendage, the opening of the invagination, according to Kishinouye being away from the median line. This sac, the future pulmonary cavity, continues to increase in size, while the appendage proper soon becomes obsolete. In this way the pulmonary sac, with its external opening or stigma, is developed. After the pulmonary sac is formed there begins on its anterior wall,—*i.e.* on the continuation of the posterior wall of the appendage,—a series of foldings of the ectoderm, the lung-leaves. As the animal increases in size, new lung-leaves are added at the inner or proximal end. In short, as the adjacent diagrams show, the homologies between the two types of organs are perfect.

In *I* we have a condition which will apply equally well to the young of either *Limulus* or *Scorpio*. At the right side the appendage is just budded out, and on the left the sinking

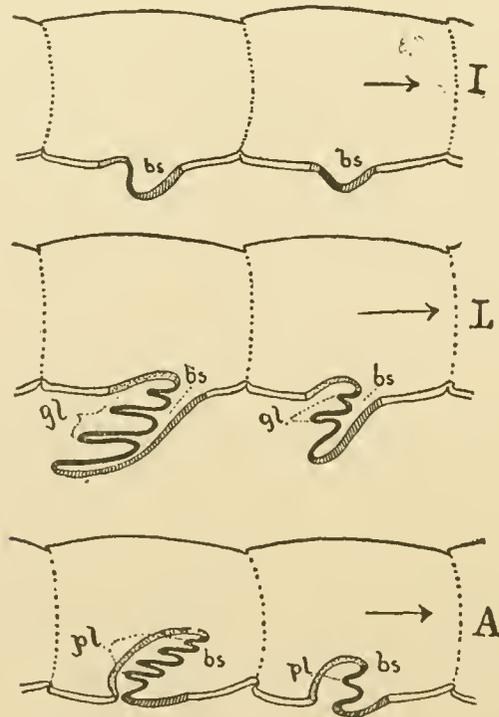


Diagram of the respiratory organs in (*A*) an Arachnid; (*L*) in *Limulus*, and (*I*) in an intermediate condition. The arrows point toward the head, the cross-lined portion is the anterior, the black, the posterior surface of the appendage, the dotted surface that part of the ventral surface of the somite, which is invaginated to form the posterior wall and roof of the pulmonary sac. *bs*, blood space; *gl*, gill-leaves; *pl*, pulmonary leaves.

in, behind it, has begun. In *L* we have the modifications of *I*, which result in the formation of the gill-book. On the posterior face of the appendage the gill-leaves are budding out. In *A* we have the Arachnid modifications of *I*. On the right side the post appendicular insinking has resulted in the formation of the pulmonary sac from the anterior wall of which,—and which is plainly the posterior surface of the appendage—the pulmonary leaves are being produced (cf. Laurie, '90, pl. XVII, Fig. 47). At the left side of the figure the same conditions are carried further, and the opening of the pulmonary sac is now reduced to the narrow spiracle. In all the figures the anterior surface of the appendage is crossed-lined, the posterior is black, the invaginated portion of the ventral surface dotted, the rest of the ventral surface is white. The arrow points towards the anterior end of the animal.

When the comparison is made in this way the similarities, as to appendages and lamellæ, are seen to be very close. When viewed from the histological standpoint the resemblances are so exact that the description of the pulmonary organ of the spider or of the scorpion will apply almost, word for word, as shown above, to the gill book of *Limulus*. This, taken in connection with the fact that the very appendages which in the scorpion (IX–XII) are converted into the lung-books, are in the *Limulus* the bearers of gill-books, and that appendages VIII of the scorpion (the pectines) have a structure also easily reducible to the gill-book of the corresponding somite of the horse-shoe crab, place the homologies of the organs in such a light that few identities of structure and of phylogeny are more certain.

The consideration of the relations existing between the lungs and the tracheæ of the Arachnids will be taken up later.

#### THE RELATIONSHIPS OF LIMULUS.

It would seem hardly necessary to review in detail the discussion of the systematic position of *Limulus* since it has been done by almost every author who has treated of its anatomy or ontogeny or who has studied the spiders. Yet some space must be devoted to it because of the new facts

brought out by the present investigations and especially because some of the arguments advanced by the advocates of the arachnidan affinities of the horse-shoe crab do not seem to be understood by several recent writers.

Notwithstanding the early suggestion of Strauss-Durckheim (*teste* Lankester) and the later one by the younger Van Beneden ('71) there was no serious question of the relationship supposed to exist between *Limulus* and the Crustacea until the publication of Lankester's paper ('81) "*Limulus* an Arachnid." Previous to that date there was a general agreement that the Arthropods were divisible into two great classes:—Tracheata and Branchiata—the division being based primarily upon the method of respiration; and this view was greatly strengthened by Moseley's discovery ('74) of tracheæ in *Peripatus*, thus apparently providing for a line of descent for the Tracheates without the necessity of any close association between these and the Crustacea. The Arachnids were of course included in the Tracheata for in most of the group were found tracheæ, apparently built upon the same plan as those of the Hexapods, while Leuckart had shown long ago ('49) that the pulmonary sacs of the spiders and scorpions were clearly homologous with the tracheæ of the other Arachnids.

Although not primarily based upon the respiratory system Lankester's conclusions were in substance that the lungs of the Arachnids were homologous with the gills of *Limulus*; and the deduction necessarily followed that all Tracheates must have come from a Limuloid ancestor or that the group "Tracheata" must be polyphyletic in origin and that the similarities of the tracheæ in Hexapods and Arachnida must be due to homoplasy rather than to community of descent.

Lankester's paper produced no little discussion and the points presented by the numerous papers upon the subject as well as those based upon the present investigations may be presented in categorical order as follows :—

I. *Limulus* agrees with the Crustacea and differs from the Arachnida in :—

1. A branchial respiration.
2. The possession of biramous appendages.

3. The absence of Malpighian tubules.
  4. The absence of salivary glands.
  5. The absence of embryonic envelopes.
  6. The presence of compound eyes.
- II. *Limulus* and the Arachnids agree in, and both differ from the other "Tracheates" (Hexapoda and Myriapoda) in :—
7. The primitively postoral condition of appendage I and its later transfer to a prestomial position.
  8. The six-jointed appendages II–V.
  9. The existence of a metastoma (chilaria) upon the sixth somite.
  10. A regional division of the body behind somite VI.
  11. The openings of the genital ducts upon appendage VII.
  12. The subservience of appendages IX to XII (*Limulus* VIII–XII) to respiration.
  13. The possession of a post-anal spine.
  14. The formation of deutova.
  15. The formation of the entoderm by delamination.
  16. The early appearance of metamerism in the body.
  17. The occasional later appearance of appendage I and its somite.
  18. The existence of a well-marked cœlom (schizocœle) extending at first into the appendages.
  19. The extension of the cœlom of somite I into the pre-oral region.
  20. The presence of a chitinous entosternite.
  21. The presence of a posterior artery from the heart.
  22. The possession of a pair of sternal arteries passing, one on either side of the œsophagus, to unite below in —
  23. A longitudinal arterial canal upon or surrounding the nervous system.
  24. The possession of blood colored blue by hæmocyanin.
  25. In the possession of reticulate genital ducts.
  26. In the method of oviginesis.
  27. In the possession of nephridia in somite V.
  28. In the pitted origin of the nervous system.
  29. In the concentration of the postoral ganglia in a circumœsophageal nerve ring.

30. The invaginate character of the median eyes.
31. The long stomodæum.
32. The large mesenteron.
33. The large midgut glands (hepatopancreas) emptying by metameric ducts.
34. The short proctodæum.

III. *Limulus* and the Arachnids agree with the Crustacea, and differ from the "Tracheates" in points 14, 21, 27, 31, and also in :

35. The absence of any differentiated head.
36. The position of the genital ducts in the appendages near the middle of the body.
37. The development of the respiratory organs in connection with the appendages.
38. The paired sexual openings.

Several of the foregoing points need but little discussion since they have already been considered both by Prof. Lankester (81) and by myself. It is, however, to be noted that this enumeration of resemblances and differences omits all reference to characters which are common to all great groups of Arthropods, and also to those which are peculiar to any one group, except so far as they are, apparently, based upon misconceptions. It must also be mentioned that *Peripatus* is omitted from the discussion, since, notwithstanding the recent researches of von Kennel, Sedgwick, Sclater and Miss Sheldon, its position in the Arthropod phylum is not beyond question. So too with the chilognathous Myriapods, since for reasons which will appear later, their relations to the chilopods are exceedingly doubtful.

I have already discussed in the previous part of this paper the evidence presented by the ovigenesis, in which there is a close parallel between the Arachnids and *Limulus*, the egg in both passing into a follicle formed by the separation of the *tunica propria* from the germinal epithelium. I have also considered the matter of the origin of the entoderm in both *Limulus* and the Arachnids (and Pycnogonids) by delamination, and the early segmentation of the body, closely parallel in both groups, before the appearance of the legs. A farther point

of similarity is in the tendency toward a late appearance of appendage I in both groups, it having been noticed by both Metschnikoff and Laurie in the scorpion and by Birula ('92) in *Galeodes*. This, however, has less weight than it otherwise would have were it confined to these forms alone, Grobben having noticed ('79) a similar delay in the appearance of the antennulæ in *Moina*.

To several other points exceptions may be adduced. Thus a chitinous entosternite has been noticed in several Crustacea, *c.g.* in *Apus* and by Claus ('92) in Ostracodes. Deutova<sup>1</sup> occur in both Arachnids and *Limulus* but as Zaddach described long ago ('41) they are found in *Apus* as well. In the American *Limulus*, as in the Arachnids, the coelom at first extends into the appendages (Kishinouye says it does not in the Japanese species) but similar conditions have lately been shown to occur in the Hexapods. Reticulate genital ducts occur in the Phyllopods. I cannot agree with Kishinouye that the metastoma of *Limulus* possesses a separate somite, and as I have already pointed out ('92, p. 60) his figures can receive another interpretation. There is no somite and no neuromere for the metastoma, and as it occurs upon somite VI which is already provided with appendages, its appendicular nature is not apparent. Metastomal structures occur in other Arthropods; the exact serial similarity between that of *Limulus* and that of the Arachnids is the important point.

The possession of a post-anal moveable spine (telson in *Limulus*, sting in the scorpions, multiarticulate whip in *Thelyphonus*) is not paralleled outside of these forms. It is to be regarded not as a somite or a series of somites — the position of the anus settles that — but as an articulated outgrowth of the supra-anal region of the terminal somite of the body.

The foregoing disposes of points 9, 13, 14, 15, 16, 17, 20, 25 and 26, while no discussion need here be given to items 8, 24,

<sup>1</sup> I have used this term, introduced by Claparède, for those molted cuticula or "Blastodermhauten" which serve as protective envelopes — Packard's "vicarious chorion" — after the splitting of the chorion, and before the young is turned free to shift for itself. Henking has called ('82) the same structures in the Arachnida "apoderma."

29 and 30, as they have either been treated of sufficiently in previous papers or they are based upon conditions not described in the present series. For the discussion of a large number of the remaining points it becomes necessary first to review briefly our knowledge of the homologies between the somites in the principal groups of Arthropods.

Until we have more evidence than we now possess of the total disappearance of a somite from the anterior portion of the body of any arthropod it will be necessary in making our comparisons between the regions in the different groups to proceed upon the assumption that the metamERICALLY repeated portions are, somite for somite, the same in the whole phylum, and are to be compared throughout upon the serial basis, the first being equivalent throughout, and so with the second, and so on, the comparison ceasing only with the hinder region of the body, where the budding zone occurs and behind which is the terminal or caudal lobe. Upon no other basis can any comparison be made.

The great difficulty with this is in the recognition of the metameres in the anterior end of the body for there we find a tendency toward the obliteration of parts and the obsolescence of those features by which the existence of the somite is made most apparent. The nervous system seems, at present, to afford us the most certain means of recognition of the somites and upon this we must place the most dependence, since in some cases the cœlom of the somite may disappear, its mesoderm becoming fused with that of its neighbors while the appendages may totally fail to develop. In the Hexapods various authors have expressed the idea that the so-called brain was a compound structure, and of these the later writers — Tichomiroff (*teste* Cholodkowsky) Patten ('88) and Cholodkowsky ('91) represent it as composed of three neuromeres, and Carriere (in Chalicoderma) has recognized still another in the cephalic region. Of these the most anterior, the protocerebrum, is apparently prestomial and hence is to be regarded as homologous with the annelid cerebrum, and the descendant of the "Scheitelplatte." All recent observers — Patten, Heider, Wheeler, Graber, Carriere, Cholodkowsky *et. als.* — have amply

confirmed the early observation of Weismann ('63) that the Hexapod antennæ are postoral, and have shown that they are innervated from the second or deutocerebral neuromere. Between this and the mandibular ganglion is the third component of the brain, the tritocerebrum, and in a few forms this has been shown to have a small embryonic appendage which apparently becomes obsolete in the later stages. This has been observed by Tichomiroff (*teste* Cholodkowsky<sup>1</sup>) in the silk-worm; by Carriere ('90) in the bee *Chalicoderma*<sup>2</sup> while in a note Dr. Wheeler sends me an account and a drawing of the embryo of the Collembolan, *Anurida maritima*, in which the appendage between the antenna and the mandible, the tritocerebral appendage, is well marked. Apparently Wheeler had seen traces of the same in *Doryphora* ('89, p. 337 Fig. 44). Regarding these neuromeres and appendages the conclusion is inevitable that they belong to the primitively postoral series. They arise in the same line and agree in all respects with those further back. The only other supposition would be that they are preoral and temporarily wander backwards to be immediately returned to their proper position, a supposition of very doubtful value. In the light of these observations we must regard the Hexapod head as composed of at least six elements, the procephalic lobes and five postoral somites, each of the latter having appendages.

In the Arachnida our evidence is much less abundant and much less detailed. Schimkewitsch ('89) describes in the spiders ocular and rostral ganglia (Pl. XXI, Fig. 3) in advance of the ganglion of the chelicerae, while in the schematic Fig. 5 of his Pl. XXIII he represents the cerebral ganglion (in front of the rostral ganglion) as three-lobed. Patten ('90) describes the brain of the scorpion as composed of three pairs of ganglia,<sup>3</sup> while Jawonowsky ('92) figures four postoral somites in front of the cheliceral somite, the posterior of which bears a pair of

<sup>1</sup> In his preliminary paper Tichomiroff merely says "Es existirt bei dem Seidewurm eine echte untere Lippe . . . und die als der allbekannten Oberlippe der Insecten homolog betrachtet werden darf." His later paper is unfortunately buried in an outlandish tongue.

<sup>2</sup> Carriere recognizes *four* cerebral somites and finds a preantennal appendage.

<sup>3</sup> See also Metschnikoff ('70), Pl. XV, Fig. 14.

appendages, possibly the same as that of Croneberg ('80) in *Dendryphantes*. No preoral ganglion is indicated in his extremely unsatisfactory figures, while his evident desire to find the Hexapod antennæ in the Arachnids has possibly influenced his observations.<sup>1</sup> Locy has also figured ('86, Pl. XI, Fig. 70) a distinctly three-lobed brain in *Agelena*. I regret that I have not been able to consult the original plates of Morin's account of the development of the spiders, but as copied by Korshelt and Heider ('92, Fig. 383B) the brain of *Theridium* consists of four lobes, the posterior of which is apparently the cheliceral ganglion. Kishinouye ('90) also describes the brain of *Agelæna* as three-segmented.

In *Limulus* both Patten and myself have recognized a three-ganglioned cerebrum in front of the ganglia of the chelicerae. None of these cerebral ganglia have been seen by me in a postoral position, but their relation to the ventral chain is such as to justify the supposition that here, as in the Hexapod, there is a very early shifting.

In the Crustacea I know of no observations of evanescent appendages or neuromeres, unless, possibly in the case of the metastoma. So far as observations go the series is, apparently: first, the procephalic lobes; second, a pair of ganglia in front of the antennulæ, figured by Bumpus ('91, Pl. XVII, Fig. 1) then, antennulæ, antennæ, etc. As to just where the line between preoral and postoral is to be drawn is uncertain. That the antennulæ of the Crustacea are to be classed in the primitively postoral series is evidenced by several facts. In the first place, they are placed by all observers at first in a paraoral if not a postoral position, and in a direct continuation of the postoral appendages. Secondly, the evidence presented by *Apus* is clearly understood upon the basis of a complete transfer of the ganglia and appendages to a preoral position. In the adult the ganglia of the antennulæ (*cf.* Pelseneer, '85) are fused with the cerebrum, while the course of the nerves (see Zaddach, '41, Pl. III, Figs. 1 and 5) shows distinctly a transfer of the structures

<sup>1</sup> In the light of the observations of Carriere and Wheeler his discovery does not help matters, for there is still a somite lacking, to make the parallel exact from his standpoint.

at either end of the cord. I do not quote my own observations upon the postoral position of the antennulæ of Crangon because their accuracy has lately been denied by Weldon<sup>1</sup> ('92) and by Herrick ('92), and hence they need confirmation.

It is evident, I think, from the foregoing *résumé* that we cannot with much confidence compare, somite for somite, the bodies of even the most studied Arthropods, but we may present the following tentative statement, merely remarking that as far as Arachnid and Limulus are concerned, the correctness of our assumption receives confirmation from other sources discussed in this essay.

	HEXAPOD.	ARACHNID.	LIMULUS.	CRUSTACEA.
Neuromere I	No Appendage	No Appendage	No Appendage	No Appendage
“ II	Antennæ	No Appendage	No Appendage	No Appendage
“ III	Appendage	No Appendage	No Appendage	Antennula
“ IV	Mandible	Chelicera	1st Leg	Antenna
“ V	Maxilla	Pedipalpus	2d Leg	Mandible
“ VI	Labium	1st Leg	3d Leg	Maxilla 1
“ VII	1st Leg	2d Leg	4th Leg	Maxilla 2
“ VIII	2d Leg	3d Leg	5th Leg	Maxilliped 1
“ IX	3d Leg	4th Leg	6th Leg	Maxilliped 2

It will be noted that this comparison brings the end of the Hexapod thorax, and the hinder margin of the cephalothorax of both the Arachnids and Xiphosures into correspondence. Further, if we insert into the Crustacean line a segment for

<sup>1</sup> Professor Weldon and myself are apparently at variance upon several points with regard to the embryology of Crangon, but the points in dispute cannot be settled except by renewed observation. I would, however, point out that in several places he has attributed to me views which I do not hold and which he would not have obtained had he read my papers carefully or had he availed himself of his opportunity to talk over the points of difference while his paper was in press. Thus he says (*l. c.* p. 349) that I claim that the blastopore closes completely. I have ('89, p. 6) repudiated this view. He refers to my “remarkable Fig. 32 . . . in which a black dot placed between the optic lobes is called the mouth” as representing my evidence as to the postoral nature of the antennulæ. The figure is distinctly stated to be a diagram to illustrate the plane of the sections; Fig. 11 is the one to which he should have referred. I have no desire for controversy, but would respectfully suggest that possibly Weldon's figure (7),

the metastoma (which numerous authors have regarded as an appendage homodynamous with the others) the result will be to bring the third thoracic foot of the Hexapod into homology with the first rather than the second maxilliped of the Crustacea, and there is no little evidence to show that here, if anywhere, the line between head and thorax in the Crustacea is to be drawn. We are, however, more concerned at present with the serial comparison between *Limulus* and the Arachnids, and the studies of the nervous system warrant the comparisons made above.

Although we are fully justified in the recognition of somites in both *Limulus* and Arachnids in front of the segment of the first appendage, I have, from convenience, followed the old nomenclature in the following discussion, and have numbered the somites according to the appendages, somite I being that of the first appendage. From the foregoing it will be seen that point 7, the transfer of appendage I from a postoral to a prestomial position has but little value in deciding the affinities of *Limulus*, while, if our comparisons be correct, point 10, the existence of a regional division of the body behind appendage VI occurs in the Hexapods and possibly in the Crustacea as well. Point 35—the absence of any differentiated head—is closely allied to this last. In the Arthropods the terms “head” and “thorax” must be used with physiological rather than morphological values. The cephalothorax of *Limulus*, as well as that of the Arachnids, is co-extensive, so far as our present knowledge goes, with both head and thorax

upon which he relies to support his statement that “the first antennæ are evidently preoral from the very earliest period at which the mouth is visible,” represents not the first antennæ, but the optic lobes alone. Herrick says ('92, p. 442) that he cannot agree with me in saying that Reichenbach “has all the appendages at first distinctly postoral.” Reichenbach figures (Fig. 7*a*) the condition to which I referred. A leader (*lb.*) goes to the “labrum,” a thickening of cells some distance in front of his antennulæ (*E.ii*). His sections show that there is behind this but (if I read his description aright) still in front of the antennulæ, a mass of cells, his “Vorderdarmkeim.” If this be so I am certainly justified in my reference to Reichenbach as showing the mouth in front of the first pair of appendages. There is as yet no functional mouth found, but the collection of cells indicated by Reichenbach marks the point of the later stomodæal invagination.

of the Hexapod, and — if we be permitted to recognize a metastomal somite in the Crustacea — with the “head” of the Tetracapods and also of that of the Decapods as limited by Milne Edwards, but not with the Decapod head as understood by Huxley. The argument which Huxley draws for placing the division between head and thorax in the Crustacea between appendages V and VI, is largely based upon his views of the cervical suture of the crayfish which Dana long before ('51) showed to be untenable and which Ayers ('85) has more lately reviewed.

Upon the standpoint we have taken the cephalothorax of the Arachnids and *Limulus* must be regarded as equivalent to the combined head and thorax of the Hexapod. In the forms first mentioned we find no tendency towards a differentiation of this region except in the case of the Solpugids, a knowledge of whose embryology would prove so interesting. As the Solpugids are not primitive forms, and as no such regional divisions occur in the more ancestral types, we would rather suspect that the apparent existence of the Hexapod thorax in the group was secondary and adaptional rather than derived from a common ancestor. Thorell's view that the Solpugids are Hexapods is not tenable.

In this connection the multiarticulate character of the anterior appendages of both *Limulus* and the Arachnids is interesting. In the Hexapods and Myriapods the mandibles are at no time of either embryonic or adult life multiarticulate, a fact which would apparently indicate that this appendage had obtained its present form and function at an early period. It is, as Lang has suggested, hardly to be supposed that the well segmented corresponding appendage of the Arachnids has been derived from the specialized mandible of the Hexapods. The modification of the basal joints (coxa) of several appendages in both *Limulus* and scorpions for manducatory purposes should be alluded to here as well as the persistence of the same number — six — of articles in the legs of these animals.

It would hardly seem necessary to review in detail the arguments for the homology of the respiratory organs of

Limulus and the Arachnids were it not that the question is frequently misunderstood. The difficulty, at least in some instances, seems to lie in the failure to recognize the possibility of the tracheæ of the Hexapods and those of the Arachnids being homoplastic rather than homologous organs. Thus to these persons the attempt to derive the tracheæ of the Arachnid from the gill of some branchiate form seems to imply the promotion of the Arachnids to the position of the "Stammform" of the Tracheata, a conclusion which no one would care to defend at the present day.

There exists at present no question of the accuracy of the view of Leuckart ('49) that the lungs of the scorpions and spiders on the one hand and the tracheæ of the Araneina and other Arachnids are homologous, but these organs differ in one important respect from the tracheæ of the Hexapods which would prevent their close comparison. In the Hexapods (as also in the Chilopodous Myriapoda) the stigmata are placed outside or dorsal to the appendages and they never develop in connection with the legs. The observations of Chun ('75) followed by those of later writers would tend to show that the Hexapod tracheæ were derived from dermal<sup>1</sup> glands. In the Arachnids, observations are as yet lacking as to the ontogeny of the tracheæ, but several students have described the development of their homologues, the pulmonary organs.

The lungs of scorpions — Metschnikoff, ('71); Kowalevsky and Schulgin ('86) Laurie ('90) — and those of spiders — Bruce ('87); Locy ('86) — develop in connection with the abdominal feet in the embryo. The lung-leaves arise as outgrowths upon the posterior faces of these appendages, concomitantly with the formation of a pit behind the appendage and the sinking of the appendage itself. In this it is, making allowance for the position of the organ — freely projecting in the one, sunken in the other — closely comparable to the gills of *Limulus*, and in the later stages, the resemblance extends to the minute histological details.

<sup>1</sup> The recent speculations of Bernard ('92, '93) in which the endeavor is made to trace all tracheæ — Hexapod and Arachnid — to the setiparous glands of the Chætopods should possibly be referred to here.

Between the lungs of the scorpion and the gills of *Limulus* the resemblances are closest. In *Limulus* the gills are borne on appendages VIII–XII, in the scorpion upon appendages IX–XII and in no Arachnid do tracheæ occur behind this point.<sup>1</sup> Farther, appendage VIII in the scorpion — the pecten — shows plainly its homologies with its homologue in *Limulus*, the teeth of the comb being the gill-leaves. This is exactly what we should expect upon our hypothesis, for the scorpions, where the resemblances are closest, are admitted by all to be the most primitive of the Arachnids and which naturally should possess the most ancestral type of respiratory organs. The other view, that the lungs are modified tracheæ,<sup>2</sup> leads into considerable difficulties for we then find the oldest stock, — the Stammform of the Arachnida — possessing the most highly differentiated organs of breathing, while in the most aberrant groups the tracheæ have been retained in an unmodified condition. Again the Arachnida as a class, according to the observations of Plateau ('86) and Berteaux ('90), show an entire absence of those visible respiratory movements of the body wall which are so characteristic of Hexapods and Chilopods, a fact in full accordance with the thesis here maintained but not easily explained upon the standpoint of a common origin of all Arthropod tracheæ.

Farther, the conversion of the gills directly into tracheal tubes is at present going on in the case of the Oniscid Crustacea where we have tubes lined with a chitinous intima penetrating to the interior of the organ and conveying air to the blood.

<sup>1</sup> Bernard claims ('93) to have found traces of stigmata in the Pseudoscorpions behind this point, but apparently his discovery is not a new one for von Siebold pointed out, over forty years ago ('53, p. 370) that Bernard's predecessors had also mistaken the cutaneous insertion of muscles for stigmata.

<sup>2</sup> This view is held by Sinclair ('92) who seems to ignore the possibility of there being two kinds of tracheæ; and, influenced by his observations upon the peculiar dorsal tracheæ of *Scutigera*, states his opinion "that we have a series from the simple tracheæ found in *Peripatus* up to the complete lungs of spiders which is incapable of explanation in the present state of our knowledge, except as representing the stages of development of tracheæ into the pulmonary organ of spiders." A few lines lower he seems to think that the derivation of the lungs of scorpions from gills implies a difference between spiders and scorpions greater than has been supposed.

Cases like this clearly show us that tracheæ may arise in different ways from different sources.

The presence of the so-called spiral thread in the tracheæ of both Arachnids and Hexapods has been adduced as an argument in favor of the homology of the organs in both groups. As in both cases the tracheæ are formed as invaginations of the external integument it is natural that they should consist of tubules of ectoderm lined with a chitinous intima, and the thinner this intima the easier the transfer of gases through it. But if it become too thin the tube is liable to total collapse by the pressure of the various viscera upon it and so the chitinous layer is developed into folds or corrugations, which when regularly arranged form the spiral "threads." In many spiders they are not regular and show clearly their origin in response to the mechanical conditions presented.

The greatest difficulty in connection with this view of the origin of tracheæ from gills through the lungs is that presented by the Solpugids and certain Acarina where tracheal stigmata occur in the cephalothoracic region, where they should not occur according to our thesis. Yet until we know more of the structure and ontogeny of these organs the full weight of this objection cannot be properly estimated. A full history of *Solpuga* would settle many questions of arthropod morphology.

To summarize: The lungs of the scorpion arise in the same way and on the same somites as the gills of *Limulus*. In the one they sink into a pit, in the other they remain free. The homologies between the lungs and tracheæ of Arachnida were demonstrated by Leuckart. Hence the lungs, the fan tracheæ of authors, are to be regarded as the primitive, the bush-like the derived form, and these tracheæ have no relation to those of Hexapods.

The comparisons between the nephridia of *Limulus* and those of the Arachnida have been made upon a previous page. The observations made by Laurie, Kishinouye, and myself have clearly shown that we have to deal here with structures homologous with the nephridia of the worms, although but a single pair may persist in its unmodified condition. We find, how-

ever, nephridia occurring either in the young or the adult of other Arthropods, and hence a more accurate review of our knowledge becomes necessary. In Peripatus the investigations of von Kennell ('84), and especially of Sedgwick ('88), have shown that in each somite, except the posterior one, the cœlom on either side divides into dorsal, lateral and ventral moities; the dorsal becomes converted into the gonad, while the ventral portion becomes converted one part into the nephridium and the lateral into the funnel and end sac. The connection between the dorsal and ventral portions of the cœlom persists in the posterior somite, and from the cavity thus formed the genital ducts are developed, in other words, the posterior nephridia of Peripatus become modified for reproductive ducts. In the Hexapods Heymons ('90), Graber and Cholodkowsky ('91)<sup>1</sup> have described a similar division of the cœlom into three portions, the gonad developing in connection with the dorsal portion; the formation of genital ducts, much as in Peripatus; and the development of the third division into a temporary structure to be regarded as the homologue of the nephridium of Peripatus, and which later disappears. In the Crustacea nephridial structures also occur. Our knowledge of them and of their relations to the cœlom are most detailed in Decapod. Here Weldon ('89, '91) has described a large cœlomic dorsal sac, extending back to the heart and the gonads and connected ventrally with the green (antennal) gland, the character of which as a nephridium is thus placed beyond question.<sup>2</sup> The position of the so-called "shell gland" is less certain, though all evidence goes to show that this is also to be regarded nephridial. As I pointed out several years ago this organ, opening in the Crustacea at the base of the second maxilla is apparently exactly homologous with the coxal gland of Limulus. Although recent researches (*vide supra*) have changed our views

<sup>1</sup> There is not full agreement between these authors as to the details of the process.

<sup>2</sup> Both Grobben ('79) and myself ('89) have shown that the green gland of the decapod is mesodermal. Richenbach in his first paper upon *Astacus* ('77) stated that it was derived from the ectoderm. Although he was corrected in this by Grobben he reiterates his account in his later paper on the crayfish ('86) and ignores Grobben's correction.

of the somites of the Arthropods, still if the metastoma be admitted as an appendage in the Crustacea, the correspondence between the opening of the duct of their shell gland and that of the coxal gland is exact. Another fact which goes to show that the shell gland is nephridial is, that it and the antennæ gland but rarely coexist in the same individual (*Nebalia*, Claus).

From the evidence presented by the nephridia therefore, we are justified in the close association of the Arachnids and the Xiphosures. We are also apparently led to associate these two groups more closely with the Crustacea than with the Hexapods.

The correspondence between the genital ducts of *Limulus* and those of the Scorpion is as close as that of the respiratory organs. In *Limulus* the genital ducts in both sexes open upon the posterior surface of appendage VII. In the scorpions Narayanan ('89) has shown that the genital operculum is a paired organ in both sexes and that the genital ducts open up on what is morphologically its posterior surface. Farther, Laurie's observations upon the development of the ducts show beyond a question that they belong to somite VII. In *Limulus* I have failed to see the development of the duct, it being apparently delayed even longer than in the scorpion.

There is to-day little doubt that the genital ducts of all Arthropods are to be regarded as modified nephridia. I have alluded, just above to the method of development of these structures in the Hexapods and in *Peripatus*. In the scorpion Laurie's account of their development would indicate that here, too, they are to be classed in the same category, the later appearance of their external opening being the greatest objection to such a view.

In the Crustacea we have, so far as I am aware, no direct observations upon the ontogeny of the ducts, but the facts of comparative anatomy are all but conclusive. Thus the relations of the gonads to the persistent cœlom are such as would be required were the ducts segmental organs, while the varying position of the ducts themselves in the two sexes of the same species and the fact that in abnormal instances two pairs of ducts may occur in the same individual, show that they

must have been derived from some metameric structure connecting the cœlom with the exterior, and the nephridia of the annelids are the most probable if not the only ducts which answer the conditions.

Limulus, the Arachnids, the Crustacea and the Chilognaths agree in having the genital ducts some little distance in advance of the posterior end of the body while in the Hexapods and Chilopods they are sub-terminal, but how much weight is to be given this point is not yet apparent.

The reticulate and anastomosing character of the genital ducts in Limulus and the Arachnids has been commented upon by Lankester. Such conditions are not paralleled in the Arthropods except in certain Phyllopods. Again the existence of motile spermatozoa in both Limulus and Arachnids and their absence from all Crustacea except the Cirripedia has a certain value as cumulative evidence.

The correspondences between the circulatory systems of Limulus and the scorpions are remarkably close. In both there is the same median anterior aorta which divides and passes downward, as a pair of sternal arteries—one passing on either side of the œsophagus—which unite below in a ventral vessel in close connection with the ventral nerve chain. In the scorpions this ventral vessel consists of an artery<sup>1</sup> lying *upon* the nervous system, and following not only the ventral cord but the various metameric nerves which arise from it. This condition, which is characteristic of the adult scorpion is found in the earlier stages of Limulus. Later the neural artery completely envelopes the ventral cord and its nerves in the manner first pointed out by Owen ('55, p. 310) and later so elaborately described by the younger Milne-Edwards ('72).

This relation between the neural artery and the ventral nerve chain is not confined to the Arachnids and Limulus; a large supra-neural vessel occurs in the Isopods and a smaller one in the Amphipods, each connected with the dorsal vessel

<sup>1</sup> Houssay ('87) claims that this vessel in the scorpion is lacunar rather than arterial, a view which is negated by its well-marked walls and its lack of connection with the other extra-vascular spaces of the body.

by similar paired sternal arteries. A similar supra-neural vessel was described long ago by Newport ('43) in several Myriapods. The supra-neural vessel of the Chætopods will naturally suggest itself in this connection. In the Hexapods, on the other hand, the sternal arteries and the neural artery have disappeared, possibly as a result of their richly developed tracheal system. In *Peripatus* also no supra-neural vessel is found, the ventral vessel first described by Balfour ('83) lying in the body wall and the "blood spaces" shown in Sedgwick's monograph lying near the ventral cord, are lacunar rather than arterial.

The alimentary canal of *Limulus* and the Arachnids agrees in the fact that nearly the whole tract is composed of stomodæum and mesenteron while the late appearing proctodæum is short. They also agree in the metameric nature of the lobulation of the hepatopancreas, the lobes being at first outlined by the ingrowth of the mesodermic septa into the yolk. In the Hexapods on the other hand the proctodæum appears much earlier and is comparatively long, at least equalling the stomodæum in this respect. In the Crustacea on the other hand the mesenteron plays but an inconspicuous part in the formation of the digestive tube, it being mostly restricted to the so-called liver.

The possession of an entosternite which characterizes both *Limulus* and the Arachnids, the structure and relationships of which has already been discussed by Lankester ('84) is only paralleled outside these forms in a few Crustacea (certain Ostracodes, Claus, '92).

The argument for the association of *Limulus* with the Crustacea and its separation from the Arachnids, based upon the possession of biramous appendages, has been accorded more weight than seems justifiable. At no stage of development do we find a biramous condition in the cephalothoracic appendages of *Limulus*, while that of the abdominal appendages may prove to be far different from that of the Crustacea. It appears much later than in the Crustacea, is characterized by a hypertrophy of the exopodite, and lacks the evident segmentation found in most Crustacea.

On the other hand, we must not lose sight of the fact that numerous observers have recorded a biramous condition in the appendages of various "Tracheates." Among others we would mention the biramous pedipalps in *Dendryphantès* recorded by Croneberg ('80), the biflagellate antenna of an Indian *Lepisma*, and of an embryo *Blatta javanica* by Wood Mason ('79), the bifid condition of the antenna of *Blatta* by Wheeler ('89), while Patten ('84), in the same form describes the maxillæ and labium as "formed respectively of two and three branches, the second maxillæ thus attaining the typical trichotomous structure of the Crustacean appendages." Neither must we forget the peculiar antennæ of the Pauropida in this connection.

The so-called Malpighian tubes (point 3.) have a far different bearing upon the classification of the Arthropods from that which they were supposed to have a few years ago. In fact, two entirely different structures have been included under the one name, and the existence of excretory tubules in both Hexapods and Arachnids, instead of proving the close relationship of the two groups, is, in view of our present knowledge, an argument against it. In the Hexapods these organs have been shown by numerous observers to be of proctodeal, and, therefore, of ectodermal origin. In the Arachnida the supposed homologous organs, to which the same name has been given, are, in all probability, outgrowths from the mesenteron, and hence entodermal. This has been shown by Loman ('86-7) for both the tetra- and the dipneumonous *Araneina*, and by Laurie ('90) for the scorpion.<sup>1</sup> Hence these organs, — ectodermal in the one group, entodermal in the other — instead of indicating community of descent for Arachnids and Hexapods, must rather be regarded as indicating that the group Tracheata as usually limited is polyphyletic in origin.

<sup>1</sup> Kishenouye ('90) claims that in the *Araneina* both the Malpighian tubules and the stercoral pocket are derivatives of the mesoderm, the cavity of the latter being the cœlom of that region of the body. This is on its face improbable. It would seem that the failure of many investigators to recognize that these tubules are entodermal in origin was due to the fact that since they were known to be ectodermal in the Hexapods, they have been used as regional tests, the fact that they arose from a certain part of the alimentary canal being sufficient reason for regarding that portion as proctodeal. Beddard's view ('89) that the Malpighian tubes are derived from nephridia secures no support in the Arachnida.

Again, as I previously argued, the existence of similarly placed tubules in certain Amphipods can be advanced as an argument for the closer association of the Arachnids and the Crustacea. Still exact knowledge of these tubules in the Amphipoda is lacking. Nebeski ('80) regards them as diverticula of the hind-gut, while Spencer ('85), upon histological grounds, regards them as outgrowths of the mesenteron and hence, like those of the Arachnids, entodermal. It must be said, however, that this evidence is not conclusive, as the limits of the hind-gut are not clearly ascertained, and the assumption that these are entodermal is based upon the absence of a chitinous cuticle (Spencer, '85, Pl. XIII, Fig. 2) and by a break in the character of the epithelium in the alimentary canal at the point of origin of these tubes, the tubes themselves apparently belonging to the anterior portion.

All, then, that can be argued from the various structures known as Malpighian tubules is that homoplastic and analogous organs, rather than exact homologues, are included under this name; that their existence in both Arachnids and Hexapods is an argument against the close association of these forms and that their absence in *Limulus* can only be used as a negative argument of little weight. In this connection the conditions figured on Pl. XIII, Fig. 88, deserve more detailed study in later stages.

The presence of salivary glands in the "Tracheates" and their absence from the "Branchiates" (Crustacea, *Limulus*) is possibly to be explained by the different method of life of the members of the two groups—aquatic in the latter, terrestrial in the former. It is, however, to be noted that salivary glands have been recognized in *Astacus* (Lang, '89, p. 344), while renewed studies must be made of the so-called salivary glands of the Arachnida before we are certain of their homology with those of the Hexapods. Several organs which have been called salivary glands among the spiders and their allies have been shown to be coxal glands (*i.e.* nephridia) or poison glands, and it is possible that all of these organs may have different homologies than those indicated by the name usually applied to them.

There is one point of resemblance between the Arachnids and the Hexapods which may have no inconsiderable weight. In the Scorpions as in the Hexapods, the embryo develops those as yet unexplained foetal membranes which so closely simulate those of the higher vertebrates. It may be that here, as in other places, we have similar but not identical organs. The accounts of their development in the Arachnids by Metschnikoff, Kowalevsky and Schulgin, and Laurie differ considerably, and until we know something of the ancestry and real meaning of the structures which are united under this head we cannot be certain of the taxonomic value to be placed upon them. It may be noted here that the structures described by Bruce ('87) as occurring in the spiders are in all probability not amnion and serosa, but either the invaginations in connection with the brain or the inpushing to form the median eye.

#### THE CLASSIFICATION OF THE ARTHROPODA.

As a result of my studies it would seem as if the Arthropoda must be divided in some such manner as that here given :—

- Phylum Arthropoda.
  - Sub-Phylum Branchiata.
    - Class Crustacea.
    - Class Acerata.
      - Sub-Class Gigantostraca.
      - Sub-Class Arachnida.<sup>1</sup>
  - Sub-Phylum Insecta.
    - Class Hexapoda.
    - Class Chilopoda.
  - Sub-Phylum Diplopoda (Chilognatha).

#### *Incertæ Sedes.*

- Pauropoda.
- Pycnogonida.
- Trilobitæ.
- Tardigrada.
- Malacopoda.

<sup>1</sup> The attempt by Haller ('81) to separate the Acarina as a distinct class hardly seems warranted.

While the present is not the proper opportunity to support the above classification in detail, a slight amount of explanation seems necessary with regard to some of the novelties introduced above.

It has been shown, I think conclusively, that the relationship existing between the Arachnida and the Xiphosures is very close; that they have more affinities with each other than, on the one side, the Arachnida have with the other "Tracheates," or than *Limulus* has, on the other hand, to the Crustacea. For the class formed by the union of these forms I proposed, eight years ago, the name *Acerata*,<sup>1</sup> a modification of the term *Acera* applied by Latreille to the Arachnida alone. For the sub-class containing the Xiphosures and the Euryptera I have followed Dohrn in modifying and adopting the term *Gigantostraca* of Haeckel. For essentially the same group Packard has proposed at different times the names *Palæocarida* and *Podostomata*, while Steinmann and Döderlein (*Elemente der Paläontologie*) have applied to the same association of forms the term *Palæostraca*.

The resemblances between the *Acerata* and the Crustacea are much closer than those between either and any of the other groups of Arthropods, and from the fact that in each respiration is effected by gills or by their homologues, developed in all cases as membranous expansions of the limbs, the older term *Branchiata*, used with enlarged scope, seems most applicable to the group or sub-phylum formed by their union.

The so-called Myriapoda seems to be a heterogeneous association of forms, polyphyletic in origin, and only associated together through the possession of many locomotor appendages. On the other hand, the resemblances between the Chilopods and the Hexapods are far more numerous and of far more sig-

<sup>1</sup> Since this use of the term *Acerata* Lankester has employed it ('90) as equivalent to the term *Branchiata* as limited in this article. Cholodkowsky ('91) objects to my group *Acerata* apparently more upon the inapplicability of the term than from any objection to the association of the Arachnids with the Xiphosures. As I think I have strengthened the ground for such union in the present article a name for the group becomes necessary, and as in both the Xiphosures and the Arachnids functional (if not morphological) antennæ are entirely lacking, I may be permitted to continue the use of the term.

nificance than those between Chilopods and Diplopods (Chilognaths). This was pointed out some years ago by Mr. Pocock ('87), of the British Museum, while I, independently ('88), stated similar conclusions.

Until we know more of both the structure and the ontogeny of the Myriapod forms the correctness of this view cannot be regarded as settled, but in the present state of our knowledge the following facts seem important:

The Diplopod head bears, besides the antennæ, but two pairs of appendages, — a pair of mandibles and a lower lip, composed of a pair of coalesced maxillæ.<sup>1</sup> In the Chilopod the conditions are as in the Hexapod, two pairs of maxillæ being present.

In the Chilopods as in the Hexapods, each somite bears a single pair of appendages, while in the Diplopods the majority of the segments bear two pairs of appendages, and the researches of Heathcote show that each segment is in reality composed of two coalesced somites, a condition without parallel elsewhere in the Arthropoda. In the Chilopods there is a wide sternum separating the coxæ of the ambulatory appendages; in the Diplopods the coxæ are approximate, and the sternum is exceedingly narrow, or even entirely absent.

In the Chilopods the stigmata, a pair to a somite, are lateral (dorsal in *Scutigera*), and are placed above and outside the insertion of the limbs, exactly as in the Hexapods. The tracheæ which arise from them are branched, and the intima is thrown into a well developed spiral thickening as in the six-footed insects. In the Diplopoda, on the other hand, the stigmata are beneath or even in the coxæ, while the tracheæ (except in the *Glomeridæ*) are tufted and unbranched, and the thickening of the intima is poorly developed.

In the Diplopods there are well developed foramina repugnatoria upon the sides of each somite of the body. Such

<sup>1</sup> The attempt made to show that this lower lip is a "gnathochilarium" composed of the two coalesced lower jaws, or first and second maxillæ of the Chilognaths receives no support from the embryology of *Julus* (Heathcote '88), where there is but a single somite when the hypothesis calls for two. Further the innervation of the sense organs of the lower lip (*cf.* vom Rath. '86, Pl. XX, Fig. 1) shows that but a single pair of appendages is concerned in the part.

structures are absent from the Chilopods (as from the Hexapods), except in a few Geophilidæ, where repugnatorial glands occur, opening by foramina in the mid-ventral line.

In the Chilopods the reproductive organs consist of paired<sup>1</sup> gonads situated above the alimentary canal and opening to the exterior by ducts which are at first paired, but which later unite into a common tube which leads to a single external opening situated in the penultimate segment of the body. In the Hexapods the conditions are almost exactly the same; the gonads are dorsal, the genital ducts unite (except in Epheméridæ), and there is a single external opening, always at the posterior end of the abdomen. In both Hexapods and Chilopods the spermatozoa are motile. In the Diplopods there is a single unpaired gonad situated beneath the alimentary canal, and the genital duct, passing forward, divides into two, each of which has its own opening at the bases of the legs of the second post cephalic segment. The spermatozoa are quiescent.

We know so little of the embryology of the Myriapods that the aid of development can be had to only a slight extent in our comparisons, but the facts which it affords seem important. In the Chilopods the embryo escapes from the egg with numerous ambulatory appendages, a pair to each somite. The same is true of the typical Hexapods, all later observers agreeing that a polypod precedes a hexapod condition. The young Diploped escapes from the egg in a hexapod condition, and the presence of these six legs has been seized upon as a proof of the near association of these forms. An exact comparison, however, seems to show that the two are in reality very unlike as appears in the following table.<sup>2</sup>

<sup>1</sup> Single in Scolopendra.

<sup>2</sup> As nothing is known of the existence of a tritocerebral segment in the Diplopods, the comparison can only be made upon the basis of the appendages of the adult. If the tritocerebral segment should prove lacking in the millepedes the contrast will prove stronger than it now is. The statement of the Diploped appendages is based upon Heathcote ('88).

	HEXAPOD.	DIPLOPOD.
Appendage I	Antenna	Antenna
“ II	Mandible	Mandible
“ III	Maxilla 1	Lower Lip
“ IV	Maxilla 2	Foot 1
“ V	Thoracic Foot 1	Absent
“ VI	Thoracic Foot 2	Foot 2
“ VII	Thoracic Foot 3	Foot 3
“ VIII	Abdominal Foot 1	Absent
“ IX	Abdominal Foot 2	Absent

The result of these comparisons is sufficient, I think, to justify the dismemberment of the old group Myriapoda and the association of the Chilopoda with the Hexapoda in a group to which the much abused term Insecta may be applied, while, until more definite knowledge be obtained, the Diplopoda must be allowed to stand alone. The position of the Pauropoda is as yet very uncertain as we are almost entirely ignorant of their internal structure. In the tendency towards a fusion of somites, in the lack of a second pair of maxillæ, and in the positions of the external paired openings of the genital ducts at the base of the second pair of ambulatory appendages they show undoubted affinities with the Diplopoda; but the peculiar triramous antennæ and especially the characters of the hexapod young (if Ryder's (79) figure of the young of Eurypauropus be correct) militate against this view.

The Malacopoda<sup>1</sup> (Peripatus) are also frequently placed in close association with the Myriapods, but it may be that their status as members of the Arthropod phylum is not beyond question. In the following points they differ from all “Tracheates” and also from all Arthropods, while in just these same points they show affinities with the Annelids:—

The presence of functional nephridia in each body segment; the presence of well developed coxal glands (*cf.* setiparous glands of Annelids); the existence of an outer circular muscular

<sup>1</sup> Malacopoda, Blanchard 1847; Onychophora, Grube 1853; Protracheata, Moseley 1874.

layer in the body wall; the absence of striation from all muscles except those of the mouth parts; the presence of cilia in the alimentary canal and in the nephridia; the situation of the antennæ as outgrowths from the primitively preoral region (*cf. supra* p. 232); the muscular nature of the pharynx, unlike that of any Arthropod and strikingly like that of certain Chætopods. The eyes too are unlike the visual organs of any other Arthropod but as figured by Balfour they closely resemble these organs in *Autolytus*. It is noticeable that Balfour has described ('83) a pair of problematical organs upon the lower surface of the brain of *P. capensis* (the auditory organs of Grube, '53). Sedgwick has shown that these organs are developed by an invagination of the cerebral surface while the slight account given by Balfour of the adult structure at once suggests a degenerate eye formed upon the same plan as the functional one. In *Autolytid* worms a second pair of eyes occur at the same point.

On the other hand the Arthropod structures are not to be ignored; the tracheæ; the appendicular jaws; the setting aside of a pair of nephridia for genital ducts; the heart, with several paired ostia, enclosed in a pericardium; the lacunar circulation, and the reduced cœlom.

To the discussion of the position of the Pycnogonids and the Tardigrades I can add nothing. Morgan ('90) has, it seems, shown that the Pycnogonids present certain features both in ontogeny and in adult structure which can only be paralleled in the Arachnids, while the Tardigrades may have no other claim upon a position in the same group than that afforded by their eight ambulatory feet.

For many years the general consensus of opinion has been to the effect that the Trilobites are closely related to the Xiphosures. We unfortunately know but little regarding the structure of the Trilobites aside from the features presented by the dorsal surface. For our knowledge of the appendages we have to thank the papers of Billings ('70) and Walcott ('81 and '84). From Billings' paper (and from electrotypes of his specimens which I have studied) we can learn but little except the presence of jointed appendages. Walcott's researches tell much more, but the facts which they have added all are

opposed to the close association of the Trilobites with *Limulus*. The body of *Limulus*, it must be remembered, possesses an anterior cephalothorax bearing six pairs of circumoral chelate appendages without differentiation into exopodite and endopodite,<sup>1</sup> and with no trace of gills. In the Trilobite but *four* pairs of appendages occur in this region. Hence, if the "head" of the Trilobite is to be compared with the cephalothorax of *Limulus* we must assume — for which we have as yet no evidence — that two pairs of appendages have been lost from the Trilobite. The abdomen of *Limulus* bears six pairs of broad leaf-like appendages, the posterior five pairs having lamellate gill books upon the posterior surface. In the corresponding region of the Trilobite, the thorax, we have an indefinite number of somites, each of which bears the *typical Crustacean foot*, consisting of basiopodite, exopodite, and endopodite, and, outside the exopodite, occupying the same position as the gill in the Decapod, a straight or curiously coiled structure interpreted by Walcott as the gill. In the horse-shoe crab the abdominal region extends to the anus, behind which comes the non-segmental tail. In the Trilobites the thorax is followed by a segmented pygidium on which the series of appendages<sup>2</sup> is continued to the end, and there is no evidence of a supra-anal telson.

The necessary conclusion is that *the appendages of the Trilobite vary in number and differ totally in structure from those of Limulus*, and the association of the Trilobites with the Xiphosura is not warranted by our present state of knowledge. The trilobites would appear to be true Crustacea, the sessile eyes and general shape of the body allying them to the Isopods,

<sup>1</sup> The flabellum of the sixth appendage cannot be considered as a representative of the exopodite since it develops later than the rest of the limb, develops independently of it and only in the later embryonic stages does the base of the leg enlarge so that it is included.

<sup>2</sup> Walcott continues the series of ambulatory appendages through this region, but Professor Mickleborough ('83), who found the specimen forming the basis of Walcott's second paper ('84), thinks that these pygidial appendages were lamellar. Henry Woodward ('70) describes what he considers as the jointed palpus of one of the "maxillæ" of *Asaphus* with seven articulations beyond the basal joint.

while the biramous appendage and the epipodial gills would rather indicate relationships between the Phyllopoets and the lower Podophthalmia. It will prove a profitable field for some student of Arthropod morphology to repeat Walcott's earlier investigations. It will be noticed that I place the grounds for rejection of the association of *Limulus* and the Trilobites upon different grounds from those advanced by Owen ('72, pp. 491-493) and the older Milne Edwards ('81).

TUFTS COLLEGE, MASS.,  
Mar. 12, 1893.

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## EXPLANATION OF THE FIGURES.

## REFERENCE LETTERS.

<i>a.</i>	Anus.	<i>mu.</i>	Muscles.
<i>an.</i>	Neural artery.	<i>n.'-n.</i> <sup>8</sup>	Segmental nerves.
<i>ar.</i>	Artery.	<i>nd.</i>	Nephridial duct.
<i>av.</i>	Ventral (circumneural) artery.	<i>nc.</i>	Nephridium.
<i>bs.</i>	Blood sinus.	<i>n. a.</i>	Neural anlage (in Fig. 54, the anterior loop of the nephridium).
<i>c. 1-c. 9.</i>	Cœlomic cavities.	<i>no.</i>	External opening of nephridium.
<i>ce.</i>	Cerebrum.	<i>nst.</i>	Nephrostome.
<i>ct.</i>	Cuticula.	<i>oc.</i>	Ocellus.
<i>cc.</i>	Ectoderm (in figures 50-52 edge of carapax).	<i>a.</i>	Œsophagus.
<i>en.</i>	Entoderm.	<i>ac.</i>	Œsophageal commissure.
<i>ent.</i>	Entapophysis.	<i>on.</i>	Nerve to ocellus.
<i>es.</i>	Entosternite.	<i>op.</i>	Operculum.
<i>ev.</i>	Excretory vesicle of nephridium.	<i>pd.</i>	Proctodæum.
<i>g.</i>	Ganglion.	<i>pe.</i>	Pavement epithelium of cœlom 5.
<i>gl.</i>	Gill-leaves.	<i>pr.</i>	Proventriculus.
<i>h or ht.</i>	Heart.	<i>ps.</i>	Pericardial sinus.
<i>hep.</i>	Hepatic duct.	<i>pst.</i>	Primitive streak.
<i>i.</i>	Intima of heart.	<i>sg.</i>	Sympathetic ganglion.
<i>iv.</i>	Invaginations of nuclei in neural anlage ( <i>cf.</i> Fig. 29).	<i>sn.</i>	Sympathetic nerve.
<i>l.</i>	Liver and liver lobes.	<i>sp.</i>	Splanchnopleure.
<i>lac.</i>	Lacunæ.	<i>ss.</i>	Segmental sense (?) organs.
<i>m or mc.</i>	Mesoderm.	<i>st.</i>	Stomodæum.
<i>mes.</i>	Mesenteron and in later stages, intestine.	<i>so.</i>	Somatopleure.
<i>ml.</i>	Middle line of section.	<i>t.</i>	Telson.
<i>mg.</i>	Marginal groove.	<i>y.</i>	Yolk-entoderm.
<i>mo.</i>	Mouth.	<i>ys.</i>	Yolk spherules.
<i>mt.</i>	Metastoma.	<i>yx.</i>	Yolk cells.
		<b>I-X.</b>	Somites and appendages.



## EXPLANATION OF PLATE X.

FIG. 40. Longitudinal section of an embryo with cephalic and caudal areas and one intermediate somite developed. The arrows mark the limits of the somite.

FIG. 41. Through primitive streak in advance of mouth, Stage C, early.

FIG. 42. Transverse through the posterior primitive streak, Stage C, early.

FIG. 43. Transverse through the anterior end of the primitive streak ("mouth"), showing cœlom of somite I, Stage C, early.

FIG. 44. Through primitive streak and marginal groove, Stage C, early.

FIG. 45. Longitudinal section through an embryo of Stage C, late.

FIG. 46. Longitudinal section (a little oblique) through an embryo, with eight somites developed, showing a cœlomic cavity developed in each of the anterior seven somites.

FIG. 47. Obliquely transverse section, Stage C, showing the neural anlage, cœlom I, and the segmental structure (? gland) of somite II.

FIG. 48. Through somite V, Stage C, late. (Through a misunderstanding on the part of the lithographer this and Figs. 49 and 52, representing only one side of the body, are so turned upon the plate that the median plane is oblique.)

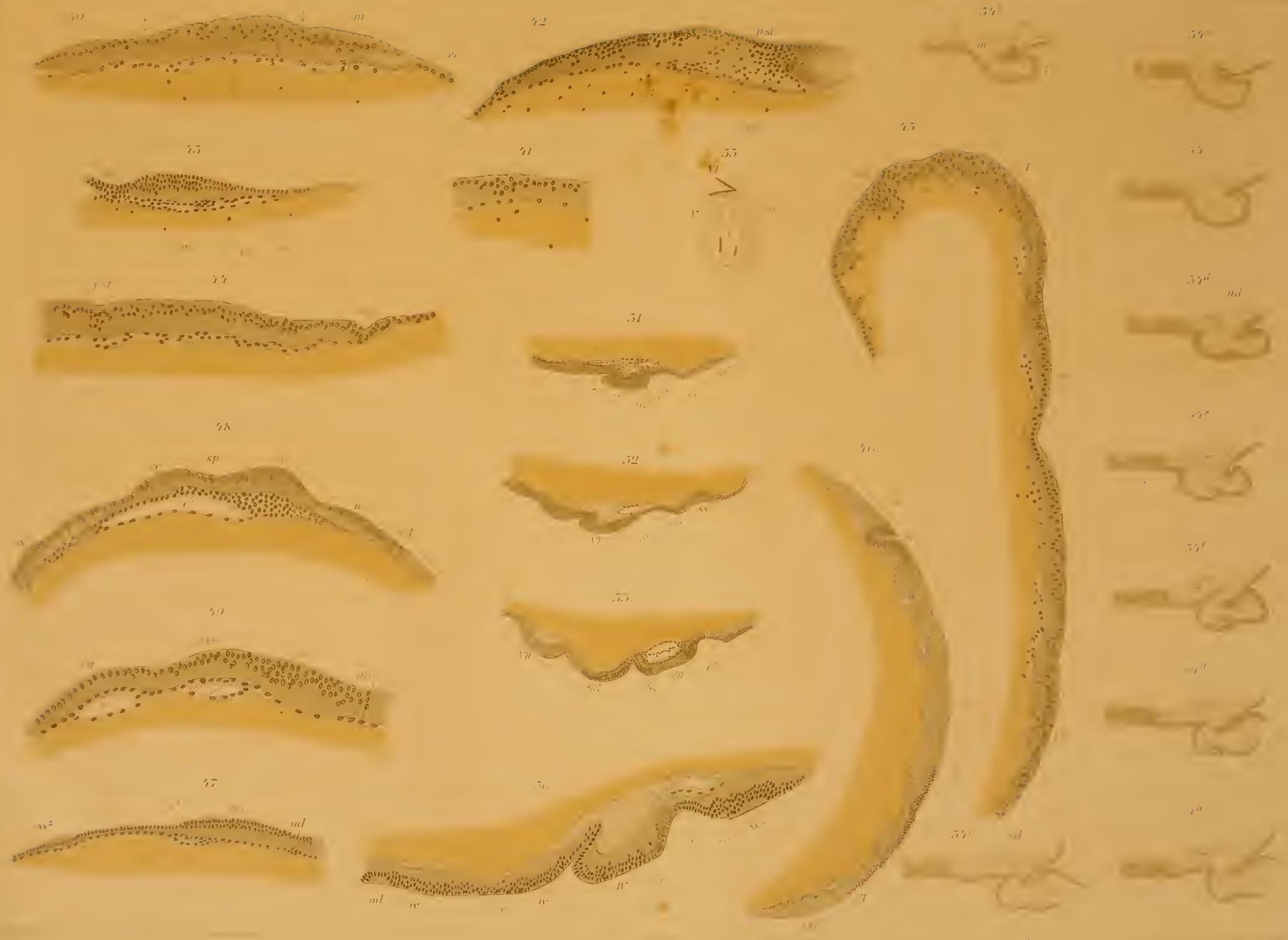
FIG. 49. Transverse through the abdomen, Stage C, late.

FIG. 50. Transverse through somite IV, showing the segmental structure (dorsal organ) of that somite, Stage D.

FIGS. 51, 52, 53. Transverse sections through somites V, VI, and VII, Stage E, showing the cœlomic cavities in each and the segmental structures (? glands) in V and VI.

FIG. 54 *a-i*. Modifications of the cœlom of somite V, Stage H, into the nephridium; Fig. 54 *i* is the most anterior. In this *n. a.* refers to the anterior bend of the nephridial tube.

Fig. 55. Reconstruction (by plotting) of the nephridium of Fig. 54.







## EXPLANATION OF PLATE XI.

FIGS. 56, 57, 58, 59 transverse sections of the nephridium, about Stage H. Fig. 56, is most anterior.

FIG. 60. Longitudinal section of the nephridium after the duct is open to the exterior. The external opening is plainly on the posterior surface of the basal joint of the fifth appendage while the loop of the duct extends nearly to somite III.

FIG. 61 *a-c*. Horizontal sections through the nephridium, Stage I.

FIG. 61 *f* and *g*. Reconstructions (in wax) of the nephridium represented in Figs 61 *a-e*.

FIG. 62. Reconstruction (in wax) of the nephridium of Stage L. To be compared with Gulland's ('85) Fig. 2. (Is exaggerated in transverse diameter.)

FIG. 63. Longitudinal section of a portion of the abdomen showing the early appearance, by splitting, of somatoplure and splanchnoplure in that region. In the neural anlage (*n.a.*) can be seen the inpushing of nuclei for rapid cell proliferation producing the pitted appearance shown in Fig. 29.

Figs. 64 and 65. Transverse sections of the heart Stage H, Fig. 65, being the more anterior and the plane of section 64 passing through appendage V.

FIG. 66. Heart, transverse, Stage I.

FIG. 67. Transverse section through abdomen, the section passing through the operculum, Stage I.

FIG. 68. From the same series as Fig. 67, but more posterior.

FIG. 69. Transverse through œsophagus, œsophageal commissures and sternal artery, Stage I.







## EXPLANATION OF PLATE XII.

FIG. 70. Section posterior to that shown in Fig. 69. The sternal arteries have reached the nervous system and lie upon it. Beneath may be seen the anterior extension of the ventral part of the neural artery.

FIG. 71. More posterior, showing that the neural artery is paired above and below and also showing connection of dorsal and ventral parts of neural artery.

FIG. 72. Horizontal section, Stage L, through proventriculus, sternal arteries and anterior end of mesenteron with ducts of the hepato-pancreas.

FIG. 73. From the same series as 72, but passing through heart and showing the bifurcation for sternal arteries, *an.*

FIG. 74. Section from same series as Figs. 69-71, passing through fifth pair of legs.

FIG. 75. Through the abdomen, Stage L.

FIG. 76. From the same series as Fig. 74, showing the connection of sternal arteries with the heart and then splitting for the stomodæum.

FIG. 77. Reconstruction (wax) of the anterior end of the heart, sternal arteries and neural artery, Stage K. At the left is shown the cavity for the ventral cord; the dark spots mark the places for the exit of nerves.

FIG. 78. Long section through the abdomen, Stage G, showing the pit-like invaginations behind appendages VII and VIII.

FIG. 79. Operculum and anterior gill-bearing appendages, Stage I. (VII and VIII should read VIII and IX respectively.)

FIG. 80. First and second gill-bearing appendages, Stage L.

FIG. 81. Longitudinal median section, Stage L, in which the connection between the mesenteron and proctodæum is not yet made.

FIG. 82. Longitudinal section, Stage I.

70



71



72



73



74



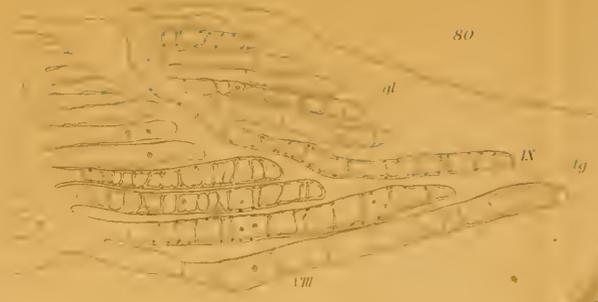
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77



80



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78



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82



81







## EXPLANATION OF PLATE XIII.

FIG. 83. Horizontal section, Stage I, to show the relations of the mesenteron and lobes of the hepatopancreas.

FIG. 84. Transverse through an embryo of Stage L, to show the conversion of yolk cells into the epithelium of the alimentary canal.

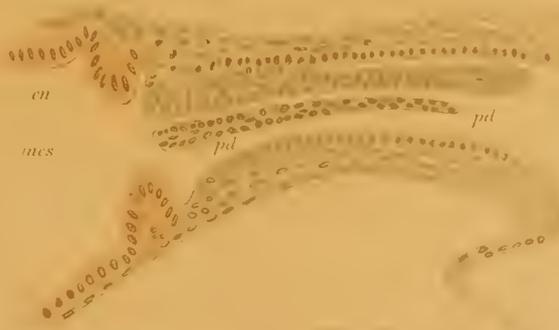
FIG. 85. Longitudinal section through the junction of stomodæum and mesenteron, Stage L, before the connection of the lumens, to show the transformation of yolk cells into the columnar epithelium of the midgut.

FIG. 86. Late Stage L showing the first hepatopancreatic duct.

FIG. 87. Late Stage L through the third pair of appendages.

FIG. 88. Longitudinal section through the oldest larva studied, showing the junction of mesenteron and proctodæum. The section was not quite median and hence cuts off the folds in the proctodæal region.

FIG. 89. Section of a leg, Stage L, to show the nerve surrounded by the artery.





# THE HABITS AND DEVELOPMENT OF THE NEWT (*Diemyctylus viridescens*).

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## TABLE OF CONTENTS.

	PAGE
Introduction .....	270
I. Natural History .....	270
1. <i>Habitat</i> .....	270
2. <i>Food</i> .....	271
3. <i>Spermatophores</i> .....	273
4. <i>Receptaculum seminis</i> .....	274
5. <i>Egg-laying</i> .....	275
6. <i>Transformations</i> .....	279
II. Maturation of the Ovum .....	280
1. <i>Technique</i> .....	280
2. <i>Origin and development of the ovum as a whole</i> .....	282
3. <i>Yolk-nucleus</i> .....	284
4. <i>Yolk formation</i> .....	291
5. <i>Germinal vesicle as a whole</i> .....	294
6. <i>Nucleoli</i> .....	296
7. <i>Chromosomes</i> .....	299
8. <i>General considerations</i> .....	300
9. <i>Atrophy of the germinal vesicle and formation of the polar bodies</i> .....	301
III. Fertilization .....	309
1. <i>General phenomena</i> .....	309
2. <i>Polyspermy</i> .....	315
IV. Cleavage .....	319
V. Formation of the Blastopore .....	325
1. <i>Technique</i> .....	325
2. <i>Segmentation cavity</i> .....	327
3. <i>External appearances</i> .....	328
4. <i>Invagination</i> .....	328
VI. Fate of the Blastopore .....	334
1. <i>Method of closure of the blastopore</i> .....	336
2. <i>Neuropore and anus</i> .....	340
3. <i>Primitive streak</i> .....	341
VII. Germ-layers and Notochord .....	347
1. <i>Germ-layers</i> .....	347
2. <i>Notochord</i> .....	351

THE work embodied in this paper was carried on at Clark University during the winters of 1890-91 and 1891-92, and during the summers of the same years at the Marine Biological Laboratory at Wood's Holl.

I gladly avail myself of this opportunity to acknowledge my great indebtedness to the University authorities for the privileges accorded me, and for the readiness with which every facility for work has been freely granted. I am under particularly deep obligations to Professor Whitman, in whose laboratories my studies have been pursued, and who has constantly furthered my work with inspiring suggestion and advice.

Through the kindness of Mr. A. C. Eycleshymer I have enjoyed the valuable privilege of comparison of certain stages of my work with his unpublished results upon *Amblystoma*. Such comparison has been of great service to me and I gladly acknowledge my indebtedness to him. To my friends Drs. Wheeler and Watasé I am likewise deeply indebted for many friendly courtesies.

#### I. NATURAL HISTORY.

The smaller North American newt or "water-lizard" (*Desmognathus viridescens*, Raf.) is widely distributed throughout the northern and eastern part of the United States, and may be obtained easily and in great abundance.

My investigations have been made chiefly upon newts collected in the neighborhood of Worcester, Massachusetts. The animals in that locality can be readily captured with blind sweeps of a net in small shallow pools of a few feet in diameter, and also in ponds and lakes of considerable size. They frequent, for the most part, places with a soft, muddy bottom and an abundance of water plants such as *Anacharis*, *Fontinalis* and *Ceratophyllum*; in such spots they usually conceal themselves under fallen leaves and among the tangle of water weeds. On warm, sunny days in early spring, however, they bask openly in the sunshine in the shallow water close along shore. They are not as a rule found in swiftly running water; and in the large ponds they appear to prefer small bays and coves sheltered from the wind.

The males considerably outnumber the females ; of 426 individuals taken from many different localities at different times 280 were males.<sup>1</sup> The females are slightly larger and in the breeding season considerably heavier than the males. The five largest individuals measured were three females, respectively 11.3 cm., 11 cm., 10.6 cm., in length, and two males 10.5 cm. and 10.2 cm. The newts obtained from ponds of some size are as a rule larger than those from smaller bodies of water.

*Food, etc.*—The newts in their natural habitat are carnivorous. They are exceedingly voracious, and when freshly captured almost invariably have their stomachs distended with partly digested prey. They feed chiefly upon insect larvae and small mollusks which they swallow bodily. Among the most common objects in the stomachs of the newts are the mollusks, *Bythinella*, *Valvata*, *Planorbis* and *Cyclas* ; orthopteran and dipteran larvae ; small water-spiders ; encased Phryganid larvae ; small crustacea and the like.

The females will take food eagerly all through the time of egg-laying. In this respect they differ from *Necturus* and from *Amblystoma*, since in the breeding season these larger urodeles refuse to take food. The longer duration of the period of oviposition in the newt as compared with many other amphibia may perhaps be correlated with this absence of the fasting habit.

In captivity, newts may thrive for several weeks without being fed, although they are kept in best condition by feeding about three times a week with earth-worms or with finely chopped beef. They become very tame in confinement, and exhibit a lively interest in the preparations for feeding them, even jumping an inch or two out of water to catch a piece of meat held over them by the forceps. They do not, however, discriminate between the meat and the point of one's finger, and will often leap into the air after the bare forceps. They

<sup>1</sup> In the breeding season both sexes, particularly the males, lose some of their shyness and may be observed in their natural habitat without difficulty. Each female is usually surrounded by several — sometimes as many as ten or twelve — suitors.

appear generally to recognize the presence of food by smell or taste rather than by sight. If a few bits of chopped meat are put quietly into an aquarium the newts at first take no apparent notice of its presence, but after a few seconds they begin to stir around uneasily and are soon moving slowly over the floor of the aquarium with noses close to the bottom like hounds on the scent. As soon as a morsel of meat comes in contact with the snout it is snapped up with a quickness that is in marked contrast to the newt's previous ignorance of its precise whereabouts. When roused or very hungry, however, the newts will catch bits of sinking meat, rapidly swimming insect larvae, water beetles, and other moving objects. It seems reasonable to suppose that there are tactile corpuscles on the end of the snout which impart this ability to detect objects in motion. It often happens that when several newts are being fed together in the same aquarium, the legs and tails of some of them are snapped at by their indiscriminating fellows with quite as much avidity as are pieces of meat. The individuals thus attacked frequently emit a faint cry or squeak resembling the sound made by drawing a wet finger rapidly over a plate of glass.

In such cases, where the stimulus of the neighborhood of food causes the newt to snap at any moving object without regard to the object's connections, it certainly seems as if tactile and not visual sensations must be predominant. Bateson ('89) has shown that many fishes, such as the skate, dogfish, sole and sterlet, habitually rely upon scent (taste?) and to some extent upon touch in seeking their food, while sight plays little or no part. The newts appear in this respect to resemble closely these fishes. In the newt, as in other aquatic animals, there is probably little distinction to be drawn between taste and smell. The stimulus must necessarily be a special molecular vibration that informs the animal of the presence of a distant object. The sense is like that of "smell" in obtaining cognizance of bodies at a distance, and like that of "taste" in obtaining it through the medium of a solution.

With proper food and care newts are easily kept for a long time in captivity. I have kept 250 of them in aquaria from

October to May with only ordinary care and attention, not one of them dying during this time. I have also been able to obtain them directly from the ponds as late in the season as the 16th December and as early as the 22d February.

*Spermatophores, etc.* — I have described in an earlier paper ('91) the chief phenomena attending the deposition of spermatophores, and need here add little to that description. Gage ('91) has recently observed the deposition of spermatophores in the autumn, which occurs "exactly as described for the spring," and the same interesting occurrence is also noticed by Zeller ('91). This curious anticipation of the normal breeding season I had previously observed, and at first regarded as due simply to the forced association of the two sexes in captivity at a somewhat higher temperature than that to which they are accustomed in their natural habitat, but I have since seen reason to think that production of spermatophores may take place sporadically in the autumn under natural conditions. I have on several occasions in the autumn months seen pairs in the ponds engaged in the preliminary "*Liebesspiel*," but have at no time observed any approach to such an outbreak of sexual susceptibility as occurs in early spring. The observations of both Zeller and Gage were made solely upon specimens in confinement where the influences I have indicated above must be potent. There is no doubt, however, about the fact that spermatophores are sometimes discharged in the fall, whereas, in the mid-summer months, so far as my observations go, this discharge does not occur, even in individuals in captivity.

The seminal receptacles of females captured in the autumn usually contain spermatozoa, although not in the same abundance as in spring. One may account for the presence of the spermatozoa by supposing either that they are the acquisition of a recent mating, or that they have been kept over from the preceding spring. It is possible that both explanations are valid in individual cases.

It is not very uncommon to see two males in the aquaria pass through all the stages of the *Liebesspiel*, even to the discharge of spermatophores, exactly as if they were of

opposite sexes, but I was somewhat surprised to witness the same singular occurrence in the ponds and to repeat this observation on several occasions. The males in the aquaria are frequently attracted (by the odor?) toward a male that has just left a female and often succeed in frightening the female away; they then temporarily usurp her function, and by vigorous pushing against the cloacal region of the unsuspecting male in front cause him to discharge fruitlessly several spermatophores.<sup>1</sup>

*Receptaculum seminis, etc.*—At about the same time that my paper upon the spermatophores appeared, Alfred Stieda ('91) published a description of the receptaculum seminis of the female European triton which renders superfluous any detailed description of this structure in *Diemyctylus*. Stieda's description appears to me fairly adequate, and agrees in all essential particulars with my own observations upon our North American genus. Stieda charges Heidenhain ('90) with having overlooked the function of the receptaculum in calling it a "rudimentary gland," but in making this charge lays himself open to the suspicion of having made an oversight of his own. The "rudimentary gland" referred to by Heidenhain (p. 201, *et. seq.*) as the homologue of the "Bauchdrüse" of the male exists, as Heidenhain states, in addition to the receptaculum. It seems probable that Stieda completely missed this structure. It is hardly likely that any one could mistake the female receptaculum for a "rudimentary gland."

Stieda apparently does not believe that the cells lining the receptaculum seminis have any secretory function whatever, although his figures assuredly do not forbid the assumption that such is the case. I have not found in *Diemyctylus* any convincing evidence that these cells are truly secretory, but if we regard them as mere passive linings to the walls of the tubules, it is difficult to account for the entrance of the

<sup>1</sup> Fig 2, Plate XV. represents a spermatophore just after deposition. It was stated in my earlier paper, through an oversight, that the gelatinous base of the spermatophore measured about *six* millimeters in diameter; the correct statement should read "about *nine* millimeters," though the size of the spermatophore is quite variable and depends on the size of the male discharging it.

spermatozoa. If I understand Stieda correctly he does not regard the receptaculum of the female as corresponding to any of the glandular structures so strongly developed in the male. A direct homology of the receptaculum with the pelvic gland of the male has, however, been affirmed without hesitation by Blanchard whose opinion I have quoted elsewhere ('91), and of whose work Stieda seems to have been ignorant. As regards *Dicmyctylus*, the male pelvic gland certainly seems to occupy a position precisely equivalent to that of the receptaculum, but it is clearly futile to pronounce upon the absolute homology of the two structures without some study of their development, and this I have not made. The question is probably a barren one.

Fischer ('91) has recently discovered the receptaculum in *Geotriton*, and, like Blanchard, homologizes it with the pelvic gland of the male, "da es nämlich die entsprechende Lage einnimmt." The receptaculum in *Geotriton*, according to Fischer, is unpaired. The tubules of the individuals that he examined contained no spermatozoa.<sup>1</sup>

*Egg-laying, etc.* — Females that have been kept in confinement all winter are not so apt to lay eggs in the spring as are individuals freshly captured. In some few cases I have obtained eggs from females kept in my aquaria during the winter, but, as a rule, only females taken during the spring months can be relied upon to furnish eggs in any number. Egg-laying begins about the 10th April and is brisk until the first of June, then slowly falls off, and practically ceases by the first of July. It is probable that for a single individual the egg-laying season lasts for at least seven or eight weeks. The longest time over which I have actually observed the laying of a single

<sup>1</sup> Fischer has drawn a curious conclusion from the presence of the receptaculum: "Wenn man sich noch einen Augenblick die Lage des receptaculum vor Auge hält, wie es gegenüber den Oviducten liegt, wie hier der Samen jeden Augenblick bereit steht, um zur richtigen Zeit in den Uterus zu gelangen, so ist wohl richtig, was oben schon als wahrscheinlich bezeichnet wurde, dass der *Geotriton fuscus* zu lebendig gebärenden Amphibien gerechnet werden muss." (Fischer ('91), p. 24.) It is not at first glance apparent how the presence of a receptaculum renders any greater the probability that *Geotriton* produces living young! A receptaculum exists in many egg-laying amphibia — to say nothing of other animals — and its presence or absence is not correlated in any way with viviparity.

individual to extend is four weeks, but in this case the ovaries still retained large pigmented eggs, and under perfectly normal conditions egg-laying would undoubtedly have continued for some time longer. The appearance of the ovaries of freshly captured females which I examined at frequent intervals throughout the laying season fully justifies this view.

The largest number of eggs that I have observed a single female to lay in captivity is shown in the following table :—

*Eggs laid by one female.*

1891.	No. Eggs.		No. Eggs.		No. Eggs.		No. Eggs.
April 20,	1	April 26,	9	May 2,	2	May 9,	12
“ 21,	2	“ 27,	1	“ 3,	1	“ 10,	3
“ 22,	1	“ 28,	5	“ 4,	0	“ 11,	16
“ 23,	8	“ 29,	9	“ 5,	0	“ 12,	0
“ 24,	8	“ 30,	4	“ 6, 7,	10	“ 13,	<u>1</u>
“ 25,	2	May 1,	13	“ 8,	0	Total,	108

In this particular instance the female was not in company with a male after the 24th April, yet she continued, for nineteen days after her separation, to lay eggs that developed normally. If she had been under perfectly natural conditions it is probable that she would have still continued to lay, since her ovaries were tolerably well filled with large pigmented eggs. It will be noticed that the number of eggs—ninety-six—laid after separation from the male is considerably larger than that observed by Gage who found indications “that for a single mating about six eggs may be internally fertilized, about the number found in the oviducts at one time.” (‘91, p. 1091). Numerous other observations corroborate my figures above. Furthermore, I have reason to think, as I shall state in the sequel (p. 309), that fertilization of the eggs does not take place in the oviducts. When egg-laying ceases prematurely it seems to me that it must be for other reasons than lack of male elements, since the supply of spermatozoa in the receptaculum is practically inexhaustible. It does not seem to me *necessary* that more than one mating should occur in a single season, but I agree with Gage that in a state of nature several matings of the same female may and frequently do take place.

The eggs are laid singly as a rule, but occasionally two, and rarely three, may be deposited successively in the same nest. The eggs are laid between folds of a leaf of *Anacharis* or some similar water plant, or in a bunch of *Fontinalis* leaflets. (Fig. 1, Plate XV). I have found eggs in the ponds wrapped in the same elaborate fashion as those laid in my aquaria. The eggs in the ponds are laid upon water plants in the localities usually frequented by the newts, and may sometimes be obtained in considerable abundance. The selection of a suitable spot for the laying of an egg often gives the female some little concern, since she is rather fastidious in this particular, and will wander from plant to plant until a thoroughly satisfactory place is found. She then bestrides the chosen spray of water plant, and gathers in with her hind legs the surrounding shoots, pressing them close around her cloaca. She next turns on her side or occasionally on her back, and, with forelimbs outstretched and rigid, with hind-limbs and twigs completely hiding her cloaca, usually remains perfectly motionless for about six to eight minutes. At the end of this time she slowly leaves the "nest," which now holds an egg well protected by a tangle of shoots glued together by the gelatinous secretion poured out of the cloaca.

I have observed a female begin to lay an egg five minutes after laying the preceding one, but this is the shortest interval that has come under my notice; usually the time elapsing between the deposition of two eggs is considerably longer than this. The largest number of eggs that I have seen laid by one individual in the course of twenty-four hours was seventeen.

The ripe ovaries of adult females consist of two paired sacs applied more or less closely to the dorsal wall and extending nearly the whole length of the body cavity. In an individual measuring about 9.1 centimeters from tip to tip, the ovaries, when in position, are about 1.7 centimeters long, but they are flexed considerably upon themselves so that, when straightened, they are fully twice that length. These sacs are composed of two folds of epithelium, between which lie the eggs; Schultze ('87) has appropriately called the outer of these layers the "Peritonealepithel," and the inner the "Innenepithel."

The number of eggs in each ovary and the weight of the ovaries, are indicated in the following table :

Length of Animal.	Weight.	No. Eggs		No. Eggs	
		Wt. Rt. Ovary.	Rt. Ovary.	Wt. Lft. Ovary.	Lft. Ovary.
9.1 cm.	3.20 gm.	.17 gm.	216	.20 gm.	236
9.0	2.54	.13	248	.12	193
9.2	3.24	.21	254	.17	174
9.6	4.32	.33	326	.15	162

The relative weight of the mature ovaries to the weight of the whole body is about one to nine. The "number of eggs" given in the table is the number plainly visible to the naked eye ; the number visible with even a low power of the microscope is of course far greater than this. The number of large pigmented eggs measuring approximately one millimeter in diameter is about forty per cent. of the number given in the table. All of these pigmented eggs are in ordinary circumstances deposited in a single season ; the number of eggs laid by a good-sized female every year is consequently about 180-250.

The ovaries of females captured at the close of the breeding season contain no large pigmented eggs, and are stringy, light-colored and greatly shrunken. The weight of the ovaries has greatly decreased as shown in the following table :

Length of Animal.	Weight.	Weight of Right Ovary.	Weight of Left Ovary.
8.2 cm.	1.86 gm.	.03 gm.	.03 gm.
9.1	2.03	.02	.02
8.9	2.01	.01	.01
10.0	2.31	.01	.01

That is to say, about one-tenth of the body weight is discharged in the form of eggs every year. It may be noticed also that the weight of females captured at the close of the laying season is distinctly lower than that of females taken in early spring. The average weight of the former is 2.05 gm. ; that of the latter, less the average shrinkage of the ovaries, 2.74 gm., while the average length of the individuals composing the two groups is about the same. This result is substantiated by other weighings and measurements which it is not necessary to give in detail. There is hence a loss of body-weight, in addition to the direct loss of egg-substance, during

the laying season, amounting possibly to one-fourth of the total weight. This may be partly due to the not inconsiderable amount of substance that goes to form the protective membrane of the egg, and partly to the conversion of body-substance into yolk during the laying season. One might be tempted to explain this loss of body-weight by increased katabolism of the tissues, due to higher temperature, were it not for the fact that the males, although strikingly thin and exhausted at the end of the period of "*Liebesspiel*," gain rapidly in plumpness and weight as the summer advances. The true explanation seems to lie rather in the directions first indicated.

*Transformations, etc.* — Gage ('91) in his recent interesting paper, describes and figures the external developmental stages that the newt passes through, and arrives at the important conclusion that the well-known red, terrestrial form — the so-called variety *miniatus* — is merely a stage in the life-history of the species, and is not to be regarded as a distinct variety. Although I have not had these animals under observation long enough to warrant me in expressing a very decided opinion on this point, I feel fairly confident, from what I have seen, that Gage's main contention is correct. One of the first things that strikes the collector of the aquatic form is the comparative absence of all newts below a certain general grade of development. These young and immature individuals are not to be found in water, by using a net with finer meshes, but may be discovered on land by turning up the stones and logs on the shores of the pond, and one is hence tempted to infer what Gage has concluded, *vis.* that the red, terrestrial form is merely an immature condition of the common aquatic newt. Gage has given us an interesting discussion of the causes that may have led to this curious change of color and habitat.

I do not yet feel prepared to say that I regard the assumption of the terrestrial habit as a necessary stage in the development of every individual. It is quite possible that certain individuals attain maturity without ever leaving the water, although perhaps the great majority of newts pass their *Wanderjahre* on land.

I may add that Gage has appended to his paper an annotated bibliography that must win the gratitude of future students of the natural history of the newt.

## II. MATURATION OF THE OVUM.

The phenomena of maturation may be taken in their broadest sense to include all the progressive changes in the ovum from its youth up, and cannot strictly be limited to the nuclear changes immediately preceding fertilization. Indeed one may hold with some reason that the early stages of oögenesis are no less significant than the later.

In amphibian ova two general stages of development may conveniently be distinguished: the early, constructive, or as it may be called, anabolic period, during which the yolk is elaborated and the full size of the ovum reached; and a second period, beginning with the remarkable regressive metamorphosis of the germinal vesicle, and culminating in the expulsion of the polar bodies. The accumulation of yolk may be considered quite as essential to "ripeness" as the extrusion of nuclear substance, since, so far as we know, both are invariable preliminaries to the normal union of male and female pronuclei. It is a fact especially worthy of notice that the germinal vesicle undergoes a progressive development up to the time when yolk-formation is practically completed, and only then begins to show signs of degenerative change; this significant fact must be taken into account in all conjectures as to the functional rôle of the amphibian egg-nucleus.

The maturation phenomena in amphibian ova have been so often and so thoroughly studied, that, where my observations are in full harmony with those of my predecessors, I shall either refrain from all reference to the fact, or shall content myself with bare mention of it without entering into superfluous details.

*Technique.*—I have found the two most satisfactory killing agents for the ovarian eggs to be hot water (80° C.), and Flemming's chrom-aceto-osmic mixtures. In my experience I have found no advantage in leaving the eggs longer than two hours in Flemming's mixture, and though I have used a solu-

tion of the strength recommended by O. Schultze ('87) I have not reached his successful result with so long an immersion as twenty-four hours. This may perhaps be due to the comparatively small size of *Diemyctylus* eggs and their consequent speedier penetration by the fluid.

After an immersion of two hours in Flemming's mixture the eggs are washed repeatedly with distilled water and 35 per cent. alcohol, and then transferred to 50 per cent. alcohol, where they remain for an hour, after which they are hardened in 70 per cent. alcohol for from twelve to eighteen hours. They are finally passed successively through 95 per cent. alcohol, absolute alcohol and xylol (or turpentine), not remaining above an hour in each, and are imbedded in paraffine. After repeated trials of Schultze's method of leaving the eggs for twenty-four hours in each of the different grades of alcohol, I was obliged to abandon it as eminently unsatisfactory for *Diemyctylus* eggs. A short stay in the alcohols afforded me much better results.

I have stained *in toto* by putting the eggs, after their stay in 70 per cent. alcohol, into borax carmine or alum cochineal for twenty-four hours, and have in this way obtained excellent preparations, particularly with the cochineal. For staining on the slide I have used Mayer's method of fastening the sections to the slide with albumen fixative.

If the eggs are killed with hot water and then passed slowly through rising grades of alcohol the results are quite as satisfactory as those reached with Flemming's mixture. In some respects, even, they surpass the latter, since shrinkage of the germinal vesicle is less frequent with hot water than with any other method. The eggs are dropped into water of about 80° C. for a few seconds, and then passed at once into weak alcohol and slowly up to the higher grades.

Perenyi's fluid and Kleinenberg's picro-sulphuric mixture do not seem well adapted for use on the ovarian egg. The latter, in particular, badly distorts nuclear appearances.

For study of the young living egg I have relied chiefly upon examination of perfectly fresh specimens in physiological salt solution, though I have found both silver nitrate and methyl green very helpful in determining certain points.

*Origin and Development of the Ovum as a Whole.*—I have not yet completed my study of the differentiation of the germ-cells in the larva, and shall confine myself here to a brief description of the formation of the ova in the mature female. In the adult, as has been stated, the ova arise between two folds of epithelium and originate directly from the epithelial cells of the outer or so-called germinal epithelium (Fig. 4, Pl. XV). My observations on this point agree substantially with those of Iwakawa ('82), and I have frequently found in silver nitrate preparations appearances like those figured by him in Figs. 3, 5, and 6, Pl. XXII. The epithelial cells that give rise to ova are to all outward seeming exactly like the other peritoneal cells. As soon as the germ-cell becomes recognizable as such, other epithelial cells are seen crowded around it, and eventually some of these form the follicle cells of the young ovum.

I have not been able to determine to my own satisfaction whether follicle cells and ovum originate immediately from a single germinal epithelium cell, or whether the young ovum is a lineal descendant from a primordial ovum and the follicle cells from primordial follicle cells. Considered cytogenetically, however, both follicle and ovum, if the line of cell descent only be pushed back far enough, must have had a common origin from a single cell somewhere in the developmental history of the larva. There is no valid morphological support for a belief in an early analysis into definite ova on the one hand and into definite follicle cells on the other, and I can see no inherent improbability in the view that the analysis into ovum and follicle occurs at a comparatively late stage in the development of the germinal epithelium.

At the same time, however, I incline to the view taken by Iwakawa ('82) and Hoffmann ('86) that the follicle takes its rise from the germinal epithelium contemporaneously with the ovum, and that follicle and ovum do not have immediate origin from the same cell. Fig. 4 shows a well developed ovum, with the follicle cells just beginning to grow over it from the superjacent epithelium, and indicates a condition similar to that described by the observers just cited. The cells of the

germinal epithelium abutting on the germ-cell appear to accompany the latter in its passage below the surface, and to become its enveloping sheath. But I cannot regard these appearances as conclusive and must still leave open the possibility of a late "analysis" of one of the "primordial ova" into true follicle and ovum. It is an hypothesis not altogether without warrant that all the germinal epithelium cells may at the start be very similar, or even identical, and that local conditions may bring it about that some shall develop into ova and others into follicle cells.

Starting with the young ovum just sinking beneath the surface of the other germinal epithelium cells we may conveniently separate its history into the two periods already mentioned, the period of the accumulation of food-stuff, and the period of the changes that qualify the female nucleus for union with the sperm nucleus. The first period is marked by the advent of new elements in the cytoplasm, and by rapid increase in size of the ovum; the second by the comparative cessation of cytoplasmic phenomena, and by striking internal changes in the germinal vesicle.

The germinal vesicle in a young egg, before any yolk spherules are apparent, is about half the diameter of the egg itself, measuring .07 mm. in an egg .13 mm. in diameter. Fig. 4 shows that in an even younger stage the germinal vesicle occupies a slightly larger proportional share of the egg space. The egg as a whole increases in size though not in complexity up to a diameter of about .3 to .4 mm. when the first traces of yolk begin to darken the field. (Fig. 7, Plate XV.) The germinal vesicle up to this time has increased *pari passu* with the whole egg, and in an egg of .4 mm. in diameter measures .2 mm. From now on, however, while the yolk is gradually forming, the growth of the whole egg far outstrips that of the nucleus. In an egg of 1.3 mm., which is very nearly the average size of mature eggs, the germinal vesicle is only about .3 to .4 mm. and rarely exceeds that proportion. In other words the volume of the germinal vesicle compared with the volume of the whole egg before yolk formation is about 1:8; in the mature egg it does not exceed 1:35.

The stage of pre-eminently cytoplasmic phenomena to which we first turn is distinguished in amphibian ova by the appearance and elaboration of the yolk, and by the advent of certain enigmatical structures, the so-called yolk-nuclei.

*Yolk-nucleus.*—The so-called yolk-nucleus (*Dotterkern*) of the amphibian egg appears to have been first observed by Cramer ('48) who discovered it in the ovarian eggs of *Rana temporaria* in 1846. He describes it as a "little ball of granules," which, as the egg becomes larger, spreads out into "an elegant half-moon" around the germinal vesicle, and, finally degenerating, mixes completely with the substance of the egg. In the previous year a similar body had been seen by v. Wittich ('45) in the ovarian eggs of spiders, but whether this concentrically ringed structure is in any way comparable with the body found in the amphibian egg was then and is still to this day an open question. Further studies by v. Wittich, v. Siebold and Carus failed to shed much light on the significance of this concentric body in spiders' eggs. v. Wittich ('49) found it upon the surface of the yolk in freshly laid spider eggs, and speaks of it as "a hollow, thick-walled capsule filled with liquid." Carus ('50) was the first to apply the name *Dotterkern* to this element of the egg. He refers to Cramer's observations upon the similar body in the egg of the frog, and is inclined to homologize the two structures.

Practically nothing was added to these early and fragmentary observations upon the yolk-nucleus until the investigations of Lubbock and of Gegenbaur in 1861. Gegenbaur ('61) discovered in the avian egg "einen fast scharf umschriebenen runden Fleck . . . mit dem Keimbläschen hat er keine Beziehung, denn dieses liegt immer entfernt von ihm." Gegenbaur does not commit himself to any definite view on the significance of this "constant element" of the egg, but is evidently inclined to regard it as in some way connected with the origin of the yolk granules.

Lubbock ('61) described and figured a body which he observed in young Myriopod eggs of several genera, but was doubtful as to its homology with the concentric structure observed in Arachnid eggs, and prudently refrained from at-

tributing any special importance to it. "I have," he writes, "several times been inclined to look upon it as a mere accidental agglomeration of yelk ; but it is, I think, too regular and too constantly present."

Since 1861 the yolk nucleus has been described and figured by nearly every worker upon Arachnid, Myriopod or Amphibian ova. An analogous body has also been observed in Crustacea (Reichenbach, '77), in Mollusca (v. Ihering, '77), and many other groups, but it is doubtful if there is any community of relation whatever between these structurally different bodies. With few exceptions these numerous observations have done little towards explaining the significance of the "yolk nucleus" in any one of the groups of animals in which it appears.

Balbiani ('64<sup>a</sup>, '64<sup>b</sup>) was the first to observe the yolk-nucleus in the egg of Myriopods (*Gcophilus*), where it appeared to him as "une petite vésicule," much smaller than the germinal vesicle, and lying close to the periphery of the egg. He describes circumstantially the formation of a layer of granules around the vésicule and the subsequent migration of these granules to distant parts of the egg. It was chiefly this phenomenon that led him to believe that the small vesicle was the formative center for the nutritive elements of the egg. He also advanced the view that the substance of these eggs consists of a "partie germinative fondamentale et d'une partie nutritive, chacune de ces parties se constitue isolément et pour son propre compte." This separation between the germinative and nutritive elements is, according to Balbiani, primordial, and dates from the first stages of the egg.

In a paper published some time afterwards, Balbiani ('73) modifies his views regarding the "cellule embryogène" as he now prefers to call it. From his observations upon the Teleostean egg he concluded that the cellule has its birth in the epithelium of the follicle — in some such way as van Beneden and Julin ('87), and Morgan ('90) claim is followed in the formation of those strangely similar bodies, the Ascidian test-cells. In some Teleosts Balbiani finds a straight canal or pathway leading from the follicle into the

egg substance, and his embryogenic cell lying at the bottom of this canal. He regards the external origin of this body as strengthening his view of a fundamental difference between it and the germinal vesicle and as an additional reason for insisting on the separation of the nutritive and germinative elements of the ovum. Balbiani does not agree with v. Bamberke that the embryogenic cell disappears before the egg reaches maturity, but holds that it persists even after fertilization.

In a later paper Balbiani ('83) brings forward some new observations, which, if confirmed, are certainly important. He finds that the eggs of *Gcophilus*, when treated with dilute acetic acid show a process of the germinal vesicle which projects into the body of the egg. There are sometimes several of these processes, each process consisting of a double tube, the outer tube communicating with the nucleus, the inner with the nucleolus. The "vitellin protoplasm" at the outer extremity of these tubes becomes denser, and presumably receives substances through the tubes from the nucleus and nucleolus, and this vitelline protoplasm so comes to contain "tous les éléments d'une cellule"! These "corps intravitellins" then break away—though this part of the process was not directly observed—and can thereafter be recognized as true "noyaux vitellins." These "noyaux vitellins," according to Balbiani, now become follicle cells! Whether Balbiani's views have undergone any further modification since the appearance of this last paper I have no means of knowing, but must confess to some bewilderment at the kaleidoscopic comprehensiveness of the views just stated.

Schütz ('82) at about this time published a brief account of his work upon the Dotterkern—principally in Arachnids—and after summing up the work of previous investigators concludes that the Dotterkern has a nutritive significance. Schütz, however, appears to have overlooked what was in many respects the soundest utterance on the amphibian Dotterkern. O. Hertwig ('77) observed the Dotterkern in the eggs of *Rana*, and concluded that very great morphological significance could not be attributed to it since it was not found in all amphibian ova.

“Mir scheint er einzig und allein mit der Bildung der Dottersubstanz in Beziehung und eine eigenthümliche locale Ansammlung von Nährstoffen darzustellen.” Hertwig suggests the name “Dotterconcrement” as preferable to that of Dotterkern, and justly criticises the term Dotterkern as applied to the body observed in the amphibian egg. I have, however, retained the name *yolk-nucleus* because of the wide currency that this name has already obtained, and because the designation “*yolk-concrement*” is open to the construction that this body is a mere *concretion* of yolk.

Sabatier ('83) describes the origin and fate of the yolk-nucleus as he observed it in the Arachnids: “né dans le voisinage immédiat de la vésicule germinative, il s'en éloigne progressivement. . . . Il devient plus granuleux et se désagrège progressivement. Ses éléments divisés en petits globules indépendants sont en partie résorbés par le vitellus, ou s'introduisent en partie entre les grosses sphères vitellines et viennent sourdre à la surface de l'œuf pour se mêler au protoplasme granuleux superficiel.” From these observations he draws the remarkable conclusion that the “*noyau vitellin*” is to be regarded “comme un élément de polarité mâle, qui se détruit comme tel pour accentuer et compléter la sexualité de la cellule femelle.”

Will ('84) advances a rather improbable view of the origin of these bodies in the amphibian and insect ova and appears to regard the phenomena in these two groups as practically identical. According to his observations some of the nucleoli in the germinal vesicle of the amphibian egg reach a comparatively large size, but do not differ from the others in their behavior to staining fluids or other reagents. One or more of these enlarged nucleoli pass out into the body of the egg and there as “*yolk-nuclei*” take part in the formation of the yolk. Will finds some cases in which the nucleoli change into epithelial cells, but, since in the long run these share in the formation of the yolk the “*Endproduct*” is the same.

Stuhlmann ('86) describes the origin of the Dotterkern in the arthropod egg and seems to regard it as originating independently in the cytoplasm. “Niemals aber konnte ich eine

Entstehung aus dem Keimbläschen constatiren, wie Balbiani dies für *Geophilus* und Will für den Frosch angiebt." Stuhlmann agrees with Hertwig and Schütz as to the significance of the Dotterkern. "Der Dotterkern stellt eine Concretion von dem besonderem, von dem gewöhnlichem Dotter verschiedenem Nahrungsmaterial dar, das zu irgend einer Zeit vom Ei resorbirt wird."

The more recent observers of the Dotterkern have for the most part contented themselves with passing notices of its presence and general characteristics, and have not added materially to our knowledge of its relations and significance.

I have given this brief historical sketch of the observations on the "Dotterkern" as well for the purpose of calling attention to the interesting problems connected with this body, particularly in its occurrence in the Myriopod and Arachnid ova, as for emphasizing the entire absence of homology between the structures usually classed as yolk-nuclei.

In the newt the yolk-nuclei first become distinctly visible about the time of beginning yolk-formation, at which time several of these bodies appear nearly simultaneously in different parts of the egg. I have always found at least two and sometimes as many as nine in eggs of about the stage shown in Fig. 7. The number of yolk-nuclei in eggs of a more advanced development is no greater, and on the whole averages somewhat less than the number in eggs of this particular stage. I have no conclusive evidence of coalescence of these bodies, but am confident that some of them disintegrate. They all disappear completely some time before fertilization of the egg, and I have never been able to detect any trace of them in eggs that contained a maturation spindle.

In eggs of the stage shown in Fig. 7 the yolk-nuclei appear first as small, finely granular bodies, irregularly shaped and with their outlines poorly defined from the surrounding cell-substance (Fig. 11). They stain more deeply than the cytoplasm, but still very feebly as compared with the developing patches of yolk. At a still earlier stage I have observed an appearance which can be best interpreted as a localized condensation of cytoplasm and a consequent greater avidity for

staining fluids (Fig. 10, Pl. XV). Whether or not these condensations are the immediate progenitors of such undoubted yolk-nuclei as that represented in Fig. 11, I am unable to decide, but it seems to me highly probable that such is the case. Between the latter and such complex bodies as that shown in Fig. 13, Pl. XV. are found all gradations. I think it exceedingly probable that in the newt the yolk-nuclei always arise first as condensations of the cytoplasm and subsequently increase in size and complexity with the growth of the egg.<sup>1</sup>

At first they lie about half-way between the germinal vesicle and the periphery of the egg and often maintain this relative position for some time, but eventually they draw nearer to the germinal vesicle and in large eggs are usually found in close contact with it. It is not unlikely that they sometimes fuse with the substance of the vesicle, but from sections it is of course impossible to obtain absolute proof of complete fusion. Certainly a more common fate would seem to be the gradual disintegration of these bodies and the dispersion of the derived granules around the germinal vesicle in the manner described by Cramer ('48) and Schultze ('87). This breaking up of the yolk-nuclei appears to be their ordinary fate, and I have often observed such a disintegration as is figured by Schultze ('87) in Figs. 4*a*, 4*b*, 4*c*, 4*d*. As the yolk-nuclei approach the stage at which this resolution into granules takes place, they generally assume a somewhat complex structure. They become more sharply marked off from the surrounding yolk (Figs. 10, 11, 12 and 13, Pl. XV), and are usually differentiated into two elements, a coarsely granular portion staining feebly and resembling ordinary cell protoplasm, and a more compact portion of varying extent which stains deeply with carmine or alum cochineal. This highly stainable constituent often occurs irregularly disposed in two or more patches in a single yolk-nucleus as in Fig. 13, Pl. XV, and Fig. 16, Pl. XVI. This fact might perhaps be thought to countenance the view that these large yolk-nuclei arise from the union of several of the smaller bodies, but it is

<sup>1</sup>I have never seen anything that would indicate that the yolk-nucleus arises from a large nucleolus which has migrated into the cytoplasm in the manner described by Will, and on this point am in full accord with Stuhlmann ('86).

the only observation I have made that would tend to such an inference, and cannot in itself be regarded as convincing.

The yolk-nucleus in the egg of the newt, then, arises from the cytoplasm, and usually disintegrates in the cytoplasm. It is likely, however, as I have already intimated, that yolk-nuclei are sometimes fused with the germinal vesicle. I am strongly inclined to believe that such fusion actually occurs, although I am unable to produce any very conclusive evidence in favor of such a belief. The fact that a considerable number of yolk-nuclei in late stages of the egg are sunk in a hollow of the vesicle, and in some cases almost surrounded by its substance, gives strong ground for such an opinion. In Fig. 16, Pl. XVI, is shown a yolk-nucleus apparently coalescing with the germinal vesicle. The union in this instance is less pronounced than is sometimes the case, and I have frequently obtained preparations which show the yolk-nucleus engulfed to half its extent.

Not uncommonly the yolk-nucleus in these later stages appears to be split up into irregular polygonal fragments (Fig. 13), but as this does not appear to be invariable no great significance can be attached to it. It is very likely a mere sign of approaching dissolution.

It is evident, I think, if we compare the observations above recorded with those upon the structure and history of the "yolk-nuclei" in other groups of animals, that if all the observations of different authors are to be credited, any real homology between these bodies is out of the question. It is incredible that bodies so widely diverse in origin, structure and fate as the "yolk-nuclei" of Arachnids, Myriopods, and Amphibia should be homologous in any ordinary sense of the word. The only respect in which some of these bodies resemble one another is in appearing in the cytoplasm at some stage in the development of the ovarian ovum. It seems to be true, furthermore, that the yolk-nucleus is not of general occurrence in all species of the same group of animals. Although it is present in many amphibian eggs, according to Götte ('75) it is altogether absent in the ovarian eggs of *Bufo* and *Bombinator*. Indeed, it is said that yolk-nuclei are not always found in all the eggs of the

same animal; this is stated by Iwakawa ('82) to be the case with *Triton pyrrhogaster*. These facts incline me strongly towards the view, also taken by Leydig, that the various structures usually grouped together under the name Dotterkern have nothing but the name in common.

I have here nothing to say upon the significance to be attributed to the curious bodies found in Myriopod and Arachnid eggs, and I cannot attempt to pass upon the peculiar views of Balbiani and Sabatier, but can only repeat that their explanations, however valid they may be for these particular groups of animals, are by no means satisfactory if applied to the amphibian Dotterkern.

We can from the nature of the case only surmise as to the physiological meaning of the bodies found in the amphibian egg. It is a seductive hypothesis that the amphibian yolk-nuclei are in some way concerned with the formation of yolk. The number, size, constancy, and complexity of structure of these bodies in the egg of the newt, their appearance about the time of beginning yolk-formation, and their disintegration or absorption after yolk-formation is complete, point to them as serving some physiological purpose during the building up of the cell. Whether they are, as Hertwig believes, "peculiar local gatherings of nutrient substances," or whether they are true formative centers, is a difficult question to determine. The fact that they have not been found in all amphibian ova is certainly against regarding them as of high importance, but it is premature to urge an objection based on negative observations. It is possible, and I think probable, that future investigation will show the yolk-nuclei to be of general distribution in all amphibian ova of certain stages of development. It appears to me certain that where the yolk-nuclei do occur they are not mere accidental agglomerations, but have a real physiological significance, probably related to the construction of yolk. I am strongly inclined to believe that the yolk-nucleus bears the same relation to the cytoplasm that the nucleoli do to the germinal vesicle.

*Yolk-formation.* — The yolk first appears when the ovum has reached a diameter of about .4 to .5 millimeters. A vacuolation

of the cytoplasm, such as shown in Fig. 7, precedes the formation of the yolk itself. This is not a change due to reagents for it is shown in eggs killed with Flemming's chrom-osmo-acetic mixture and other reliable reagents, and moreover appears only in eggs of a certain stage, younger eggs not displaying this vacuolated appearance. A similar arrangement of the protoplasm has been observed by Leydig ('88) in the young eggs of *Triton taeniatus*. The yolk is laid down upon these trabeculæ of the cytoplasm and in consequence of this arrangement often has in sections a distinctly reticulated appearance. Sometimes the yolk is formed first at one pole of the egg (Fig. 7) and then spreads to other parts, but I have not been able to discover any constancy in this polarity, or what relation such polarity bears to the pigmentation poles of the adult egg. More commonly the yolk appears nearly simultaneously in different parts of the cytoplasm and forms a more or less continuous ring. This ring of forming yolk is considerably nearer to the periphery than to the germinal vesicle, but never, at this earliest stage, lies in immediate contact with the follicle cells. The yolk patches are early distinguished by their great avidity for staining fluids, and by their high index of refraction and coarse granulation in unstained eggs. I have been able to discover nothing that would indicate that the yolk spherules increase by division; everything on the contrary indicates that they arise from points of independent origin. With a high magnification (1000 diameters) the beginning yolk patches show as in Fig. 5, Pl. XV.

I do not feel like speaking at all confidently as to the agencies concerned in the production of yolk. I have frequently observed appearances which may be interpreted as the stepping out of very minute granules from the germinal vesicle into the cell-body. These granules I have been unable to distinguish on the one hand from numerous small stainable granules (nucleoli?) inside the germinal vesicle or on the other from the granules of the forming yolk. Whether it is safe to regard this appearance as an actual migration of minute nucleoli which *in persona propria* become yolk spherules seems to me doubtful. There is, however, less objection to the view that certain

deutogenic substances are formed in the vesicle, perhaps through the agency of the nucleoli, and are then sent forth to share in the building up of the cell. The peripheral position of the nucleoli in amphibian eggs, and their subsequent migration to the center after yolk construction has practically ceased may be best explained by supposing that in some way they take part in the management of deutoplasmic affairs. That either the germinal vesicle, as represented by the migrating granules, or the cytoplasm, as represented by the yolk-nuclei, is exclusively concerned in yolk-formation does not seem to me probable. If it be desired to formulate an hypothesis one might suppose that granules from the germinal vesicles serve as starting points, centers of attraction or stimulation as it were, while the cytoplasm, perhaps through the mediation of the yolk-nuclei, elaborates and supplies the requisite deutoplasmic material out of nutritive elements furnished it by the follicle cells.

I may add that there is some support for such a view in the recent cytological work of Macallum ('91). From studies upon the ovarian ova of *Necturus* and *Rana*, principally with the indigo-carmin stain, he draws the following conclusion: "The peripheral nucleoli generate a substance, therefore, which diffuses gradually through the nucleus, then into the cell protoplasm, the point of time of the latter occurrence corresponding with the formation of the yolk spherules. . . . I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm. . . . The formation of yolk spherules in the cell protoplasm is analogous to the formation of zymogen granules in the pancreatic cells, and both are accompanied by changes in the nucleus and an increase in the cell protoplasm. It is most natural to conclude that the processes underlying the formation both of the yolk spherules and of the zymogen granules are in a general way alike." That there is some such sharing of nucleolar substance in cytoplasmic activities as Macallum here avers is a familiar and well-supported opinion.

Korschelt ('89) thus expresses a widespread conviction: "Ich muss es nach meinen Erfahrungen, die an Eiern und anderen

Zellen gemacht wurden, als zweifellos hinstellen, dass eine Auflösung der Nucleolarsubstanz stattfindet. Die Erklärung dieser Erscheinung fand ich darin, dass die Nucleolarsubstanz in und vielleicht auch ausserhalb des Kerns zur Verwendung gebraucht werden sollte" (p. 112). My own observations upon the position and behavior of the amphibian egg nucleoli lead me to endorse this view.

*Germinal vesicle as a whole.*—The germinal vesicle is derived from the nucleus of the germinal epithelium from which the egg first springs. I have never seen any appearances which would indicate that it arises from the union of several epithelial nuclei.

The germinal vesicle, while still very young, shows a differentiation of its substance into three elements—nuclear sap, nucleoli, and chromatin threads or, as I may call them at the outset, chromosomes (Fig. 4). The history of these two important chromatic elements will be dealt with in detail presently.

A nuclear membrane is visible in the young egg and comes out particularly well in fresh preparations treated with methyl green. This membrane cannot be detected in sections through late stages of the vesicle, but I have not been able to discover the precise period at which it disappears. Its disappearance seems, however, to be contemporaneous with the general vesicular atrophy.

The amphibian germinal vesicle sometimes presents, as observed by O. Schultze, Will, Götte and others, an irregular and often ragged contour. This has been considered to indicate amoeboid movements on the part of the vesicle similar to those witnessed in the nucleus of insect eggs. Schultze ('87) has given several figures illustrating this condition, — Figs. 5, 18, 19, 20. In *Dicmyctylus* I have observed this appearance only in rather young eggs, and in these the germinal vesicle has assumed the form shown in Schultze's Fig. 5, with sharp projections and jagged contour. Germinal vesicles in newts' egg of the same age as the ones shown in Schultze's Figs. 18 and 19, rarely, if ever, display these "amoeboid movements." The outer boundary, on the contrary, is smooth, even, and at

most slightly crenated (Figs. 14, 15, 16). In eggs killed with hot water I have never found such an appearance as Schultze depicts in Fig. 18. With other methods of killing, however, I have sometimes seen a similar pseudopodia-like outline to the germinal vesicle. Since the penetration of any irritating fluid into an amphibian egg must necessarily be far from rapid, it seems possible that the germinal vesicle may be stimulated to slight amoeboid movements; so that while in one sense the "pseudopodia" may be an artificial product, in another sense they may be the expression of potential amoeboid capabilities. While I incline, therefore, to the belief that amoeboid movements in the older vesicles are not, in *Dicmyctylus* at least, usual and normal occurrences, I am in full accord with Schultze as to the reality of these appearances in young eggs, and can confirm his statement as to the presence of both amoeboid and resting nuclei side by side in the same preparation. It is impossible to set a precise limit to the ceasing of these amoeboid phenomena, but in a general way I can say that it is extremely rare to find germinal vesicles with pseudopodia after migration of the nucleoli has begun.

I have never seen the pseudopodia of the vesicle stretch out into the cell and engulf bits of nutrient substance as some observers (Brass) claim to have done, but I nevertheless agree with Korschelt ('89) that the amoeboid movements of the young vesicle are of nutritive significance, and possibly aid in the diffusion of fluid from the nucleus into the cell. In the eggs of *Rana palustris* I have frequently observed the appearance depicted in Fig. 17. The peculiar amoeboid structure at the center of the vesicle with nucleoli clustered around it might almost suggest that the nucleoli had been drawn to the center rather than migrated there independently. I have, however, never observed such an amoeboid center in the germinal vesicle of the newt.

The curious reticular structure of the germinal vesicle which Iwakawa ('82) has figured in Figs. 19 and 27 is, I am inclined to believe, delusive. I have obtained a similar appearance with the use of picro-sulphuric mixture, but not with hot water, corrosive sublimate or Flemming's mixture. One would

expect truer pictures of nuclear structure with the use of the latter reagents than with the former, but one cannot completely exclude the possibility that, as regards this particular character of the nucleus, the micro-sulphuric mixture preserves the natural aspects better than the other killing fluids named. I do not, however, believe that such is the case, and I consider the reticular appearance as probably due to the action of the reagent.

With regard to the accumulation of fluid around the germinal vesicle in advanced stages I agree fully with v. Bambeke, Hertwig and Schultze, that it is not a natural condition, but is caused by osmotic action during the processes of killing and hardening. When the egg is hardened slowly in rising grades of alcohol this escape of fluid does not occur, and the germinal vesicle impinges directly upon the yolk. (Figs. 15, 16, 17).

*Nucleoli.*—The nucleoli in the young egg appear arranged along the chromatin threads, and possibly originate from the thread substance. It is very difficult to determine just what relation the nucleoli bear to the threads in the earliest stages. The fact that the two structures are in the closest contact does not exclude the possibility of distinct and independent origin. The nucleoli are at first, to all appearances, integral parts of the threads, at least as much so as the highly refractive granules of the threads shown in Fig. 14, Pl. XV. I must leave it an open question just how far it is safe to regard the early association of nucleoli and threads as indicating a common origin.

In eggs somewhat younger than Fig. 4 the nucleoli exist in this intimate association with the threads, but they soon lose their connection, increase rapidly in number and size, and come to lie close to the periphery of the vesicle. O. Schultze ('87) regards the increase in the number of nucleoli as due to division of those first formed, but he does not raise the important question as to how the first nucleoli originate. My own observations have led me to conclude that the nucleoli never multiply by division. I have repeatedly sought in vain, both in fresh eggs and in sections, for nucleoli in the act of fission. Schultze states, in support of his view (p. 195), that the nucleoli in very

young eggs somewhat surpass in size those in slightly advanced stages. I have not found this to be the case in *Diemyctylus*. In a germinal vesicle some  $60\mu$  in diameter and containing 199 nucleoli, the larger nucleoli measured from  $1\mu$  to  $3\mu$ , the latter dimension being never exceeded. A germinal vesicle about  $90\mu$  in diameter contained about 442 nucleoli, the larger of which measured  $3\mu$  to  $4\mu$ , while another vesicle  $131\mu$  in diameter contained 1123 nucleoli of about  $4\mu$ . The preponderance of large nucleoli is, if anywhere, on the side of the large eggs. Furthermore, if multiplication of the nucleoli by division were the ordinary occurrence, we might expect to find a certain uniformity in the size of these bodies, and a certain critical bulk, so to speak, at which division takes place. This is not so, but on the contrary, the nucleoli attain their maximum size shortly before their centripetal movement, which is just the condition that might be anticipated if we assumed a steady development and growth from the beginning. It is, moreover, not difficult, particularly in the early stages, when the number of nucleoli increases most rapidly, to find all gradations from the largest to the smallest, the smallest being indistinguishable from the more conspicuous granules in the threads. These facts would lead to the suspicion that the origin of the nucleoli is in some way intimately associated with the chromatin threads, that in fact the larger granules of the threads, such as seen in Fig. 14, break loose from the threads and pass over into true nucleoli. I incline to believe that such is the case. Leydig ('88) holds a similar view as to the origin of the nucleoli in *Triton taeniatus*: “. . . in der Mitte des Keimbläschens ein zartes, dichtes Reticulum sichtbar ist, dessen Knotenpunkte von Keimflecken der kleinsten Form nicht zu unterscheiden sind, so dass ein Uebergang der einen in die andern unmöglich gezeugnet werden kann (Fig. 94).” If the nucleoli in the growing egg thus arise from chromatin threads, it strengthens the probability of a like origin for the first-born nucleoli.

Nucleoli are often said to arise as “much-thickened knots of the network of threads.” I cannot regard such an expression as appropriate in the present instance, since there is no true “network,” and since the designation of the young nucleolus

as a "knot" is not particularly descriptive, as a glance at my figures will suffice to show.

At the time when the egg reaches nearly its full size, when the yolk is formed and the yolk-nuclei have attained their maximum complexity, when the nucleoli are largest and most numerous, there begins that remarkable centripetal march of the nucleoli which foreshadows their speedy disintegration. The first sign of this retrogressive movement is the peculiar appearance of the nucleoli while still at the periphery. They stain feebly and very unevenly, their outlines display the greatest roughness and irregularity and they are plainly in every respect experiencing degenerative change (see Fig. 16, Pl. XVI). The centripetal movement is not simultaneous on the part of all nucleoli. Some are to be found at the periphery while others are in the last stages of dissolution in the center. They usually straggle severally to the center and there fall apart, as shown in Fig. 15, Pl. XVI, where several nucleoli are seen disintegrating *en route*. Subsequent stages are well shown by Schultze ('87) in Figs. 20 and 23.

I must strongly dissent from certain of Schultze's conclusions as to the fate of the nucleolar substance. After describing the disintegration of the nucleoli into small granules he adds: "Man überzeugt sich, dass die Körnchen, die ich jetzt Mikrosomen nennen darf, allmählich zur Erzeugung eines *Fadenknäuels* zusammentreten, der also *nicht aus einem präformirten Kerngerüst entsteht, sondern sich direkt aus den winzigen Keimkörperchen herausbildet.*" (p. 198.) I cannot agree with Schultze that the granules from the disintegrating nucleoli go to build up the "Fadenknäuel." The granules resulting from the nucleolar dissolution are simply strewn throughout the central part of the vesicle (Fig. 15), and to the best of my observation ultimately mix with the rest of the egg substance at the time of the dissipation of the germinal vesicle. There is indeed no preformed "nuclear network," but since there never is a very clear network in the amphibian egg this is beside the question. *There are, however, chromatin threads or chromosomes distinctly traccable through the whole history of the germinal vesicle.* These preformed chromosomes seem to have been overlooked

by Schultze. They do not arise from the disintegration of the nucleoli, but exist before the nucleoli begin to migrate centrewards (see Fig. 16, Pl. XVI). To the consideration of these important structures we must now turn.

*Chromatin threads or Chromosomes.* — I have hitherto referred to the filamentous chromatin as if it were in the form of isolated and independent elements. I freely admit that no convincing proof can be adduced to show that there actually exist distinct individual chromosomes rather than a single looped and twisted skein. I have found it impossible to determine this point, but I am so far influenced by the appearances observed in my sections as to incline to the belief that the filamentous chromatin is in the form of detached threads rather than in one continuous coil. I shall accordingly use the term "chromosomes," premising, however, that I do so rather for the sake of convenience than for any strong belief in its absolute accordance with the facts.

In sections through young eggs the chromosomes present the appearance shown in Figs. 4 and 14. They are strings of cohering granules of varying dimensions, and in the youngest eggs these granules are indistinguishable from the small nucleoli. I have sometimes found threads somewhat resembling those figured by Flemming ('82) in *Siredon* (p. 134) though his figure of the egg of *Rana* (Fig. 78, Pl. V) is much closer to the usual appearance in the egg of the newt. Leydig ('88) has given for *Triton* (Fig. 93, Pl. XV) a representation very much like what I have observed in *Diemyctylus* except in the disposition of the threads. I have never seen chromosomes of the curious filamentous structure found by Rückert ('92) in the Selachian egg; the chromosomes of *Diemyctylus* are always of a loosely granular texture. I have also never observed any association of the chromosomes — if such they be — in pairs in the highly suggestive manner figured by Kastschenko ('90) and Rückert ('92) in the Selachian egg.

The most striking difference between the chromosomes of the stage shown in Fig. 4 and those of Fig. 16 is in the relative susceptibility to stain. The young chromosomes stain comparatively readily and are in consequence easily detected. They subsequently appear to undergo a gradual change in their

stainability, and stain more and more feebly until it is only with the closest scrutiny that they can be observed at all (Fig. 16). This fact, I think, accounts for the overlooking of these structures by Schultze. The apparent feeble reaction of the older chromosomes to stains may be due either to chemical change in the chromatic substance or to the mechanical separation of the chromatin granules. About the time that the nucleoli break up, the chromosomes become more deeply stained; whether this indicates a welding together of the chromosome substance or a different molecular constitution I must regard as an open question. The problem of the number of chromosomes at different stages in the history of the ovarian egg, which has been raised by the interesting researches of Rückert ('92) on Selachians, may safely be left until the actual existence of independent elements is definitively demonstrated.

*General considerations.*—The history of the germinal vesicle and its parts, as thus far outlined, brings us to an estimate of the general significance of the phenomena observed. It is a time-honored hypothesis that the substance of the egg-cell of every organism is differentiated into two radically unlike protoplasmic parts, into a part concerned wholly with the transmission of hereditary qualities, and a part occupied solely with the development of the egg-cell. The principal bearer of the hereditary tendencies is considered by many to be the nuclear chromatin network, composed of the so-called Idants of Weismann, which are handed down from generation to generation practically unchanged by somatic influences.

There is not this consensus of opinion, however, regarding the seat of the ovogenetic or histogenetic element. This ovogenetic element has been variously regarded as resident in the yolk-nuclei, as an attribute of the cytoplasm itself and as inextricably mingled with the germ plasm. The now abandoned hypothesis according to which the ovogenetic substance was thought to be sundered from close association with the germ plasm and then expelled as the first polar globule is too familiar to need comment. A more recent view is that put forth by

Rückert ('92) who finds a remarkable increase in bulk of the chromosomes in the Selachian egg during the growth of the egg, and an equally striking decrease premonitory of vesicular dissolution. Rückert considers this general atrophy of the chromosomes as due to the withdrawal of the ovogenetic or "somatic" substance. The chromosomic material remaining after the dissolution of the ovogenetic portion corresponds to Weismann's Keimplasma.

Rückert draws an interesting comparison between the phenomena observed by him in the Selachian egg and the nuclear phenomena discovered by Maupas and R. Hertwig in the infusoria ('92, p. 134). In the behavior and functions of the infusorian macronuclei and micronuclei he sees a close analogy the phenomena displayed by the Selachian chromosomes.

While admitting that such an interpretation may perhaps hold for the Selachian egg I think that in the amphibian ovum a somewhat different explanation must be sought after. The chromosomes here undergo no extensive changes in bulk; *the nucleoli, however, do increase enormously in number and size during the anabolic period of maturation.* I therefore believe that in this case it is between the nucleoli and the macronucleus of infusoria that a comparison, if such a comparison have any value, can be most aptly drawn.

It is now well established, thanks to the brilliant researches of Maupas and Hertwig, that the infusorian micronucleus is the bearer of the hereditary tendencies while the macronucleus possesses a nutritive and regenerative significance. At the time of the conjugation of ciliate infusoria the macronucleus degenerates and totally disappears. The micronucleus takes part in conjugation, and the daughter micronuclei give birth to new macronuclei.

A close parallel to this series of events is found in the history of the amphibian nucleoli. (1) They arise if not from, at least in intimate connection with the chromatin threads, the presumable bearers of the germ plasm. (2) Their function is almost certainly one of nutrition. (3) When the nucleoli have ended their ovogenetic duties and the ovum is fully formed, they degenerate and are dissipated throughout the vesicular

substance, taking no visible part in the process of fertilization. In all these respects the nucleoli resemble the infusorian macronucleus.

(1) It is universally admitted that cell nucleoli in general, even if they do not arise directly from the chromatin network, lie in the meshes of the chromatin coil and at their inception are always in the most intimate association with it. Many good observers insist that the nucleoli originate immediately from the substance of the chromatin threads, but on this point the evidence is still insufficient to produce conviction.

(2) The general functional significance of nucleoli is still very obscure, although many ingenious surmises on the matter have been advanced. Strasburger and Pfitzner, as is well known, regard the nucleolus as a place of storage of "reserve substance." On all sides there is a growing tendency to look at the nucleoli as concerned either in the storage or the elaboration of nutritive substance. In the amphibian egg, as we have seen, it is difficult to avoid regarding the nucleoli in this light. In a general way, also, it is true that the larger the egg, the larger the quantity of nucleolar substance, seeming to point to a direct ovogenetic relation. There is, on the whole, therefore, substantial reason for looking upon nucleoli, wherever found, as concerned in one way or another with the active metabolism (anabolism?) of the cell. The fact that different types of nucleolar-like bodies have been found by Carnoy and others does not militate against this view. Indeed, the behavior of the "plasmasomes" described by Ogata (83) which wander out into the cell and there become the so-called "Nebenkerne," playing an important part in the regeneration of the cell, confirms the essential similarity in function between these and other nucleoli. One may legitimately suppose that these bodies which behave thus differently towards stains—whether "karyosomes," "plasmasomes" or "hyalosomes"—are in different stages of nucleolar development, or even that a fundamental nucleolar substance has differentiated according to the metabolic needs of the cell and has assumed various allotropic forms. One is not constrained to regard these bodies as fundamentally unlike; the presumption may be rather the other way when we

remember the sharp differences in chemical reaction dependent on apparently slight differences in chemical constitution.

(3) There is much greater unanimity of opinion concerning the fate of the nucleoli of the egg-cell than concerning either their origin or significance. The nucleoli always degenerate and disappear before the process of fertilization.

Thus in origin, function, and fate many of the bodies called nucleoli constantly suggest community of relationship with the infusorian macronucleus. We are, however, hardly at present in a position to know whether this resemblance is superficial or fundamental.

The interesting case brought forward by Rückert, and already touched upon, may be considered as consonant with the analogy just drawn. It may be supposed that the fluctuations in the bulk of the Selachian chromosomes are due to a certain proportion of ovogenetic (nucleolar?) substance remaining attached to the chromosomes and never becoming separated as true nucleoli. This modification of his view allows it to be brought into harmony with other ovogenetic observations. It is worthy of notice that Rückert observes and remarks upon the close parallelism of development between Selachian nucleoli and chromosomes.

*The atrophy of the germinal vesicle and the formation of the polar bodies.*—The principal changes that take place in the germinal vesicle before the egg leaves the ovary have been already described. The germinal vesicle in Fig. 15 has nearly reached the limit of the changes which it undergoes while the egg is still in the ovary. The oldest nuclear appearance that I have found in an ovarian egg very closely resembles that shown in Fig. 15, except that the chromosomes are slightly stouter and more distinct, and are massed together in the center, while at the same time the borders of the vesicle show an incipient invasion of yolk spheres. Between such an appearance and the well-defined maturation spindle, shown in Figs. 18 and 21, I have found no intermediate stages, although I have made careful serial sections through a number of eggs showing a light spot externally and apparently just ready to break loose from the ovary. The transition is evidently an

abrupt one. It seems likely that the vesicle remains for some time on the verge of dissolution, and that the shock of separation from the ovary precipitates the sudden change. All the eggs that I have found, both in the body-cavity and in the oviducts, contain a completely formed spindle.

It is manifest, then, that in *Dicmyctylus*, at about the time the egg falls from the ovary into the body-cavity, the greater part of the germinal vesicle is distributed throughout the yolk. With a high power (Zeiss, 2 mm. immersion) the débris of the vesicle can be easily recognized surrounding the maturation spindle near the upper pole of the egg. The semi-fluid nuclear sap containing minute granules lies between the yolk spheres, and in it are strewn fragments of half-digested nucleoli. In some cases a portion of the vesicular substance appears to ooze out upon the surface of the egg, where it has been described as the perivitellin by Hertwig, Schultze, and others. The perivitellin of the newt resembles the perivitellin of *Sirvdon*, as figured by Schultze (see Schultze, '87, Fig. 29). Schultze also recognized the remains of the nucleoli, — “kleine, kreisrunde Chromatinkörperchen, offenbar die Reste der nicht völlig gelösten Keimkörperchen.”

This admitted fate of a portion of the nucleolar substance seems to me to indicate the fate of all. It does not appear to me probable that, as Schultze maintains, a part of the material of the nucleoli goes to form chromosomes while another part degenerates and is lost, but rather that all the nucleolar substance shares alike. Dissolution and dissipation seems the likely end of all the nucleoli, as it is here unquestionably the end of some.

Besides the disintegration of the vesicle, when the egg is loosed from the ovary, there is another highly important but very obscure change. This is the transformation of the chromosomes from numerous, long, thin, faintly staining threads to a few stout rods (Fig. 18). I have not been able to discover how this welding of the chromosomic substance takes place. The change from the delicate filamentous structure to the massive compact one is apparently the work of a short time. There seems to be a reduction in the number of individual

chromosomes during this process, but owing to the extreme difficulty of delimitation (see *c.g.* Fig. 15) this may be only apparent.

Coincident with this abrupt internal change there becomes visible on the upper pole of the egg the well-known "light spot" of amphibian ova, the so-called "Cicatricula" of Prévost and Dumas, the "Keimpunkt" of v. Baer and the "Fovea germinativa" of Max Schultze. This area has been figured for the axolotl egg by v. Bambeke ('70), and his figures resemble essentially the fovea of the newt. There is (Fig. 3) an outer ring of brown pigment shading off on the outside into the normal pigmentation of the upper pole, and on the inner side sharply defining the limits of the light area. This light area (*cl. sp.*) encloses within itself a small circular spot of still greater whiteness, in the middle of which is a minute dark spot. This superficial marking is usually found on all eggs taken from the body-cavity and oviducts, and persists for about two hours after the egg is laid. It then slowly disappears and the surface of the egg resumes its original uniformity.

The internal changes that give rise to this constant external appearance are in the main identical with those that have been described by O. Schultze in *Siredon* and other amphibia. The germinal vesicle towards the end of maturation slowly presses nearer to the periphery of the egg, and before the egg leaves the ovary the position of the vesicle is betrayed by the "light spot" due to contrast of the vesicle with the surrounding egg substance. The pigment and yolk are of course pushed to one side by the advancing vesicle. Then steps in the abrupt vesicular dissolution above described.

Sections through eggs from the body-cavity (Fig. 21) show a well-formed spindle which often exhibits a striking accumulation of pigment at the two poles. The spindle itself is undoubtedly the basis of the small white spot seen in the midst of the light area (Fig. 3), while the pigment at the peripheral pole of the spindle causes the minute central point of black. The neighborhood of the spindle is occupied by the débris of the vesicle together with small invading yolk spheres. Except in immediate proximity to the spindle, pigment gran-

ules are conspicuous by their absence, and this absence of pigment, or the presence of the vesicular débris, or both, unquestionably gives the appearance of the large light area (Fig. 3).

Schultze (*l.c.*, p. 205) seems inclined to adopt the view that the absence of pigment in this region is due to the fact that the germinal vesicle once filled this area, and that, notwithstanding the vesicular atrophy, the pigment granules still remain pressed out in a ring. It does not seem to me, however, that this explanation, simple as it is, is quite satisfactory. Since yolk granules are found in considerable numbers throughout this region, one is tempted to ask, if the yolk granules have thus invaded the former site of the vesicle, why not pigment granules also? The mantle of pigment granules that often envelops the spindle, and is particularly dense at the two poles, is a partial answer to this question. It shows that pigment granules have entered the vesicular region along with the yolk spherules, and have been attracted to the close neighborhood of the spindle. The comparative lack of pigment in the surrounding territory may well be due in part to this concentration around the spindle. But this cannot account for all the phenomena. Shortly after the egg is laid the pigment begins to show a more even distribution over the upper hemisphere of the egg, and eventually regains its original uniformity of disposition. This takes place in a few hours. Now, since the white spot has in some cases remained practically unchanged for at least forty-eight hours, — as I shall show later — and since during all this time the outer ring of pigment has been forced to keep its distance, it cannot be mere lapse of time that brings about the redistribution of the pigment after the egg is laid. The principal events of the period that follows the deposition of the egg are changes in the nuclear substance (including by that phrase the whole spindle-chromosome mass), and the migration of the female pronucleus away from the periphery. Since the female pronucleus carries with it in this migration a comparatively insignificant amount of protoplasm, one is led to infer that it is either the nuclear metamorphosis or the nuclear

migration that permits the rearrangement of the pigment on the surface of the egg. It is not therefore altogether unwarrantable to suppose that the disturbing influence of the nucleus may account in part at least for the existence and position of the outer pigment ring. After the nucleus leaves its peripheral position, but not before, the pigment becomes uniformly distributed over the surface. The nature of this repellent influence, if we choose to regard it as such, must for the present remain obscure. It is sufficient to remember that similar phenomena, viz., the attraction of small particles into close proximity to an object and the repulsion of other like particles to a distance, are not wholly unknown.

The massing of the pigment at the poles of the spindle would seem to indicate the presence there of attracting bodies, and I was therefore led to search quite carefully for centrosomes, but without success.

The spindle in all the eggs of this stage lies close against the periphery of the egg (Figs 18-22). There is sometimes a slight depression in the surface of the egg at the bottom of which lies the spindle (Fig. 21), but this would seem to be the exception rather than the rule.

The axis of the spindle in thirteen oviduct eggs was approximately radial; in two it was tangential. Schultze ('87) has advanced an ingenious explanation of this varying disposition of the spindle axis in the ripening eggs of *Siredon*. He considers in brief, "dass auch hier die abwechselnde Einstellung der Spindel kurz gesagt aus dem Kampfe zwischen dem karyokinetischen Gesetz und dem Gesetz der Kernstreckung hervorgeht." For a searching discussion of this interesting question I must refer to his analysis.

I am unable to determine with certainty from my preparations the precise time of expulsion of the first polar body, since I have not succeeded in discovering a polar body on the surface of the oviduct egg. There is, however, a certain amount of circumstantial evidence which appears to indicate that the first polar body may be formed while the eggs are in the upper portion of the oviduct. Eggs from the body-cavity and from the

upper part of the oviduct exhibit the disposition of chromosomes seen in Figs. 18 and 21. This must be taken as indicating the metakinetic separation of the chromosomes, and apparently foreshadows the speedy formation of a polar body. This view is strengthened by the fact that eggs in the lower part of the oviduct are all in the quiescent "equatorial plate" stage as shown in Fig. 19. In this condition they remain until after the egg is deposited and spermatozoa have entered. About one hour and a half to two hours after deposition the second polar body is expelled (Fig. 20). The minute size of this body in the newt, as indicated in Fig. 20, is probably the reason for my failure to discover it after expulsion.

The eggs of the newt often remain in the oviducts unchanged for at least forty-eight hours. I have elsewhere stated that newts freshly captured from the ponds during the months of May and June are almost invariably found to have eggs in the oviducts. These individuals when brought to the laboratory and placed in aquaria do not as a rule deposit their eggs until the second day after the capture; the eggs thus tardily deposited develop normally. The number of eggs that is usually laid in the course of twenty-four hours (see p. 276) seems to indicate that at least twenty-four hours is required for the passage of an egg down the oviduct. Not more than ten or twelve eggs are found in an oviduct at one time, even in a large female. The formation of the protecting membrane takes place along the whole length of the oviduct, but is most active in the middle and lower thirds.

NOTE.— Since finishing this paper I have received Born's paper upon the maturation of the amphibian ovum (*Die Reifung des Amphibieneies und die Befruchtung unreifer Eier bei Triton tæniatus*, *Anat. Anz.*, VII., 1892, p. 772). Born's account of the continuity of the chromatin filaments fully corroborates what I have found in *Diemyctylus*, and renders completely untenable the view that the "skein" in the older eggs is constructed out of fragments of disintegrated nucleoli.

The only mention of a yolk-nucleus is on p. 777. Until Born certainly identifies this "finely granular, oval body" with the yolk-nucleus of other authors it would be unfair to criticise his singular statement that "in diesen Gebilden das Archoplasma und die Centrosomen des Keimbläschens zu suchen sind."

Born finds that in *Triton taeniatus* the first polar body is expelled while the egg is in the upper part of the oviduct. I had strongly suspected that this was the case in *Diemyctylus* (see p. 307), but was unable to present definite proof. Born's results on this important point furnish a welcome confirmation of my surmise.

I do not feel it necessary to enter at this time into a fuller discussion of the minor points of accord and discord between Born's results and my own.

### III. FERTILIZATION.

The fertilization of the egg takes place just before the egg is extruded. The spermatozoa, which have long been in waiting in the tubes of the receptaculum seminis, are either attracted from their resting-places by the passing egg or forced out by contraction of the surrounding muscles. I have made repeated and careful search for spermatozoa in the oviducts, but have never succeeded in finding one. Neither have I ever found in sections any indication that spermatozoa enter oviduct eggs, although eggs often lie for some time in the mouth of the oviducts. Fertilization, then, would seem to take place only after the egg has left the oviduct and passed into the cloaca.

It sometimes happens that non-fertilized eggs are dropped by females in captivity. It is an interesting fact that these non-fertilized eggs are not deposited in a carefully prepared nest, but are dropped, apparently without concern, on the floor of the aquarium. Sometimes, but very rarely, an egg thus loosely dropped develops normally, showing that fertilization has taken place. The explanation of the dropping of non-fertilized eggs seems to be that in females with full oviducts, an egg is occasionally pressed into the cloaca by the mere elasticity of the oviduct walls and without the special cognizance of the newt. This egg then passes out like so much excreta without the performance of a voluntary act of oviposition. The fact that these eggs are, for the most part, unfertilized indicates an expulsion of spermatozoa from the receptacle during the normal process of egg-laying. This view is strengthened by the fact that the act of oviposition usually takes an appreciable time, often eight or ten minutes, pointing to essential accompaniments of the mere process of extru-

sion. There is good reason to think, then, that during the process of oviposition (see p. 277) the egg passes from the oviduct into the cloaca, where it is fertilized by spermatozoa pressed out of the receptacle, and thence is extruded along with a quantity of gelatinous secretion that glues together the leaves of the "nest." All of these actions are undoubtedly to a certain degree coördinated under voluntary control. The occasional fertilization of one of the excreted eggs is probably due to the chance presence of a stray spermatozoon in the cloaca.

Since it is true, then, that the fertilization of the egg does not take place until the egg reaches the cloaca, the spermatozoa which strive for entrance there find themselves confronted with a formidable egg-membrane. The consistency of this membrane can be adequately realized only through repeated attempts to remove it from the living egg. So improbable did it appear to me that spermatozoa should be able to pierce this dense and leathery covering, that I sought long and carefully for a micropylar opening. The search, however, was fruitless, and this negative result, together with the fact that spermatozoa ordinarily penetrate the egg at different and widely separated points, oblige me to admit, though somewhat reluctantly, the probability that spermatozoa somehow succeed in making their way through the membrane.

The points of entrance of the spermatozoa may be seen as slightly depressed, pigmented areas on the surface of the living egg freed from its membrane, one to two hours after oviposition (Fig. 6). There are several of these points, as a rule, indicating the penetration of several spermatozoa, an inference which, as we shall see, is fully sustained by the evidence from sections. There is no fixed and predetermined point of entrance; the spermatozoa may invade the egg in the neighborhood of the "light spot" (Fig. 6), or they may force an entrance near the equator of the egg. Most frequently they enter the upper (pigmented) pole, but I have several times observed them entering the lower hemisphere. Roux ('87, p. 174) has established a similar indeterminateness in the point of penetration of the spermatozoa of *Rana*.

The progress and maturation of the spermatozoa after they enter the egg may be best studied by sections through eggs from two to five hours old. The advance of the spermatozoon into the egg is signalized by the often described streak of pigment (Fig. 23C). This pigment streak, though it is not so prominent in the egg of the newt as in more deeply pigmented eggs, enables the path of the spermatozoon after entrance to be easily traced. It has been thought by some observers (see *e. g.* Hertwig, '77, p. 49) that the pigment is mechanically carried in from the cortical layer by the onward movement of the spermatozoon. While this may be in part true, there is another factor that undoubtedly assists in the production of the pigment streak. The eggs of the newt when viewed from the surface show the points of entrance of the spermatozoa *each distinctly marked by a spot of pigment*. The pigment accumulation at these points forces one to conclude that the male element exerts an attractive influence upon pigment granules comparable to that shown by the poles of the maturation spindle (Fig. 21). This attractive force appears to diminish as the sperm penetrates deeper into the egg, and the pigment granules discarded along the way then form the familiar pigment trail. There is, therefore, some force connected with both male and female elements that, in certain stages, attracts pigment granules; this force at other stages appears to lapse into quiescence.

There frequently arises a considerable accumulation of a semi-fluid reticular substance (protoplasm?) around the head of the male element. This accumulation often assumes the form of a mushroom, showing crescentic in sections (Figs. 23B, 23C). The head of the sperm occupies the middle of this area, and the whole effect is such as almost to convey the impression that the yolk granules have been driven out of this region by movements of the spermatozoon. I am loth, however, to believe that such is the case, since there is good reason to think that the active movements of the sperm cease after it penetrates the egg. Occasionally a faint filament stretches off behind into the pigment streak and strongly suggests the presence of the tail of the spermatozoon (Fig. 23B, 23C). I am unable, however, to identify it certainly as such. It is not to be seen when

the spermatozoon has penetrated some distance into the egg substance.

I have not succeeded in discovering the earliest stages in the metamorphosis of the sperm-head into the male pronucleus. When the spermatozoon first enters the egg (Figs. 23B, 23C) the head does not appear to be sharply defined, and the earliest stage at which I have been able to recognize a well-differentiated portion is shown in Fig. 23D. Here the head is already far on the way towards becoming a true pronucleus. The most obvious point of difference between this stage and the mature pronucleus shown in Fig. 23A is one of size. This is somewhat exaggerated by the difference in magnification of the two figures, but even making the necessary deduction for the difference in magnification the increase in size will be seen to be considerable, and amounts to at least a doubling in diameter. Sometimes during the maturation of the male element the yolk granules in the neighborhood assume a radial arrangement, but this astral phenomenon is not of invariable or indeed frequent occurrence. The membrane of the pronucleus in the early stages is less distinct than it afterward becomes (*cf.* Figs. 23A and 23D).

My friend Dr. Watasé ('92) has determined by Auerbach's method of differential staining that the sperm-nucleus of the newt reacts differently at different stages of its maturation period. When it first enters the egg it is, like the original sperm-head, strongly cyanophilous, but the reaction gradually alters until, just before union with the female pronucleus, *both male and female pronuclei stain identically.*

The rapidity of movement of the male element when once inside the egg does not seem to be great. The spermatozoa enter the egg from one to two hours after deposition, and although they sometimes enter in close proximity to the female element (Fig. 6), union does not usually occur until from four to six hours later. It is obvious that the fallacy incident to all studies upon preserved eggs lurks in this estimate of rapidity of movement. Since, however, Fig. 23C shows a spermatozoon in an egg two hours old, and since Fig. 23D is from an egg four hours old there would seem to be a fair basis for the assumption of slow penetration. It is of course likely that the

male and female elements busy themselves during this period more in preparation for union than in actual movement. It is even fair to suppose that speedier metamorphosis of the nuclei would imply — or, perhaps, cause — greater rapidity of movement. This indeed is indicated by the course of the phenomena in the frog's egg where the course of events is more rapid. The hindering effect of the yolk must be practically the same in both cases, and one is justified therefore in regarding the slower movement of the male element in the newt's egg as the expression of a slower maturation of that element. In other words it is not the external obstacles, but the internal changes that determine the rate at which the two pronuclei approach each other.

The path taken by the sperm is similar to that described by Roux ('87) for the frog's ovum. The spermatozoon moves at first centripetally, describing the "penetration-path," and later describing the "copulation-path," curves more or less abruptly towards the female pronucleus. The initial change from the centripetal to the nucleopetal direction is shown in Figs. 23C and 23D. It is impossible not to agree with those who hold that this change in direction is due to some sort of nuclear attraction.

The female element, which is the objective point of the male pronucleus and is hence the cause of the change of direction of the latter, is by no means a mere inactive spectator of the entrance of the spermatozoon. About two hours after the egg is laid the polar body (second?) is expelled (Fig. 20). It is clear that this expulsion of the polar body is caused directly by the penetration of the sperm into the egg, since before this event the female element has remained perfectly passive, it may be for upwards of forty-eight hours. The nature of this telepathic influence is far from clear. After this event the female pronucleus is formed by a series of changes which I have not been able to trace out completely, but which appear to be essentially parallel to those of the sperm. Indeed I have been in most cases unable to distinguish between the male and female pronucleus after the formation of the latter. This is owing partly to the fact that the male pronuclei are usually numerous, partly

to the fact that in the newt's egg only a slight quantity of pigment attends the path of the sperm, but chiefly to the absence of any essential divergence in structure between the two pronuclei. It occasionally happens that one may pick out from the rest a pronucleus which is perceptibly larger or is surrounded by a greater quantity of protoplasm than the others, and I have naturally been inclined to regard such a body as the female element. This, it will be recognized, is a very inadequate basis for such identification, and careful examination of all the pronuclei found in an egg has served only to convince me more strongly of the unsatisfactory nature of such a criterion.

The union of the two pronuclei takes place ordinarily about six to eight hours after the egg is laid. The place of meeting does not seem to be constant or predetermined. It is never in the geometric center of the egg, but is nearer the upper pole, and is usually, so far as I have been able to determine, in the neighborhood of the position occupied by the germinal vesicle before dissolution. The general phenomena attending the union of the pronuclei are substantially such as described by Hertwig ('77). The pronuclei that I have figured in Fig. 23A present a typical appearance. The inner one (*f*) may perhaps be regarded as more probably the female since it is surrounded by the larger quantity of protoplasmic substance, and since the other (*m*) has apparently just impinged upon it from the side. Such identification, however, is based on pure analogy and on no sure recognition-marks. The question here arises as to whether the female pronucleus exercises any power of selection from among the several suitors at her disposal or whether it is a mere matter of proximity that determines which male nucleus shall unite with the female. I may here state, what to some will doubtless seem superfluous, that I have never seen any evidence whatever that more than one male element unites with the female. Why the one individual so favored should be preferred above his fellows is somewhat problematical. It is obvious, however, that other things, such as proximity, etc., being equal, the sperm first entering would have the advantage. Its earlier maturation would bring about a reciprocal movement of both male and female which would soon

place it ahead of all competition. Proximity and order of entrance are undoubtedly the two factors that determine the success of the sperm's quest.

*Polyspermy.* — I have already mentioned that several spermatozoa normally enter the egg of the newt. I may now state that I have sectioned twenty eggs from two to ten hours old and found in every egg superfluous male elements. The number of these varied from one to thirteen and was in most cases from six to eight. If it be objected (Born, Roux, Hertwig) that such polyspermy indicates pathological conditions, I may reply that these twenty eggs were all laid in a normal way, by different females and at different times; that abnormalities are very rare in newt development and that such a succession of non-developing or abnormally developing eggs would in my experience be unprecedented; and finally that I have in the living egg watched several spermatozoa enter the upper pole and normal development succeed in every case. There is thus every reason for regarding such physiological polyspermy in the newt as a natural, normal, and, in fact, usual occurrence.

What now is the further history of these superfluous spermatozoa which normally enter the egg of the newt? In the first place it is to be remarked that these accessory sperm nuclei (Nebenspermakerne) undergo a progressive development after their entrance. They increase in size, acquire a double-contoured membrane, and show precisely the same affinity for stains as the male element that fuses with the female pronucleus. They are in all objective respects quite as competent aspirants to union with the latter as is the male nucleus (Hauptspermakern) actually honored. They owe their non-preferment not to any intrinsic inferiority, but to the time and place at which they entered the egg. These accessory sperm nuclei are most numerous in the region of the upper pole, but are quite often found stranded among the large yolk spheres of the lower hemisphere. In the latter case they are usually near to the periphery of the egg.

The accessory sperm nuclei ultimately degenerate. Shortly after the fusion of the two chief pronuclei the superfluous male

elements begin to show signs of degenerative change, and in eggs ten hours old—just before the beginning of the first cleavage plane—they are in a condition of well-marked atrophy. This is evinced by the disappearance of the membrane and by the mixing of the chromatin through the egg substance. I have been able to identify degenerating accessory nuclei in the four-cell stage, but not beyond.

v. Bambeke ('70) was one of the first to affirm that several spermatozoa normally entered the amphibian egg, but his statements were on the whole regarded as relating to abnormal conditions, and were not given the credence to which I think they were entitled. Since his observations were made upon the eggs of certain Urodela, and since none of his critics have repeated his observations on these particular species, but have reasoned themselves into incredulity solely through their own negative results upon the eggs of other animals, there seems no good reason for refusing our acceptance of v. Bambeke's observation. Negative observations upon the eggs of one animal hardly furnish an adequate basis for scepticism regarding positive phenomena in the eggs of related animals. Kupffer ('82) also has affirmed the normal entrance of several spermatozoa into the amphibian egg.

Physiological polyspermy has been observed, moreover, in the eggs of other vertebrates. Two of the most important cases I may briefly mention. Rückert ('91, '92<sup>a</sup>, '92<sup>b</sup>) has described some remarkable phenomena of this character in the eggs of Selachians (*Pristiurus*, *Torpedo*). A number of spermatozoa normally enter the egg, develop like the chief male pronucleus, and, at the formation of the first cleavage nucleus, *divide caryokinetically, and give rise, in part, at least, to the parablast nuclei, or "Merocytenkerne"!* These Merocytenkerne which arise thus independently from accessory spermatozoa are distinguished from the nuclei of the "Furchungskern" by a smaller number of chromosomes—"höchstens die Hälfte"! "Alle Merocytenkerne der jungen Furchungsstadien, welche eine reduzierte Zahl von Chromosomen besitzen, sind Abkömmlinge von Spermaköpfen. (Rückert, '92<sup>b</sup>, p. 329.)

Polyspermy has been described also by Opper ('92) in the reptilian egg (*Anguis, Tropidonotus*). His results agree strikingly with those of Rückert, although presenting many interesting points of difference. Here, as in the Selachians, a number of spermatozoa normally penetrate the egg. They develop into full-fledged sperm nuclei which divide caryokinetically, though very sluggishly and irregularly, and soon abort.

There is, hence, wide variation in the phenomena of fertilization among vertebrates. (1) Only a single spermatozoon normally enters the egg [*Teleost* (Trout), Böhm; *Amphibia* (*Rana*), Born, Roux]. (2) Many spermatozoa normally enter the egg, in which case they may (*a*) develop into nuclei which divide caryokinetically and regularly (*Selachians*, Rückert); or (*b*) they develop into nuclei which divide slowly and with many abnormalities, and soon degenerate (*Reptilia*, Opper); or (*c*) they develop into nuclei which speedily degenerate without undergoing division (*Diemyctylus*).

One thing is certain: such physiological polyspermy as has been described here is a usual occurrence in the eggs of many vertebrates. This establishes the important fact that the mere entrance of several spermatozoa into an ovum exerts no injurious influence upon development. This being true, it follows that the more frequent case of the admission of only a single sperm is probably to be regarded as due to the conditions of nuclear attraction rather than to any necessity for barring the way against superfluous spermatozoa. It is not that an excess of spermatozoa is harmful, but that the female nucleus is satisfied, as it were, with the entrance of one, and no longer acts so attractively upon those outside. The fact that several spermatozoa enter an egg which has been weakened by rough treatment or overripeness shows that the internal conditions of the egg cell have been altered, but shows nothing more. The abnormal development that follows cannot be regarded as purely the result of the superfertilization, but both events, the abnormal development as well as the entrance of several spermatozoa, are direct consequences of the internal disturbances in the egg. The normal

entrance of a varying number of spermatozoa into an egg merely indicates unessential variations in the intensity and duration of nuclear attraction.

#### IV. CLEAVAGE.<sup>1</sup>

It is not difficult to obtain the egg of the newt immediately after the female has deposited it, and the order of appearance of the cleavage planes can then be accurately timed. I have preferred this mode of procedure to artificial fertilization since in this way the reproach of departure from the normal is not incurred.

The first cleavage plane begins from ten to twelve hours after the egg is laid. The furrow is vertical and divides the egg into two nearly equivalent halves, but usually falls more or less to one side of the exact mid line (Fig. 24, Pl. XVII). The furrow reaches the lower pole forty-five to sixty minutes after it first becomes visible at the top of the egg.

The second furrow is likewise vertical, and usually cuts the first at right angles near the mid-line, thus dividing the egg into four equivalent portions (Fig. 25). The second furrow appears from one and a half to two hours after the beginning of the first, and cuts its way to the lower pole with the same rapidity as the first.

With the completion of the second furrow all consistent regularity is at an end. The third furrow is often horizontal, but is very variable in position and is usually differently disposed in each of the four quadrants. Frequently it can be designated as equatorial only by courtesy, and occasionally it is distinctly vertical. This latter important fact I hope to bring out more clearly presently.

The point that I wish to touch on first is the great irregularity in the early cleavage stages. I have made a careful study of a large number of preserved eggs, and have depicted some of these in Figs. 26 to 32, Pl. XVII. These figures con-

<sup>1</sup> I should here state that I reserve a more detailed consideration of the facts and significance of cleavage for a joint paper soon to appear by Mr. A. C. Eycleshymer and myself.

sist of camera drawings of eggs seen in different positions. Each cell has its own individual number in order to facilitate recognition in the different points of view. The eggs chosen for this study were those especially favorable specimens which permitted identification of the first two cleavage planes. They are therefore to be regarded as more regular than the great majority of newts' eggs, since in most cases the first two planes cannot be recognized with any fair degree of certainty. In the eggs I have chosen to represent there is a minimum of uncertainty. The lines indicating the first two planes are printed more heavily than are the others. Since the magnification of all figures on this plate is the same, the series from Fig. 26 to Fig. 32 serves to illustrate the considerable variation in size of the newt's egg. The size appears to be in the main dependent on the size of the female, the larger individual as a rule depositing the larger egg.

Figs. 26A–26D represent an egg of eight cells seen in different positions. 26A is a view from the upper pole, 26B and 26C are views from the side, and 26D is from the bottom of the egg. The general disposition of the cells is very remote from the usual eight-cell stage, one cell—3—extending all the way from the upper to the lower pole. Figs. 27A–27D are views of an egg of a more advanced stage, 27A and 27D being respectively the upper and lower poles. The two upper right hand octants, 3–4 and 5–6, have divided in diagram fashion from the intersection of the first two planes, but the other two, 1–7 and 2–8, have divided at very different angles. Only one of the lower pole cells (Fig. 27C, 9–12) has begun to divide. Figs. 28A–28D show a still wider departure from the orthodox type of cleavage. The upper pole (28A) presents a far from regular appearance. Figs. 29A<sup>1</sup>–29D picture the most regular egg of this stage that I was able to find. Even here the lines of the third vertical do not start from the intersection of the first and second planes. Figs. 30A–32B illustrate top and bottom views of three eggs in advanced stages of

<sup>1</sup>The upper pole of this egg bears a close resemblance to the egg figured by Rauber ('83) in Fig. 35, Pl. XII.

cleavage. The total absence of any regularity in the arrangement of the cells is the most conspicuous feature.

It cannot reasonably be held, I think, that these eggs depart from the "normal" or the "type." They fairly represent the average and common course of cleavage in the newt, and would undoubtedly have resulted in normal development. I am convinced of the truth of this latter assertion through careful following out of a large number of living eggs.

The difficulty of following the cleavage in the egg of the newt is much enhanced by the opacity of the enveloping membrane. I have, therefore, usually removed the egg from the membrane soon after deposition. The removal of the membrane is accomplished with sharp needles and fine scissors, but is attended with considerable difficulty, and often results, even with the best of care, in the destruction of the egg. I have succeeded, however, after some practice, in extricating without injury a number of eggs and in following their subsequent development.

Figs. 33A-33F illustrate successive stages in the development of a living egg. Fig. 33A shows the upper pole at the beginning of the *third* set of cleavage furrows. These furrows appeared in this egg at the time when the "equatorial" plane was normally due, and must hence be regarded as "homologous" with the usual "first equatorial plane" of the amphibian egg. Fig. 33B is taken ten minutes later and exhibits an interesting and remarkable bilaterality. The two furrows on the left have united near the equator of the egg, and, except for the unusual nearness of their proximal ends to the middle of the upper pole, would be regarded as part of a typical equatorial. The two furrows in the right half of the egg, on the contrary, cut down to a point near the middle of the lower pole, as is shown by the aid of an underlying plane mirror (Fig. 33C). By comparison of these views of the upper and lower poles it is clearly seen that in one half of this egg the *third* cleavage plane is an undoubted vertical, while in the other half it is a dubious equatorial. The fourth rhythmical nuclear division cleaves the egg into the cells seen in Fig. 33D. Comparison with Fig. 33C will show the distortion of the earlier cleavage

lines, and this distortion is carried still further by the next set of furrows (Fig. 33F). Fig. 33E portrays the lower pole of the stage shown in 33D and emphasizes the truly vertical character of the two furrows in the right half, which have here advanced so that they strike the center of the lower pole; the equatorial tendency of the furrows in the other half of the egg is also confirmed. This egg developed into a perfectly normal embryo, with no visible deviation from the normal course of development.

I may here anticipate the forthcoming paper by Mr. Eycleshymer and myself so far as to state that we have convincing evidence that the whole third set of cleavage furrows in the amphibian egg may at times be vertical instead of horizontal and yet result in normal development. This fact has obvious and important bearings upon the question as to the "homologies" in amphibian and teleostean cleavage.

*Axis of the Embryo, etc.* — The relation of the first cleavage plane to the axis of the embryo is a point of some interest, although in the light of recent cytogenetic studies it cannot be held to be a matter of such vital importance as was at one time supposed. The egg of the newt, as I have stated elsewhere, is enclosed at the time of deposition in an oval, thick-walled membrane. The egg thus enveloped has been submitted while in the oviducts to such compression that for several hours after it is laid it is very much elongated in the direction of the long axis of the capsule. The long diameter of the egg at this time is often double the transverse diameter. Before the formation of the first cleavage plane the egg resumes its spherical form. The first cleavage plane invariably passes through the egg at right angles to the axis of the capsule and therefore at right angles to the direction in which the egg was elongated by compression. If the egg be left undisturbed in its capsule it adheres to the bottom of the capsule so firmly that it may be turned bottom upwards without rotating. After repeated observation of the living egg, I am convinced that when the egg thus fixed remains undisturbed and under normal conditions, rotation cannot occur. By the direction of the convexity of the crescentic blastopore (Fig. 35) and while the form of the egg is

still spherical the axis of the embryo is seen to lie in the long axis of the capsule, and hence at right angles to the first cleavage plane. There is usually variation, often amounting to as much as  $45^\circ$  or  $50^\circ$ , but, in by far the great majority of the eggs I have examined, the axis of the embryo is approximately at right angles to the first plane of cleavage.

In the egg of the frog, as is well known from the researches of Newport, Pflüger, Roux and others, the direction of the axis of the embryo coincides with the first cleavage plane. Throughout the animal kingdom there can be discovered no constancy in the relation of first cleavage plane and embryonic axis. There are several well-established instances where, as in the frog's egg, the two planes coincide, and recently cases have multiplied where the opposite is true and the planes are at right angles (*Nereis*, Wilson; *Crepidula*, Conklin; *Jæra*, McMurrich). Miss Clapp ('91) has shown that out of twenty-three embryos of *Batrachus*, three showed coincidence of the axis of the embryo with the first cleavage plane. "Fourteen of the embryos had the head directed towards the right of the first line of cleavage, the axis of the body being at an angle with the first cleavage plane of from  $30^\circ$  to  $70^\circ$ . In the remaining six the head was to the left of the first cleavage plane, the angle varying as before." It is a plausible hypothesis that when planes and axes do coincide or cross at right angles it is because both are determined by the same external conditions and not necessarily because there is any causal nexus between the two. It is possible that the same external factors that determine that the first spindle shall lie in a certain axis determine also the direction of the embryonic axis.

The most recent work in experimental embryology seems to confirm this inference and to demonstrate that great stress cannot be laid upon the relations of early cleavage planes and embryonic axes. When we know that four perfect gastrulae of *Amphioxus* can be produced from the first four quadrants of the egg, there is no basis for regarding the direction of the first cleavage plane as a matter of supreme importance. The early variations in the position of the nuclei in the newt (Plate XVII), and the irregular torsions of the first cleavage planes

(Fig. 33F) indicate sufficiently well that the early stages of cleavage do not, in this case, rigidly mark out the future development of the embryo. The course of embryonic development in some animals is not dependent on the position or indeed the existence of all the early cleavage nuclei. We are consequently led to the conclusion that the parts are not essential to the integrity of the whole, that indeed the whole may be deprived of some of its parts and yet remain the whole. There is no qualitative sorting out of nuclear substance in the early cleavage stages; on the contrary a large proportion of nuclear substance can be displaced or even sacrificed without impairing the individuality of the embryo, or rendering it incapable of normal development. Wilson ('92) in his brilliant research upon the cell-lineage of *Nereis*, reached the conclusion that "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" (p. 455). Such a conclusion, drawn from annelid cleavage where the cell formation follows definite lines and the differentiation of important organs is strikingly precocious, is certainly applicable with added stringency to the other extreme of amphibian cleavage where the initial cleavage stages exhibit as a rule chaotic irregularities and variations.

Turning now to quite a different question, let us ask why the first cleavage plane should fall as it does in any individual case. Why should the plane pass through any particular meridian rather than through any other?

In the newt the most interesting fact about the first cleavage plane is its determinateness. It cuts the egg *invariably*, so far as my observations go, at right angles to the long axis of the enveloping capsule. In removing the egg from the capsule I have unfortunately not been able to preserve the orientation of the egg, and so have not succeeded in determining whether the egg when freed from the capsule still cleaves as it would have done inside. This constancy of direction of the first cleavage plane in the encapsuled egg puts an obstacle in the way of accepting for the newt the view that Roux has advanced for the

frog, namely that the path of meeting of the male and female pronuclei defines the first plane of cleavage. In the newt we should have to suppose, to uphold this theory, that the pathway was always coincident with the median transverse axis of the capsule. Since the point of entrance of the spermatozoon is indeterminate this supposition of an invariable pathway of approach is not justified. Furthermore, since several spermatozoa normally enter the egg there are several pathways in different directions, and some of the spermatozoa travel a greater distance through the egg-substance than does the spermatozoon that unites with the female pronucleus. The sundering of the egg-substance may therefore be of greater extent along the path of an unsuccessful sperm-nucleus than along that of the "Hauptspermakern." It is obvious, then, that Roux's explanation of the direction of the first cleavage plane, however valid it may be for the egg of the frog, does not admit of application to the egg of the newt, and that here we must seek for some determining cause other than the path taken by the male and female pronuclei

As regards Schultze's view that the first cleavage plane is determined by the position of the germinal vesicle in the unfertilized ovum I must agree with Roux in considering the evidence for such a view inadequate.

In the case of the newt the very tempting explanation suggests itself that the direction of the first cleavage plane is due to the compression and elongation to which the egg has been subjected in the oviduct. It may be legitimately maintained that the arrangement of the egg substance has been so affected by this compression that the first spindle can place itself with its long axis parallel to the elongated axis of the egg more readily than it can in any other axis. In other words the spindle takes the direction that it does because the egg has been elongated in that direction.

Another slightly different explanation of a similar mechanical nature is perhaps still more plausible. The egg soon after it is laid resumes its spherical form and is then in close contact with the capsule on all sides except in the direction of the long axis of the capsule. In this direction there is

room for expansion ; on all other sides the egg is hemmed in and is sometimes slightly compressed, as in cases where the capsule is so narrow as not to permit the egg fully to regain its spherical form. It is a curious coincidence, if nothing more, that the expansion of the egg incident upon the first cleavage should occur in just that direction in which it is most free to expand, that in other words the spindle takes up that position in which it can act most freely.

I am inclined to accept provisionally some such mechanical interpretation of the determinateness of direction of the first cleavage plane in the newt's egg. I do this the more readily since the effect of pressure in influencing the direction of cleavage planes is well known through the researches of Pflüger, Roux, Driesch, and others. Roux's experiment of elongating frogs' eggs by compression in a narrow glass tube showed that such elongated eggs divided at right angles to their long axis, a fact that bears directly upon the case in hand.

By attributing the direction of the first cleavage plane to Sachs' law I do not in the least wish to be considered as implying that in the eggs of some animals other causes may not often take the place of pressure as a determining factor and may indeed override it. I wish simply to state my belief that mechanical compression operates as the determinant of the direction of the first cleavage plane in the newt.

If we admit, as I think we are bound to do, that the earliest cleavage stages of these eggs have little or no morphological significance then I see no escape from the conclusion that they must depend on environmental or mechanical causes of one sort or another. Precocious differentiation may invest the early cleavage-stages of some eggs with a factitious significance. In others the early spindles may change the direction of their axes under the influence of various slight mechanical causes.

#### V. FORMATION OF THE BLASTOPORE.

*Technique.*—The difficulty of extricating the living egg uninjured from the membrane has been so great that I have as a rule preferred to kill the egg while it was still in the envelopes.

This has not only saved much time, but has given me very satisfactory results for all studies of preserved specimens. I have found the most satisfactory killing agents to be Perenyi's fluid and the micro-sulphuric mixture of Kleinenberg, particularly the former, which I have come to use almost exclusively. The eggs at the desired stages are thrown into Perenyi for 24 to 48 hours, and then removed to a solution of hypochlorite of sodium (Eau de Labarraque), which so softens the membrane that it may easily be teased off with needles. I have usually found it necessary to leave the eggs in the Labarraque for at least four to five minutes, in order to affect the membrane sufficiently. The eggs must be watched carefully during their stay in the hypochlorite, since both the strength of the commercial fluid and the resistance of the membrane vary widely; over-exposure to the action of the fluid renders the egg valueless. When the process of membrane softening has gone far enough—a point that only experience can determine—the hypochlorite is decanted and the eggs are very carefully washed with distilled water. After repeated washings the membrane is removed with needles, and the eggs are then transferred to 70 per cent. alcohol, where, after a few washings, they remain until wanted for examination. In this way I have obtained excellent specimens, which stain without difficulty and cut smoothly in paraffine. Eggs killed with the micro-sulphuric mixture are better for surface study, but are apt to be rather brittle for sectioning.

I have used for staining *in toto* chiefly borax carmine and Czokor's alum cochineal, both of which have given very satisfactory results.

For the surface study of preserved eggs I have used to advantage a background of white paraffine, in a hollow of which the egg lies, covered with 95 per cent. alcohol and lighted by direct sunlight with the aid of a condenser. By this method, which is substantially that followed by Erlanger ('89, p. 240), the surface of the egg may be exhaustively studied, and camera drawings made of all the external features.

I have relied not so much, however, upon the examination of preserved eggs as upon the following out of the surface

changes in the living egg. Despite the considerable difficulty of removing the membrane I have succeeded in a number of cases in performing this operation, and in tracing the external changes in the egg thus freed. The development of such eggs is normal, and the time of appearance of the different external features is the same as in eggs on which the membrane is allowed to remain. The first stages of the formation of the blastopore are best observed with the aid of a plane mirror placed underneath the flat-bottomed watch-glass in which the egg rests. The reflected image of the lower pole may be easily studied with the dissecting microscope and the successive changes accurately determined. For the study of the blastopore when it shifts to the side, I place the egg in a thin-walled glass chamber in which it can be examined with the microscope-tube in a horizontal position.

*Segmentation cavity.*—The segmentation cavity is formed at the division of the egg into eight cells, and enlarges progressively up to the time of beginning invagination (Figs. 46–48). The roof of the segmentation cavity is shown by median sections to consist of a single layer of cells, while the floor is composed of a mass of large yolk-cells with ill-defined boundaries. The cells of the roof are somewhat columnar and, up to the time when invagination begins, divide for the most part vertically. Scott and Osborn ('79) state that in *Triton tæniatus* the roof of the segmentation cavity is only one cell thick, in which respect it agrees with that of the egg of *Petromyzon* and differs from that of *Rana*. v. Bambeke ('80) and Hertwig ('82) have not been able to confirm this statement. They maintain that there is no essential divergence in this respect between the frog and the newt. However this may be with regard to the particular species considered by them, I am confident that in *Diemyctylus* the roof of the segmentation cavity up to a comparatively late period is only one cell thick. In other words, the horizontal division of the roof cells does not occur so early in the newt as it does in the frog. This is clearly seen if we compare Fig. 48, Plate XVIII with Götte's figures of *Bombinator* (Entwicklungsgeschichte, Figs. 28 and 29, Pl. II), or with other figures of Anuran eggs at this

stage. The section through the egg of *Triton tæniatus*, depicted by Hertwig (Fig. 1, Pl. XIII) does not at all correspond with what I find in median sections through the eggs of *Diemyctylus* at this period.

*External appearances.*—Beyond a slight increase in the diameter of the egg, due, doubtless, to the formation of the segmentation cavity, there is for some time no visible external change except decrease in size of the cells consequent upon their multiplication. The first signs of gastrulation appear about fifty-four hours after the egg is laid, the temperature of the water being about 18° C. There is first a slight depression of some of the cells in the middle of the lower pole; this becomes more and more pronounced, and finally results in an irregular pit, such as shown in Fig. 34. This pit grows deeper and its edges become more sharply outlined till there is a deep, nearly oblong depression extending in the direction A-B, Fig. 34. This depression is now at right angles to the future axis of the embryo. Almost as soon, however, as the depression assumes this form, faint sunken lines may be seen running off from the ends of the depression at an obtuse angle. The point of junction of these lines of sinking with the original area of depression is at first perfectly clear, and the angle is well marked, but very soon the angle is obliterated and the depression takes on the rounded semi-lunar appearance which has become familiar by repeated portrayal (Fig. 35). The horns of this half-moon gradually approximate, and in about twenty-four hours after the appearance of the depression the circle of the blastopore is complete (Fig. 36).

The more intimate phenomena of the formation of the blastopore must now be considered.

*Invagination.*—The view that the archenteron of the amphibia is formed by the infolding or ingrowth of cells that originally were external has been the one very generally upheld by the students of amphibian development. This conventional opinion has, however, been opposed by Moquin-Tandon ('76) and by Houssay ('90), and has recently been attacked with spirit by Robinson and Assheton ('91). These dissenters from the invagination hypothesis believe in its stead, "that the archen-

teron is formed *in situ* by splitting amongst the yolk-cells, and that it is entirely surrounded by modified yolk cells." (R. and A.) The entoblast on the latter interpretation takes its origin wholly from the so-called yolk-cells; on the invagination hypothesis it comes in part from infolding of the cells once covering the exterior and is continuous with them at the lips of the blastopore.

It is evident at the outset that the question of invagination or no-invagination can not readily be determined from the inspection of sections. The most that can be derived from sections will be only circumstantial evidence of greater or less significance. In the opinion of some investigators there is to be found in sections certain positive evidence that favors the invagination hypothesis; their opponents deny the validity of such evidence and regard the appearances observed as accounted for more easily on the splitting hypothesis.

If we examine some of the evidence that has been supposed to indicate invagination it cannot be said to carry conviction. Some stress has been laid upon the fact that the cells lining the dorsal wall of the archenteron, and lying opposite to the cells of the yolk-plug, are smaller than those of the ventral wall, and that by this character they show their affinity to the ectoblast cells, with which, moreover, they are in direct connection. It has been shown by Robinson and Assheton, however, that in *Rana* this difference in cell size does not appear in the early stages of the blastopore, but becomes manifest only after the blastopore is well established. At first there is practical equality in the size of the cells around the blastopore and only later can a difference in size be detected. This is also the case in *Dicmyctylus*. Later on, however, there is a striking inequality of size (Fig. 52). The advocates of the splitting hypothesis (v. Houssay, '90, p. 165) attribute this inequality to the greater activity of the cells forming the dorsal wall of the archenteron. That is to say, the dorsal archenteric cells divide more rapidly than the cells below them, and hence their number is greater and their average size less; this differentiation is supposed to have taken place *in situ*. It is obvious that this is a plausible or at any rate

possible explanation of the difference in cell size; the fact that the dorsal archenteric cells are smaller than the ventral, and resemble in this respect the ectoblast cells, is no proof of their direct genetic connection with the latter: for aught that is known to the contrary a secondary resemblance may have been acquired *in situ*.

The presence of pigment in the borders of the cells supposed to be invaginated has likewise been taken as proving their external origin, but this cannot be regarded as a more conclusive argument than that drawn from the size of the cells. The pigment, even in early segmentation, is not confined to the cells of the upper pole, but darkens to a greater or less extent the borders of all the cells. At the time the archenteron begins to be formed pigment is found in the cells on both sides of the archenteric cleft, and as the cleft widens and deepens the black borders of the lining cells become more and more pronounced. Furthermore, as is well known, a double line of pigmented cells extends forward beyond the limit of the actual cleft, and this occurs at a stage when it is exceedingly unlikely, if not impossible, that epiblast cells should have invaginated to that distance. The fact that the pigment in this region is found in the opposed borders of *two* rows of cells cannot be regarded as strengthening the opinion that the presence of pigment signifies invaginated ectoblast cells. The presence of pigment in certain cells, therefore, should by no means lead us to refer those cells to an ectoblastic origin. It might on the contrary be quite as reasonably supposed that the pigment marks physiological activity, and that the less heavily pigmented cells of the ventral wall of the archenteron owe their relative lack of pigment to the more sluggish metabolism attendant upon less rapid cell division. That is to say, the presence of pigment may be directly correlated with the small size of the cells containing it.

The size of the yolk granules has sometimes been taken as affording a criterion of invagination. The yolk granules in the dorsal archenteric cells are much smaller than those in the ventral, and this characteristic is thought to relate the dorsal cells to the ectoblast cells and distinguish them from the cells

of the yolk-plug. This distinction, however, does not exist in early stages of the archenteron, as has been well shown by Houssay for the axolotl. I have observed a similar condition in *Diemyctylus*: at the beginning of the formation of the archenteron the yolk granules in the surrounding cells are practically identical in size, and it is only in later stages that the yolk granules are smaller in the dorsal cells.<sup>1</sup> This reduction in the size of the yolk granules may perhaps depend, like the size of the cells and the presence of pigment, upon the activity of the cells themselves and may have been due to the differentiation of cells *in situ*. The size of the particles of yolk is consequently not a sound criterion of the source of the cells.

No one, of these characteristics of the cells of the dorsal wall, therefore, can be regarded as in any way satisfactory evidence for infolding: neither the size of the cells nor that of the yolk granules, and assuredly not the accumulation of pigment. All of these appearances may be explained perhaps equally well by supposing a differentiation of the cells *in situ*. Houssay regards the difference between the dorsal and ventral walls as due simply to a difference in rapidity of development, and believes that the ventral cells pass through the same stages as the dorsal, but more slowly. Why there should be this striking disparity he does not attempt to explain.

The evidence thus far adduced for invagination is, to say the least, inconclusive. Let us next examine that brought forward

<sup>1</sup> Houssay, taking his cue from Nuel ('81), believes in the subsequent regeneration of these reduced yolk granules! "Il y a plus. Ces cellules de la paroi dorsale, qui avaient d'abord une apparence identiques aux cellules vitellines, puis qui ont acquis une forme voisine de celle des éléments épiblastiques, vont de nouveau perdre cet aspect, acquérir de nouveau une grande taille et de gros granules, en passant de l'état d'activité qui préside à la formation de l'intestin, à l'état de repos où elles demeurent tant que dure la formation du système nerveux" (p. 170). Houssay seems to ignore the grave physiological difficulties that lie in the way of accepting such an independent existence for yolk granules; and it is certainly difficult to appreciate the precise advantage to the embryo of removing nutritive material from one portion of the developing egg to deposit it in the form of large yolk granules in the cells of the dorsal wall! It is hardly necessary to suggest the fallacy involved in comparing sections of different stages, especially in such a respect as the size of yolk granules, and assuming continuity of development.

in support of the splitting hypothesis. This evidence is, unfortunately, almost wholly of a negative character, and concerns itself with showing that the appearances observed in sections do not necessarily indicate invagination, but tally equally well — or, as is believed, better — with the hypothesis of delamination. The direct evidence for delamination seems to me singularly inadequate. I confess that it is difficult for me to understand why so much stress should be laid by the advocates of delamination upon the fact of the equality of cells, yolk granules, and pigment at the first origin of the archenteric cleft. While this fact does perhaps leave open the possibility of a subsequent differentiation of these elements *in situ*, it in no way indicates the probability of such an occurrence. It might be urged by the upholders of invagination, with perfect justice, that, although the first step in the formation of the archenteric cleft may be a split between the yolk-cells, there is no reason why one should not suppose invagination immediately to follow. There is not a shred of evidence to show that the large cells at first surrounding the mouth of the blastopore are not subsequently pushed in by the ingrowth of ectoblast cells. No positive evidence whatever exists to prove either the impossibility of invagination or the likelihood of no-invagination. I find it difficult to gather the reasons that have influenced Houssay, and Robinson and Assheton to adopt the view that invagination does not occur. Even if some of the usual arguments for invagination have no weight and have been carelessly advanced, that fact would hardly prove that the process of invagination did not occur.

I must here advert to the elementary fact that there are really two processes of “invagination” concerning which it is important to distinguish clearly. “Invagination” may mean either that the upper small (ectoblast) cells grow down over the large cells of the yolk-plug, or that the cells around the rim of the blastopore are turned in and rolled under to form the wall of the archenteron. Now either one of these invaginative processes may occur alone, or both may occur together. If I understand Robinson and Assheton correctly they maintain that the small cells neither grow down nor fold in (*Rana*).

“For during the formation of the blastopore the epiblast does not grow over the yolk-cells, enclosing them by a process of epibolic invagination” (p. 461). While I think there is good reason for holding that the process of ectoblast formation is not one of simple overgrowth, but is modified by the advancing differentiation of cells *in situ*, I do not see any reason for the wholesale denial of such overgrowth. There can be no question that the roof of the segmentation cavity contains much more material in the early stages than in the later (*cf.* Figs. 46–48). The disappearance of such a mass of substance may be best accounted for by supposing that cell proliferation has carried it further towards the lower pole. In fact, as I shall state presently, there is conclusive evidence that such overgrowth does occur. Robinson and Assheton, furthermore, fail to see that denial of this epibolic invagination involves them in serious difficulties when they attempt to explain the closure of the blastopore.

I have fortunately succeeded in obtaining decisive ocular evidence that the small cells around the lips of the blastopore are actually infolded. With the aid of the underlying plane mirror previously mentioned, I have repeatedly watched in the living egg the slow rolling in of the small epiblast cells. This I have seen in the newt and in *Rana palustris*, and, through the kindness of Mr. A. C. Eycleshymer, I have repeated his observations upon *Amblystoma punctatum*, where the large size of the cells renders the demonstration peculiarly convincing. The small cells roll down over the others (epibolic invagination), and at the same time the cells around the edge of the blastopore turn in and disappear from view (embolic invagination?). It is obvious that the appearance of embolic invagination may be in part delusive. The small cells at the rim of the blastopore may simply remain stationary, while the other cells grow down over them. In other words, it may not be wholly cell proliferation that forms the “invaginated hypoblast,” but a simple modification of the process of epibole. In the one case, the “invaginated hypoblast” is active, in the other, passive. I do not at present see how it is possible to differentiate sharply the two processes, and distinguish between

the parts played by each. Epibole and embole certainly run into each other at the lip of the blastopore, and I see no reason for supposing that the process of cell proliferation, which is so active in the exterior cells, ceases when the cells have been engulfed by the epibolic overgrowth. Of the existence of the latter process there can be no question. Two hours spent in watching the marginal blastoporic cells in the living amphibian egg will enable any one to determine this to his complete satisfaction.

#### VI. FATE OF THE BLASTOPORE.

It is with some diffidence that I take up the question of the fate of the blastopore, since for a long time the literature on the amphibian blastopore has been increasing in quantity if not in cogency. It would be a superfluous task to review *in extenso* the far from concurrent opinions, since an excellent summary of the literature has been quite recently given by Erlanger ('89), and by Robinson and Assheton ('91), to mention only two of the most recent.<sup>1</sup> On one point only is there practical agreement among all the later investigators, namely that the amphibian anus is either a remnant of the blastopore opening, or a breaking through in that region of the embryo at one time occupied by the blastopore. Whether it is in all cases a persisting portion of the original blastopore, or is in some cases a new opening of the old blastopore raphe, is, however, a much disputed question.

It seems necessary for the sake of clearness, if for no other reason, to distinguish between the Anuran and Urodelan blastopore. There seems good cause to suspect that the phenomena attending the closure of the blastopore differ in the two groups; at any rate, nothing is to be gained by a hostile criticism of the results obtained from the study of one group upon the basis of results obtained from the study of the other.

<sup>1</sup> It seems a curious oversight on the part of Robinson and Assheton that they make no mention either of Hertwig's papers (*Jenaische Zeitschr. f. Naturwiss.*, 1882 and 1883), or of Schanz's (*Jenaische Zeitschr.*, 1887), since these papers must be regarded as among the most important contributions to the literature of the blastopore (cf. Erlanger, '91).

The anus in the Anura is apparently, in some forms at least, a new formation in the region of the primitive blastopore. Although my own observations have thus far been exclusively upon the newt, and my belief has consequently the weight only of an *obiter dictum*, I think the careful observations of Schanz ('87), Erlanger ('91), and Robinson and Assheton ('91), are deserving of acceptance. Even if we admit the possibility that the fate of the blastopore is different in other members of the same group, I see no way of escape from the facts adduced by these authors in the specific instances which they consider. It is possible, nay probable, that variations even among individuals of the same species may account for the discordant results of other investigators. Viewed in the light of Erlanger's suggestion ('89, p. 251), that the Anuran anus should be regarded as a secondary formation, I see no reason why we should not expect occasional and perhaps frequent reversion to the more primitive condition. I may here suggest that the interesting observations of Ziegler ('92) on the living egg of *Rana* may perhaps be referable to some such explanation. Ziegler's observations, moreover, although highly interesting and important, afford no conclusive proof of the actual persistence of a portion of the blastopore opening. There is nothing in his figures that proves that the depression pictured communicates at all stages with the archenteron. That, I take it, could be demonstrated only by sections, though it is a supposition which the surface indications render plausible.

In the case of the Urodela there seems to be greater unanimity of opinion. The work of Miss Johnson ('84), Schanz ('87) and Morgan ('89) have practically established the fact that the Urodelan anus is a remnant of the blastopore which has remained open from the first. I shall show presently that my own observations on *Dicmyctylus* support this view.

This main fact, however, admits of many variations of opinion as to essential details. What portion of the blastopore is it that persists as the anus? What are the relations of the so-called neuropore to the blastopore and to the anus? How is the primitive streak related to the blastopore? What is the method of closure of the blastopore? It is clear that

the answers to these questions involve large theoretical considerations.

In my description of the closure of the blastopore in the newt, I shall, in this section, confine myself chiefly to a discussion of the questions already advanced and shall defer to a later section all extended reference to the origin and development of the germ-layers.

*Method of closure of the blastopore.* — There are several conceivable modes by which the blastopore might close. It might close either (1) by a convergence of all the marginal cells towards a central point, or (2) by a sort of peristaltic wave of approximation of the lateral cells from behind forwards or from before backwards, or (3) by a similar growth of the cells dorsally or ventrally, or (4) by a combination of any or all of these processes. Surface views afford most valuable evidence on this point, and for these I have learned to rely chiefly on the study of the living egg, since prolonged study of preserved specimens has served to convince me of the confusing nature of the evidence derived from comparison of different eggs in different stages.

The process in the living egg, as I have observed it in the newt, is usually as follows. When the circle of the blastopore is completed (Fig. 36) there ensues an approximately uniform convergence of cells from all sides. This convergence at first takes place at a nearly uniform rate, as shown by observation of the cell movement at different parts of the blastopore rim. It continues for a very varying length of time, but seems in all cases to be the first step in the dwindling of the blastopore. This method of closure by centripetal convergence of the marginal cells is substantially the method witnessed in some Annelids (*Nereis*, *Rhynchelmis*). In the newt, however, such uniform convergence does not continue to take place up to the end. Before the blastopore is obliterated, and usually while its diameter is still considerable, the cells at the ends of a line drawn through the blastopore parallel with the mid-axis of the embryo lag behind the cells at the sides. These latter press rapidly in towards the middle, and the result is the formation first of an oval (Fig. 38), then of a longitudinal slit (Fig. 40). This movement brings it to pass that the yolk-plug is covered

first at the ends of the mid-line and remains exposed longest in the middle. (Fig. 38.)

There are, however, important variations in the process above outlined. Such a one is depicted in Fig. 37. Here, it will be observed, the closure has been chiefly from behind forwards as indicated by the faint groove running off posteriorly. Sections through the region of this groove show a fusion of the layers, and I have found by the reconstruction of embryos in this stage that this fusion of layers is of greater extent than the fusion at either the anterior or the lateral lips. This condition is comparable with that described by Robinson and Assheton ('91) in the eggs of *Rana temporaria*. In the newt the posterior groove seems to be more clearly marked than in *Rana* and the visible remnant of the yolk-plug is oval rather than circular in outline. I agree with these authors in believing that in eggs showing such a structure there has been a coalescence of the posterior lips of the blastopore, but I cannot regard this as illustrating the "typical" method of closure for the blastopore of the newt. It must, I think, be considered rather as the usual preliminary to a stage similar to that shown in Fig. 38, though I have unfortunately been unable to determine this point in the living egg. I am certain that in the newt the whole blastopore rarely, if ever, closes by the progressive coalescence of the posterior lips alone, although I admit that such posterior coalescence does sometimes anticipate to a greater or less degree the movement of the anterior cells. The simultaneous approximation of the lateral cells *both anteriorly and posteriorly*, as shown in Fig. 38, is the more usual occurrence in the newt.

Hertwig ('82, Pl. XII, Figs. 3 and 4) has figured stages in the closure of the blastopore of *Triton taeniatus* that agree very closely with what I have observed in *Diemyctylus*. Fig. 4 (Hertwig, '82) shows the lines of closure stretching off both in front of and behind an oval yolk-plug. I infer that this must indicate the same general method of antero-posterior closure which occurs in *Diemyctylus*. Morgan ('89, Pl. XLII, Fig. A) also has figured a similar stage for *Amblystoma*, but attributes the appearance (p. 356) to the elongation of a circular blasto-

pore. I am unable to convince myself that in the newt there is anything more than a continuation of the process of shrinkage.<sup>1</sup>

Another not uncommon condition is illustrated in Fig. 39. This is somewhat older than the stage shown in Fig. 38 and is marked by the still circular outline of the very small blastopore. The lines running off behind and in front indicate the seams of closure.

The closure of the blastopore in the newt, then, may be considered to take place in the following manner. There is first a convergence at a uniform rate of cells from all parts of the margin. This process becomes gradually modified, sometimes very early in the blastopore history, sometimes very late, by the more rapid movement of the cells on either side of the axial mid-line. At this point considerable variations arise. (1) The movement of the posterior cells may anticipate that of the anterior, in which case the blastopore "closes from behind forwards." This occurrence I believe to be quite frequent in the newt. About ten per cent. of the preserved eggs that I have examined seem to be closing in this manner. The point of great interest in these cases, and one which my study of the living egg unfortunately leaves unsettled, is whether a closing also "from before backwards" may not immediately succeed the stage figured (Fig. 37). It is worthy of note in this connection that the blastopore in eggs of the stage showing only a posterior groove is usually of about the same size. When the eggs are in the stage in which the blastopore opening is nearly obliterated there are, I think invariably, lines running in both directions (Figs. 38 and 39). (2) There is the possibility that the movement of the anterior cells may anticipate that of the posterior. This, if it happens at all in the newt, is not common. That it occurs occasionally is, I think, probable. Only in this way can I explain certain puzzling cases in which such a line as shown in Fig. 57 is seen running off *anteriorly*. (3) The movement of the anterior and posterior cells may be approximately synchronous, in which case the blastopore may be

<sup>1</sup> Dr. Morgan has since informed me that he did not intend to imply actual elongation, but simply the narrowing to an oval of the earlier circular outline.

considered as "closing" in both directions. This I think is what usually happens in the newt.

The lateral cells continue to press in towards the mid-line until the blastopore exists only in the form of a longitudinal slit. This at first opens into the archenteron along its whole length, but soon the ingrowing lateral cells meet along the middle of the slit, leaving a small circular orifice at each end. The anterior of these openings is the evanescent neuropore; the posterior remains open as the permanent anus (Fig. 42).

It is evident from the facts above adduced that very great significance cannot be attached to the manner of closure of the blastopore. The work of other observers points to the same conclusion. The blastopore in amphibia, and in other vertebrates also, has been described as closing both from behind forwards and from before backwards, as well as in both directions simultaneously. The closing of the Urodelan blastopore in the middle has been observed by Schanz ('87) and Morgan ('89), and is a constant occurrence in the newt. Whether this mesial coalescence is in all cases preceded by a coalescence at either end of the blastopore varying in character and extent, or whether the mesial coalescence sometimes steps in directly upon the cessation of the uniform convergence of the marginal cells seems to me an open question. I am convinced that in the newt, at least, occasional variations cover several of the possibilities of antero-posterior coalescence. The mesial approximation of the lateral lips, resulting first in the formation of a longitudinal slit and then of an anterior neuropore and a posterior anus seems invariably to follow the diversity of the earlier conditions.

Variation in the manner of blastopore closure in the same group of animals, or even in the same species, is not uncommon. E. B. Wilson ('89) observed in *Lumbricus* "a considerable variation in the closure of the blastopore, owing to differences in the rate of folding between the sides and the posterior margin of the blastopore. As a rule, the sides fold in more rapidly than the hinder lip, thus giving rise to a slit-like blastopore, but in some cases the reverse is true, so that the blastopore never appears as a slit, but always as a rounded opening"

(p. 400). In *Lumbricus* the blastopore closes from behind forwards, in *Rhynchelmis* and *Clepsine* from before backwards.

To what, then, shall we attribute the variations in the closure of the blastopore? Evidently they can be due to no phylogenetic necessity, but rather to slight, unimportant individual differences in the rapidity of cell proliferation and movement at different parts of the blastopore rim, and may perhaps in the final analysis rest upon trivial mechanical causes.

One important fact, however, is brought out into sharp relief by the work of all investigators of the amphibian blastopore, namely, that the blastopore is closed at least in part *by closing in of the lateral lips along a median line*. I cannot regard it as an objection against the theory of concrescence of the vertebrate embryo that the blastopore does not always close by retrogressive fusion of its lips. On the contrary, I think variations such as we have noticed are distinctly the outcome of secondary and fluctuating conditions. Whether the blastopore closes "from behind forwards" or "from before backwards," or in both directions, does not appear to have great significance, and does not in the least affect the salient fact of coalescence of the lateral lips along a mid-line.

*Neuropore and Anus*.—It is evident, from what I have already stated, that the relative position of neuropore and anus cannot be constant. I am thoroughly convinced, after a careful examination of many preserved eggs, and after observation of the blastopore closure in the living egg, that the anus may lie in almost any portion of the original mid-line through the blastopore. Fig. 42 shows the greatest separation of neuropore and anus that I have ever observed. The two openings usually lie much closer together, at a distance represented by about one-third of the interval shown in Fig. 42. The examination of preserved specimens inclined me at first to believe that the neuropore was pushed back from some such extreme initial position as that represented in the figure, but study of the living egg showed me that such is probably not the case, and that the gradations I observed were individual variations and not consecutive stages. The neuropore and anus, then, have their position, relative and

absolute, determined by the extent of antero-posterior coalescence. The longest space that I have observed between the two openings is represented in Fig. 42, and it must be noticed that this extreme case affords no support to the view that there is an elongation of a circular blastopore. In such an egg as that shown in Fig. 39 the other extreme is indicated; in this instance the distance separating neuropore and anus would obviously be slight.

The neuropore is quite transitory, remaining open but a few hours, and closing long before the meeting of the medullary folds. The position of the neuropore is more constant than that of the anus; it always lies near the anterior end of the blastopore, and the fused area in front of the neuropore is, in all specimens I have examined, less than the fused area behind the anus. Fig. 63 represents a horizontal section through Fig. 40, in which the blastopore has narrowed to a longitudinal slit. Fig. 62 shows a sagittal section through a stage similar to that shown in Fig. 42. The neuropore lies dorsally (anteriorly) and the anus below, and between these is shown the mass of ingrowing lateral cells which have just met in the mid-line. Regarding the significance of this strangely persistent ancestral structure, conjecture is at present idle. We are not even, I think, able to determine whether this anterior portion of the blastopore primitively opened freely to the exterior as the neuropore, and has been only secondarily caught in as the "neurenteric canal" by the precocious closing of the medullary folds, or whether the reverse is true.

The posterior opening remains as the permanent anus. The backward extension of the medullary folds brings the anus to lie just behind them (Fig. 43). The external appearances do not differ from those pictured by Ziegler ('92) for the frog, while sections show that this opening in the newt maintains its integrity throughout, and eventually functions as the anus. The sections figured by Miss Johnson ('84) adequately represent in this respect the conditions I have found in the later stages of the newt.

*Primitive Streak.*—The term primitive streak, originally applied to a certain darkened region in the posterior part of

the area pellucida of the chick blastoderm, has since come to obtain a wider significance. Homologous areas have been alleged to exist in the early stages of other vertebrates, and some of these cases have been looked upon as throwing light on the meaning of this primitive and persistent structure. The macroscopic appearance of a "streak" is simply the outward expression of an inner thickening of the embryonic area, *due to the fusion of the three primary germ-layers*. This fusion must be regarded as the most characteristic mark of the primitive streak and can evidently be discovered only from sections, so that observations of a "primitive streak" based on surface views alone are of doubtful value. Taking as a criterion of the primitive streak, then, the fusion of germ-layers, what is the hidden significance of this early union of the three layers in a linear mass or streak, *the long axis of which invariably coincides with the long axis of the embryo?*

It is a fact of no small importance that the rim of the blastopore is the region where the fusion of layers first makes its appearance in many vertebrate embryos. This fact alone tempts one to believe that the primitive streak is the result of the fusion of the lips of the blastopore in the mid-line. The comparison of the line of fusion of the lips of the Elasmobranch blastopore with the primitive streak of the Amniota was made long ago by Balfour, who, however, expressly disclaimed any implication of homology.

Assuming that in the newt the primitive streak is the linear mass of fused germ-layers formed by the bringing together of the blastopore lips, let us determine its extent and character without, for the instant, any reference to possible homologies. Figs. 52 and 55 represent median sagittal sections through early blastopore stages. The only region of fusion is that immediately around the yolk-plug. This fused area is seen in these sections to be slightly greater at the posterior (ventral) lip, but this fact does not come out so clearly as in reconstructions. The lateral lips of the blastopore begin to approximate shortly after the stage shown in Fig. 55, and a horizontal section through Fig. 40 in the line 63-63 (Fig. 63) shows the lips almost touching. A median sagittal section through the next

stage (Fig. 42) affords us a good view of the primitive streak at its period of maximum extension (Fig. 62,  $x$ ,  $s$ ). The lateral lips have just met in the mid-line, and the fused area (Figs. 42 and 62) reaches from a point in front of the neuropore ( $x$ ) to a point some distance behind the anus ( $s$ ), the only breaks in its continuity being the small perforations of neuropore and anus. If we adhere to our determination to designate the whole extent of fused area as primitive streak, then there is obviously a portion of the streak that lies in front of the neuropore and another portion that lies behind the anus. There is, however, an important consideration that tends to qualify the acceptance of such a view. This is the question of the thickness of the fused blastopore rim, — the germ-ring. For it is obvious that a portion of this fused mass will in any event lie in front of the neuropore, and, unless it exceeds in extent the thickness of the original germ-ring at this point, cannot be strictly regarded as formed by fusion of the lateral lips. Now comparison of Fig. 62 with Figs. 52 and 55 shows that this is actually the case, that the fused area in front of the neuropore is no greater than the thickness of the germ-ring, and that therefore the neuropore in this instance probably lies at the anterior end of the blastopore. If we apply the same test to the fused area behind the anus (*cf.* the lower lip in Fig. 62 with Figs. 52 and 55) we discover that the thickness is considerably greater than the thickness of the original blastopore rim, and we must therefore conclude that the anus does not lie at the posterior end of the original blastopore, but at some distance in advance of it. These facts come out still more clearly on comparison of the thickness of the lateral lips (Fig. 63) with that of the anterior and posterior lips (Fig. 62). The primitive streak, then, is that fused mass formed by the median meeting of the lateral lips plus the thickness of the anterior and posterior lips of the germ-ring.<sup>1</sup>

The early fusion of layers, both anteriorly and posteriorly,

<sup>1</sup> In making this statement I do not wish to be understood as implying a constant thickness for the germ-ring from the moment of its first inception. On the contrary I believe that cell proliferation usually modifies the original thickness, although to a comparatively slight extent.

is shown in Figs. 49-51. Here the primitive groove is deep, and is interrupted in the middle by the still exposed yolk-plug. Fig. 58 is a horizontal section through a stage somewhat older than that shown in Fig. 42, and represents the primitive streak and primitive groove. This condition results, it is evident, from the fusion of the lateral lips depicted in Fig. 63. A still later aspect of the primitive streak is represented in Fig. 65. This is a section through the hind end of an egg slightly younger than that shown in Fig. 44, and shows the somewhat dorsal position of the streak at this time. The neuropore has by this time vanished, and the primitive streak in consequence extends with unbroken continuity in front of the anus. The section figured is the twelfth in front of the anus, and there are ten sections more before the anterior limit of the primitive streak is reached. Fig. 59 is the eighteenth section anterior to Fig. 65, and Fig. 53 is the thirty-seventh anterior to Fig. 59.

The primitive streak and groove as they appear in surface views are shown in Fig. 42 as the dark line between the neuropore and anus. Later these surface features cannot be detected; the groove is completely obliterated, but sections reveal to us the presence of a primitive streak running both backwards and forwards from the anus (Fig. 65).

It is this primitive streak in front of the *anus* which was looked upon by Miss Johnson ('84), so far as I can gather from her figures, as lying in front of the *blastopore*. The two statements carry quite a different signification, as will be evident to those who have followed my description. I am unable, however, to account for the great forward extension of the primitive streak represented in some of Miss Johnson's figures. In *Dicmyctylus* the fusion of layers by no means reaches so far forward as "the middle of the embryo." No more can I reconcile my observations with those of O. Schultze ('88) on *Rana fusca*. Schultze depicts a primitive streak which "von der dorsalen Urmundlippe sich nach dem Kopfe hin allmählich ausdehnt, und wächst also der Primitivstreif auch bei den Amphibien von hinten nach vorn." (p. 330.) However it may be in these animals, in *Dicmyctylus*, I am confident that

there is no such forward extension of the fused mass of layers.

The length of the primitive streak in *Dicmyctylus* never exceeds or indeed equals the diameter of the original blastopore. This, too, is despite the probable additions to the fused area due to cell proliferation. I attribute the differences in the length of the primitive streak of *Dicmyctylus* to the variations in the amount of centripetal convergence of the marginal cells. Early meeting of the lateral lips will produce a long primitive streak, late meeting a short one.

Surface views, as I have already stated, do not show in the newt any trace of a true primitive streak in front of the blastopore. There is, however, a wide, shallow groove on the dorsal aspect of the embryo ("Rückenrinne," "sillon médian") which might perhaps be taken for the primitive groove. There is no fusion of layers in this region (Fig. 54, *n.g.*), and since I must persist in regarding the fusion of layers as the criterion *par excellence* of the true primitive streak and groove, I must consider this second groove as at least in some degree an independent structure. I shall designate it accordingly as the neural groove (Figs. 40-42, *n.g.*). The neural groove at its first appearance is not connected with the primitive groove which lies posterior to it, but is in the early stages separated from it by a ridge of cells (Figs. 40-42). Later on the neural groove extends backward to the primitive groove so as to appear continuous with it. The neural groove appears in sections as a shallow depression of the ectoblast, the layers beneath not being involved (Figs. 53-54). Since the formation of the notochord takes place at about this period it seems possible that the uplifting of the notochord cells may have something to do with the external appearance of a "streak" which is sometimes described. More often only the "groove" is apparent externally.

I may perhaps be allowed to suggest that the depression of the ectoblast may in itself account for the "fusion" described by Schultze ('88). It may possibly have come about that the sinking in of the neural groove ("*pr.*" in Schultze's figures 5, 6, 7) changes the earlier condition of separate layers (Schultze's

figures 1, 2, 3) into a stage of apparent fusion. I put this forward, however, only as a mere suggestion to be tested by future observation of Anuran embryos of this stage. Schultze's figures do not convince me that the "fusion" along the median dorsal line in *Rana* is in any way the equivalent of the primitive streak in *Dicmyctylus*. (Cf. e.g. Figs. 58 and 65 with Schultze's Figs. 5, 6, 7.)

The neural groove, which is always quite shallow along most of its extent, becomes, soon after its appearance, much deeper in the extreme anterior portion. This deepening is evidently the "pit" mentioned by Miss Johnson ('84, p. 662). There is, as will be seen in Fig. 64, an apparent fusion of layers at the base of this pit. I hesitate, however, to attribute any special significance to this fusion since it is isolated and limited in extent, and since I am convinced that the appearance of fusion is due chiefly to the dipping in of the superficial ectoblast.

The meaning of the neural groove is, I confess, very puzzling. I am inclined to think the groove may be caused by the mechanical stresses that attend the development of the neural folds, but am poorly satisfied with so nebulous an explanation. I am compelled, nevertheless, to accept some such interpretation rather than any that would relate it to the primitive groove, from which I believe it to differ utterly in origin, structure and significance. It will be seen, then, that I regard the primitive streak of Amphibia as formed by fusion of the blastodermic layers, and that this fusion is given a linear direction by the coming together of the lateral blastopore lips. In this respect I agree with Schwarz ('84), Goette,<sup>1</sup> and Robinson and Assheton ('91). I agree also with these last-named authors that there is no reason for not including the ventral lip of the anus within the primitive streak. The whole germ-ring, the fused rim of the blastopore, is responsible for the formation of the primitive streak, and the latter structure is simply the result of the fusion of the blastopore lips along the axial

<sup>1</sup> I have not been so fortunate as to see Goette's paper (Abhandlungen zur Entwicklungsgeschichte der Tiere, V. Heft, Leipzig, 1890) and know it only by abstracts.

line. Any one portion of this fused area therefore is just as much entitled to be considered primitive streak as any other.

I must dissent, however, from the singular conclusion of Robinson and Assheton that the primitive streak of *Bombinator*, *Triton* and *Petromyzon* is not homologous with the primitive streak of *Rana temporaria*. I do not think that variations in direction of formation or in the relative position of the anus are sufficient reasons for the rejection of the homology of this fused linear mass of germ layers. As I have endeavored to show, the method of closure of the blastopore and the position of the anus are not constant, even in the same group of animals, and hence cannot be looked upon as dependent on purely primary causes.

I consider, therefore, that the primitive streak, wherever found, is the expression of the fusion of the blastopore lips. (Prostomanant). That in different groups of animals the primitive streak should vary widely in character and extent is not surprising.

## VII. GERM-LAYERS AND NOTOCHORD.

Since I believe that the prevailing uncertainty regarding the origin and extent of the germ-layers in amphibia can be successfully met only by a comparative study, I shall not attempt at this time any extensive discussion of my results upon the newt. It does not seem to me likely that the events in a single animal, however illuminating in themselves, can be regarded as "characteristic" or "typical" of the whole group. For this reason I shall for the present simply describe the early development of the germ-layers in the newt and shall draw none but the most obvious inferences from my work.

The ectoblast in the newt is derived chiefly from the small cells of the upper pole, although cells split off from the upper end of the yolk-cells are at first constantly added to these. There is always a certain area of yolk-cells—the yolk-plug—which, although originally superficial, is ultimately covered in by the downward growth of small ectoblast cells. I have else-

where (pp. 333, 334) indicated my reasons for stating that this epibolic invagination occurs.

The roof of the segmentation cavity is for a long time only one cell thick. About the time invagination begins, the nuclei of the roof cells assume an alternate arrangement like that described by Scott and Osborn ('79), and the formation of a double-layered ectoblast proceeds in the way they describe. This doubling is undoubtedly in part due to delamination, and in part also, as Scott and Osborn suggest, to the alternation of wedge-shaped cells which draw in their edges and come to lie one row above the other (Fig. 54). Sometimes the single layer of ectoblast cells persists to quite a late period (Fig. 55). Whatever the significance of this persistent condition of single-layered ectoblast there is no question that it is radically different from the several-layered condition seen in Anuran embryos of this stage. (See *e.g.* Schultze's figures of *Rana fusca*, '88.)

The thinning out of the ectoblastic roof of the segmentation cavity and the concomitant thickening near the rim of the blastopore are, I think, genetically related to the corresponding phenomena attending the formation of the germ-ring in Teleosts.

The entoblast in the newt has in a sense a double origin. It comes in part from invagination of the primitive ectoblast cells and in part from the yolk-cells. Along the dorsal wall of the archenteron it is impossible to distinguish the cells derived from one source from those derived from the other. In such an egg as that shown in section in Fig. 55 we can safely assert only that the most anterior entoblastic cells have been differentiated *in situ* and that the most posterior cells have been invaginated. Between these two extremes there is no cell that we can point to and surely identify as the foremost invaginate cell. The cells derived from the two sources grade so insensibly into each other that the transition point cannot be detected. It does not seem likely that at the early stage shown in Fig. 55 the invaginate cells reach as far forward as the middle of the embryo, but such a belief must obviously be grounded on other considerations than those afforded by sections alone. The ventral and a considerable part of the lateral walls of the archenteron are formed from the yolk-cells (*cf.* Figs. 55, 56,

62, etc.). The entoblast cells which line the archenteron come in later stages to assume a columnar form (Figs. 57, 62).

I have not been able to discover a stage where I could distinguish only two germ-layers. At the time of beginning invagination there is always an indifferent fused mass of cells around the blastopore rim. It is from this indifferent region that the mesoblast is first formed. The line between ectoblast and entoblast at this point must always be an arbitrary one, since individual cells, which at one time lie externally, are a few hours later rolled under the edge and thus converted into entoblast. Whether we shall regard the mesoblast in this region as coming from the inner or outer germ-layer is largely a matter for personal predilection to determine. It is in a sense true that the mesoblast comes from both layers since it unquestionably develops in just that indifferent, indeterminate region where ectoblast is being continually transformed into entoblast by the rolling under of the outer cells. It has on the whole, however, a much more intimate relation with the invaginated cells than with the cells just on the outer edge.

The mesoblast grows forward in this way from the blastopore rim and has, even in very early stages, a greater forward extension dorsally and ventrally than on the sides. Precisely how much of this is due to forward proliferation and how much to differentiation *in situ* I have not been able to determine. Except for the comparatively limited fused area immediately in front of the blastopore, the mesoblast is deficient along the median dorsal line (Figs. 54, 55, 59). In the immediate neighborhood of the dorsal line and at a somewhat variable distance in front of the region of fusion the mesoblast comes into close and unmistakable relations with the entoblast. The mesoblast exists here in the form of small, scattered cells which can in some cases be sharply distinguished from the entoblast cells, and in other cases run into them by imperceptible gradations. Fig. 59 shows one of these localities where mesoblast and entoblast are differentiating from the same mass of cells. This conversion of yolk-cells into mesoblast appears to take place first in the hinder part of the embryo and progresses anteriorly.

The process of mesoblast formation in the extreme anterior end of the embryo is of a particularly interesting nature; the way in which it here develops is represented in Fig. 64. Here the archenteron is in free communication with diverticula of mesoblast on either side. The mesentoblast in this locality is in a similar condition in all eggs of this stage that I have examined although it rarely presents such a strikingly pouched arrangement as is shown in the section I have figured. I have purposely illustrated the clearest case of mesoblastic diverticula that has come under my notice, but the same general arrangement of cells, although often masked and obscure, exists always in this region and almost always suggests diverticula from the archenteron. That such a connection of mesoblast and entoblast indicates an origin for the mesoblast of the newt similar to that typified by the serial gut-pouches of *Amphioxus* seems to me probable. Such a condition of the mesoblast as exists in the newt tends unmistakably to support Hertwig's well-known coelom theory. Fig. 59 shows a similar state of mesentoblast in the hinder part of the embryo although here the pouches are less pronounced. I have not been able to discover any very clear serial arrangement of these archenteric diverticula, but hesitate to say that such metameric arrangement does not exist. If present at all in the newt, however, it is decidedly obscure.

The mesoblast, then, appears first around the margin of the blastopore and is consequently in this region in close connection with the entoblast. This connection of mesoblast and entoblast is ruptured somewhat irregularly and is usually broken first near the middle of the embryo. Precisely as in the case of the entoblast I have not found it possible to distinguish between the mesoblast derived from invagination and that derived from the yolk-cells. This connection of mesoblast and entoblast is confined to a limited area on either side of the dorsal median line; laterally the two layers are not in contact. The mesoblast appears to originate chiefly, if not solely, in this locality (Figs. 59, 60), and to proliferate ventrally. In later stages it completely encircles the yolk in the hinder part of the embryo (Fig. 56), but more anteriorly (Fig. 57) the sheets of mesoblast are still separate and do not yet meet in the median ventral

line. This condition is probably due to the fact that the anterior mesoblast develops more tardily than the posterior, and is therefore really at an earlier stage in Fig. 57 than in Fig. 56.

The mesoblast in the newt has in this sense, then, a double origin. It arises like the entoblast in part from the blastopore rim, and in part from the yolk-cells along either side of the median dorsal line. Like the entoblast, too, the mesoblast from these two sources is separated by no well-marked line, but to all appearances is completely continuous. It is, from the outset, however, separated by the chorda-entoblast along the median dorsal line into two lateral sheets, and consequently may be said to have along the greater part of its course a paired origin.

The origin of both entoblast and mesoblast, therefore, points to a fundamental unity of origin for both layers, and indicates that the distinction of mesoblast as "gastral" or "peristomal," although justified perhaps by considerations of convenience, is not grounded on an essential difference. Instead of regarding the mesoblast as having in reality a "double origin," I think we may suppose that the splitting off from the entoblast of the lateral mesoblastic leaves is simply a continuation into the embryo, as it were, of the process of invagination. It is possible that the two modes of mesoblast formation were originally alike and that differentiation has stepped in only secondarily. The continuity of entoblast and mesoblast is certainly a point in favor of this view, as is also the progressive formation of mesoblast from behind forwards. It is also significant that the mesoblastic diverticula are, as in *Amphioxus*, most pronounced in the anterior part of the embryo (Fig. 64).

*Notochord.*—The notochord in the newt is, along most of its length, exclusively entoblastic. The early stages of the notochord near the middle of the embryo are shown in Figs. 53, 54. The cells of the median dorsal wall of the archenteron assume a somewhat columnar form, and are gradually pushed up and pinched off until they are completely separated from the entoblast and come to lie above it in the mid-line. The gap left in the wall of the archenteron is soon closed up by entoblast cells, and all trace of the line of origin of the notochord is thus obliterated. The size of the notochordal cells

soon diminishes, whether by division or by actual loss of substance I do not know; but the effect is to produce cells of about the same dimensions as the mesoblast cells that lie on either side and often come into contact with the notochordal cells (Figs. 56, 57). In many cases, and particularly in the more anterior and posterior portions of the embryo, the notochord begins to be formed while the connection of entoblast and mesoblast still persists in the immediate neighborhood. It thus comes about that both notochord and mesoblast take their origin simultaneously from the same mass of entoblastic cells, and the boundaries of the different elements are consequently hard to define (Figs. 59, 64). The notochord, like the mesoblast, becomes separated from the entoblast in the middle of the embryo while the connection is still intact in the more posterior portion (Figs. 60, 61). The small size of the mesoblastic cells, as compared with the notochordal cells in this region (Figs. 60, 61), renders impossible any confusion as to the origin of the notochord at this point.

The notochord is formed along its posterior portion to an unknown and probably varying extent by "invaginate entoblast," and in its more anterior portion by "yolk-entoblast." Posteriorly the chord runs into the fused mass of cells known as the primitive streak, and anteriorly into a body of cells poorly defined, but probably entoblastic (Fig. 64).

Since Schultze's ('88) observations were made upon *Rana fusca* and not upon a Urodelan amphibian, I do not feel called upon to criticise his results. I can merely remark that the large number of cells in the dorsal mid-line of *Rana* is quite different from the condition existing in the newt. It is possible that this large number of cells may tend to obscure somewhat the real course of events. I must also point out the fact that certain of Schultze's figures (as Fig. 12a) do not forbid the assumption that in *Rana*, as in *Dicmyctylus*, the entoblast plays an important part in the formation of the notochord. The fact that both notochord and mesoblast form simultaneously from the same region of entoblastic cells has also doubtless its share in producing delusive appearances.

August, 1892.

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## REFERENCE LETTERS.

<i>a.</i>	Anus.	<i>nd.</i>	Notochord.
<i>chr.</i>	Chromosomes.	<i>n. f.</i>	Neural folds.
<i>cl. sp.</i>	"Clear spot."	<i>n. g.</i>	Neural groove.
<i>dk.</i>	Yolk-nucleus.	<i>np.</i>	Neuropore.
<i>ec.</i>	Ectoblast.	<i>prg.</i>	Primitive groove.
<i>en.</i>	Entoblast.	<i>prs.</i>	Primitive streak.
<i>f.</i>	Female pronucleus.	<i>sp.</i>	Spermatozoon.
<i>g. v.</i>	Germinal vesicle.	<i>y.</i>	Yolk.
<i>m.</i>	Mesoblast.	<i>y. p.</i>	Yolk-plug.
<i>mes.</i>	Mesenteron.	<i>y. sph.</i>	Yolk spherules.
<i>n.</i>	Nucleoli.		



## PLATE XIV.

[Figs. 1-3 were drawn from nature by Mr. R. Takano.]

FIG. 1. Egg just after deposition; accidental break in membrane ( $\times 7$ ).

FIG. 2. Spermatophore just after discharge; gelatinous base with projecting spine which bears tuft of spermatozoa ( $\times 2$ ).

FIG. 3. Portion of upper pole of egg at time of deposition, showing "clear spot" ( $\times 48$ ) [*cf.* Fig. 21].

FIG. 4. Section through young egg; follicle just forming ( $\times 500$ ).

FIG. 5. Portion of section showing beginning yolk-formation ( $\times 1000$ ).

FIG. 6. Surface view of portion of the upper pole of living egg soon after deposition; *sp.*, spermatozoon entering egg in close proximity to the "clear spot," *cl. sp.* ( $\times 20$ ).

FIG. 7. Section through young egg showing yolk formation; vacuolation of cytoplasm in upper half of section ( $\times 90$ ).

FIG. 8. Egg membranes — *Necturus* ( $\times 500$ ).

FIG. 9. Egg membranes — *Diemyctylus* ( $\times 500$ ).

FIGS. 10-13. Sections through yolk-nuclei of different stages, *dk.* ( $\times 375$ ).

FIG. 14. Section showing germinal vesicle in young egg; egg, .31 mm. in diameter ( $\times 1000$ ).

[The outlines of Figs. 4-14 were drawn with the Zeiss camera.]



Fig 14.





## PLATE XV.

FIG. 15. Section of germinal vesicle; nucleoli migrating to the center and disintegrating ( $\times 500$ ).

FIG. 16. Section of germinal vesicle (*g. v.*); yolk-nucleus (*dk.*) in contact with it; peripheral nucleoli disintegrating. In the yolk-nucleus are three coarsely granular areas which stain more deeply than the rest of the Dotterkern ( $\times 375$ ).

FIG. 17. Section of germinal vesicle of *Rana palustris* ( $\times 225$ ).

FIG. 18. Maturation spindle from egg found in body-cavity. Stained with acid fuchsine. ( $\times 140$ ).

FIG. 19. Maturation spindle from egg found in oviduct ( $\times 140$ ).

FIG. 20. "Second polar body"; one and one-half hour after the egg was laid; several spermatozoa in the egg ( $\times 140$ ).

FIG. 21. Maturation spindle from egg found in body-cavity. Unstained preparation ( $\times 140$ ).

FIG. 22. Maturation spindle from an egg found in the upper part of the oviduct; the egg possessed a very thin enveloping membrane ( $\times 140$ ).

FIG. 23A. Union of male and female pronuclei in egg eight hours after laying. *m.*, male pronucleus (?); *f.*, female pronucleus (?) ( $\times 375$ ).

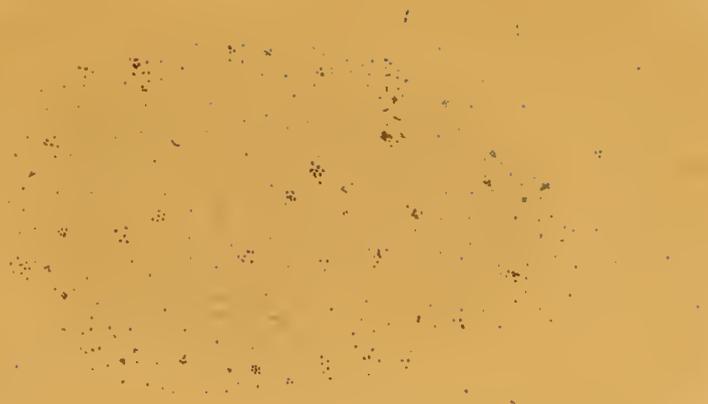
FIG. 23B. Section showing spermatozoon entering egg; two hours after laying. A spindle in the same stage as that in Fig. 20 is present in the egg ( $\times 140$ ).

FIG. 23C. Spermatozoon entering egg; two hours after laying. A spindle of the stage shown in Fig. 20 is present ( $\times 140$ ).

FIG. 23D. Spermatozoon in egg; four hours after laying ( $\times 140$ ).

[The outlines of all these figures were drawn with the camera.]

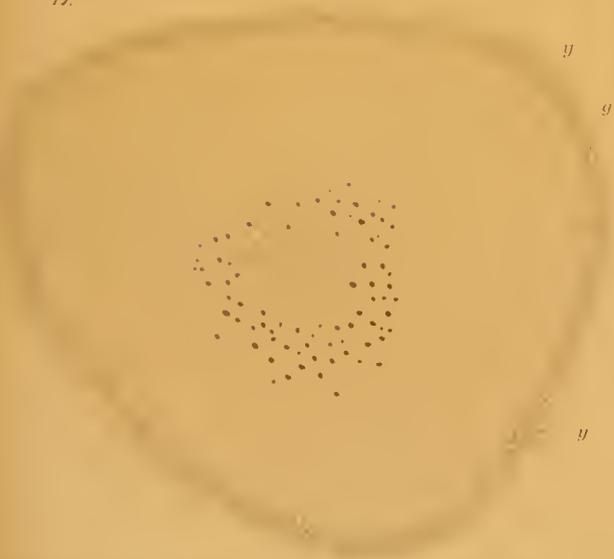
15



18



17



25C

y

gv

n

y

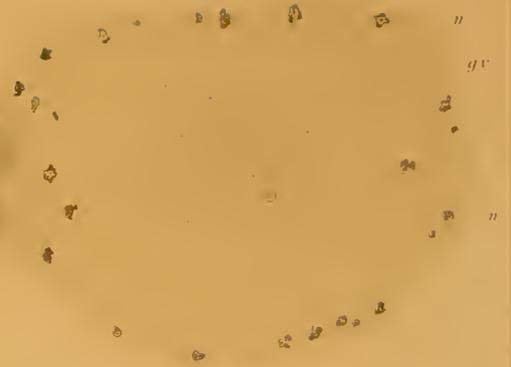
21



gv

dk

16



n

gv

n

25B



25A



f

n

22



19



20



25D







## PLATE XVI.

FIGS. 24-25. Views of upper pole of living egg; first two cleavage planes.

FIGS. 26A-26D. One egg seen from different points of view. 26A, upper pole; 26D, lower pole.

FIGS. 27A-27D. One egg from different points of view. 27A, upper pole; 27D, lower pole.

FIGS. 28A-28D. One egg from different points of view. 28A, upper pole; 28D, lower pole.

FIGS. 29A-29D. One egg from different points of view. 29A, upper pole; 29D, lower pole.

FIGS. 30A-30B. One egg from different points of view. 30A, upper pole; 30B, lower pole.

FIGS. 31A-31B. Upper (31A) and lower (31B) pole views of one egg.

FIGS. 32A-32B. Upper (32A) and lower (32B) pole views of one egg.

FIG. 33A. Upper pole of living egg, four hours and three-quarters after the appearance of first cleavage plane; 1-1, first plane; 2-2, second.

FIG. 33B. Same egg, ten minutes later than Fig. 33A.

FIG. 33C. Lower pole of same egg, one hour later than Fig. 33A.

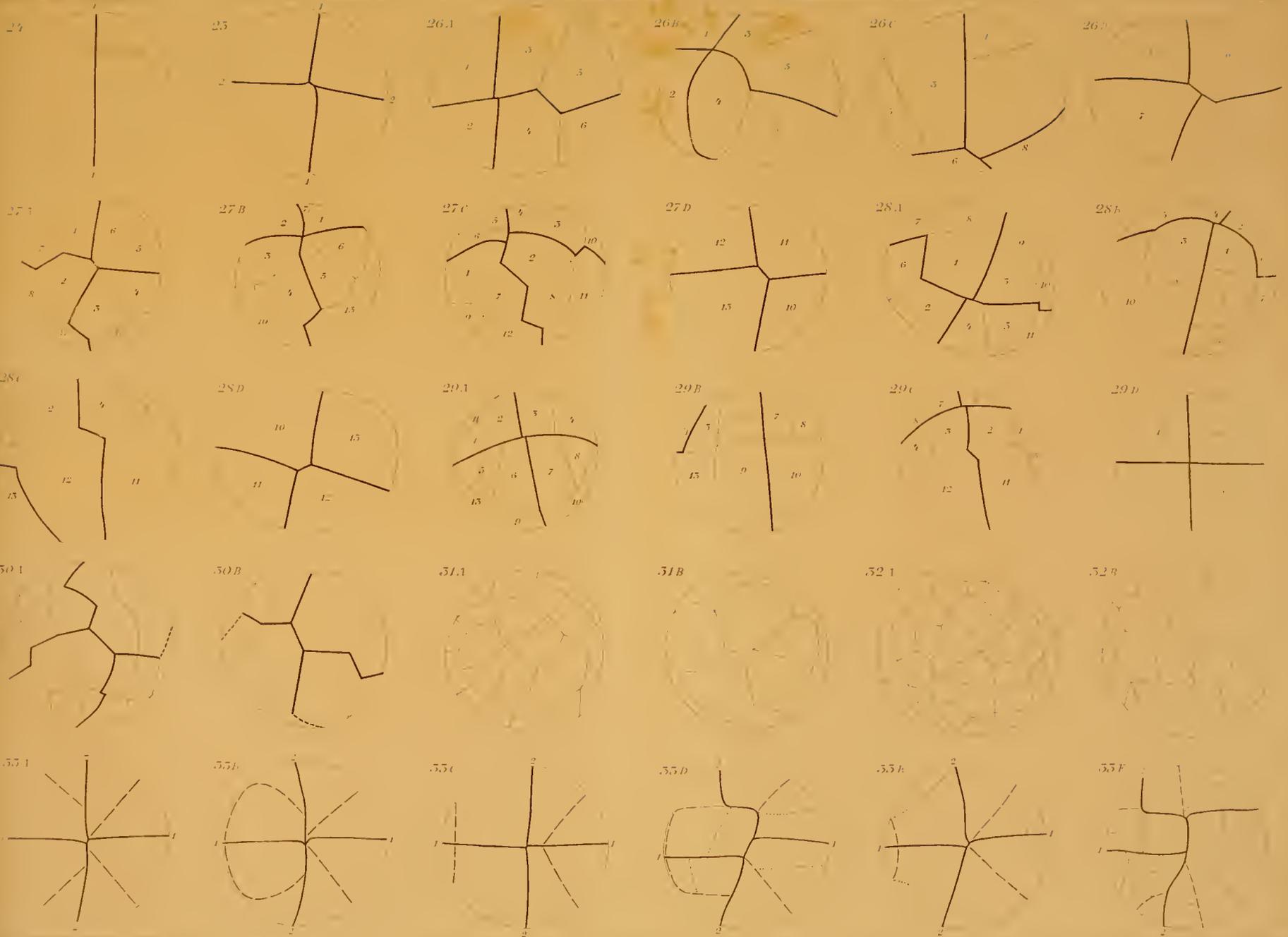
FIG. 33D. Upper pole of same egg, two hours later than Fig. 33A.

FIG. 33E. Lower pole of same egg, thirty minutes after Fig. 33D.

FIG. 33F. Upper pole of same egg, fifty minutes after Fig. 33D.

Figs. 26A-32B were drawn with the camera from preserved specimens. All  $\times 20$ . The heavy lines indicate the position of the first two cleavage planes.

Figs. 33A-33F were drawn freehand from the successive stages of a living egg. The first two cleavage planes are designated by heavy lines, the third set by long dashes, the fourth by short dashes, and the fifth by light full lines.







## PLATE XVII.

- FIG. 34. Surface view of living egg, showing depression of yolk-cells ( $\times 20$ ).
- FIG. 35. Surface view of preserved egg, showing semi-lune blastopore.
- FIG. 36. Surface view of preserved egg, showing unusually large circular blastopore, *yp.*, yolk-plug.
- FIG. 37. Surface view of living egg, showing blastopore closing "from behind forwards."
- FIG. 38. Surface view of preserved egg, showing blastopore "closing in both directions."
- FIG. 39. Surface view of living egg, in somewhat later stage than Fig. 38.
- FIG. 40. Surface view of preserved egg. The blastopore (*prg.*) — or primitive groove — is now a narrow slit opening into the mesenteron.
- FIG. 41. Top view of same egg as that shown in Fig. 40.
- FIG. 42. Surface view of preserved egg. First appearance of neural folds. Neural groove terminates in front of the neuropore, *np.* The blastopore has closed in the middle, leaving open, however, an anterior neuropore and a posterior anus, *a.*
- FIG. 43. Surface view of preserved egg. The anus lies at the posterior limit of the neural folds.
- FIG. 44. Dorsal view of preserved egg. Neural folds widely separated.
- FIG. 45. Dorsal view of preserved egg, in somewhat later stage than that shown in Fig. 44. The neural folds have approximated, and the neural groove has deepened.
- FIGS. 46-48. Vertical median sections through eggs in different stages of cleavage; before invagination has begun ( $\times 48$ ).
- FIGS. 49-51. Horizontal sections through egg shown in Fig. 37, in planes 49-49, 50-50, 51-51.
- [Outlines drawn with the camera. Figs. 34-45,  $\times 20$ ; Figs. 46-51,  $\times 48$ .]



Figs 34-51





## PLATE XVIII.

FIG. 52. Sagittal section through egg shown in Fig. 36 ( $\times 48$ ).

FIG. 53. Transverse section through egg shown in Fig. 44. Thirty-seven sections cephalad to Fig. 59 ( $\times 200$ ).

FIG. 54. Transverse section through egg shown in Fig. 44. Thirty sections cephalad to Fig. 53 ( $\times 200$ ).

FIG. 55. Transverse section through an egg with circular blastopore; blastopore, however, much smaller than that shown in Fig. 36 ( $\times 48$ ).

FIG. 56. Transverse section through egg shown in Fig. 45; near posterior end of the embryo ( $\times 48$ ).

FIG. 57. Transverse section through egg shown in Fig. 45; thirty-two sections anterior to Fig. 56 ( $\times 48$ ).

FIG. 58. Horizontal section through stage between egg shown in Fig. 36 and that in Fig. 37. Section is ventral to blastopore ( $\times 48$ ).

FIG. 59. Transverse section through egg shown in Fig. 44. Eighteen sections cephalad to Fig. 65 ( $\times 48$ ).

FIG. 60. Transverse section through a stage between egg in Fig. 44 and egg in Fig. 45. Hind end of embryo.

FIG. 61. Transverse section at about the stage of egg in Fig. 45. Same region as Fig. 60 ( $\times 48$ ).

FIG. 62. Transverse median section through egg shown in Fig. 42 ( $\times 48$ ).

FIG. 63. Horizontal section through egg shown in Fig. 40 ( $\times 48$ ).

FIG. 64. Transverse section through egg of same stage as that depicted in Fig. 44. Extreme anterior end. Position of cells forming "pouches" were located with the camera ( $\times 48$ ).

FIG. 65. Transverse section through egg shown in Fig. 44. Hind end ( $\times 48$ ).

[The outlines of all the figures were drawn with the camera.]



Figs. 52-65.



# THE FORMATION OF THE MEDULLARY GROOVE AND SOME OTHER FEATURES OF EMBRYONIC DEVELOPMENT IN THE ELASMOBRANCHS.<sup>1</sup>

WILLIAM A. LOCY.

IN studying the mode of formation of the body of Elasmobranch embryos,<sup>2</sup> I have noticed some points regarding the formation of the medullary groove, the construction of the cephalic plate, and the early formation of the eyes, that seem to me of sufficient interest to justify a preliminary communication, inasmuch as the publication of my memoir dealing with these and other topics of Elasmobranch development will be considerably delayed.

The early formed groove which appears in very young embryos of Elasmobranchs—in Balfour's Stage B—has long been known, and the method of its formation has been many times correctly described (Balfour, His, and subsequent writers). This groove is conventionally stated to be formed by the "Concrescence of the embryonic rim." It is consistently referred to in Balfour's various publications as the medullary groove, and he has been followed in this respect by other writers, including the Zieglers<sup>3</sup> in 1892.

A careful comparison of sections arranged in close sequence of development has led me to the conclusion that the early formed median furrow—or "Concrescence furrow," as it might be called—is not the medullary groove.<sup>4</sup> It occupies the same

<sup>1</sup> Read before the American Morphological Society at Princeton, New Jersey, December 28, 1892.

<sup>2</sup> This line of study was taken up at the suggestion of Dr. C. O. Whitman, and was carried on at the Wood's Holl Biological Observatory during the summer of 1892.

<sup>3</sup> Archiv für Mikroskopische Anatomie, Bd. 39, S. 56-98.

<sup>4</sup> Kastschenko expresses substantially the same conclusion when he says: "Die Primitivwülste sind mit den Medullarwülsten nicht identisch" etc., (*Anat. Anz.*, Bd. III, 1888, s. 455); and Adam Sedgwick in his article of last June refers, in a passing way, to the early fissure as a transitory structure (*Q. J. M. S.*, June, 1893).

position, in the median line of the embryo, as the latter, but soon after its formation it becomes very much reduced in depth and in longitudinal extent; then it disappears and is replaced at a later stage by the true medullary groove.

Figs. A', B', C', C'', and E' represent five successive stages of embryonic growth and show the way in which this median furrow disappears and gives place to the medullary groove in the neck region of the embryo. I designate the point from which these sections come the neck region, although it is back of the front third of the embryo and considerably behind the expanded cephalic plate. The figures are all (except C'') taken as nearly as possible from corresponding regions of the body, and they represent the conditions as fairly as they may be represented by such a limited number of sections.

In Fig. A', which is the youngest of the group, the median furrow is well developed; the ectoblast is moulded over the two lateral thickenings of the axial mesoblast and dips down between them, coming in contact with the endoblast it pushes that layer downwards in the median line. The section B' is taken from a slightly older embryo and shows the median furrow relatively more shallow. In Fig. C', which is older than the one just examined, the median furrow has disappeared and the medullary folds (*mf.*) have made their appearance as lateral expansions of the dorsal wall (medullary plate). Fig. C'' shows a section of the same embryo taken about twenty sections behind C'. It is introduced in place of a figure D', naturally to be expected in this position, because I have not at present any available section in the right region of embryo D.

In Fig. E', which is considerably older than any of the foregoing figures, the medullary folds are elevated and in the process of uniting to form the medullary canal. The space enclosed between them is that which corresponds to the medullary groove of other vertebrates, and it is to be carefully noted that *this groove is a newly formed one and is not a continuation of the median furrow which is so well marked in Fig. A'*. On the contrary, that furrow has disappeared during the intervals of growth, the medullary folds have been produced

as lateral expansions, and the newly formed groove (Fig. E', *m.g.*) is due to the upgrowth and approximation of the medullary folds. The Stage usually characterized as marked by the "first appearance of the medullary groove" should be placed considerably later. As we shall presently see the medullary folds are not yet formed at Ziegler's Stage C.

The surface changes that accompany those shown by the sections are partly represented in Figs. 1, 2, 3, 4, 5 and 6. These figures are to be used later for another purpose. They are not chosen to illustrate especially the different phases of medullary groove formation, and there is, therefore, no precise correspondence between them and the sections. The appearance of the embryo from above during the first stage of the median furrow is well known — it is not shown in my figures. It is, however, that stage designated "B" by Balfour, and is just after the body of the embryo begins to appear by the concrescence of the embryonic rim. Fig. 1 represents approximately the stage from which the sections B and B' are taken. The median furrow in this period of growth is rather deep near the middle of the embryo, but is shallow in front and behind. Fig. 2 shows a slightly younger stage than the embryo from which the sections C, C' and C'' were taken. The median furrow in this period of growth becomes obliterated with the exception of a very abbreviated portion of it located in the mid-dorsal region. My material does not enable me to say at present whether this remnant of the median furrow entirely disappears or not. I have found the surface study of embryos in this stage very deceiving as to the depth, or even the presence of the median furrow. The central part of the embryo is occupied by the gastrular cavity, and its anterior extension, the archenteron, and this extensive cavity beneath the surface lights up in such a way as to create the impression of a well marked median furrow when there is none; but, of course, the sections are conclusive on this point.

The manner of formation of the medullary folds is very unusual — so far as I know it is unlike that of any other animals whose embryology has been recorded. Glancing again over the figures indicated by the letters B', C', C'' and E' we shall

see how the medullary folds are formed in the cervical region of the embryo. Fig. B' shows that the cells of the medullary plate are much thickened laterally, and, since the base of the body has become more narrow than the top, the thickened edges project beyond it (Fig. B', *mf.*). This is quite different from the preceding figure (A') in which the base is the broadest part of the embryo. In Fig. C' we see that the thickened edges of the medullary plate have been transformed into the medullary folds (*mf.*) which are expanded laterally beyond the body, and are also ventrally curved. When completely formed the medullary folds constitute wing-like expansions running along each side of the body of the embryo and gradually fading out behind.

In the anterior head region the lateral expansion of the folds is greatest (Figs. C and D'', *mf.*), and behind the cephalic plate they become narrower as we follow them backwards. This decrease in width of the folds is well shown by comparing Figs. C' and C'' which are sections of the same embryo drawn to the same scale; but the section C'' is considerably behind the section C'. Behind the section C'' the folds show relatively the same amount of lateral expansion, to a point about seven eighths the length of the whole embryo. Back of that point, the concrescence of the embryonic rim is progressing, and the condition of the medullary folds in that part of the body requires a further account illustrated by sections, which I shall give at a future time.

There is one region, immediately behind the much expanded cephalic plate that is deserving of especial mention. Here, the medullary folds are abruptly folded downwards for a considerable space and come almost into contact with the sides of the body. Fig. G represents a section through this particular region, and the position of the bend in the medullary folds is indicated by the shading in Figs. 2, 3, 4 and 5 (*bd.*). This abrupt bending of the medullary folds makes its appearance as soon as the cephalic plate is formed and it persists through several stages of growth. Just in front of the bend on each side the cephalic plate becomes raised into eminences (Fig. 3, x; Fig. 4, 3).

During the process of upgrowth of the medullary folds, and also, while the medullary groove is being converted into the medullary canal, the folds in this particular region respond slowest to the new direction of growth and the canal remains widely open above this region for a long time after it has closed behind and in front of it.

The question naturally arises whether such unusual ventral folding as that shown in Fig. G is not artificially produced, either by the reagents used or by some process of the technique. I have completely satisfied myself on that point. The abrupt folding appears with constant characteristics in all preparations I have ever seen if they are at the right age. I have used a variety of reagents: Davidoff's fluid, picrosulphuric acid, picro-nitric acid, Fleming's stronger solution, Perenyi fluid, corrosive sublimate and picric acid. All the preparations show this characteristic. In most of my own preparations of these stages the eggs were hardened *in toto* before the blastoderm was removed, and as the eggs kept their form, the embryos were not subjected to any considerable artificial tension.

The formation of the medullary folds as described above takes place between Ziegler's<sup>1</sup> Stages C and D, and my studies indicate that it is essential to recognize stages of growth at shorter intervals. If one studies sections of the conventional Stage C, and then follows with sections of the Stage D, as designated by Ziegler, they will completely miss the laterally expanded condition of the medullary folds shown in my figures C' and C''. My sections of the stage designated D by Ziegler show precisely the same condition of the medullary folds and the medullary groove that he has so well represented in his figures of that stage.

Balfour<sup>2</sup> first pointed out the ventral curvature of the head-folds of the Elasmobranchs, but he was apparently not acquainted with the complete history of the head-folds, and with the method of formation of the medullary folds in the dorsal

<sup>1</sup> *L.c.* Fig. 4, p. 68; Fig. 7, p. 77.

<sup>2</sup> Monograph on the Development of the Elasmobranch Fishes, Plate IX, Fig. 5.

region. As before indicated, he consistently refers to the median furrow as the medullary groove.

Figs. A, B, C, D, E, F and D'' show certain characteristic stages in the formation of the medullary folds in the region of the cephalic plate, and, also, certain steps in their upward growth to form the medullary groove. The first five of these sections are taken from the same embryos that afforded the sections already described. The same letters indicate in every case the same embryos, and the marks (' and '') attached to the letters serve to indicate the different regions of the body from which the sections are taken.

Fig. A shows a section through the head region before any changes have occurred looking towards the formation of the medullary folds. It will be remembered that further back in this same embryo (Fig. A', *cf.*), the median furrow is well marked.

In Fig. B we have a faint indication of the median furrow; on the sides are seen lateral thickenings of the medullary plate (*mf.*) which become transformed into the medullary folds.

Fig. C is an interesting one; it shows the position of the future groove occupied by an eminence (*e.*). The medullary folds are far removed from the median plane; they are unusually wide and have the characteristic (at this period) ventral curvature. A pencil point in following the outline of this section from the outer margin of the medullary folds, will mount in passing towards the median line, and will reach a position on the summit of the central eminence, that is more than one-half the full vertical axis of the embryo above the medullary folds. Yet in subsequent growth changes of such a nature take place, that the medullary folds become elevated, and, arching above the median part they join some distance above it.

Fig. D affords a contrast with Fig. D'',—which is taken from the same embryo near its anterior tip—showing the condition of the medullary folds, at this stage, in the anterior (D'') and the posterior (D') parts of the cephalic plate.

Fig. F. shows a stage in which the medullary folds are growing upwards, and have risen a little above the horizontal plane.

In Fig. E, the medullary folds are elevated into nearly a perpendicular position, their upper margins are still slightly rolled ventrally.

The medullary canal is produced from the medullary groove in the usual way, by the coalescence of the folds in the median plane. In the process of closing, the walls of the groove come together first in the extreme anterior tip of the head in front of the eyes and they unite a very little in this region : they are then approximated in the cervical region and the closing of the groove progresses both anteriorly and posteriorly but it does not close throughout the length of the embryo by a continuous extension of this union. In the posterior dorsal region a new point of contact is made considerably behind the point reached by the backward growth of the first cervical union, and, in this way, an open sinus is formed between the new point of contact and the canal already formed in front of it. This sinus closes gradually but after it is completely closed, the medullary canal is still open behind that region by a continuous posterior slit as Ziegler has shown in his Fig. 8.<sup>1</sup>

In the meantime the closing of the groove in front progresses even more slowly, and, after the posterior sinus is completely closed, that region of the brain lying above the abrupt bend of the medullary folds, already described, is conspicuously open and the margins of the medullary folds are ventrally rolled. In front of this, between the eyes, there is a distinct neuropore that closes last of all.

The decided tendency, as recorded above, of the rudiments of the dorsal nerve cord to grow ventrally when they are first formed is suggestive, but I shall indulge in no speculations at present as to what may be the theoretical bearing of these facts.

In addition to the unique features of medullary groove formation, there is to be noted in my preparations several interesting points as to the formation of the cephalic plate and its transformation into the head of the embryo.

In an early stage of growth the anterior tip of the body from which the cephalic plate is to be formed shows no

<sup>1</sup> *l.c.* p. 81.

especially noteworthy features. It consists of a semi-circular fold of the embryonic rim, which curves around the end of the median furrow and gradually passes into the sides of the body; these in turn shade off into the rim of the blastoderm.

The first observable change in the form of the head-end arises from its becoming widened laterally, at the same time the anterior median portion of the head grows somewhat forwards, forming a blunt protruding tip. The head region becomes through these changes, divisible into a central portion (Fig. 1, T) and two lateral portions (Fig. 1, L). The two lateral portions expand rapidly sideways, overhanging the blastoderm, and the form of the cephalic plate changes so that its transverse axis becomes the major axis (Figs. 2 and 3). The rest of the embryo is much narrower, and the whole taken together may be spoken of as club-shaped. I apply the term cephalic plate only to the much expanded anterior part of the embryo, but this does not correspond with the brain region, which extends further back into the narrower part of the embryo. The posterior limit of the cephalic plate is marked by two eminences (Fig. 3, x; Fig. 4, 3) and behind these is the region, before mentioned, in which the medullary folds are so abruptly bent downwards.

A tongue-like elevation, which is very noticeable in surface views, soon makes its appearance in the median line (Fig. 2, T). When it is first clearly outlined it extends from the anterior tip of the head backwards about one half the length of the cephalic plate; it is wedge-shaped in outline and its upper surface is convex. This structure tends to mark off more clearly the central portion of the cephalic plate; it is separated from the lateral portions by two distinct furrows. Although it becomes less prominent in later stages, the tongue-like process is readily distinguishable, until the head-folds finally arch over it and hide it from view.

The anterior part of the cephalic plate becomes the seat of an infolding in which is formed both the infundibulum and the optic vesicles. The first surface indication of this depression is such as might be produced by pressing very lightly with rounded dies on each side of the tongue-like process. Two

faint circular depressions that are concave upwards, show even at as early a stage as is represented in Figure 2. [It should be stated for clearness that Figures 2, 3 and 4 represent stages that occur between those designated C and D by Ziegler. I have met with no figures of corresponding stages in any authorities I have been able to consult.] The circular depressions meet in the median line and encroach upon the tongue-like process and it becomes separated from the extreme anterior tip of the head. The infolding started in this form continues, but it does not take place so rapidly in the median line as on each side of it, and it thus happens that the anterior tip of the head and the front end of the tongue-like process are connected by a narrow isthmus, which becomes gradually reduced, and just before its disappearance it forms a very thin connecting plate between these two points (Fig. 3).

Fig. 2 shows the central tongue-like process continuous with the anterior tip of the head and Figs. 3 and 4 show it (T') after its separation from the latter.

The optic vesicles are formed in the lateral parts of the depressed region; they are circular in outline and concave from within while externally they form corresponding elevations. In Fig. 3 the head-folds are so much expanded laterally beyond the optic vesicles that these structures seem, at first sight, very much out of place; but the broad folds, which in the figure are at the side of the vesicles, grow upwards and unite in the median plane, and by this process the head becomes narrow so that the eye vesicles are then laterally situated with reference to the rest of the head. It thus appears that the optic vesicles are formed very early and almost at the extreme anterior tip of the head. They do not begin to move backwards until the anlage of the nose is formed. In Figs. 3 and 4 that part of the cephalic plate which is behind the optic vesicle is all on a higher plane than the anterior part. In Fig. 4 this elevated part of the cephalic plate is divided into concentric ridges (1, 2, 3) and furrows. I shall reserve a more detailed description of the morphology of these structures for my forthcoming publication.

The growth of the cephalic plate becomes more complicated, for a frontal flexure progresses simultaneously with the other changes, and while the head folds are undergoing various transformations they are at the same time being drawn downwards in a new plane by the progress of this frontal flexure. The "frontal flexure" has already begun in Fig. 2, and can be seen in all the succeeding figures. It occurs so much earlier than the flexure which has heretofore been designated the cranial flexure that I use a definitive term for it. The central tongue-like process, having become entirely separated from the anterior tip of the body, is carried downwards by the infolding to form the infundibulum and it becomes sickle-shaped in side view. The head-folds begin to rise around the depressed region in the forward part of the plate, while still retaining their ventral flexure in the middle and hinder parts, and this serves to further complicate matters.

After the medullary folds become elevated in the head region there is formed upon their margins a number of epiblastic thickenings. I have not as yet traced their relation to the central nervous system.

The observations recorded above were made mainly upon embryos of *Squalus (Acanthias) vulgaris*.

There is one other point to which I wish to direct attention. I have noted an invagination in the eggs of *Galeus* which appears at a much earlier stage than any invagination that has been recorded for other elasmobranchs. The involution occurs at a stage that might be designated mid-segmentation period (following the boundaries assigned by Balfour). It appears at the posterior edge of the blastodisc, where the embryo finally makes its appearance.

Seen from above this invagination is crescentic in form and covers rather less than 90° of the edge of the blastodisc.

It resembles very strikingly the early invagination in the bird's egg as figured by Kollar and also by Duval, and the invagination in the reptiles as figured by Kupffer and Benecke.

I have seen this invagination many times on living eggs of *Galeus*, and my sections show that it is a true infolding.

Kastschenko,<sup>1</sup> in a preliminary paper mentions a "cleavage cavity" in Torpedo eggs which at an early stage is imperfectly covered and shows the food yolk through the very thin covering. He could not find an external opening in his sections, but inclines to the belief that such an opening exists. It is barely possible that this "cleavage cavity" of Kastschenko is the same structure as the crescentic involution which I have noted above.

LAKE FOREST UNIVERSITY,  
January, 1893.

<sup>1</sup> *Anat. Anz.*, 1888, Bd. iii, S. 445.

## DESCRIPTIONS OF THE FIGURES.

FIGS. A, B, C, D, and E represent sections through the head region of five *Acanthias* embryos of different ages; A being the youngest stage, B the next older, and so on.

FIGS. A', B', C', and E' represent sections through the cervical region of the same embryos. D' is not represented in this series, but in place of it the section C'' is inserted. C'' is a section of embryo C made much further back in the body than C'.

*mf.*, medullary fold; *cf.*, "concrecence" or median furrow; *mg.*, space enclosed by the medullary folds, corresponds to the medullary groove of other vertebrates. It should be carefully noted that the space indicated by *mg.* is an entirely different formation from the median furrow (*cf.* in Fig. A).

FIG. D'' represents a section of embryo D, in the depressed anterior region just after the head-folds have begun to grow upwards.

FIG. F represents a section through the middle of the cephalic plate after the head-folds have begun to grow upwards, and have reached a position a little above the horizontal plane.

FIG. G shows a section through the region just back of the cephalic plate, where the medullary folds are abruptly bent downwards.

All drawings of the sections were outlined with the aid of the camera, and all are magnified about 75 diameters — Zeiss AA, 2 oc.

FIGS. 1, 2, 3, 4, 5, and 6 are to show especially the transformation of the frontal region into the cephalic plate and certain stages in the growth of the latter. The figures are taken from photographs, and all are magnified about 20 diameters.

FIG. 1. An embryo showing the head region divided into a central and two lateral parts.

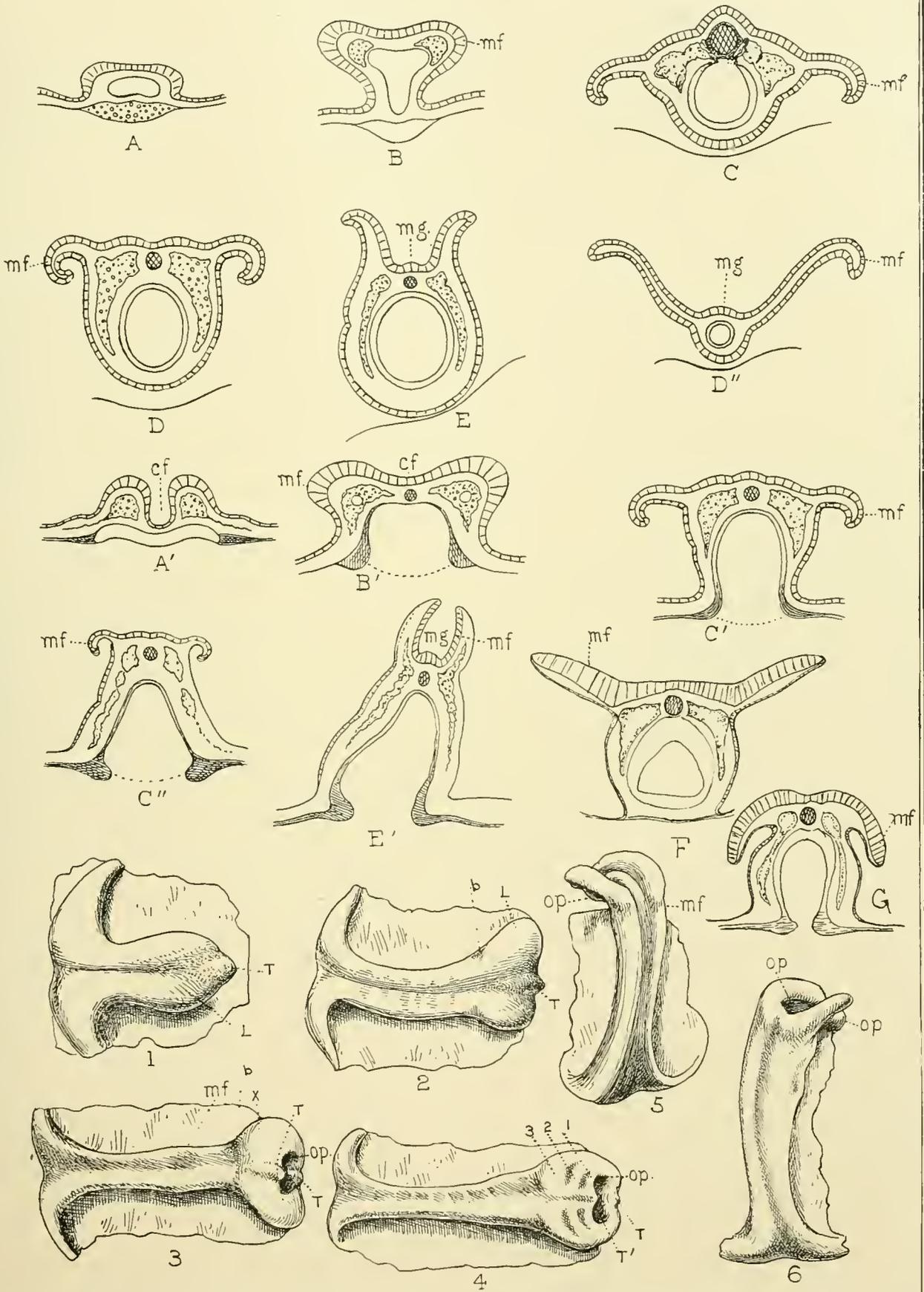
FIG. 2. Older embryo showing considerable expansion of the lateral parts (L) of the head region — these are expanded folds ventrally curved. In the central part the tongue-like process T is conspicuous, and is in connection with the anterior tip of the head. At the point *b* is shown the place where the medullary folds are so abruptly bent downwards.

FIG. 3. Older stage showing the beginning of the infolding to form the infundibulum and the optic vesicles. The tongue-like process (T) is separated by this infolding from the anterior tip of the head (T'). At *op*, the beginning of the optic vesicles is indicated; *x*, one of the two posterior eminences marking the posterior limit of the cephalic plate.

FIG. 4. A still older stage showing the point mentioned for the previous figure. It also shows the concentric elevations (1, 2, 3) mentioned in the text.

FIG. 5. Stage in which the medullary folds have begun to rise. The frontal flexure is considerably advanced. At *op'* are shown the external elevations of the optic vesicles.

FIG. 6. Embryo viewed from such a position as to show the optic vesicles externally (*op'*) and internally (*op*). The tongue-like process is bent downwards and backwards in the median plane, and is hidden from view by an edge of the head-folds.





## SOME NERVE-MUSCLE EXPERIMENTS ON THE FROG (*Rana Catesbiana*).<sup>1</sup>

HOWARD AYERS.

THE experiments herein recorded have to do with the influence of the *rate* of stimulation upon the propagation of the results of electrical stimuli in peripheral nerves. This topic has been the subject of recent investigations by several physiologists, among whom are Dr. F. H. Hooper<sup>2</sup> and N. Wedensky,<sup>3</sup> who, as a result of their researches, consider *the rate of stimulation* to be as important a factor affecting the propagation of nerve force as the *intensity* of the stimulus is conceded to be. I shall first describe the experiment on the frog's larynx, then those on the antagonistic muscles of the leg of the animal.

For an account of the physiology of the mammalian larynx the reader is referred to Dr. Hooper's excellent résumé in the above mentioned paper (1) where the experimental results of all the studies of this structure — reaching back some centuries — are recorded. From Dr. Hooper's papers it is clear that even closely related tracheate vertebrates, *e.g.* the dog and the cat, do not agree in the responses made by the laryngeal muscles to similar electrical stimuli, all other conditions of the experiments being so far as known (and they were intended to be) the same.

Owing to this condition of the subject, these experiments, which were begun at the suggestion of Prof. Bowditch, are

<sup>1</sup> Performed in the Physiological Laboratory of the Harvard Medical School, 1888-89.

<sup>2</sup> F. H. Hooper, M.D. The Anatomy and Physiology of the Recurrent Laryngeal Nerve. The N. Y. Med. Journ., 1887.

The same. Effects of Varying Rates of Stimulation on the Action of the Recurrent Laryngeal Nerves. *ib.*, 1887.

<sup>3</sup> N. Wedensky. Ueber die Ursachen des Ritter-Rolletschen Phänomens am Fusse des Froches. Centrblt. für Physiologie, I, 1887.

important in that they make it possible to institute some comparisons between the highly differentiated mammalian larynx and the much simpler amphibian organ which we have every right to expect will show us the primitive mode of action of this specialized set of muscles, and in this way throw some light on the points still to be elucidated.

The question of the influence of the rate of stimulation on the action of nerves is the outgrowth of attempts to explain a much older question — the so-called Ritter-Rollet phenomenon. Ritter discovered, and later on Rollet<sup>1</sup> established more firmly, that (1) when the sciatic nerve of a frog is acted on by a *weak* tetanic electrical stimulation the flexor group of muscles contract, producing flexion of the leg and adduction of the toes, and that (2) when such a stimulus of greater strength is applied to the nerve the extensor group of muscles contracted producing extension of the leg and abduction of the toes. Since then it has been shown that the same is true of other vertebrates, including mammals. Experiments on invertebrates show that this peculiar difference between the antagonistic muscles of the appendages must lie in a fundamental quality of nerve-muscle mechanisms, for the Lobster's claw has been found to open in response to the stimulation of weak electrical currents, while closing upon increasing the strength of the stimulus. It has been usual to account for these phenomena by the hypothesis of "*differing irritabilities.*" Fick and Bour, however, explained the phenomena on the basis of the differences in the length, thickness and position of the antagonistic groups of muscles. Wedensky, on the other hand, thought it could be accounted for on the basis of the *rate* or frequency of stimulation. His experiments with the aid of the telephone show, 1st, that with indirect tetanus of the muscles, it often happens that the muscle does not contract upon the application of the proper stimulus, but only in response to low rates, and 2d, that any variation of the intensity of the stimulus is, in a certain sense, equivalent to a similar variation of the rate, for indirectly tetanized muscles, so that the same result

<sup>1</sup> 4. Sitzber. d. Wiener Akad. Bd. LXX bis LXXII. 3te Abth. Richet and Luchsingers.

is obtained whether one varies the intensity of stimulation while using a given maximum rate, or varies the rate while using a given maximum intensity. It is obvious that Fick and Bour's explanation is inadequate, for if no other elements than the length and size of nerves and muscles, or differences in their position, entered into the problem, the stronger group of muscles would ultimately overpower the weaker with any strength of the stimulus. Differing irritabilities is a "blanket" explanation, and may be said to include the third explanation which attributes these results to differences in the rates of stimulation required by the two sets of muscles. The latter explanation presents the matter in a specific manner, so that we should be able to determine by experiment whether it is a *causa vera* or not.

The apparatus used consisted of a Du Bois Reymond induction apparatus for controlling the intensity of the Faradic current, a Bowditch interruptor for controlling the rate of stimulation.

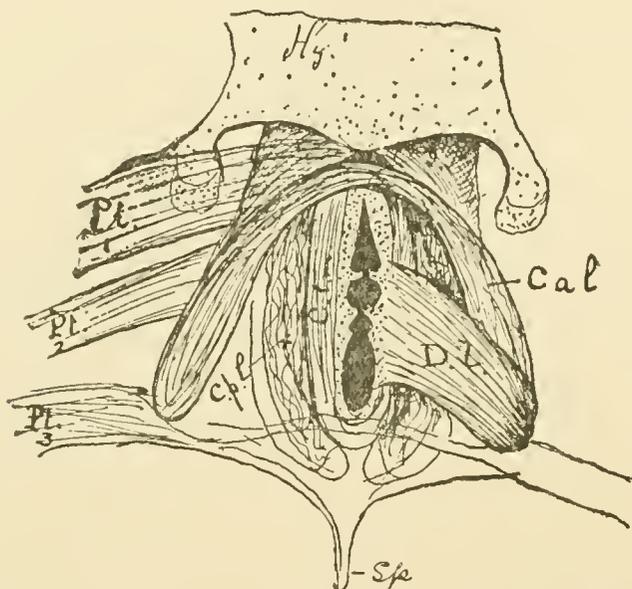
The Frogs were prepared by destroying both the brain and spinal cord and dissecting out the laryngeal nerve, or the sciatic nerve, as the experiment required. In a part of the experiments shielded electrodes were placed on the nerve, from one to one and a half centimeters from the larynx, and in another set simple hook electrodes were used to hook up the nerve at any point of its course.

To determine whether or not a closure of the glottis was called forth by stimulating the laryngeal nerve, a delicate celluloid paper spring was placed in the *rima glottidis* so as to slightly open the laryngeal passage.

The larynx of *Rana* is composed of three<sup>1</sup> cartilaginous plates formed into a complicated mechanism, the parts of which I will not here describe. The ground plan is simple enough and consists of a basal ring formed by the cricoid cartilage which has an equatorial loop projecting ventrad. This loop is so placed relative to certain processes of the ring that the roots of the lungs, which are attached to it (a trachea

<sup>1</sup> The thyroid cartilage is absent, but the posterior cornua of the hyoid assumes its functions.

being absent), are kept constantly open. The two remaining cartilages form the pair of relatively large arytenoids. They fill in the opening of the cricoid ring and project dorsad in the form of an elliptical cupola, whose upper surface may be seen forming the glottidian eminence in the floor of the Frog's mouth. Within this dome the vocal cords are stretched in such fashion that the slit between them lies directly over and parallel with the median plate formed by the coalesced walls of



*Cut 1.*—A dorsal view of the Frog's larynx dissected to show the cartilages and muscles. *Hy.*, Hyoid Cartilage; *C.l.*, *Cal.*, *C.p.l.*, Constrictors; *D.l.*, Dilator.

the adjacent lung roots (the equivalent of the carina of human-anatomy.)

To this cartilaginous skeleton are attached three pairs of muscles. A dilator laryngis and two pairs of constrictors. These muscles are all very nearly the same in mass and, since it contains two sets of muscles, the constrictor group is twice as strong as the dilator group, and serves to keep the glottis closed most of the time. Under certain conditions the dilators contract and produce an opening of the glottis. It follows from what is given in the table below that there is very probably an elastic action of the cartilages of the *rima glottidis* which aid the muscles in maintaining the normal closure of the glottis against the pressure of the air swallowed into the lungs

and there held during the prolonged submersions to which Frogs frequently subject their bodies. In the dead Frog the amount of the resistance due to the elasticity of the laryngeal cartilages was found to equal from 0.5 to 1. centimeter of water in the manometer used. There is no possibility of muscular action here for the animals were dead in all their parts. A very slight per cent of this amount may be due to the pressure of the mass of the small muscles of the larynx but this quantity is probably inappreciable.

A TABLE OF THE RESISTENCE EXERTED BY THE FROG'S LARYNX AGAINST PRESSURE FROM WITHIN IN CM. OF MERCURY AND WATER MANOMETERS.

NO. OF OBS.	ALIVE.	ETHER.	PITHED.	CORD DESTROYED AND PITHED.	CURARE.	DEAD.	SEX.
5	{ mouth open 40-6-8 } { " closed 30-9-12 } { H <sub>2</sub> O Hg. }						♂
3		6 Hg.	4 Hg.	2.5 Hg.			♂
2						0.5-1 H <sub>2</sub> O	♀
1					5 Hg.		
7		3 Hg.	3 Hg.	2.5 Hg.			
3		3 Hg.			2.5 Hg.	1.	♀

The resistance in the live frog represents the sum total of involuntary and voluntary efforts of the laryngeal apparatus.

From what was said concerning the laryngeal muscles it is evident that the dilators are capable of overcoming twice their mass of muscle when they contract, since the two pairs of constrictors are at all times stimulated to the same extent with the dilators. It is also true that the latter are so inserted as to have a decided mechanical advantage over the former and further, that under very strong stimulation they are only capable of producing a slight opening of the glottis at the summit of the glottidean eminence, *i.e.* at their point of insertion, where the action of the constrictors is exerted the least. We meet here the same conditions found in the leg of the

Frog where the weak flexor group is able under influence of weak stimuli to overcome the stronger extensor muscles : but with the increase in strength of the stimulation the extensors gain the advantage and flexion gradually passes into extension. Whether in either case the flexor muscles suffer any loss in the intensity of their stimulation during extreme stimulation of the antagonistic groups has not been determined, but it is probable that they are the more stimulated the greater the excitation of the flexors in order that the proper muscular control may be maintained at all times.

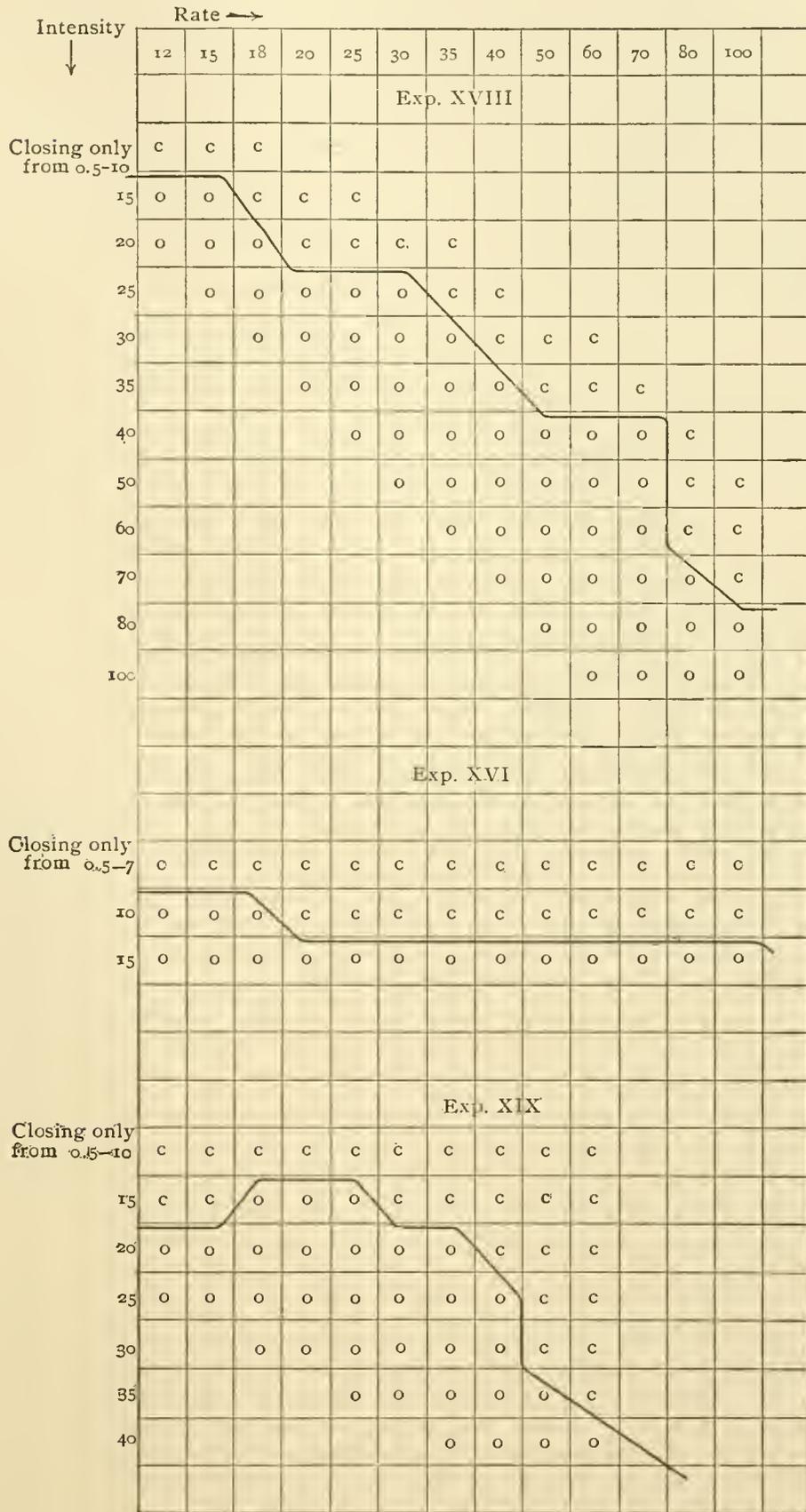
The following tables will serve to illustrate the important variations in the response made by the laryngeal muscles to electrical stimuli in all the Frogs experimented upon. The opening following the increase in rate in Exp. XIX is unusual but occurred several times in the course of the work. As to its cause no clue was obtained. A tetanizing current applied to the laryngeal nerve, when it produces opening of the glottis does not suffice to produce a continued opening, for the glottis soon returns to closure with all the muscles strongly contracted.

After describing the experiments on the leg muscles, the results of the two sets of observations will be compared and their relation to Hooper's and Wedensky's results pointed out.

In the experiments on the antagonistic muscles of the Frog's leg constant care was taken to have the nerves as nearly normal as possible. Through a cut in the skin of the leg, the peroneal and tibial nerves were severed or else the sciatic trunk was taken higher up and either separated into its component peroneal and tibial branches or stimulated as a whole. As a rule however the former method was employed for it served to modify the conditions in an important way from those adopted by Ellis in his experiments with the "ether effect," for it allowed of absolute separation of the stimuli sent into the two sets of muscles whether sent in simultaneously or consecutively and permitted the experimenter to change at will the rate or intensity, or both, of the stimulus to either set of muscles. In part of the observations the leg was suspended in a cuvette in water or salt solution, in others it was fastened by means of the femur to a "frog board" and allowed to hang

TABULATION OF THE RESULTS OF THREE EXPERIMENTS ON THE LARYNX.

(INTENSITIES FROM 0.5-5 ALWAYS CAUSED A *closing*.)



free in air. Of course care was used to keep the exposed nerves and muscles from drying, by moistening with normal salt solution or covering with thin strips of filter paper moistened in this fluid.

TABLE OF RESULTS OF ELECTRICAL STIMULATION OF THE ANTAGONISTIC MUSCLES OF THE FROG'S LEG WITH CHANGING RATES AND INTENSITIES.

Rate →	12	15	20	25	30	35	40	50	60	70	80	100	
Intensity 0.5-3 ↓	F	F											
5	e	F											
7	e	F											
10	e	F											
15	e	F											
20	e	F	F										
25	e	F	F										
30	e	e	e	F									
35		e	e	e	F								
40			e	e	e	F	F						
50				e	e	e	F	F	F				
60					e	e	e	e	e	F	F	F	
70						e	e	e	e	e	e	e	
							e	e	e	e	e	e	

This table is selected from a series, as representative of the usual response of the leg musculature to changing rates and intensities.

The following is a table of the results of some of the experiments. It shows the intensity required with a given rate to convert a flexion into an extension. Each experiment here given is taken from a fresh Frog and represents the series made on that individual.

DATE.	NO. OF EXP.	RATE.	INTENSITY FLEXION.	INTENSITY EXTENSION.	OBSERVATIONS.
19. X. 88.	I.	12	80	90	
20. X. 88.	II.	12	40	60	{ 50 mixed movements, 60 extensors gradually overcome flexors, 70 complete extension. The nerves here were extremely sensitive and a slight difference in intensity served to throw flexion into extension with this rate.
20. X. 88.	III.	30	0.5+6 cm.	0.5+5 mm.	
26. X. 88.	IV.	25	25	40	
26. X. 88.	V.	6	0.5+2.5	0.5+10 mm.	} Limit 0.5 flexion of foot and knee. } The flexion of toes, ankle and knee was complete.
		12	0.5+3.5	57	
		5		0.5+10 mm.	
		7		0.5+7.5	
26. X. 88.	VI.	25		0.5+7 mm.	{ A true case of the Ritter-Rollet and only apparently a case of Wedensky since the rate difference was due to instruments alone in that it changed the I. of currents differently.
		12	0.5+7 mm.		
26. X. 88.	VII.	12	70	85	
		12	60	75	
31. X. 88.	VIII.	4	0.4		
		16	0.3		
	IX.	12	1.2 ±	1.2 ±	{ Strong flexion passing into extension. } { Strong extension. }
		50			
	X.	50		55	} No flexion obtained with the less I. } When intensity was so low as to give no reaction quickly raising R. from 12-60?
2. XII. 88.	XI.	12	1.	55	
	XII.	25		22 cm.	} In these experiments on the fowl it was found that the flexors of the toes are quickly fatigued even with low intensity, and afterwards give nothing but extension even with flexion of tarso-metatarsus. The nerve reacted to the elect. stimuli 17 mi. after cessation of respiration. No continuous flexion was obtained with rates below 25.
		30	29		
		100	25		

In concluding his able discussion of the ether effect (Amer. Jour. Med. Sciences, 1888, Bowditch says on p. 8: "These experiments by Ellis prove beyond a doubt that the effects observed by Perkins on stimulating a nerve immersed in a solution of ether, can be obtained with other drugs, and often with very weak currents without any drug. Without ether, flexion and adduction are to be obtained only with very feeble currents, but with ether much stronger stimulation produces the same effect. In both cases, however, an increase in the intensity of the stimulus causes these movements to give place to extension and abduction; *the effect of the ether may, therefore, be said to consist in transferring the point, on the scale of intensity, at which the effect of nerve irritation changes from flexion and adduction to extension and abduction, from the*

*region of weak to that of relatively strong stimulation.*" (Italics mine.) "The 'ether effect,' as observed in the sciatic nerve of the Frog, may be best explained by supposing that a partial paralysis of the nerve by the drug converts what would naturally be a strong into a weak irritation, and that this weak irritation affects only the flexor group of muscles, because these are, for some reason or other, more irritable than their antagonists." The author, after citing the investigations of Ritter, Rollett, Bour, and Lombard on this subject, concludes with the following paragraph: "There seems, therefore, to be no doubt that the flexor apparatus of the Frog's leg responds to a feebler stimulation than the extensor, but whether the cause of this difference lies in the nerve fibres, the muscle fibres or in the mode of connection between the two, must be left for future investigators to determine," and he adds the suggestion that histological differences such as exist between the red and white muscle of Rabbits may be found to obtain in the Frog's antagonistic groups. I have quoted thus at length because this important topic as clearly and concisely stated above, is still further elucidated by an experiment which is performed in the following manner. In a pithed Frog the sciatic was exposed near the head of the femur, the nerve cut and separated into its two branches, peroneal and tibial from the point of natural bifurcation to the cut end. The nerves were then placed on separate electrodes and stimulated with equal currents, graduated by means of a telephone, and results noted through a series of variations of the intensity and rate. The main results are as follows:—

1. Stimulation of Peroneal nerve *alone* with all R and I, produced complete flexion of leg and foot and *abduction* of the toes.

2. Stimulation of the Tibial nerve *alone* with all R and I produced complete extension of the leg and foot with *adduction* of the toes.

3. Stimulation of the Peroneal and Tibial together with low I and R — produced flexion of leg and foot and *adduction* of toes but with high I and R always extension of leg and foot and *abduction* of the toes.

We thus find that the results of the separate stimulation of the nerves do not harmonize completely with those obtained from stimulation of the entire sciatic, or what is the same thing its two branches simultaneously. The difference is an important one. From simultaneous stimulation of both nerves with low I and R *adduction* of the toes always results but when high I and R are used *abduction* always takes its place. Now on the theory proposed by Wedensky the adductor *nerve* fibers should show themselves more irritable to weak stimuli than to strong and *vice versa* for the abductor. The same holds true of Bowditch's explanation in which however the seat of the irritability is not located. But the reverse is the case.

Any explanation of this phenomenon will have to account for the fact that nerve fibres contained in one trunk, *e.g.* the peroneal which is supposed to be a pure flexor nerve, can cause flexion of some parts of the limb and extension of other parts.

Primarily, of course, the vertebrate limb contains two sets of muscles antagonistic to each other, directly and wholly, as in the case of the fish fin, with movement in one plane backwards and forwards. Secondly, however, some of these muscles, especially near the end of the limb, become differentiated into sets with new functions, *e.g.* that of controlling the digits. Their nerve supply is derived from the trunk which supplied the primitive arrangement of the parts. In the complicated limbs of all vertebrates above the fishes some muscles, split off from the extensor set, acquire flexor connections, or are only used in the flexion of the limb, and *vice versa*.

The theory of nerve control proposed by Wedensky calls for a greater sensitiveness to stimuli on the part of the flexor nerves. But from what has been said above it is evident that some nerve fibres of the flexor groups may be so changed that they are sensitive to the stronger stimuli only, and they are called into action only with the extensor group, and *vice versa*, for the flexor nerves. This change is doubtless more in the nature of a modification of the central cells of origin of the motor fibres than of the fibres themselves. Whatever its nature may be it indicates that Wedensky's results do not

represent the whole truth, otherwise, upon the stimulation of the separated nerves simultaneously, the muscles would give the same reaction as when the trunk of the sciatic is stimulated.

To return to the explanation Bowditch suggests for the "ether effect," which may be said to transfer the extensor point of response further up the scale of intensities, and, of course, the flexor point of response follows it. The cause of the transfer is the partial ether paralysis of the nerve substance. An examination of the tables given for the larynx and leg shows that if I substitute the word *rate* for *ether* and *partial rate polarization* for the *ether paralysis* of Prof. Bowditch's explanation of the ether effect, these two series of experiments are brought into one category. The rate of stimulation, like sulphuric ether, exerts a special influence on nerve substance — in both cases probably of a molecular nature — which reduces the *irritability* or the capability of the nerve to transfer electrical stimuli. It renders the nerve axis cylinder more resistant to molecular change. Further than this my experiments do not give any insight into the causes of these phenomena.

The results of all observers on the larynx up to the present time may be tabulated as follows :

A stimulation of the laryngeal nerve with the Faradic current causes with

	(a) Low Rates and Intensities.	(b) High Intensities and Rate.+	(c) High Rate and Intensities.±
Man	opening	opening, and sometimes closing	closing
Dog	do.	do.	do.
Cat	closing { only; with reflex stimulation of the pneumo-gastric nerve with high R and I. }	opening with closure	opening
Frog	do.	do.	do.

It will be observed that man and the dog agree, and that the cat and the Frog, while differing from them, are in harmony with

each other. A satisfactory explanation of these facts has not yet appeared, for the action of the Frog's larynx, even if it be primitive in nature, does not appear to clear up the difficulties. It is none the less interesting to note that a mammal whose normal condition is with an open glottis, agrees in its responses to stimulation of this organ with an amphibian whose normal condition is with a closed glottis and who requires a voluntary effort to get it open.

The results of my study of the larynx and leg of the Frog (and the few observations made on the leg of the Fowl and Dog were entirely harmonious with them) clearly show that (*a*) in this animal with every *increase in the rate* of stimulation there must be a corresponding increase in the intensity of the stimulus in order to get the same response, given by these mechanisms to the initial low rate; (*b*) that the influence of an increase in the rate of stimulation is very like that of sulphuric ether and some other drugs, and (*c*) that Wedensky's conclusions are only partly sustained, for there does not appear to be a rate of stimulation, of influence so powerful that it can, like ether, entirely overcome the influence of increase of the intensity of stimulation.

The nature of the action of the rate of all kinds of stimuli upon nervous tissue is as unknown as is the nature of the influence which the rate of stimulation of the auditory nerve has upon some of the muscular structures of the body.

Of course the influence of the rate of stimulation is made manifest only when it progressively increases or decreases but any rate whatsoever, has its own specific influence on the propagation of nerve force while it lasts, so that changing the rate merely makes apparent what already exists.

That these two phenomena are intimately related, and that they are grounded in some fundamental principle governing the existence of protoplasm, cannot be reasonably doubted. I deeply regret that I have not been able to continue this study to a more profitable termination, and the only reason for publishing this fragment is the hope that it may prove suggestive to some student and lead to a renewed search after the effects of the rate of stimulation upon the nervous organization

of higher animals as well as upon the simpler forms, in the endeavor to discover the causes which lie back of them.

NOTE 1.—On p. 383, 8th line from the bottom of the page, it is stated that the two pairs of constrictors are at all times equally stimulated. In this I only mean to say that an equal stimulus is simultaneously applied to the nerves of both muscles. To show why the muscular responses vary by changing the rate is probably to solve our problem.

NOTE 2.—The statement in the first paragraph on p. 389 is for me one of fact merely, but I may say that Professor Bowditch is of the opinion that there is no physiological reason apparent to prevent us from assuming that the results of all these experiments are harmonious.

THE LAKE LABORATORY,  
MILWAUKEE, WIS., Feb. 10, 1893.

# BIOLOGICAL CHANGES IN THE SPLEEN OF THE FROG.

ALICE LEONARD GAULE.

WHOEVER has had occasion to experiment with frogs to any extent has doubtless been often interrupted in the progress of his investigations by the different behavior of the organs and tissues at various seasons of the year. A number of Gaule's pupils in the Physiological Laboratory at Zurich have made these changes, directly or indirectly, the subject of investigation. Some of the results have already been published by Ploetz<sup>1</sup> and by Leonard,<sup>2</sup> others have merely been referred to in the publications of Gaule,<sup>3</sup> of Gürber,<sup>4</sup> and of Johannsen<sup>5</sup> and others still await completion. The present investigation has to do with the effect of the different seasons, or of the natural external conditions on the spleen of the frog.

## HABITS OF THE FROG.

The two common Swiss species are the green frog, *Rana esculenta* and the brown or grass frog, *Rana temporaria*. The life-habits of the two species fall into two annual periods, (1) the period of hunger and (2) the period of nutrition. The former extends over the winter and spring months, that is, from November until the late spring when the nutritive period begins. In November when the days become dark and cold

<sup>1</sup> Ploetz, Die Vorgänge in den Froschhoden unter dem Einfluss der Jahreszeit. Archiv f. Anat. u. Phys. Supplement Band, 1890.

<sup>2</sup> Leonard, Der Einfluss der Jahreszeit auf die Leberzellen von *Rana temporaria*. Archiv f. Anat. u. Phys. 1887, Supplement Band.

<sup>3</sup> Gaule, Zahl und Vertheilung der markhaften Fasern im Froschrückenmark. Königl. sächs. Gesell. der Wissenschaften, Band XV, 1889.

<sup>4</sup> Gürber, Die Gesamtzahl der Beutkörperchen und ihre Variation. Archiv für Anat. u. Phys., 1889.

<sup>5</sup> Johannsen, Ringbänder der Nervenfasern. Archiv für Anat. u. Phys., 1892.

and the food is scarce, the frogs bury themselves in holes in the muddy banks of shallow ponds or sluggish streams and remain thus excluded from light, air and food, and are but slightly protected from the cold, for from four to five months of the year. On warm, sunny days in March or April they creep out of their holes into the water. Cleansed of the winter's mud, they are seen to be arrayed in holiday attire. The color of *temporaria* is greenish to reddish brown with sharply defined black spots; the throat, breast and under surface of the legs are of many hues of red, green and white. The male *temporaria* starts out on his courting tour immediately. He wanders away from his winter quarters, and springs and croaks with great persistency until he meets his mate. The act of copulation and the spawning having been completed, they forsake ponds and swamps for dry meadows in search of food. This fact makes it difficult to capture them during the summer months. After spending about two months on dry ground they gradually return to their winter haunts, that is, to moist land, the males earlier than the females. It is difficult to obtain males for investigation in June and July; in August it is less difficult to get males, but the females are rarer than in the preceding months. In September specimens of both sexes are numerous.

*R. esculenta* does not copulate until May. They, too, don a holiday attire for the occasion; their coats are bright green with clearly defined black spots, and the breast and lower surface of the legs are speckled with a black and creamy white. They remain on the banks of the swamps and ponds during the summer. In the late fall they assume their bright colors for the second time, an indication of the fact mentioned by Ploetz that this species is also found in North Africa where copulation occurs at the beginning of the rainy winter period, the dry summers of the south taking the place of our winter sleep and hunger period.

There are thus two chief points of difference in the external life of the two species, (1) the time of copulation, (2) the habitation of moist places by *esculenta* during the entire year.

These facts would lead us to expect better defined periodical changes in the organs and tissues of *temporaria* than in those of *esculenta*.

#### CYCLICAL CHANGES IN THE ORGANS AND TISSUES.

Observations as to the cyclical changes in other organs than the spleen will be mentioned here only in so far as they throw some light on the changes in this organ. Ploetz in "Die Vorgänge in den Froschhoden unter dem Einfluss der Jahreszeit" states that the spermatozoa of *R. temporaria* are developed in the testes between the months of June and September. The appearance of the spermatozoa in the testes remains the same from September until the middle of March. From the middle of March to the middle of April the spermatozoa pass from the testes into the *vesa afferentia*. From the copulative act until the end of May, such products as are not excreted are reabsorbed. The testes of *R. esculenta* on the other hand remain in nearly the same condition during the entire year, always containing more or less developed spermatozoa.

It is probable from material collected, though not as yet investigated, that there is an analogous difference in the development of the sexual products of the female *temporaria* and *esculenta*.

In "Der Einfluss der Jahreszeiten auf die Leberzellen von *Rana temporaria*," I described the changed appearance in the liver of male *temporaria* at different seasons. The varying character of the blood vessels, the vacillating color and size of the red blood corpuscles, the greater or less quantity of pigment and the size and coloring of the liver cells and their nuclei were the main points.

Johannsen, in "Ringbänder der Nervenfasern," describes some remarkable histological changes which take place in the finest structure of the nerve fibers of *R. esculenta* during the months of May and June.

Gaule, in "Zahl und Vertheilung der markhaften Fasern des Froschrückenmarks" says that the spinal cords of *temporaria* are in their best condition in May, those of *esculenta*

in June, because the fibers are most clearly and sharply defined at this period.

Physiologists often find it impossible to demonstrate the laws of nerve and muscle reactions with the usual degree of accuracy in the late spring and summer, whereas the demonstrations of the same laws are comparatively sure in the fall and winter.

#### EFFECT OF ARTIFICIAL SURROUNDINGS.

It is often painfully evident to those experimenting with frogs that the reaction following certain operations, or the absorption of poisons, varies perceptibly from day to day in captivity. The cause of this inconstancy may be referred to the difference in temperature and in the character of moisture in captivity and possibly to the lack or different character of the food, though it is not sure that these influences alone suffice to explain the mystery.

Gürber in "Die Gesamtzahl der Blutkörperchen und ihre Variation" proves that complete dryness produces remarkable changes in the character of the blood. He found that frogs which had been kept perfectly dry for eight days lost more than one-half of their red-blood corpuscles in that time and that the spleens of such frogs became extremely large. He says, "I believe that the effect of dryness and moisture as here demonstrated by the varying number of the blood corpuscles must play an important rôle in the household of the frog."

The question arises whether the natural dryness which *temporaria* seeks in the summer months is not perhaps essential to the life-cycle of this species. If so, we may expect to find traces of the influence of dryness in the histological structure of the spleens during the summer months.

#### METHODS OF INVESTIGATION.

As I mentioned above, both *Rana esculenta* and *temporaria*, were made the subject of study. In the earlier part of

every month, usually between the 7th and 15th, 12 frogs (3 *esculenta* ♂, 3 *esculenta* ♀, 3 *temporaria* ♂, 3 *temporaria* ♀) were selected for our purpose from the large number delivered at the laboratory. Those of as nearly the same weight as possible were selected. In order to avoid the effects of captivity the frogs were collected the day before delivery and killed on one of the two following days. The material for all twelve months was not completed in the first year. Advantage was taken of this fact in the succeeding two years not only to supply deficiencies but also to increase the material generally.

The frogs were killed by cutting through the spinal cord and then disturbing this and the brain with a needle. The abdominal cavity was then cut open, the spleen laid bare and so severed from the mesentery that it might fall directly into a bottle of blood warm corrosive sublimate (concentrated solution). The labeled bottles were placed in the warm oven (39° C.) for 2 hours, after which the preparations were rinsed in distilled water and left to stand covered with distilled water in the oven for a half hour. They were then rinsed again in distilled water and put into 70 per cent alcohol. The remainder of the method for imbedding in paraffine is described in Gaule's "Zahl und Vertheilung etc.," cited above. The imbedded spleens were cut into sections about 2.5 to 5 $\mu$ <sup>1</sup> thick with the microtome and fastened upon the slide in the manner described in the same article. The paraffine was dissolved with xylol, the preparation cleared with oil of cloves and then brought into 100 per cent alcohol. The sections were stained with the four coloring fluids, haematoxylin, nigrosin, eosin and safranin, in the same manner and order described in "Methods of Staining and Fixing the Elements of the Blood,"<sup>2</sup> except that sections require a different

<sup>1</sup> When the spleens contained large quantities of blood it was often impossible to cut whole sections of  $\frac{1}{400}$  mill. thickness. For the size measurements whole sections were necessary, for the study of histological details the thin sections, consequently I made one or two thicker sections of every spleen and many thin ones. Care was taken to make sections through the largest diameters of the spleens.

<sup>2</sup> *Vide*: American Naturalist, July, 1887, p. 677.

length of time to absorb the staining fluid. Haematoxylin, according to its freshness, demands from 5 to 10 min., nigrosin 2-5 sec., eosin 2-5 sec., safranin 5-10 min. Care must be taken not to leave the preparations in absolute alcohol too long after treatment with safranin as this color and eosin are very soluble in alcohol. The further treatment is also described in the same article. The material was then ready. A general inspection of the spleen showed that not only the size of the spleen, but also its general and detailed histological character, varied from month to month. Individual frogs, in turn, varied frequently in the same month, still the impression was that the variations were subject to certain laws, and so, owing to the vast material, — the spleens of over 150 frogs — I decided to arrange notes and measurements in the form of curves. The months of the year formed the abscissa in every case, the measurement, number, or proportion respectively the ordinates. The curves were made at first for every separate spleen being grouped under four heads *esculenta* ♂ and ♀ and *temporaria* ♂ and ♀. Later the average curve under each head was computed for every month. They were computed by adding the individual curve for every spleen for the month and dividing this result by the number of spleens investigated for that month.

#### GENERAL STRUCTURE OF THE SPLEEN.

The spleen of the frog has an outer fibrous covering which varies in thickness in different frogs. The large splenic artery enters the spleen at the same point that the splenic vein leaves it. The arterial blood is carried nearly to the centre of the spleen by the artery which then divides into endless capillaries and meshes extending nearly or quite to the fibrous capsule. Here the meshes often become wider and are sometimes seen to form a vein which can occasionally be followed along the inner surface of the capsule and probably enters the splenic vein. A large vein is also frequently seen returning along the splenic artery. The spleen of the frog has no distinct trabeculæ like those of mammals. This is about all that can be said to be quite constant in the spleen of every

frog, for the cell-elements which form the bulk of the splenic tissue are exceedingly varied in kind and quantity.

#### CELLS AND CELL-ELEMENTS OF THE SPLEEN.

These are : I. The cells of the blood, stained with the four coloring fluids mentioned above, were described in "Methods of Fixing and Staining the Elements of the Blood" already cited. The fourth staining fluid, safranin, changes the picture in but few points. The nuclei of the red blood corpuscles often show a great affinity for it and certain nucleoli, called by Gaule plasmosomes, hold the color fast. The blood corpuscles comprise, *a*, the red blood corpuscles, *b*, the white blood corpuscles or leucocytes, including the "Halena toflacts" the eosinophilous cells, the amoebocytes, the "nurse" cells or the endotheloid cells.

II. The endothelial cells and the cells of the adventitia play a comparatively insignificant part in the spleen of the frog.

III. Cells resembling those of the Malpighian corpuscles of the mammal spleen, which I shall designate *follicle cells*. Groups of these cells are so densely crowded with nuclei, that one can scarcely distinguish the protoplasm as such. They do not always occur in the forms by which the Malpighian corpuscles are usually illustrated but in very irregular forms, occasionally making a large proportion of the splenic tissue. At times the nuclei are larger and have distinct nucleoli, at others they are firmly granulated or almost homogenous in appearance. In certain spleens the cells are interspersed with eosinophilous cells or the eosinophilous cells circumscribe the group of follicle cells, in other spleens one sees cells in the process of division, in others a part of the cells are displaced by pigment. Occasionally one finds a partially developed or imperfect red blood corpuscle imbedded between the nuclei.

IV. The pigment of the spleen is black, brown, yellow or yellowish grey varying as well in color as in quantity at different seasons and in the different species.

V. The cytozoa described by Gaule in "Ueber die Beziehung der Cytozoen zu den Zellkernen"<sup>1</sup> and in his Strassburg lecture,<sup>2</sup> also play an important part in the life-cycle of the spleen.

VI. Cell division was observed in the endothelial cells, in the follicle cells and rarely in amoebocytes. There is a great difference in the character of the caryokinetic threads in the various spleens. I gained the impression that the threads were finer and more homogeneous in the spleens of the *temporaria* than in those of *esculenta*. The threads of *esculenta* figures of cell-division were often composed of tiny granules. The color of the *temporaria* figures varied from red to violet.

VII. The proportion of the protoplasm to the nuclear substance is a further element of variation and is characteristic of the two species. The protoplasm of the *temporaria* spleen stains more intensely with nigrosin than the protoplasm of the *esculenta* spleen.

VIII. Even the nuclei present an entirely different appearance at different seasons. The size of the nucleoli, their affinity for color, their tendency to wander out of the nucleus and even out of the cell, changes the whole aspect of the microscopic picture. Curves were made of the nucleoli called plasmosoma as to a certain extent typical of these changes. The nuclei as a whole vary greatly in size and in the tints of their main substance.

Other elements occurring at rare intervals, and not further investigated, were tiny spheres and crystal-like bodies, lying in the blood-vessels and capillaries, and colored homogeneously with cosin. Fragments of red blood corpuscles, undeveloped corpuscles, and megalocytes were also too superficially investigated to be especially described here.

<sup>1</sup> Archiv für Anatomie und Physiologie, 1881.

<sup>2</sup> Ueber die Bedeutung der Cytozoen für die Natur der thierischen Zellen. 58. Versammlung deutscher Naturforscher und Aerzte in Strassburg. Tageblatt 18-23. September 1885.

METHODS OF DETERMINING THE CURVES.<sup>1</sup>a. *Size Curves.*

As was stated above, care was taken to make sections through the largest diameter of the spleen. The largest section of every spleen was selected and drawn with the camera lucida upon millimeter paper. The instrument used was a Zeiss stand with eyepiece 4 and Leitz objective 1. The tube of the microscope was always of the same length and stood perpendicular to the drawing surface. The angle of the mirror was carefully regulated. The section was placed as near the center of the field as possible, before it was drawn. The number of square millimeters included within the outline of the section was then counted, the result thus obtained becoming the elements of the preliminary or individual curves.

b. *Blood Curves.*

The quantity of blood in the spleen was taxed according to the relative proportion of the red-blood corpuscles and the size of the blood-vessels and capillaries to the remaining tissue of the spleen. If the spleen was judged to contain about equal parts of blood tissue and spleen tissue, this was indicated by 10 on our scale; if the proportion was  $\frac{1}{4}$  spleen tissue to  $\frac{3}{4}$  blood tissue, we noted 15; if  $\frac{3}{4}$  spleen tissue to  $\frac{1}{4}$  blood tissue, 5.

c. *Eosinophilous Cells.*

The frequency of their occurrence was determined as follows: A large thin section was selected, and since the "eosinophilous" cells are especially abundant near the surface of the spleen, I counted the number occurring in as broad a zone near the circumference of the section as I could well oversee

<sup>1</sup> The simplest method of determining the size of the spleen, that of weighing it, could not be practically adopted, because of its minuteness and the fear of disturbing the microscopical structure. The form of the spleen is, further, so irregular that it would scarcely have been more accurate to estimate the volume from the area or diameter.

with the microscope. I used a power (Zeiss oc. 4, obj. D.) high enough to enable me to see the cells easily and yet overlook as much of the spleen at a time as possible. After counting those in the circumference, I counted those in two diameters at right angles to one another, taking care not to recount those already enumerated. When the number for a section was determined I classified them under four heads—0-5, 5-10, 10-15, 15-20, in order to simplify the curves; 0 = 0, 5 = 10 eosinophilous cells, 10 = 50 cells, 15 = 100 cells. Every number beyond 15 represented 100 cells.

d. *Follicle-Cell Curves.*

In estimating the amount of follicle cells I was obliged to rely upon the oft-repeated comparison of the spleens. An opinion was then formed for each spleen in turn, and the result noted in the form of a number, 5 representing a small proportion of follicle cells, 10 many, and 15 very many.

e. *"Nurse"-Cell Curves.*

The proportion of these cells was determined in the same way as the foregoing ones, it being impossible to count them on account of their indefinite form and variable character.

f. *Cytophaga Curves.*    g. *Pigment Curves.*

*f* and *g* were determined in the same manner as *c* and *d*.

h. *Curves of the Figures of Cell-Division.*

They were counted simultaneously with the eosinophilous cells. The resulting numbers 5 = 5 figures, 10 = 10, 15 = 20 figures.

i. *Plasmosoma Curves.*

Their proportion was determined like the curves *c*, *d*, *e*, *f*.

DESCRIPTION AND COMPARISON OF THE CURVES.

a. Size Curves.

The general features of the curves indicate that the spleens are larger in summer during the food-period than in winter. They are smallest at the close of the food period and before the commencement of the same, or immediately preceding copulation. The *esculenta* spleens are larger than the *temporaria* spleens. There is a greater difference in size between the summer and winter spleen of *esculenta*, than between the summer and winter spleen of *temporaria*. The difference in size of the summer and winter spleen is greater with the male than with the female.

b. Blood Curves.

The spleens are seen to contain less blood in the first months succeeding copulation than at almost any other time of the year, although the period of abundant food has already begun. During the remaining months of the food-period, August, September, October, contain a maximum quantity of blood.

The curves of the male and female *temporaria* are very similar, whereas those of the male and female *esculenta* are unlike. The spleens of both sexes of *temporaria* contain a small quantity of blood during the hunger period. In May, the month

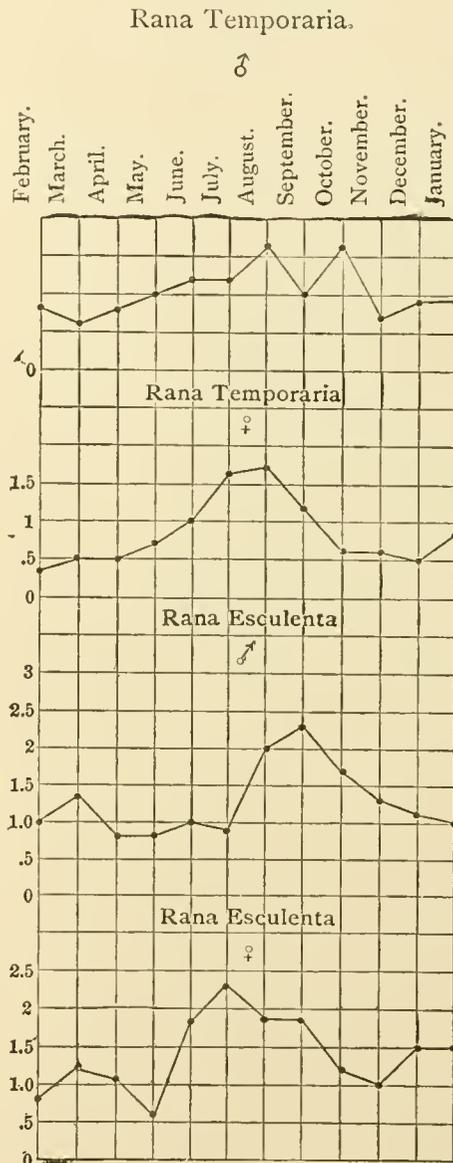


Fig. a. — Size Curves.

immediately following copulation, the spleens are better supplied with blood. A minimum quantity of blood is reached in June and July, when they wander to the dry meadows. The *esculenta* spleens contain a slightly larger proportion of blood than *temporaria*. The female spleens are larger proportion than the male spleens. If we compare Figs. *a* and *b*

we find that the size of the spleen is not alone dependent upon the quantity of blood it contains. This is notably true in the months of June and July.

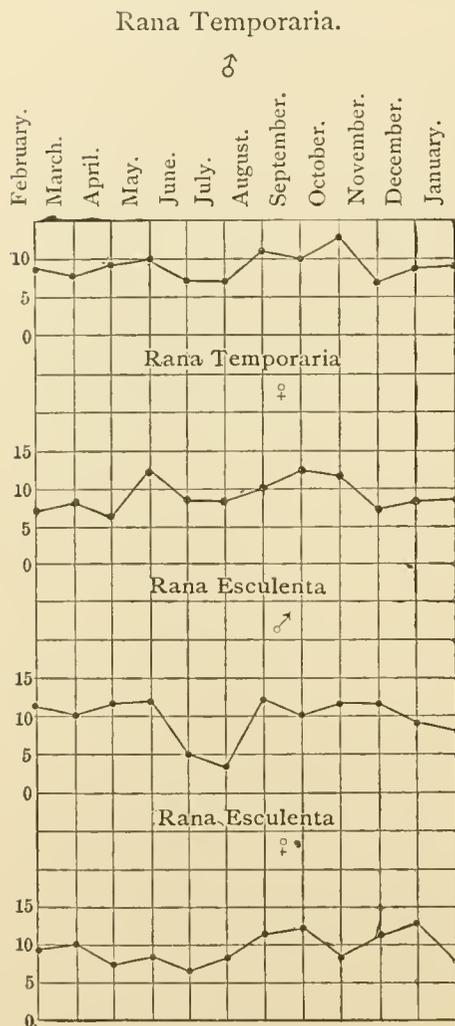


Fig. b. — Blood Curves.

*varia*, male and female, reach their culmination in February. The Fall culmination occurs in September. The Fall culmination of *esculenta* falls in November.

The curves of the same sex are more typically alike than the curves of the same species. The male spleens contain more eosinophilous cells than the female. If we compare

### c. Eosinophilous Cell Curves.

In general we learn from these curves that the spleens contain more eosinophilous cells during the period of abundant food and fewer in the hunger period or in winter. This is especially evident at the beginning of the food period in June and July. The spleen contains the fewest such cells in April and May. The *temporaria* spleens possess more of them than the *esculenta* spleens. The number of eosinophilous cells varies more distinctly in the *temporaria* than in the *esculenta* spleens. The winter curves of *temporaria*

Figs. *a* and *c* we come to the conclusion that the eosinophilous cells increase in number when the spleens diminish in size. The April and May spleens are an exception, as they contain very few eosinophilous cells, and yet the spleens are comparatively small. By a comparison of Figs. *b* and *c* we discover that the number of eosinophilous cells is very large in June and July, when the quantity of blood was found to be especially small.

d. *Follicle-Cell Curves.*

In general the spleens contain more of these cells in the hunger period than in the food period. All the curves indicate that the spleens contain a great many of these cells in July and very few in February and March.

The *esculenta* spleens contain more of these cell-groups than the *temporaria*. The male spleens contain more such cells than the female. After almost disappearing from the spleens in August, September or October, they become more abundant again in the male spleens in September or October, and in the female spleens in November. A comparison of Figs. *a* and *d* indicate that the follicle cells have something to do with the increase in size of the July spleens—especially of the female spleens—and hence probably plays an important part in its function. Figs. *b* and *d* teach us that during the summer the spleen is more abundantly supplied with blood when the number of follicle cells decrease. Figs. *c* and *d* show a similarity of general outline, the summer elevations of the follicle-cell curves being coincident with or succeeding those of the eosinophilous cell curves. If we

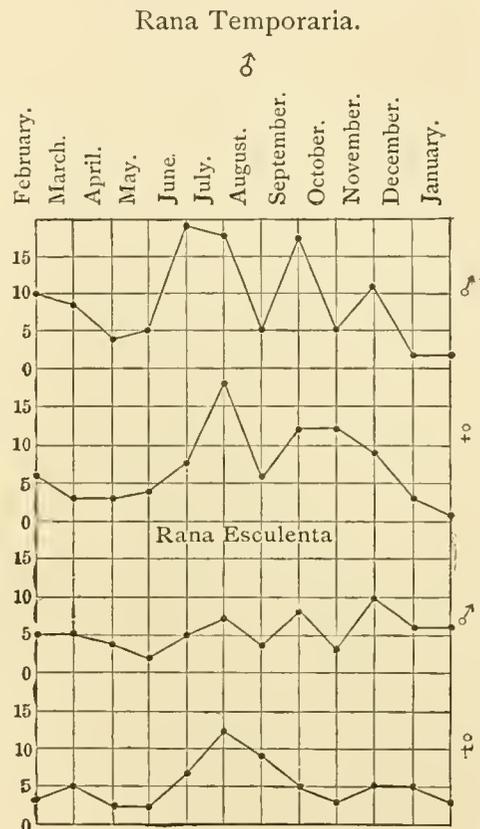


Fig. c. — Eosinophilous-Cell Curves.

recall the results of Gürber's investigation, that the white blood corpuscles increase under the influence of dryness, and then consider that the eosinophilous cells are white blood corpuscles, and that the follicle cells, if not white blood corpuscles, are closely allied to them in character, it then seems possible that the summer elevations of these two curves may

be in part the result of the dryness, but only in part, for the abundant nourishment, and possibly still another influence, must be taken into account.

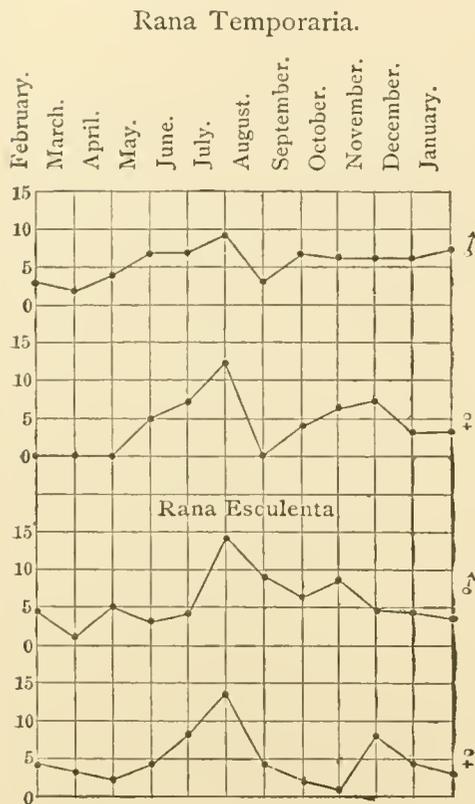


Fig. d.—Follicle-Cell Curves.

female spleens in the second month following the month of copulation. The *esculenta* spleen is richer in "nurse" cells than the *temporaria*. They are most abundant in the *esculenta* spleen in March and April, in the *temporaria* spleen in March and April. The curves of the species are typical. The male spleens contain a greater number of "nurse" cells than the female.

A comparison of Figs. *a* and *e* indicates that the *temporaria* spleens contain few of these cells when the spleens are large and more when the spleens are small. May and June spleens are an exception, for here the first slight increase in size is ac-

#### e. "Nurse"-Cell Curves.

In general there are fewer "nurse" cells in summer during the abundant food period, more in November at its close, and the most in February, March and April, immediately preceding the period of copulation. During the month of copulation there are very few of these cells in the spleen, but the number increases again in the male spleens in the month immediately following, and in the

accompanied by an increase in the number of "nurse" cells. The same is true of the *esculenta* spleens in summer, whereas in winter the elevations of the "nurse" cell curves nearly correspond to the elevations of the size curves. The comparison of Figs. *b* and *c* seems to teach that the spleens containing a small quantity of blood are richer in "nurse" cells than spleens containing a large portion of blood. In comparing Figs. *c* and *e* we find that the elevations of eosinophilous cell curves in November coincide with the elevation of the "nurse" cell curve for the same month. The May and June elevations of the "nurse" cell curves precede the June and July elevations of the eosinophilous cell curves. The elevations of the follicle cell curves (Figs. *d* and *e*) succeed those of the "nurse" cell curves, thus coinciding, in part, with the eosinophilous cell elevations, as was noticed above. If we recall the facts concerning the "nurse" cells given in Gaule's Strassburg lecture and his opinion that the "nurse" cells are developed from the other white blood corpuscles, it no longer estranges to find that the proportion of these elements varies among themselves at a time when the tide of blood in the spleen is at its ebb.

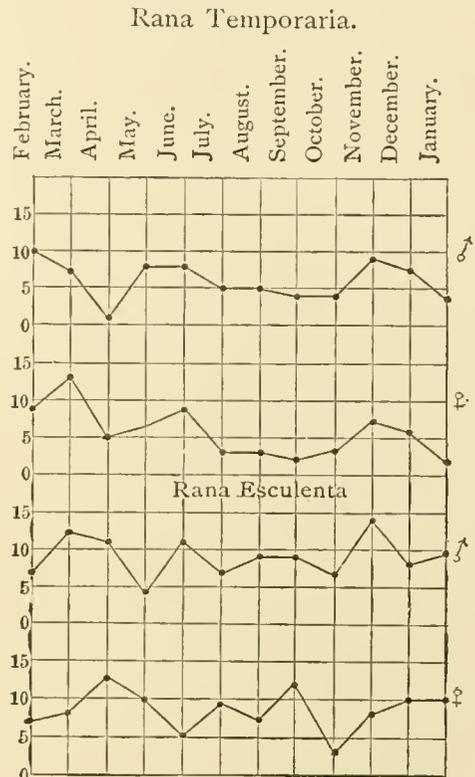


Fig. e. — "Nurse"-Cell Curves.

f. *Cytozoa* Curves.

The general conclusion to be drawn from these curves is that the cytozoa are more frequent soon after the month of copulation and after the close of the food period. They are more numerous in the *esculenta* than in the *temporaria* spleens.

They are more numerous in the male than in the female spleens. Since they are principally seen enclosed in the "nurse" cells, it is not essential to compare their curves with those of the other elements of the spleen separately.

g. *Pigment Curves.*

The general conclusion from these curves is that there is more pigment in the spleen in winter than in summer. There is less pigment in the spleen during the period of copulation than immediately before or after this act. The *esculenta* spleens have more pigment than the *temporaria* spleens, the males less than the females. Small spleens contain comparatively more pigment than large spleens. Spleens containing a large proportion of blood are poor in pigment. There is little pigment in spleens containing many follicle cells. The cytozoa and pigment curves combine to make the "nurse" cell curves, since the cytozoa and pigment are a product or element of the "nurse" cell. The predominating color of the pigment in the *esculenta* spleen is yellow, the black pigment, however, exceeds the yellow in the male spleen in the month of May. The predominating color of the pigment of the *temporaria* spleen is yellow in the summer, yellow-grey in winter. Black pigment predominates at the end of the food-period, and in the male spleen in May.

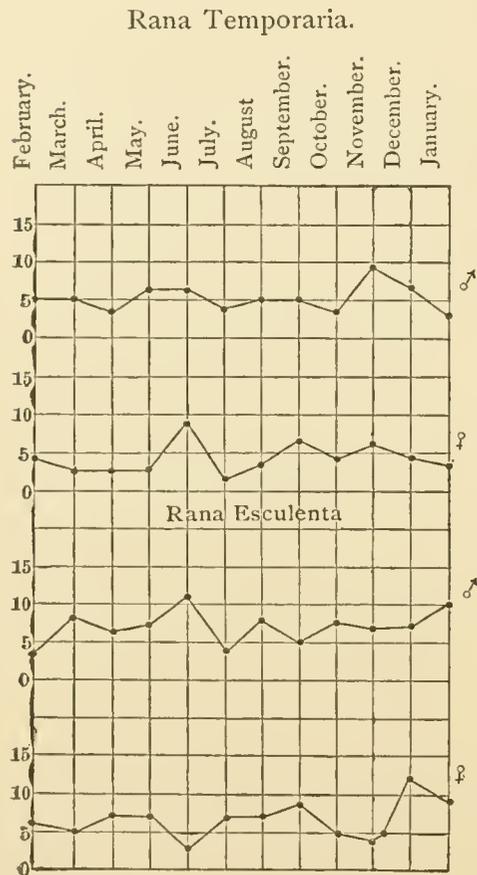


Fig. f. — Cytozoa Curves.

h. *Figures of Cell-Division Curves.*

It is evident from the curves that cell-division in the spleen is confined to a certain period, this occurring in the months of May, June, and July. They are most frequent in the female spleen in June and July; in the male in May and June.

i. *Plasmosoma.*

In general they are more frequent in winter than in summer. The presence of plasmosoma in the nuclei indicates that the nuclei are larger and more distinct in such spleens than in others, and that the structure of the nuclei has assumed a special character. Their curves must be looked upon as merely a preliminary study of the minuter structure of the cells of the spleen.

k. *Protoplasm.*

From my notes I learn that the *esculenta* spleen contains a greater proportion of protoplasm, as compared with the nucleus-substance, than the *temporaria* spleen; the male spleens a greater proportion of protoplasm than the female. From July to October the protoplasm has a net-like character, as though it contained fat or some fluid in its meshes. The protoplasm of the *esculenta* spleen is more nearly homogeneous than the *temporaria*.

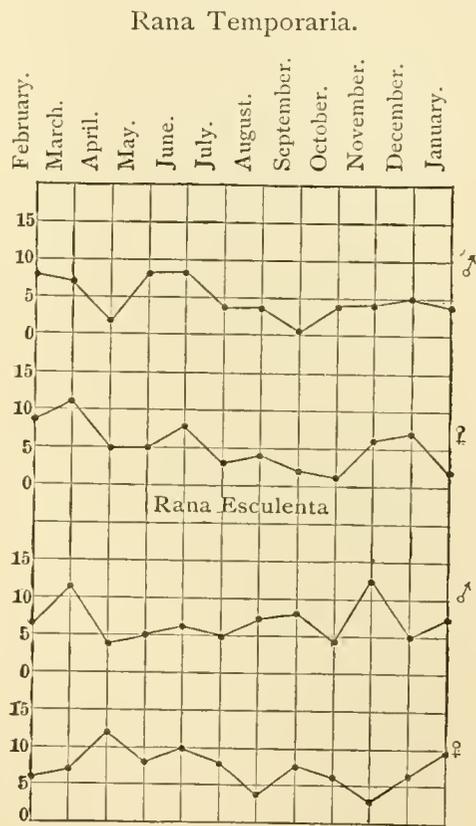


Fig. 7. — Pigment Curves.

*Summary* (compare Figures *k, l, m, n*).

If we group the curves of the single elements over one another, under the four heads, *temporaria* ♂ and ♀, *esculenta*

♂ and ♀, it is easy to get a general impression of the succession of the curve elevations and depressions and their possible relation to the two periods of the yearly cycle. We see, then, that these two periods correspond chiefly to the food or summer period, and to the hunger or winter period. The spleens are much larger in summer than in winter, the increase in size being accompanied in June and July by an increase in the number of white-blood corpuscles, that is, eosinophilous cells, follicle cells, and

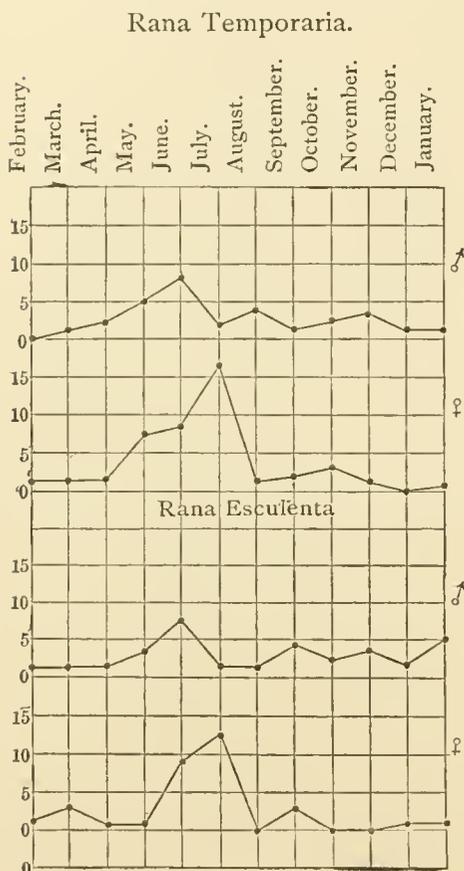


Fig. h. — Figures of Cell-Division.

of *esculenta*, and that the eosinophilous cells are characteristic of this species. The outlines of the blood, "nurse" cells, pigment, cytozoa, follicle cell, and plasmosoma curves are similar in the two sexes, which indicates that the annual chemical processes are on the whole alike in both.

The curves of *esculenta* teach that the spleens are larger than the *temporaria* spleens, and that follicle cells, pigment, cytozoa, and a greater of protoplasm are characteristic of this species. The outline of the "nurse" cell and follicle cell

follicle cells, and "nurse" cells, later, in August and September, by the increased amount of blood, as indicated by the number of red blood corpuscles and the diameter of the blood-vessels and capillaries. As the food period comes to a close, the number of white-blood corpuscles, pigment, and follicle cells increase again to fall below the average proportion during the winter. During the months directly preceding the period of copulation, the number of white blood corpuscles and the amount of pigment increases.

The curves of *temporaria* teach us that the *temporaria* spleens are smaller than those

curves are similar for the two sexes. The blood curves indicate that the quantity of blood in the spleen does not vacillate as much in the *esculenta* as in the *temporaria* spleen.

The more typical character of the *temporaria* spleen may be referred to the greater distinctness in the periods of the year's cycle, the fact that they have a dry food period in June, July and August, a moist food period in September and October, and that the sexual products, as we know for a certainty of the males, are developed almost entirely during the summer months from June to September. The *esculenta*, on the other hand, keep to the swamps and ponds for their nourishment during the entire food period, and the sexual products are in process of formation during the entire year. All these facts must have an inference upon the character of the cells in the spleen.

There is, further, a certain similarity in some curves of like sexes. Thus the male spleens are characterized by a greater number of eosinophilous cells, follicle cells, more cytozoa and more protoplasm than the female. The outlines of the eosinophilous, "nurse" cell and figures of cell-division are similar in the male spleens, the elevations coming a month later in the *esculenta* than in the *temporaria* spleen. The quantity of blood does not vary as much in the male as in the female spleen.

Characteristic for the female spleen is the greater amount of pigment and the larger amount of blood, which vacillates greatly in different months. The difference in character and mass of the sexual products partially explains these characteristics of the female spleen. The outlines of the size, eosin-

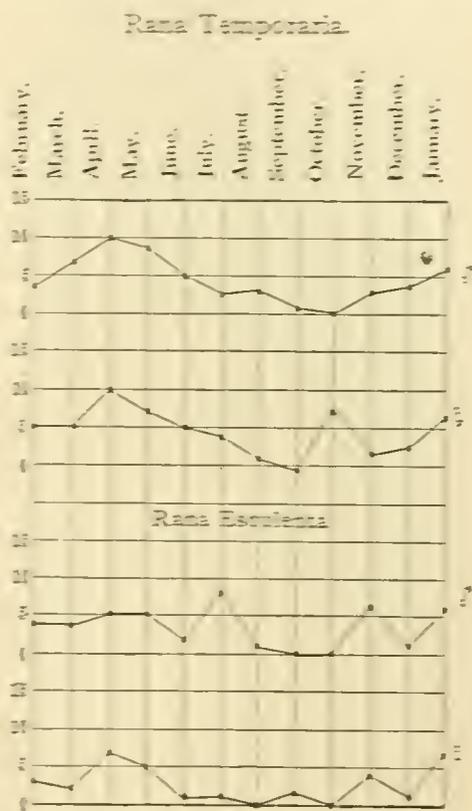
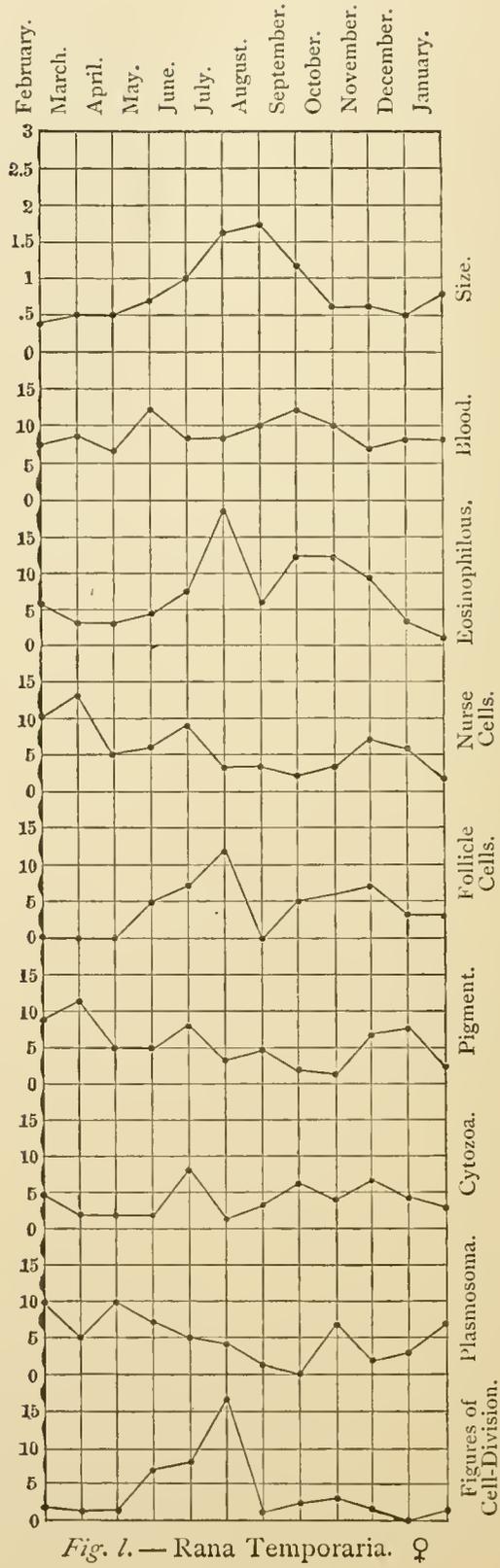
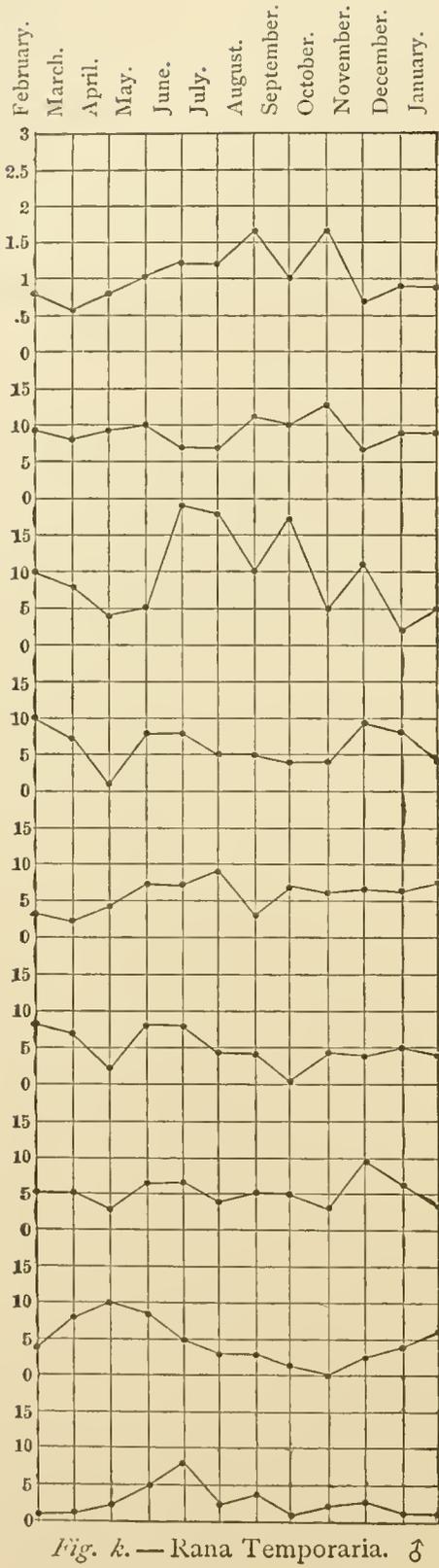


Fig. 1. — Plasmosoma Curves.



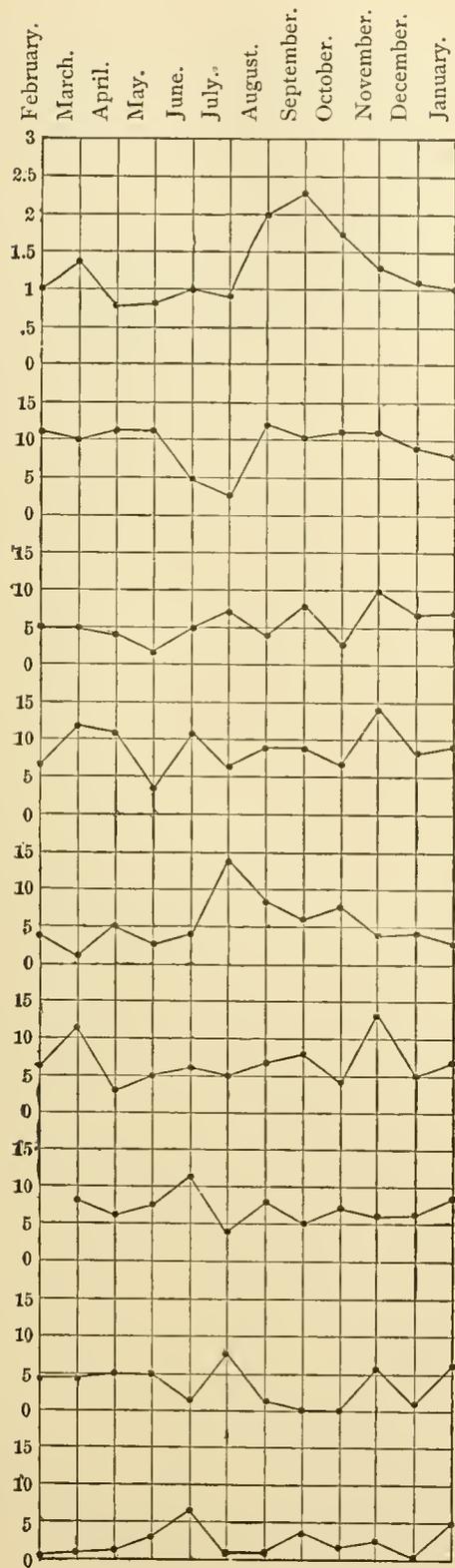


Fig. m. — *Rana Esculenta*. ♂

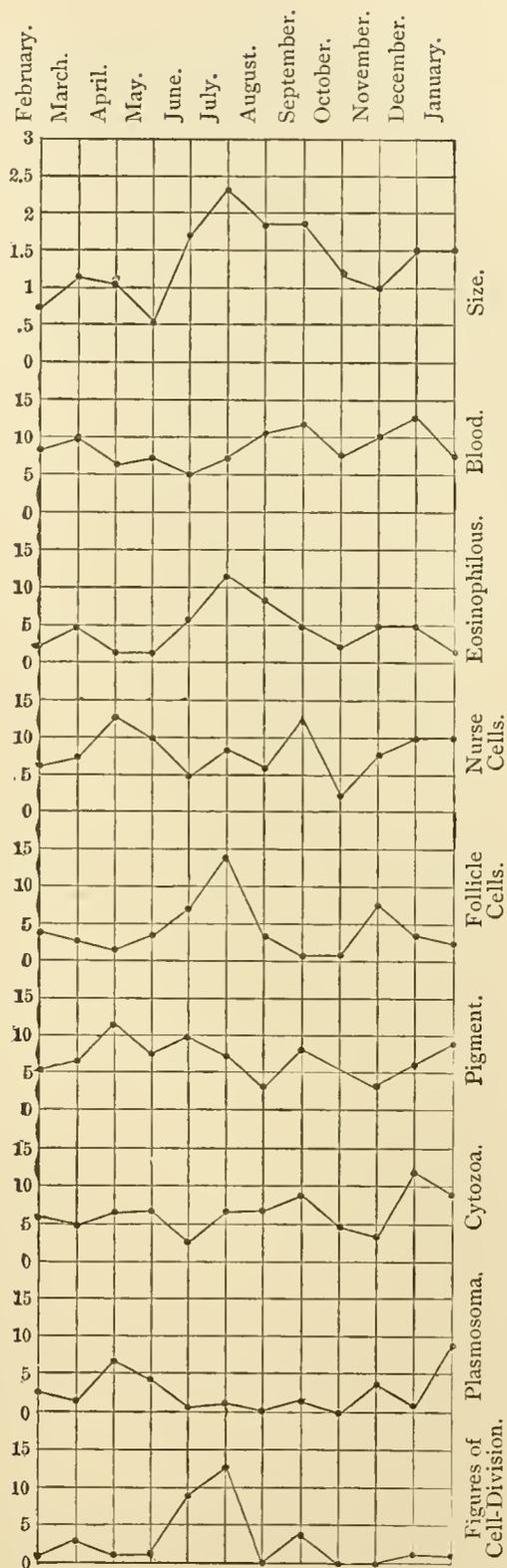


Fig. n. — *Rana Esculenta*. ♀

ophilous cell, follicle cell, pigment, plasmosoma and cell-division curves are similar.

A closer analysis of the curves than is given here is reserved for a more extended article and until a further study of the female sexual organs and the cyclical changes in other organs and tissues of the body has been completed.

The present curves, at the least, demonstrate the fact that the character of the microscopical elements of the spleen are to a certain extent dependent upon the external surroundings and habits of the frog.

ZURICH, August 8, 1892.

## HISTOGENESIS OF THE RETINA IN AMBLY- STOMA AND NECTURUS.

F. MALL.

THE various researches during the last fifty years on nerve cells and nerve fibers give us a very clear insight into the real meaning of the latter. Shortly after the cell-doctrine was propounded by Schwann, a series of studies were made upon the nerve fibers, the most prominent of which were those of Remak and of Helmholtz. Both of these observers showed that the nerve fiber is a prolongation of a nerve cell, although their studies were not upon cerebro-spinal nerves. About fifteen years later Deiters showed that the same held true for these nerves also, and from this time on histologists and physiologists accepted the discovery of the above investigators.

With these discoveries clearly in mind, and with the knowledge that the whole nervous system is ectodermal in its origin, it is no wonder that His's attention was constantly directed to the ganglion cell as the only originator of the nerve fiber. The growth of the nerve fiber had already been followed by Kölliker in the tadpole's tail, but this did not prove that the fiber arises from the ganglion cell. While investigations were being made to show that the nerve fiber is a direct continuation of a cell, a number of others were being made to show that a group of cells participate in the formation of a single axis cylinder; and it is remarkable that some of these are being published even at the present day. His was soon able, by direct observation, to show that, in its development, the fiber arises from a cell and then grows to its point of destination. Studies in nerve regeneration indicate that the same must be true. His's observations were immediately confirmed by Ramón y Cajal, who stained nerve fibers while they were developing by means of staining with a modification of Golgi's method. In addition to these two methods we have also Ehrlich's, and, by these

light is gradually being thrown upon the structure and architecture of the nervous system.

From the purely anatomical standpoint we now ask ourselves regarding every nerve, "from what cells does it arise?" In nearly all cases we find that the nerves have a mixed origin in all more complex animals, and this should be the case, for only with this mixture of nerves can the complexity of the animal be brought about. In lower animals a nerve is often confined wholly to one metamer while in the higher animals this is rarely the case. No matter how great the mixture of nerves is, the following generalizations apply to all nerves which have been carefully studied.

1. *The primitive growing point of all vertebrate nerves is in the layer of cells on the outermost side of the ectoderm and the axis of division is parallel with the ectoderm.*

I do not wish to enter into many details regarding these points in this paper, but add the following cases. It applies to the whole central nervous system, to the sense organs of the ectoderm and to the eye, ear and nose. In the latter, however, the nerve cells sink into the thickened ectoderm of the olfactory pit and then the planes of division can no longer be followed. The spinal ganglia also caused me a great deal of trouble for the relation of the plane of division to the ectoderm is lost as soon as the mass of cells separates from it. Furthermore, the ganglia of the tail of the animal do not arise directly from the neural crest but by a system of sprouts from the more anterior ganglia. In the latter case it is of course impossible to follow the relation of the plane of division to the original outside of the body.

2. *The direction of the transmission of an impulse is already determined by the position of the cell in the ectoderm.*

The afferent, or receiving pole of the cell is on its free side, *i. e.*, the side which originally communicated with the exterior of the body. The efferent, or giving pole is on the basal side of the cell. The nerve fiber from either or both poles may become very long and the nerves of the more complex organs are made up of a chain of two or three cells, in which the free side of one and the basal side of the other are more

or less highly specialized ; the middle cell is often bipolar and reaches from the first to the third.<sup>1</sup>

<sup>1</sup> The more rudimentary arrangement is when the sensory cell reaches from the ectoderm to the brain, then in turn the brain cell sends a prolongation to the muscle, thus making a very simple reflex circle. The beautiful researches of v. Lenhossék and of Retzius on *Lumbricus* show this state of things. In the olfactory organ we have a single cell reaching from the ectoderm to the brain as shown by His and other investigators. The arrangement becomes more complex in sense organs like the ear, taste-buds and eye.

In the eye the chain is composed of three cells; the rods and cones, the bipolar cells of the inner nuclear layer and the ganglion cells of the retina. Moreover we have in all probability nerves reaching from the brain to the retina as indicated by Monakow and Ramón y Cajal. In this manner we can pass through the whole system of sense organs and always find that the direction of the activity of the cell is always indicated by the pole which originally communicated with the exterior of the body.

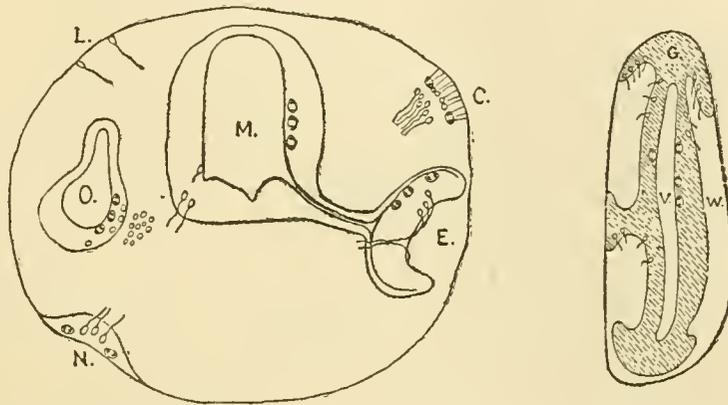


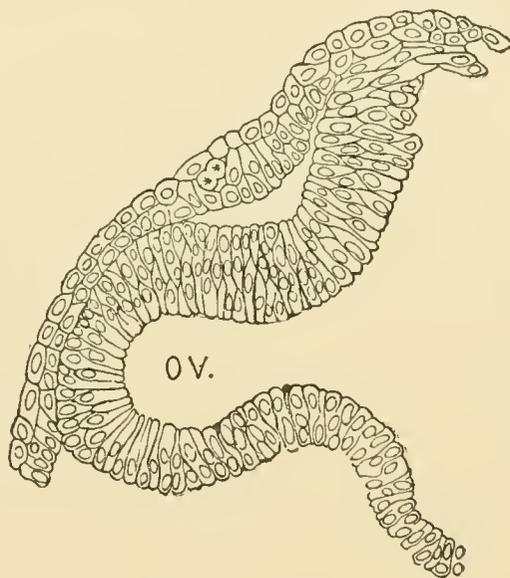
Fig. 1.—Diagrammatic transverse section of the head of an embryo, to show the growing point in the nervous system and the direction of the growth of the fiber. *M*, medullary canal; *E*, eye; *O*, ear; *N*, nose; *C*, cephalopod eye; *L*, sense cells of the skin of *Lumbricus*.

Fig. 2.—Longitudinal section of the cerebral hemispheres of *Necturus*. *V*, ventricle; *W*, white matter; *G*, grey matter extending from the ventricle to form a rudimentary cortex. The growing point and the direction of the nerve fiber is indicated.

Difficulties are met with in the formation of the gray matter, and also the ganglia of the encephalon, but I think a careful investigation of them has shown me that the same also holds true in these parts. In the gradual change of gray matter from the ventricle of the brain in lower animals to the cortex of the higher, the cell undergoes a half revolution and the side which originally pointed toward the ventricle now points toward the surface of the brain. The only serious obstacle with which I have met are the cells of the dorsal ganglia of higher animals. The cells continue to divide after they are separated from the neural crest, and moreover they become unipolar. This must be viewed as a secondary change, as originally they were bipolar. The general indication is that the pole which gives rise to the nerve fiber is the original basal end of the cell. Figs. 1 and 2 indicate in a general way the points I have emphasized. The sense cells of the skin of *Lumbricus*, as well as the cephalopod eye, are indicated in the diagram. (For literature regarding these points, see Altmann, His, Merk, Rabl and others.)

With these ideas clearly in mind it is quite easy to understand why the optic nerve perforates the retina in the vertebrate eye and passes directly to the brain in the invertebrate. They also tell us why primitive eyes are composed of but a single layer of cells sending nerve fibers directly to the brain, and why in the more complex eyes a chain of cells is used for the same purpose.

In the present study it has been my aim to follow the formation of the retina from the time the optic vesicle is well marked to the beginning of the optic nerve. Eycleshymer<sup>1</sup> has already shown us that in *Amblystoma* and *Necturus* the optic cups are well marked long before the neural canal begins to



*Fig. 3.*— Section through the eye of *Amblystoma* (3.5 mm. long).  $\times 66$  times. From a drawing by Mr. Eycleshymer. *O.V.*, optic vesicle.

close by pigmented areas which are slightly cup-shaped, and are made up of a single layer of cylindrical cells with the pigment at either end of them. As the areas grow more and more to form cups the mitoses are more numerous in them than in the surrounding ectoderm which is to form the neural canal. The pigment gradually becomes more and more diffuse and serves to a great extent in following the differentiation of the retina. "During the later stages of development the walls of the vesicles become thinner, so that just before they invaginate to

<sup>1</sup> Eycleshymer, *Journal of Morphology*, Vol. VIII.

form the optic cups they consist of but a single layer of elongated cells with their nuclei located in their peripheral ends. A continued dispersion and migration of the pigment has also taken place, and is now more abundant in the stalk than in the portion which will later form the optic cup."<sup>1</sup> Fig. 3 gives the outline of the cells of the optic cup when it is once well formed. The evagination is complete and at this stage the mitoses are not in the cells of the cup, but almost wholly at the point where the cup joins the brain. The pigment outlines the cells very well and in general is well diffused throughout the retina. The cell itself is filled with yolk

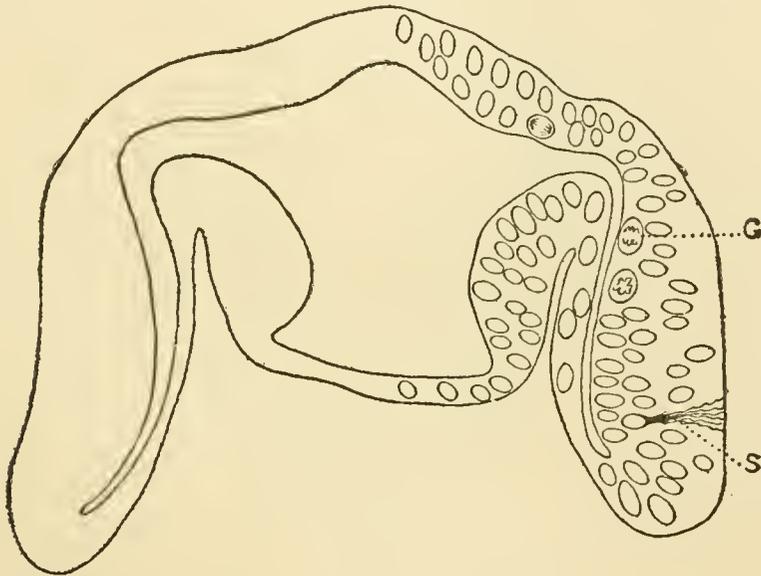


Fig. 4.— Section through the brain and eye of *Amblystoma* (4 mm. long) seventeen days before hatching. Hardened in Flemming's solution.  $\times 100$  times. S, spongioblast; G, germinating cell.

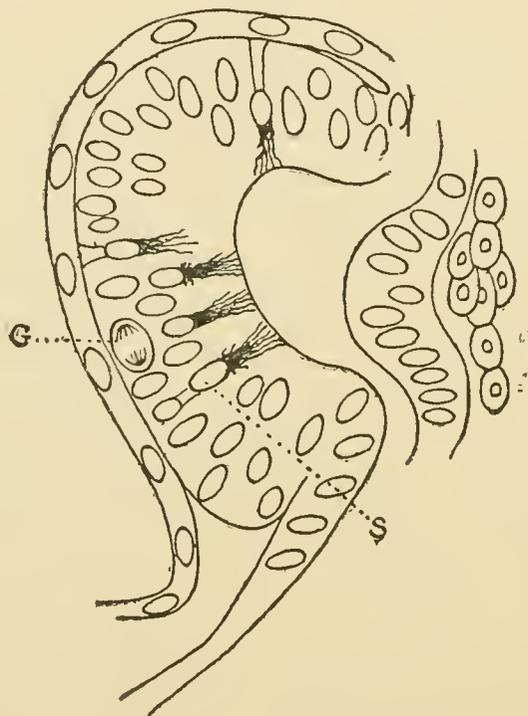
granules and its nucleus is located in its extreme peripheral end.

In an embryo which is somewhat older the optic cup is flattened and the optic stalk has become smaller than it is in the stage just described. This stage, Fig. 4, shows most beautifully the formation of the first spongioblasts and is, therefore, of the greatest value to us in our study. His<sup>2</sup> has already

<sup>1</sup> Eycleshymer, *l. c.*, p. 193.

<sup>2</sup> His, *Abhandl. d. königl. säch. Gesellsch. d. Wissenschaften*, Bd. XXVI, and *His u. Braune's Archiv*, 1889. Also, *His u. Braune's Archiv*, Supplement-Band, 1890, and *Verhandl. d. X. Internationalen Med. Congresses*, Bd. II, Berlin, 1891.

shown us how the spongioblasts are formed and his view is in general confirmed by the present study. It is quite easy to follow their formation in *Amblystoma*, because the cells of the retina are loaded with yolk and pigment granules. Just before the stage pictured in Fig. 4 is reached, the nucleus is in the free end of the cell and as it moves towards the base of the cell the protoplasm of its peripheral end is gradually broken up into a mass of fibrils. By what process this takes place is extremely difficult to determine, but as the fat globules vanish from the peripheral end the pigment adheres to the protoplasmic



*Fig. 5.* — Section through the eye of *Amblystoma* (6.5 mm. long) thirteen days before hatching.  $\times 133$  times. *G*, germinating cell; *S*, spongioblast.

filaments of these cells and soon we have spongioblasts as indicated in Fig. 5. The cell process arising from the spongioblasts often appear to communicate with one another but careful study of many specimens indicates that in all probability this is not the case. Any line of pigment granules communicates only with the body of one cell and does not continue over to that from a neighboring cell. This view is materially strengthened by the observations of Ramón y Cajal<sup>1</sup> upon the

<sup>1</sup> Ramón y Cajal, *Anatom. Anz.*, 1889.

adult retina. By means of Golgi's method he succeeded in outlining large cells extending from the rods and cones to the nerve fibers of the retina. There are no anastomoses between the prolongations and in general their outline corresponds with my Figs. 4, 5 and 6. In every respect they correspond with the Müller's fibers of the inner nuclear layer and I shall speak of them as such.

A day later the nuclei have all moved to the basal end of the cells, leaving on the free side of the retina a zone composed wholly of the prolongations of the spongioblasts. This free zone or peripheral veil of His (*Randschleier*) is not only

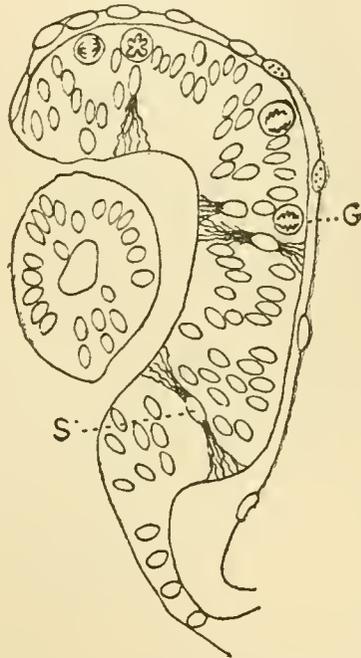
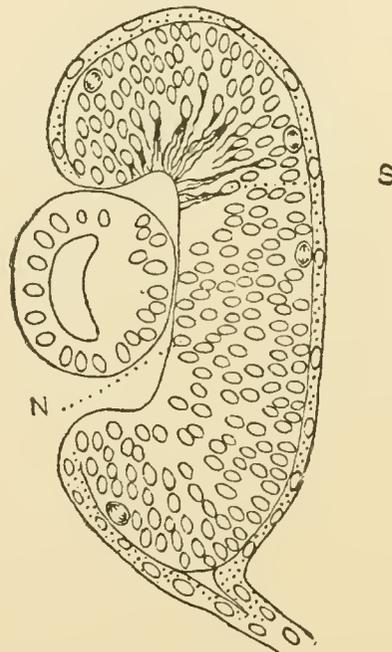


Fig. 6.—Section through the eye of *Amblystoma* (7 mm. long) twelve days before hatching.  $\times 133$  times. *G*, germinating cell; *S*, spongioblast.

present in the retinas of all classes of vertebrates but extends throughout the central nervous system, and is in all cases on the basal side of the ectoderm. Without demonstrating the point absolutely the embryological evidence is that the spongioblasts throughout the nervous system are much the same—forming Müller's fibres and the retina, and Deiter's cells in the brain and spinal cord. And as the evidence is gradually accumulating we must admit that the prolongations of the spongioblasts do not communicate directly but only come in

apposition, so that the same principle applies to them as to the nerve-cells, or to cells in general.

Fig. 5 is a stage two days in advance of Fig. 4. The fat globules in this case show how the peripheral end of the cell is breaking up into its prolongations. The process is beginning to effect the basal end of the cells also. Practically all the cells of the retina in this stage contribute to the formation of the net-work of interlocking cells, and as yet no true nerve-cells are present. In a stage later, Fig. 6, the lens is



*Fig. 7.*— Section through the eye of *Amblystoma* (8 mm. long) nine days before hatching.  $\times 133$  times. *S*, spongioblast; *N*, neuroblast.

separated from the ectoderm, and the secondary optic vesicle is more cup-shaped. The net-work of fibrils is still outlined by the pigment granules and they form such an intimate meshwork that it is impossible to separate the cells. There seems to be a complete union of protoplasm of the cells, but on account of the mode of development as well as on account of histological principles I consider this only apparent, and not real. Already in this stage some of the nuclei are beginning to move towards the free border of the retina, and this is greatly increased a day or two later (Fig. 7).

At this time the whole eye has advanced in its development, and in its centre the complete thickness of the retina is filled with cells. Cells arising from the germinal layer have moved through the fine net-work of the prolongation of the spongioblasts and are now immediately behind the lens. This is the third migration of nuclei. In Fig. 7 we have a retina representing all of the various stages in its development. In the middle it is the most advanced, and at the periphery it is just beginning. In fact this is what we have in all growing

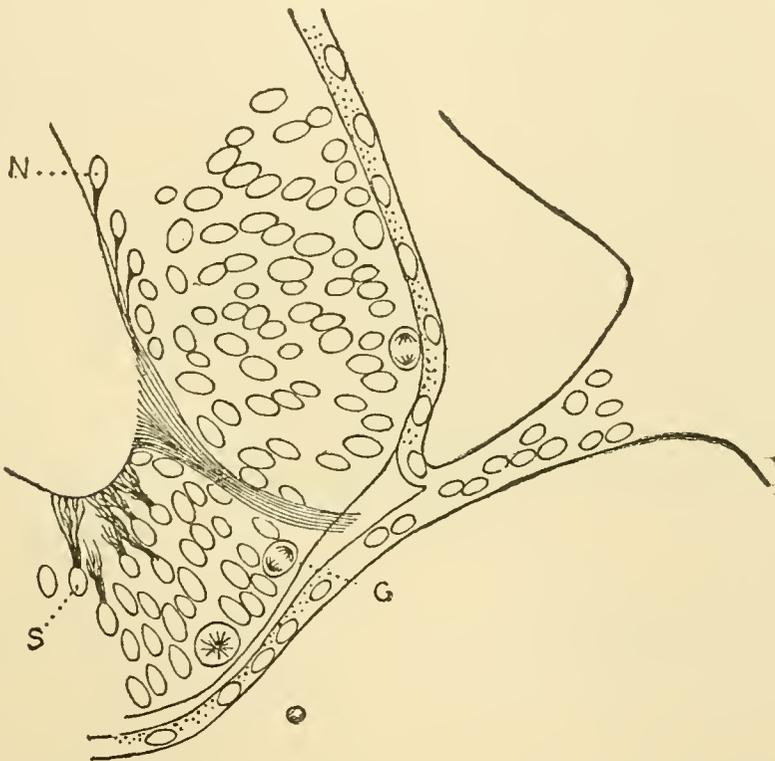
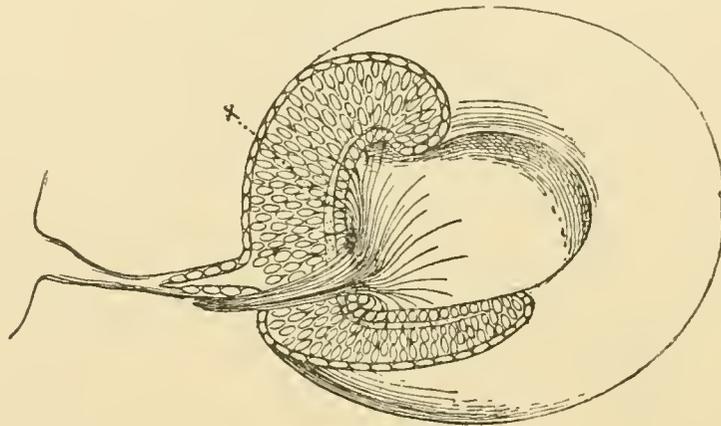


Fig. 8. — Section through the eye of *Amblystoma* (10 mm. long) five days before hatching.  $\times 300$  times. *G*, germinating cell; *S*, spongioblast; *N*, neuroblast.

retinas, the periphery always being the growing point. Following Fig. 7 from its center to the periphery, we find in the center the whole retina filled with cells; on either side the large spongioblasts completely formed; and farther towards the periphery the development of the spongioblasts. Viewed from the lens side we have in the various stages of the development of the retina, at first a central spot indicating the free ends of the spongioblasts, which in the successive stages move as a zone

towards the periphery and is gradually lost. It is lost as soon as it has once overstepped the border of the optic stem or point of the future optic nerve. Up to the present the layer of tapetum contains no pigment, but now the condition of things is reversed; the retina contains pigment only at its periphery, and the tapetum is pigmented all around as indicated in Fig. 7. Following this stage closely it is quite easy to find the origin of the first optic nerve fibers. Using the lens and inner granular layer as guides, we locate the beginning of the optic nerve in a stage between the first growth of the lens fibers, and just before the formation of the inner granular layer.

Up to the present I have been able to find some two dozen specimens in which the optic nerve is already partly formed,



*Fig. 9.* — Reconstruction of the eye of *Amblystoma* just after hatching.  $\times 100$  times. *X*, inner granular layer.

but communicates only with the retina and not with the brain. In its growth from the retina the nerve has not yet reached the brain. Fig. 8 is from a specimen in which eight or nine bundles of two to three fibers each form the beginning of the optic nerve. These bundles arise from about two dozen cells, all of which are located in what is to be the future ganglion-cell layer of the retina. The first ganglionic cell always grows toward the optic stalk, as indicated in the figure. At the point of union, between the optic stalk and retina, there is always a triangular space, and frequently the optic nerve grows through this. Along the growth of the nerve the cells are spindle shaped, and as it passes through the triangular space these cells sur-

round the nerve. The exact direction of the nerve does not seem to be constant, but, as a rule, it is towards the ventral and median side of the stalk, as indicated in Fig. 9. A section of the same stage in *Necturus* is shown in Fig. 10. The whole nerve was struck in one section, and it shows a very large triangular space, partly filled with cells which surround the nerve.

The idea that the optic nerve arises from the retina and grows to the brain was first advanced by W. Müller<sup>1</sup> although he had no direct observation on which to base his view. The various facts known at his time to histologists and embryologists were,

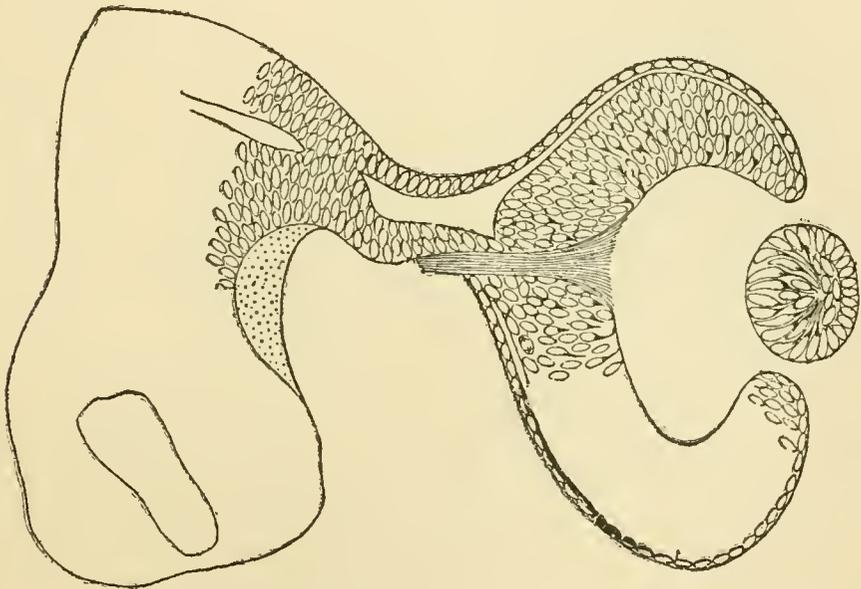


Fig. 10.—Section through the eye of *Necturus* (18 mm. long) about the time of hatching.  $\times 100$  times.

however, sufficient basis and his view was finally verified simultaneously by His<sup>2</sup> and Martin<sup>3</sup> by direct observation in mammalian embryos. The study of the latter is quite complete, and in general what I have found in the amphibian eye confirms what he found in the cat. He finds also that the optic nerve not only arises from the ganglion cells but also from the cells of the inner nuclear layer.

<sup>1</sup> W. Müller, Festgabe an C. Ludwig, 1874.

<sup>2</sup> His, His u. Braune's Archiv, 1890.

<sup>3</sup> Martin, Anatom. Anz., 1890, and Zeit. f. vergl. Augenheilkunde, Bd. 7.

From another standpoint it was also shown by Froriep<sup>1</sup> that the first fibers of the optic nerve must arise from the retina, because he found in a shark embryo an optic nerve arising from the retina but not reaching to the brain. The same has been shown to be true by Keibel<sup>2</sup> and by Assheton.<sup>3</sup>

In both Figs. 9 and 10, which are from *Amblystoma* and *Necturus* respectively, the optic nerve arises from deeper portions of the retina as well as from the ganglionic-cell layer. Especially in *Necturus* is this second growth of cells, which

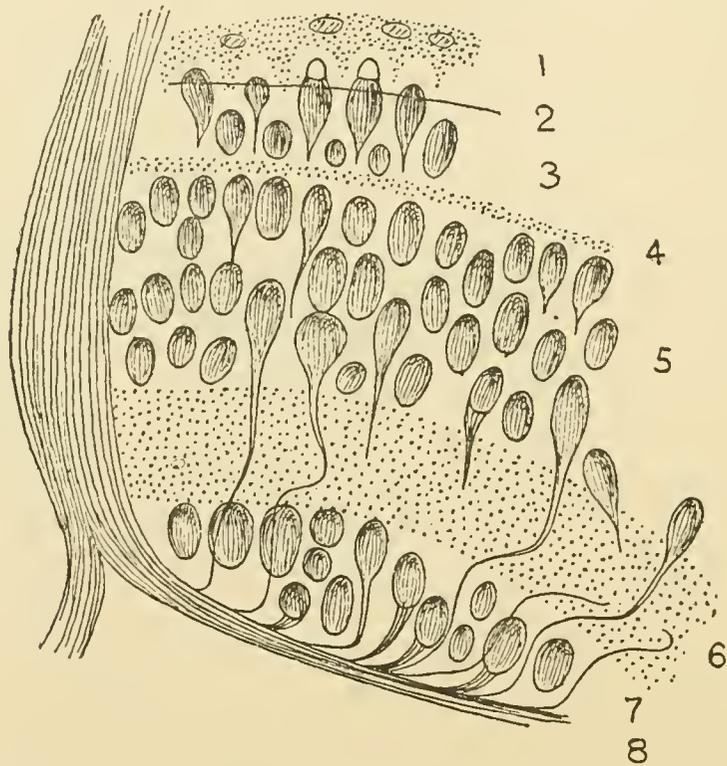


Fig. 11. — Section through the optic nerve and retina of *Necturus* two and one-half months old. Hardened in micro-chromic and osmic acids.  $\times 550$  times.

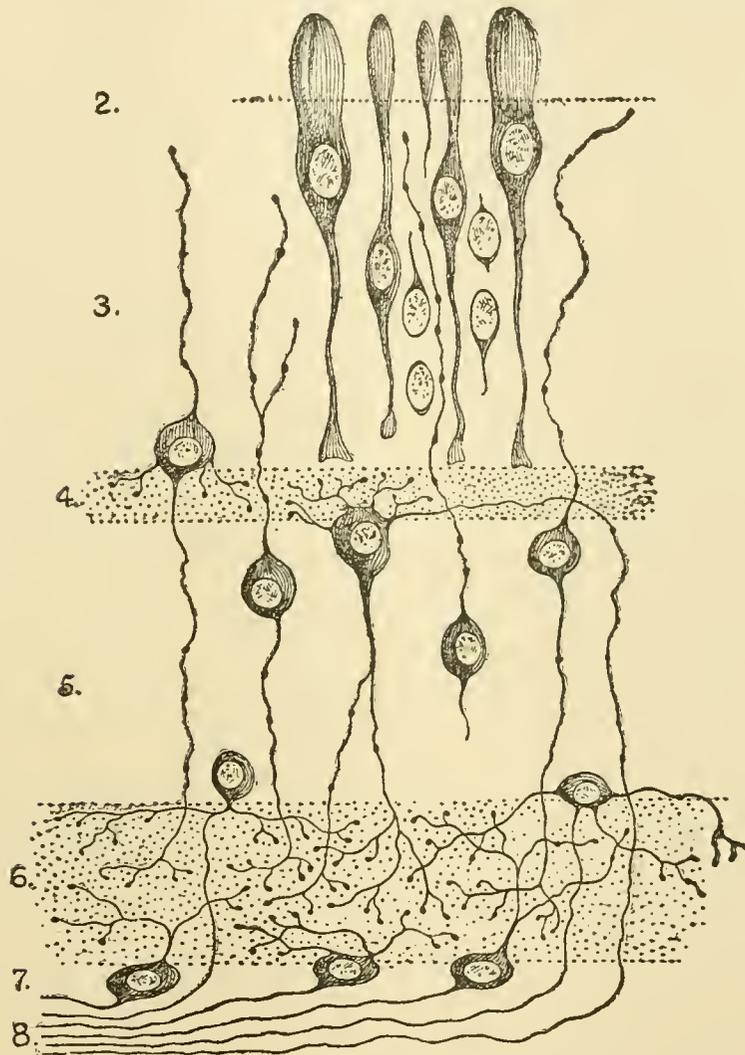
give rise to optic nerve fibers, well marked. In later stages, as soon as the inner granular layer is well formed, these large cells send a single protoplasmic prolongation almost through the granular layer and give rise to a single nerve fiber which can with the greatest of ease be followed into the optic nerve. Fig. 11 is a picture of a specimen which had been

<sup>1</sup> Froriep, *Anatom. Anz.*, 1891.

<sup>2</sup> Keibel, *Sitzungsber. d. Nat. Med. Vereins*, 1888.

<sup>3</sup> Assheton, *Quarterly Jour. of Microscop. Science*, 1892.

hardened in picro-chromic and osmic acids, a method by which the optic nerve fibers are most decidedly marked. This observation is by no means remarkable when we take into consideration the newer histology of the retina as given us by Dogiel,<sup>1</sup> by Ramón y Cajal<sup>2</sup> and by Tartuferi.<sup>3</sup> To these authors, and especially the former, we owe our knowledge of



*Fig. 12.*—Semi-diagrammatic reconstruction of the human retina from the drawings of Dogiel.

the origin of the optic nerve in the various layers of the retina. Fig. 12 is a compilation of several of Dogiel's figures and shows his interpretation of the retina obtained by the methylin blue

<sup>1</sup> Dogiel, *Anatom. Anz.*, 1888, and *Archiv. für mik. Anat.*, Bd. 38, 1891.

<sup>2</sup> Ramón y Cajal, *Anatom. Anz.*, 1889.

<sup>3</sup> Tartuferi, *Salla anatomia della retina*, Torino, 1887.

method of staining. We have the three groups of ganglion cells giving rise to nerves, two of which are situated within the inner nuclear layer. In addition we have the bipolar cells, and with Ramón y Cajal I can not wholly set aside the Müller's cells of the same layer, mainly on account of specimens obtained by means of silver precipitation, and because of the mode of development. In addition to the nerves arising from these cells we have also no doubt centripetal nerves which have free endings in the retina. This has been shown by means of physiological experiment by Monakow,<sup>1</sup> by embryological observation by Martin<sup>2</sup> and by histological methods by Ramón y Cajal.<sup>3</sup> According to this last named author they end in the inner nuclear layer of the retina.

The three groups of ganglion cells of the retina giving rise to the optic nerve, may be called, because of their location, the inner, middle and outer ganglionic layers; the first lying just beneath the outer granular, and the last just outside of the layer of optic nerve fibers. In *Necturus* and *Amblystoma* the middle group is the most prominent and sends large protoplasmic prolongation through the inner granular layer, as Fig. 11 shows. In addition to these cells there are cells lying just inside of the outer granular layer, which also seem to give rise to nerve fibers. As yet, however, I have been unable to demonstrate that in its development the optic nerve arises in part from the cells of the outer ganglionic layer of Dogiel.

Shortly after the middle ganglionic layer begins to give rise to the optic nerve fibers there appears a line of separation between the middle and inner ganglionic layers, the beginning of the inner granular layer. This rapidly increases in thickness, and just before the retina is complete the outer granular layer also makes its appearance.

Before the retina is completed, its inner and outer borders are defined by sharp lines formed by the many prolongations of the spongioblasts, or Müller's fibers. As the rods and cones develop they perforate the outer border and come in contact

<sup>1</sup> Monakow, *Arch. f. Psychatrie*, Bd. XX.

<sup>2</sup> Martin, *Zeit. f. Vergleich. Augenheilkunde*, Bd. VII.

<sup>3</sup> Ramón y Cajal, *Anatom. Anz.*, 1889.

with, and are enveloped by the pigment cells of the tapetum nigrum. The line of perforation remains sharp and in this way the membrana limitans externa is formed.

In the amphibians the retina is completed in its center before its area is very great, and its further growth is by gradual addition to the periphery. In general, then, the ora serrata is its growing point, and here its growth is by means of the same steps as was the case in its center. Therefore, the histogenesis can be studied in any growing retina, with the disadvantage, however, of no pigmentation of the cells, which is of such great value in the study of the first formation of the optic nerve. Any Necturus embryo, during the first year of its life, will show this peripheral growth. The mitoses are also in all cases in the outer nuclear layer, *i.e.*, the side which corresponds to the former outside of the body. This same process of addition to the periphery is by no means peculiar to the amphibian eye, but is present in all vertebrate, as well as many invertebrate eyes. In the cephalopod eye, which is derived directly from the ectoderm, the growing point is just beneath the bacilli ("rod and cone"), or on the lens side of the retina. This is exactly what we should expect, as it is this side which was originally the free surface of the ectoderm. Here also the retina completes its growth in its center and then becomes larger and larger by addition to its periphery. Here also the plane of the cell division is at right angles to the original ectodermal plane, as is the case in the vertebrate eye. Not only is this true in the squid, but also in various groups of arthropods. Watasê, in his excellent papers, has shown us much which brings the invertebrate eye into line with the vertebrate.<sup>1</sup> He also informs me that quite recently he has found that the eye of *Squilla* grows in the same way. Lowne<sup>2</sup> has also demonstrated that the invertebrate eye grows at its periphery.

Besides the methods of growth it is also possible to make other comparisons between the vertebrate and invertebrate eyes. The polarity of cells is identical and the chain of cells

<sup>1</sup> Watasê, Studies from the Biol. Laboratory, Johns Hopkins Univ., Vol. IV, and Johns Hopkins Univ. Circular, April, 1890.

<sup>2</sup> Lowne, Transaction of the Linn. Society, 1884.

is much the same according to the complexity of the eyes. Nowhere is the histogenesis of the arthropod eye shown better than in Watasé's paper. He gives a clear demonstration that the origin of the various cells of the ommatidium is from the same source. This also corresponds with the vertebrate retina. The corneagen cells differentiate much as, and correspond with the rods and cones. The rhabdomere corresponds with the ganglion cells; both give rise to optic nerve fibers. The former may differentiate into only one layer; the latter into three layers. The various groups of cells of the retina may be firmly bound together by what are no doubt functional cells—the ganglion cell in *Limulus*, and the bipolar cell of the inner nuclear layer of the vertebrates.<sup>1</sup> No matter whether or not we succeed in making a homology of the various histological elements of various eyes the fact remains that a cell with a single prolongation or a differentiation of cells makes the visual unit. An ommatidium on the one hand and a rod and cone, bipolar cell and ganglion cell on the other; all are complex modifications of the same simple cell with its polarity retained. In one cell (rod and cone) the receiving or the afferent side is more greatly modified; in the other cell (ganglion) the transmitting or efferent side is more highly specialized.

That the growing point of the retina is at its periphery is further demonstrated by the fact that the newt's retina may be completely regenerated from this point. Griffini and Marchinò<sup>2</sup> demonstrated, by cutting the optic nerve, that the retina degenerates completely with the exception of its periphery or ora serrata. We have seen that this is the embryonic or growing portion of the retina, and Griffini and Marchinò find that the whole retina as well as the optic nerve is regenerated from this point. This fact does not seem so remarkable when we take into consideration that the spinal cord may be regenerated by the multiplication of the cells about its central

<sup>1</sup> These bipolar cells have been well described by Emery, *Atti della Società Italiana di Scienza Naturali*, XVIII, 1876; by Dogiel, *Archiv, f. mik. Anat.*, Bd. XXII, XXIV u. XXXVIII; and by Ramón y Cajal, *Anatom. Anz.*, 1889.

<sup>2</sup> Griffini and Marchinò, *Arch. Ital. de Biol.*, XII.

canal, and a later differentiation after the same plan as in the embryo.<sup>1</sup> The structure of the ora serrata of the human retina also indicates that this must be the growing point. As we follow the retina into this point we find that the granular layers disappear — first the outer and then the inner. The two nuclear layers then run together, and finally the rods and cones disappear.<sup>2</sup> In this region there is a decided increase of Müller's fibers or spongioblasts. By passing from the ora serrata to the retina the layers appear in the order of their development in the embryo. This explains to us why the structures of the retina "flow together" as the ciliary border is approached.

I do not think it probable that the cells of the outer nuclear are liable to multiply under any normal conditions. According to Boquis<sup>3</sup> and to Falchi<sup>4</sup> direct injury of the retina causes multiplication of the cells of that part. The latter author states that the ganglion cells as well as the cells of the inner and outer nuclear layers may divide. I do not think that the papers of the above authors can bear rigid cross examination, and it is probable that most of the cell divisions were seen in the inflammatory tissue, or at the highest in the spongioblasts.<sup>5</sup> Under certain circumstances, however, it seems as if the mature retina may begin and grow anew. This is by no means an exception for the retina alone, for in all pathological new-formations the same may be true. Flexner<sup>6</sup> has described a tumor arising from the outer nuclear layer, and this whole new-formation (neuroepithelioma as he calls it) is composed of round cells and circular groups of rods and cones. We must interpret the above case as a multiplication of cells which are already highly differentiated. All of the cells of the outer

<sup>1</sup> Caporaso, Ziegler's Beiträge, Bd. V.

<sup>2</sup> Schwalbe, Anat. d. Sinnesorgane, 1887.

<sup>3</sup> Riforma Medica, IV.

<sup>4</sup> Riforma Medica, IV, and Ziegler's Beiträge, Bd. V.

<sup>5</sup> Martin states that the spongioblasts of the embryonic cat's retina multiply. Recently Kerestszeghy and Hannss (Ziegler's Beiträge) Bd. XII, have shown that when the dog's spinal cord is injured the glia cells increase to a slight extent, and that the ganglion cells do not multiply.

<sup>6</sup> Flexner, Johns Hopkins Hospital Bulletin, 1891.

nuclear layer are rod-and-cone bearing cells, and we can not admit that when they multiply that they can return to their embryonic type and regenerate all the retina structures. They must produce their kind only, *i.e.*, form rods and cones.

The above discussion shows us clearly, I think, that the histogenesis of the retina is after the plan of histogenesis of nerve tissue in general. The original growing point is on the side which was the outer border of the ectoderm. This statement applies to the central nervous system as well as the sense organs.

The optic nerve arises in great part from the dorsal end of the ganglion cells and grows into the brain. In general we can view nerve cells as having receiving and transmitting ends. The receiving end is the side which was directed originally towards the outside of the body. In the retina the receiving end is most highly modified in the rods and cones ; the transmitting end in the ganglion cells ; and an intermediate stage with both equally modified in the bipolar cells of the inner nuclear layer.

After the retina is fully formed just behind the lens, its further growth is by addition to the periphery. This secondary growth is a continuation of the primary and is in no way a modification of it.

THE UNIVERSITY OF CHICAGO,

Feb. 24, 1893

## HOMOLOGY OF THE CENTROSOME.

S. WATASÉ.

NATURALISTS are now pretty well agreed that the lately discovered centrosome with its sphere represents a highly important constituent of the cell, and that it is to be placed in the category of permanent cell-organs, as the nucleus and the cytoplasm. Indeed one<sup>1</sup> of the foremost cytologists has recently said that the discovery of the centrosome marks as important an era in the history of biological science as did the discovery of the cell-nucleus itself.

Those who have paid any attention to the subject will hardly question the physiological importance of the centrosome. But the view that the centrosome with its sphere is an organ equal in morphological importance and as permanent as the nucleus, is open to serious criticism. For the claim of the absolute novelty and the unique character of the centrosome is, after all, based on the assumption, *that among the hitherto recognized elements in the cell, there is nothing comparable to the centrosome, either in function or in structure.*

If there is any known element in the cell which can be shown to have a close affinity to, or identity with, the centrosome, its claim as a unique morphological organ in the cell must fall to the ground.

Investigators who have studied this subject have generally selected cells in which the centrosome appeared in a most conspicuous form ; and naturally, as the main object at first was to demonstrate its existence. But the possibility of discovering the homology of the centrosome among the other cell-constituents, if such exist, is rendered all the more difficult, as long as our attention is directed only to those cells in which the centrosome has reached its highest development.

Among the number of animal and vegetable cells I have studied, a most instructive illustration of this fact is afforded

<sup>1</sup> W. Flemming : *Ueber Zellteilung*. Verhandl. d. Anat. Gesellschaft zu München, 1891.

by the egg of *Unio* (Fig. 1). When the egg of *Unio* is killed with an osmo-picric mixture, and stained with acid-fuchsin, and afterward washed with the alcoholic solution of picric acid, we see the centrosome standing out with a remarkable sharpness from the rest of the cell-structure, inasmuch as the acid-fuchsin deeply stains the centrosome, while the spindle fibres and the rays of the aster remain practically unstained. On casual observation, we see nothing in the cell that can be compared with such a centrosome (Fig. 1, C.). The view that regards the centrosome as a unique organ of the cell would certainly

Fig. 1

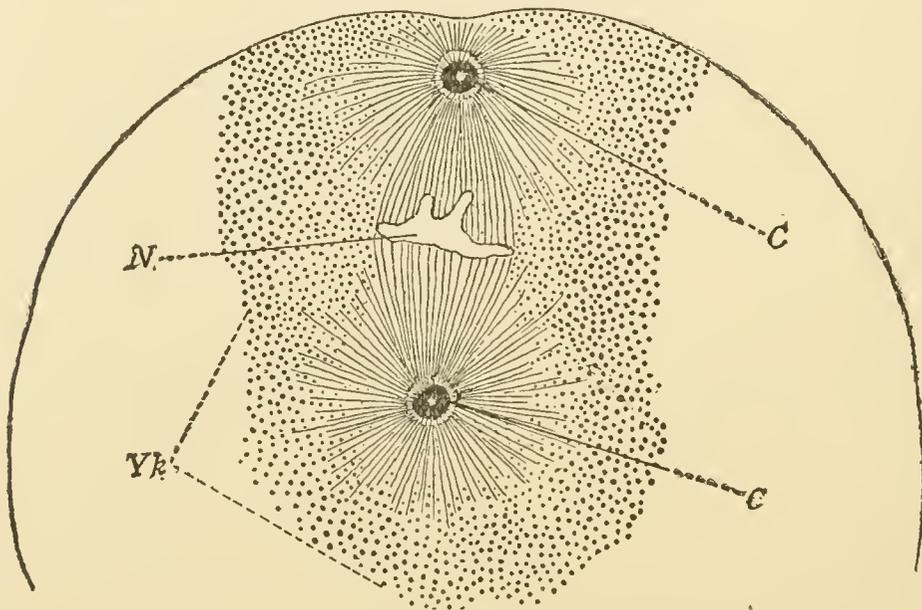


FIG. 1. *The egg of Unio complanata.* C. C. centrosome; N. nucleus; Yk. yolk-granules. The egg was killed in the picro-osmic mixture, and stained with acid-fuchsin, and afterward washed with the alcoholic solution of picric acid. The centrosome appears to be hollow, the optical section shows it as a very thick ring of deeply staining substance.

seem justified, at least, if we cannot find any element in the cell that can be directly compared with it, as in the case of the egg-cell of *Unio*.

But we must remember that we may be here dealing with an extreme case, and therefore, just the example to be avoided on account of the excessive development of the centrosome, if we wish to find any element in the cell that is to be directly compared with it.

Let us begin, therefore, with the cell in which the centrosome has not attained such an extraordinary development, as in the case of *Unio*.

Such a cell we find in the leucocyte. The accompanying illustration (Fig. 2), which is taken from the beautiful work of Heidenhain, represents the white blood corpuscle of the Salamander. In the center of the aster we find two pieces of deeply staining bodies, which are the centrosomes (*C.*). Around them, there exists a zone composed of smooth cytoplasmic filaments radiating outwards. Along the periphery of this zone we find a number of granules. These granules are considerably smaller in size compared with the centrosome. The cyto-

Fig. 2.

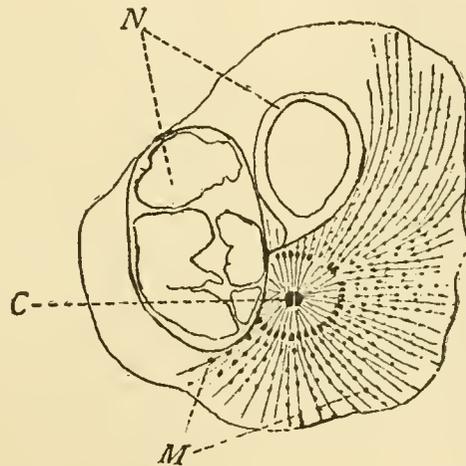


FIG. 2. *The leucocyte of Salamander*, showing the radial system of the cytoplasmic filaments, (aster) and the distribution of the microsomes (*M.*); *C.*, centrosome; *N.* nucleus. After Martin Heidenhain, *Ueber Kern und Protoplasma*, Festschrift für A. von Kölliker, 1892, Taf. X, Fig. 9.

plasmic filaments, which run from the periphery of the above-mentioned zone outward, contain a series of fine granules of varying sizes. These highly staining granules are the *microsomes* (Fig. 2, *M.*), or more strictly, the *cyto-microsomes*, and all of them stain exactly like the centrosome. The microsomes are imbedded in the substance of the cytoplasmic thread, and are different from the metaplastic products, such as the non-living granules, nutritive particles which are often quite abundant in the cell.

As mentioned above, the size of the microsome varies, and, as a general rule, becomes smaller as it lies nearer the periphery of the cell.

There are, then, two important facts to be noted, *viz.*,

1. The microsomes stain exactly like the centrosomes, and therefore both differ from the cytoplasmic thread proper in one common particular.

2. The size of the microsomes gradually increase as we pass from the periphery of the cell toward the centre of the aster.

Two questions arise, *viz.*, Are not the centrosome and the microsome essentially one and the same thing? Is not the reason why the centrosome is larger than the rest, because it is found at one end of the linear series toward which the size of the microsome gradually increases?

Considered purely from the anatomical and the micro-chemical side, I must answer these questions in the affirmative, and see no reason why the centrosome can not be regarded simply as a *microsome of gigantic size*. If this statement can be maintained, then, the absolute uniqueness claimed for the centrosome as a cellular element, must be given up, for as is well known the microsome is the universally distributed element in the cytoplasm of animal

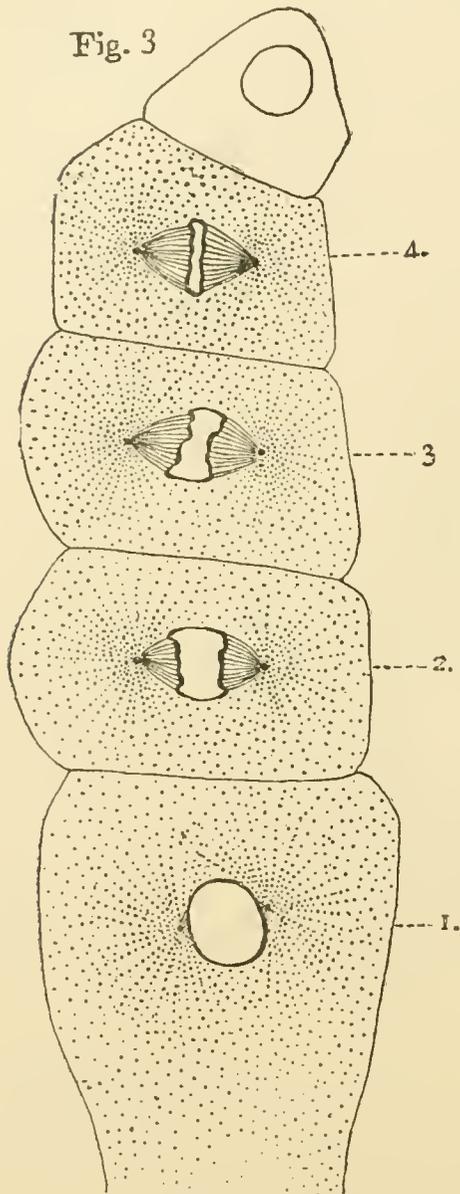


FIG. 3. Blastomeres in one of the radii in the blastodisc of *Loligo pealii*, showing the formation of the spindle filaments from the centrosome. In the segment (1), the nucleus is still spherical, and no spindle rays are yet distinctly visible; in (2) the outline of the nucleus is flattened and the spindle rays are distinctly visible; in (3) and (4) the increase in the bulk of the spindle, and the corresponding decrease in that of the nucleus go hand in hand.

and vegetable cells. The difference between the microsomes and the centrosomes according to this view, is merely that of size, and the size taken by itself is no decisive criterion by which we can differentiate one morphological element from another.

Supposing, then, that the centrosome may be considered as an enlarged form of the microsome, which exists everywhere in the cell-body, it may be further asked, How is it that a certain microsome attains such a gigantic size above all others? Is

Fig. 4

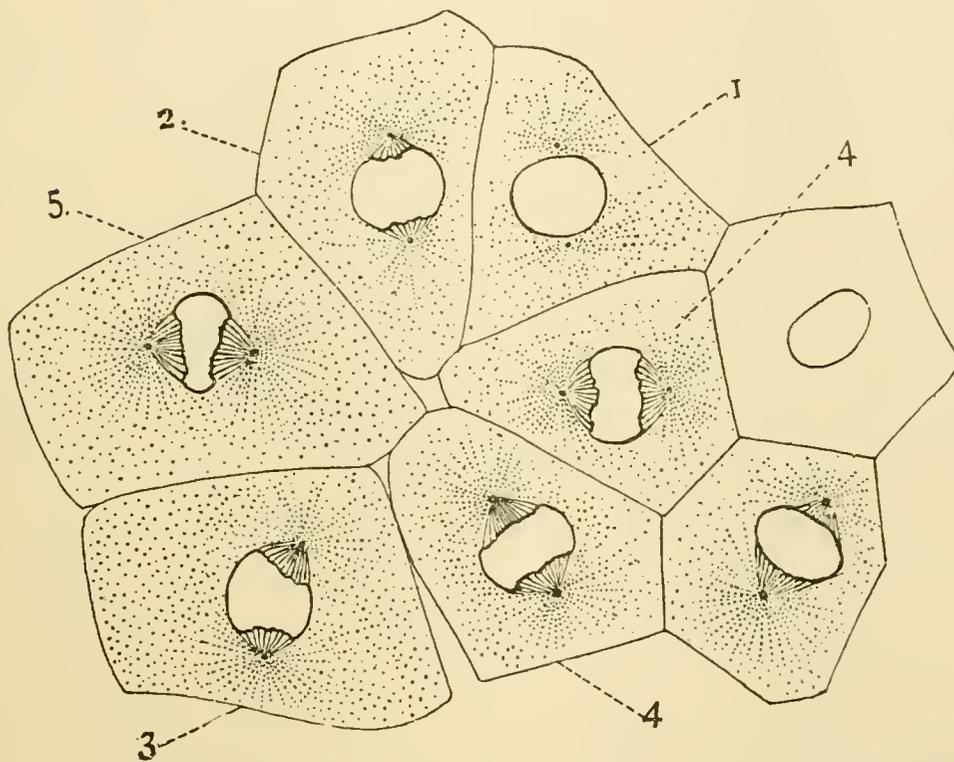


FIG. 4. Blastomeres from the central part of the blastodisc of *Loligo pealii*. The segments (1), (2), (3), (4), (5), etc. show different stages in the formation of the spindle from the centrosome.

there any special arrangement in the cytoplasm which favors the production of such a large microsome at a particular point?

The problem is a broad one, and involves the discussion of the relation existing between the microsome and the cytoplasmic thread proper. The following is my provisional answer.

The microsome of the cell-body is more often located at the junction of two or more cytoplasmic filaments. In other

cases microsome appear as varicosities on a single thread, without any visible cross-threads. This latter appearance may be due, in some cases, to the plane of the cross-threads lying in position which makes them invisible in a given section, or it may be due to the technical difficulty of preserving such extremely delicate cross-threads in a certain cell. Neither of these remarks, however, apply to those cases in which the mi-

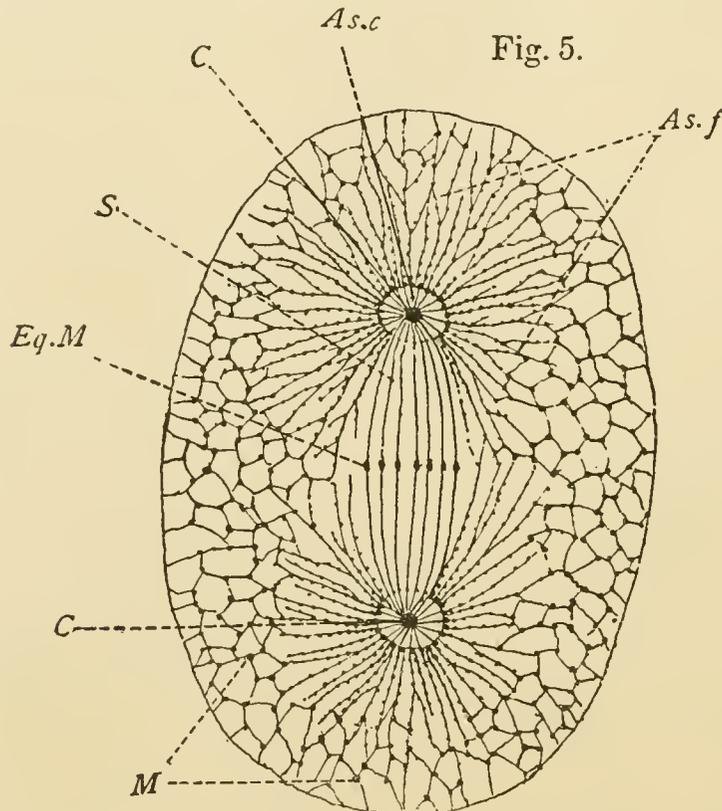


FIG. 5. *Diagram of a dividing cell*, showing the cytoplasmic framework of the caryokinetic figure and its relation to the rest of the cell-body. The chromosomes and the spindle fibres which are directly concerned with their division are omitted from the diagram. *C. C.*, Centrosomes; *S.*, Spindle filaments; *M.*, microsomes or Cyto-microsomes; *Eq. M.*, Equatorial microsomes or "cell-plate," the "*microsomes chromatiques*" of Guignard; *As. C.*, "*Astrocale*" (Fol), the "*zone médullaire*" of the attractive sphere of E. van Beneden; *As. f.*, fibres of the aster, with microsomes imbedded in the substance of each fibre, giving rise to the varicose appearance. The diagram is especially intended to show the distribution of the microsomes in different parts of the cell.

chromosomic varicosities appear in the substance of straight spindle filaments without any cross-branches. It further appears probable that the substance of the cytoplasmic thread and that of the microsome stands in a certain genetic relationship. In

short, the history of the microsome and the filament runs in a cycle. The microsome may be converted into the cytoplasmic filament, and the filament, in turn, may give rise to a microsome. The cytoplasmic filament may be called the *active phase*, and the microsome the *inactive phase* of the living cytoplasm.

If we accept the general statement that the microsome is more commonly found at the junction where two or more cytoplasmic threads meet, then the problem of the origin of the centrosome in the centre of the aster is greatly simplified. *For the centre of the aster is the point where the greatest number of cytoplasmic filaments meet with one another, and the size of the microsome produced at such a place must be correspondingly large.* In other words, the microsome produced in the centre of the aster is the centrosome.

The centrosome thus produced gives rise in turn to a new set of cytoplasmic fibres—the spindle filaments. The view that the spindle fibre originates from the centre of the aster, and not from the nucleus I have given elsewhere. A glance at a series of caryokinetic figures (Fig. 3 and Fig. 4) will show that such is really the case. The important fact that may be observed in this connection, is that those filaments produced by the centrosome are the smooth fibres which are free from varicosities, at first. The observations of

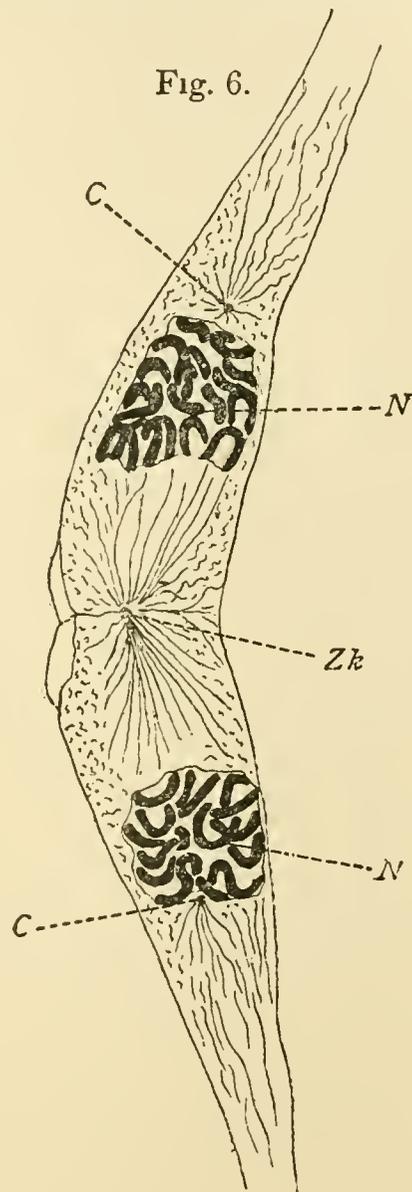


FIG. 6. *The connective tissue cells from the lung of Salamander.* C., centrosome; N., Nucleus; Zk., "Zwischenkörper." After W. Flemming: *Neue Beiträge zur Kenntniss der Zelle.* Arch. f. mikr. Anat. Bd. XXXVII, 1891, Taf. XXXVIII, Fig. 12.

Hermann,<sup>1</sup> Flemming<sup>2</sup> and others on the formation of the small but perfect spindle at a distance of the nucleus, bear the same interpretation, *viz.*, *the conversion of the material of the centrosome into the fibres of the spindle*. On the other hand, it may be incidentally observed here, that the formation of the perfect spindle in the substance of the cell-body, at some distance from the nucleus, shows pretty strongly against the view that the contents of the nucleus has anything to do with the formation of the spindle filaments.

The view that places the microsomes in the same category with the centrosome throws a further light on the obscure points connected with the formation of the "cell-plate" (Fig. 5, *Eq. M.*) and the "Zwischenkörper" (Fig. 6, *Zk.*).

The "cell-plate" which is formed in the equatorial plane of the spindle of a certain cell, is composed of a series of knob-like enlargements of the filaments. This knob-like enlargement of the filament stains deeply and shows exactly the same microchemical reaction as with the microsomes and the centrosome. This series of knob-like thickenings have been called by Guignard the "*microsomes chromatiques*" on account of their affinity to staining reagents.

I hold that these thickenings are identical with the cytomicrosomes, and are produced by the spindle filaments *in situ*. They may be called the *equatorial microsomes* (Fig. 5, *Eq. M.*). Morphologically considered, the spindle filament stretching from pole to pole of the caryokinetic figure is nothing but an extremely elongated cytoplasmic filament with but one microsome in the middle, with two huge microsomes at the extremities. In this respect the spindle filament is directly comparable with any portion of the filament of the aster, in which the microsomes are consecutively arranged, at regular intervals. There is this difference between them, however, that while in the filament of the aster the distance between the two consecutive microsomes is extremely short, in the filament

<sup>1</sup> F. Hermann: *Beitrag zur Lehre von der Entstehung der Karyokineticischen Spindel*. Archiv f. mikrosk. Anatomie, Bd. XXXVII, 1891.

<sup>2</sup> W. Flemming: *Neue Beiträge zur Kenntniss der Zelle*. II. Arch. f. mikrosk. Anatomie, Bd. XXXVII, 1891.

of the spindle it is quite long, extending from the pole to the equator of the spindle.

If the spindle fibres do not run nearly parallel with one another, as in Fig. 5, *S*, but converge to a focus in the middle plane of the caryokinetic figure, then these equatorial microsomes will fuse into one solid body and give rise to a "Zwischenkörper" (Fig. 6, *Zk.*). In other words, the "Zwischenkörper" is an intercellular centrosome, produced at the equatorial plane of the spindle in precisely the same way as the ordinary centrosome.

The present view further suggests a new explanation concerning the nature of the centrosomes in the barrel-shaped

Fig. 7.

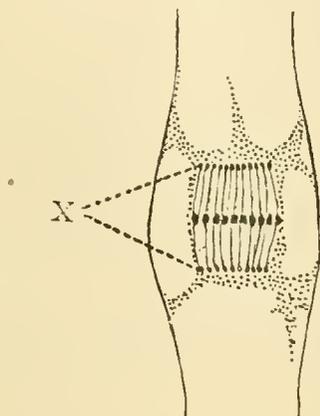


FIG. 7. The division of the secondary embryosac-nucleus of *Montropa Hypopitys*, X., Knob-like thickenings of the filaments composing the barrel-shaped spindle. These thickenings are the "polar microsomes" of the text. After Strasburger, *Zellbildung und Zelltheilung*, 1880, Taf. V. Fig. 134.

spindle, as in the *Ascaris* (maturation-spindle), *Spirogyra*, *Montropa* (Fig. 7) and several others. In these forms the caryokinetic spindle appears truncated at both ends. Instead of the spindle fibres converging to a point as they usually do, they terminate abruptly at a certain distance from the equatorial plane. The usual astral centre is wanting. If our view of the centrosome be correct, we should not find it in such a form of the spindle, but *each filament or a group of few filaments ought to have a microsome—a small, independent "centrosome," as it were—at the extremities of the filament or of*

*the group of the filaments.* I have no personal observation on this subject, nor am I aware that such a view has ever been suggested. But the figure I have copied from Strasburger (Fig. 7) seems to favor such a view. The knob-like enlargement of the filament at its extremities (Fig. 7, X.), I consider to be microsomes. Such a group of microsomes may be called the *polar microsomes*, in distinction from the *equatorial microsomes* (Fig. 5, *Eq. M.*), which are found at the equatorial plane of the spindle.

If we suppose that these free ends of the spindle filaments (Fig. 7) be brought together to a point, the thickened enlargements of each fibre will fuse with one another and form a veritable centrosome, at each end of the spindle.

The relation of the solitary centrosome at the end of the ordinary spindle to the independent microsomes at the free ends of the individual filaments of the truncated spindle, is precisely the relation existing between the "Zwischenkörper" and the equatorial microsome or "cell-plate." In the one the material of the microsome is concentrated into one solid body, and in the other it is distributed into several independent pieces.

In way of summary, we may say (1) that the *centrosome* is not a unique organ of the cell, but is identical with the *microsome* which exist everywhere in the cytoplasm. This view further explains (2) the nature of the "cell-plate," which may also be called the *equatorial microsomes*. (3) When such microsomes at the equatorial plane of the spindle fuse into one solid body, it is known as the "Zwischenkörper." (4) The knob-like thickenings at the free-end of the spindle fibre as in Fig. 7, X, are probably the microsomes. The barrel-shaped spindle possesses, according to this view, several independent microsomes, instead of one centrosome, at each pole of the caryokinetic figure.

If these views be further substantiated by a future research, the centrosome, far from being a unique structure, is one of the most familiar constituents of the cell. The *microsome*, *centrosome*, "Zwischenkörper," "cell-plate" and the *polar microsomes* all belong to the same category of cytoplasmic material.

They are different from one another only in so far as their sizes are different, but the size, as has been already stated, is foreign to the question of their homologies.

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THE AUDITORY OR HAIR-CELLS OF THE EAR  
AND THEIR RELATIONS TO THE AUDITORY  
NERVE.

HOWARD AYERS.

The present condition of this question, so far as the Golgi method has influenced it, may be epitomized as follows :

1. The hair-bearing or acoustic cells of the ear are not nerve cells ; but they represent a secondary and higher differentiation of the nerve end apparatus.

2. All fibres of the auditory nerve are apparently out-growths of the bipolar ganglion cells, one process reaching to the periphery, the other into the central nervous system. The peripheral processes sometimes terminate in small knobs below or among the hair cells ; but more frequently they end in numerous fine branches which pass up among the cells and often reach quite to the surface of the epithelium.

3. All the cells of the several auditory ganglia are simple bipolar ganglion cells and are considered to retain a more primitive condition than the ganglion cells of the spinal ganglia.

4. The acoustic cells are not homologous with olfactive cells since although hair-bearing they are not continuous with the nerve fibres and are only secondarily brought into relation (not more than a mere contact) to the free ends of the auditory

nerve to serve in the place of the primitively superficial cells (ancestral hair cells) which have become the bipolar cells of the auditory ganglia.

5. The system of spiral nerves is composed of fibres within the *cochlea* which run at right angles to the radial fibres and may or may not be all derived from the radial fibres. Very probably not, as all previous investigators who have accepted the spiral system, have maintained.

These are the conclusions of Retzius and Van Gehuchten the only investigators who have published results of the application of the *Golgi* method to the whole ear. The nerve ends of the maculae and cristae have been studied by another investigator, O. Kaiser, who has described the nerve-end as having the form of a calyx, like an egg cup, into which the hair-cell fits.

In using the *Golgi* method on the mammalian ear, I have found that success was obtained only when two conditions were observed. The tissue must be free from calcareous matter either in solution or in deposit and it must be placed in the osmium-bichromate mixture alive. Successful stains do not always reward this care, but of other precautions I have no very definite knowledge. These remarks apply to the ear of the sheep, ox and pig in adult condition, and to the ox and pig in embryonic stages, and in this case mainly to the pig. The best results were obtained from embryos of 8-14 cm. length as the creatures lie on a scale. The ears were carefully removed from the head, cleaned of superfluous tissue and put at once into a solution of *Os. O<sub>4</sub>. 1* per cent 100 cc. + *K<sub>2</sub> Cr<sub>2</sub> O<sub>7</sub>* 500 cc. As from 100 to 200 ears were prepared at a time, the work of preparation was shortened as follows. The heads were removed from the embryos to the desired number, then all were sagittally bisected and the brain removed and a third operation shelled out the internal ears which in embryos of this size are still entirely cartilaginous. From 50 to 100 ears were placed in a low but large mouthed bottle of circa 200 cc. capacity, and first well washed off with osmium-bichromate solution once used, which was replaced after a little gentle shaking by a bottle full of the fresh

solution. In this the ears remained for 24 hours without change unless the liquid grew turbid. As a rule one change is required. Having tried various *times*, from 16–56 hours, I find that if the ear stains at all, it will do so after a bichromate bath of 24 hours, and as a rule anything less gives an incomplete stain. More variation in the stain is produced by varying the time of the silver bath, but a good and uniformly satisfactory period was found to be 24 hours. Successive transfers, from the silver bath back to bichromate, were not productive of any completer stain than had resulted from the 24 hours of immersion in the bichromate and silver baths respectively. Two strengths of silver bath were used, 0.50 per cent and 0.75 per cent. If there was any difference it was in favor of the stronger solution. The silver bath was changed as often as it became turbid from suspended crystals, and ears were washed in used silver bath before being put in the full strength solution.

The penetration of the solutions is aided materially by increase in temperature, and a warm oven may be used with advantage, though it is not necessary.

It may be well to repeat that where calcareous matter was present in the ears they uniformly failed to stain, and I consider the bone tissue of the adult ear the main source of trouble in attempts to stain the adult cochlea by the Golgi method. It acts as a sure *preventive* as the method is now applied. The following paper is descriptive of Golgi preparations, and the conclusions possess only the weight which belongs to this important method. But it must be borne in mind that while this silver stain reveals details of structure in complicated arrangements with a distinctness unsurpassed, it at the same time conceals some of the relations of the very things so clearly set forth.

#### A. *The Hair cells of Corti's Organ or the Mammalian Cochlear Organ.*

The organ of Corti is a very complicated structure, but it has received during its differentiation no new elements, for the arch of Corti is not to be looked upon as a new struct-

ure, but rather as a modification of elements present in all the sense organs. Golgi preparations show many degrees of staining of the cell elements of the organ. In those stained the least, only nerve fibres are visible and these, few in number, are found in the cochlear axis and in the lamina. The supporting cells of the organ are next in order to take the stain, after which come the ganglion cells of the cochlear ganglion, and finally the hair-cells themselves. When the stain is abundantly deposited all detail is lost or rendered valueless, but where the happy mean is observed, hundreds of isolated cells are stained, and many of them show the nerve endings clearly defined. The hair-cells in such preparations show a subglobular or a pyriform body, on the top of which the hairs are only occasionally stained (Pl. I, Figs. 5 and 7). From the centre of the base of each hair-cell issues a nerve fibre which, in a favorable case, admits of being traced through a ganglion cell of the cochlear ganglion into the collection of fibres which pass on to the brain (Pl. I, Fig. 2). These fibres are by no means simple straight threads, but show many varicosities in their course from the hair-cells to the brain, the largest of which are near the hair cells of the organ of Corti, but within the territory of the Sauropsid organ (Pl. I, Figs. 2, 4, 5 and 7; Pl. II, Figs. 8-11, 15 and 17; Pl. III, Figs. 25 and 27). These varicosities vary greatly in size. The most numerous are slight enlargements in the course of the nerve thread, and are more frequently oval than spherical, but they may appear angular as the stain brings them to view. This kind of varicosity occurs in all parts of all the nerves of the ear. Another sort of enlargement consists of medium sized spherical swellings of the fibres or of their branches, and are especially numerous in the organ of Corti upon the short branchings of the nerve. They more especially belong to the "connective" fibres or hair-cell commissures which run along below each row of hair-cells for unknown distances, and enlarge below each cell into subglobular beads into which as a rule the fibres from the hair-cells penetrate. The largest varicosities are scarcely inferior to the ganglion cells in size but unlike most of the latter, several or many processes radiate from the

body of the enlargement. These bodies occur with considerable regularity, so that in surface preparations of the organ of Corti and, better still, in horizontal sections of the same, a row of the inner hair-cells is accompanied by a row of these large varicosities. I am undecided what their true nature is. One is not able with this method to determine their cellular nature, and I have no observations on this point by means of other methods which it is desirable to publish now.

A point of importance I shall mention here, though it does not belong strictly to the nerve endings. As I have already stated, the hairs on the acoustic cells of the cochlea seldom show in well stained preparations, though they frequently do in those incompletely stained. This is due to the shortness of the ends left on the cell caps, and the enclosure of these short ends by the silver deposit. In the case of the maculae and cristae the hairs do not as a rule break off, and when they do they leave a relatively large and long conical hair remnant which is not covered by the silver precipitate to such an extent as to hide the nature of the process. The hairs of the maculae and cristae are very well defined by this method, and appear about *twice as long* as by the ordinary methods of staining with balsam mount. The relation of these hairs to the otoliths I shall describe in another communication.<sup>1</sup>

Returning to the hair cells, we find that the nerve processes which leave them are not always simple, but often branching threads as the Golgi stain shows them. The branches anasto-

<sup>1</sup> In Fig. 24, Pl. III, I have sketched the stained hairs of a portion of the organ of Corti in a 20 cm. pig embryo. First of all one will notice that the hairs are not arranged in the form of a horseshoe upon the top of the cell, but they cover a more or less regular and approximately circular area. Between the row of dots marked 1 (inner hair-cells) and the row marked 2 is found the crest of the Cortian arches. The following rows are quite complete throughout all parts of the cochlea until we arrive at the fifth row which occurs for short distances only along the middle spiral turn and the distal and proximal parts of the basal and apical turns respectively. Golgi's method brings out the presence of these hairs with a clearness of definition shown by no other method. It may be noted in passing that the caps of the supporting cells are not in the region of the fifth row sufficiently well developed to form the figures composing the "reticulate membrane." Pl. II, Fig. 15, shows the appearance presented by an isolated hair-cell defined among the stained rows of hairs.

mose with the processes from neighboring cells and thus form a delicate plexus of fibres where the anastomoses are numerous, Pl. II, Figs. 11 and 17, Pl. III, Figs. 25 and 27, will serve to give a faint idea of the character of this plexus. In Fig. 27, Pl. III, the sub-epithelial commissure (*c*) is shown, while in Fig. 25 there is depicted a few meshes of the plexus connected with one of the commissural varicosities (*v*). From each of the varicosities pass off two sorts of fibres. Those which form part of the plexus, and those which serve to connect the hair-cell with the varicosity. Of the former sort are the "spiral fibres" which enter the plexus. Only a few of the spiral fibres are thus engaged.

### B. *The Radial Nerves.*

When the cochlea is viewed perpendicularly to its helical axis, the greater number of the nerve fibres, leaving the organ of Corti, run nearly at right angles to the tangent of their point of origin into the cochlear ganglion, and thence toward the helical axis, about which all the fibres are spirally twisted as they descend to the base of the helix (Pl. I, Fig. 1). Some of the fibres, between their hair cells and ganglion cells, suffer displacement in the horizontal plane, which often nearly doubles their length between these two points.

Lateral displacement in this plane occurs in greatest abundance in the organ of Corti (Pl. I, Figs. 4 and 7; Pl. II, Figs. 8, 13 and 17; Pl. III, Figs. 18, 19, 22, 23, 27 and 31), next in the region of the *Lamina ossea*, and least of all—though still to a considerable degree within the cochlear ganglion itself, where it gives rise to the appearance of bands of spiral fibres (Pl. I, Fig. 7s, and Pl. II, Fig. 12, spiral fibre), much the same as within the cochlear organ itself. The bundles in the ganglion are larger than those of the organ of Corti, a character not shown in the figures where only a single fibre is drawn in.

When viewed at right angles to the vertical or helical axis the nerve fibres form a series of incomplete funnels with the hair-cells as their thickened borders. This appearance is due to the fact that the fibres leave the organ of Corti in a nearly

continuous sheet, which stretches downward and inward, at an angle varying with the spiral gradient, until it fuses with the central core of nerve fibres.

Many of the individual nerve fibres suffer displacement in the vertical plane, both upward and downward, from the plane of the sides of the funnel, the greatest amount of displacement occurring near the ganglion (on either side) and in the region where the fibre is suddenly drawn into the vortex and spun onto the helical core. The fibres may cross the planes of each other's paths several times between the hair-cells and ganglion (Pl. I, Fig. 1).

Besides the pictures presented by the three views above described we have still to consider the fibres to be best seen in surface view of the organ (they appear in side view only in transection and consequently as black dots). These fibres are of two sorts (*a*) those simple radial fibres which, having apparently lost their way when growing toward the modiolus, have pursued a circuitous course before finding their destination, and (*b*) those fibres which arise as collaterals of the radial fibres and run for greater or less distances in more or less "spiral" direction in the lamina ossea, both within the cochlear ganglion and in the plate of nerve fibres of this lamina (Pl. I, Figs. 4 and 7), and finally the third kind (*c*) composed of the peripheral branches of radial nerves due to the division of the hair-cell without the accompanying partition of its nerve (Pl. II, Figs. 8, 9, 11 and 17; Pl. III, Figs. 24 and 27). In making a distinction between the kinds *b* and *c*, and in calling the former "collateral," I have not attempted to make a distinction based on true morphological grounds, but to direct attention to an *apparent difference* which may, in reality, be due to the same process of growth, about which in the present communication nothing more need be said.

I will add here that the varicosities on the nerve fibres in all parts of the ear appear to be cellular in nature. This does not appear from the Golgi method, nor is it apparent in the case of the finer nerves with any of the ordinary methods, but in Methylene-blue preparations I have yet to find an enlargement of an axis cylinder due to an increase in size of that

structure itself. What appears to be of such a nature in Golgi preparations is in reality due to the presence on the fibre of cellular structure, which in most cases is a sheath nucleus, resp. sheath cell.

The details and illustrations I contemplate publishing in the near future.

### C. *The so-called "Spiral Nerves."*

The system of spiral nerve fibres in the organ of Corti (only), which was discovered by F. E. Schulze, very fully described by Deiters in 1860, and which since that time has been variously identified and described by many others, is clearly defined by the Golgi method, and its claims to an independent position not sustained. This "system" of spiral nerves proves to be nothing more than portions of the radial nerve system drawn out of the radial course into oblique or short spiral courses, and is not, as previous writers have left us to infer, confined to the organ of Corti alone, but occurs frequently between the modiolus and the end organ. The most clearly defined bundle of such spiral fibres occurs in the cochlear ganglion (Pl. I, Figs. 4 and 7; Pl. II, Figs. 8-10, 11 and 12; Pl. III, Figs. 18, 19, 22, 27 and 29).<sup>1</sup>

In the case of all spiral fibres, where the fibre could be traced, the emergence of the fibre from the ganglion as a genuine radial prolongation of a ganglion cell, was found to obtain without exception. The reason why the spiral fibres exist is because during the growth of the fibres from the hair-cells toward their central connections, they grow away from the cells along the paths of least resistance, with, however, a constant tendency to enter the central nervous system. There are, apparently, only two ways in which a nerve fibre may be laid down between the cell of origin and the brain — (*a*) each cell may send its process, as a distinctly individual outgrowth, to the brain, or (*b*) all cells of later generations — derivative cells — may acquire more or less of their central process by a splitting of the fibre already belonging to the parent cell. This latter method is probably the one which takes place most

<sup>1</sup> Henle has figured one of these bundles as a part of the cochlear ganglion.

frequently, and on this assumption the intricacies of the innervation of the auditory sense organs are readily explained. When a fibre from one of the outer rows of hair-cells starts on its journey to the brain it meets obstacles of several kinds, and all along its course, so that the direction it is to take is very much a matter of accident. One process of development, above all others, determines its general course. I refer to the spiral growth of the cochlea, which, during this period, is actively going on, so that all fibres have this spiral twisting force exerted upon them, and it results in giving many of them a curved, if not spiral, course to the modiolus. As growth goes on the cell is carried away from the position occupied at the time when it started its cell process, and in this way the convexity of the curve of the fibre is turned away from the direction of growth. If the cell process fails to find passage-way through a cell-row, or past any other obstruction, it may be turned either way and grow for a greater or less distance before proceeding further radially. A fibre may suffer such an interruption of its course several times before it passes out of the organ of Corti. Another source of displacement and plexus formation, is this — after a fibre is once laid down the cells between it and its point of passage through the hair-cell row or rows may increase in number, and force the nerve fibre to grow in length in this part of its course, so that, ultimately, what was at first only a short bend in the nerve fibre becomes a long “spiral” thread.

The so-called spiral nerve tracts of Corti’s organ are, in some cases, the product of the *lateral branches* of the cochlear nerve before leaving the organ (Pl. I, Fig. 5, *sp.*; Pl. II, Figs. 11, 17; Pl. III, Figs. 25, 27). The main (system) collection of these fibres is found below and inside of the inner hair-cells, *i. e.*, in the lymph space generally formed in this region, and which has been named Nuel’s canal, a name which there is no necessity for retaining. In preparations of the fresh organ of Corti, this lymph space, with its contained structures and many of the adjacent cells, comes out as a long cylindrical structure, having the appearance shown in Pl. III, Fig. 10, of my Vertebrate Ear Memoir. On Pl. III, Fig. 28, I have sketched the

appearance of a portion of the nerve bundle within this lymph space, as defined by the Golgi stain.

D. *The Foramina nervina of the Habenula perforata.*

All previous observers have described the holes in the habenula perforata as slit-like or oval openings, and there is a unanimity of opinion (supposed to be fully justified by repeated observations by scores of observers) that the auditory nerve enters the cochlear canal in regular bundles through these holes.

It is true some observers have laid special stress upon the fact, admitted by all, that among birds and reptiles the nerves emerge from the basilar membrane, not in bundles, but singly, and also that in the upper end of the organ of Corti in Mammals the same method of entrance exists.

I held the same views until Golgi preparations taught me better. The bundles of nerve fibres from the cochlear ganglion, as they approach the habenula perforata, are not so compact as they appear in ordinary preparations of the cochlea, for the silver stain shows that there are no sharply defined *foramina nervina* for bundles of nerves, but that only an approximation to this condition obtains, while many nerve fibres may enter the cochlear canal between any two fibres of the basilar membrane, which are thus separated and which may be said to form the boundary of their foramen. Many of the nerves will be found to pass between other fibres of basilar membrane in such a way as to produce irregular groups of fibres (Pl. III, Fig. 32). In Golgi stains of this region, with the organ of Corti removed, the broken nerves appear as dots, and an inspection of Fig. 32 will make it clear that there is nearly a continuous series of fibres which find their way through the basilar membrane. Periodically reinforced by larger bundles of fibres more or less closely packed together, this nearly continuous series of fibres (the Sauropsid condition) assumes the appearance of a series of separate groups here and there continuous and with a tendency (everywhere shown) to fuse together.

E. *The Nerve Fibres in the Lamina ossea.*

The course of the fibres of the cochlear nerve in the *Lamina ossea* I have already given, but of the characters of the bundles of nerves within this region I can say little. On Pl. III, Fig. 19, are shown several bundles of fibres in this region. The figure illustrates how the fibres group themselves in bundles, leaving elongate interspaces between the bundles quite as well as any of the Golgi preparations show it, but I shall reserve for my paper on the results of the Methylen-blue stain a complete account of the structures occupying the *Lamina*.

The same causes seem to operate here that are so effective in the organ of Corti, to turn fibres out of their direct course, and as a result there is an extensive "anastomsosis" of bundles.

Part of this interchange of fibres is a simple displacement of fibres from a direct path, but apparently another part is of a much more important nature, and consists in the connecting together of different parts of the cochlear apparatus nervously, and in this way, of course, connecting many or few, more or less widely separated peripheral points in the cochlear percipient organ with single points in the central nervous system. Collateral fibres are frequent in the region of the *Lamina ossea*, but whether they occur as abundantly centrad of the ganglion I have not been able to determine. They do occur, but heretofore they have only been seen in a few instances.

F. *The Auditory Ganglia especially the Ganglion cochlearis.*

The ganglion cells of the cochlear ganglion take a very prominent place in the discussions of ear anatomy at the present time and their origin and nature is a matter of much importance to the proper understanding of the structure and workings of the acoustic apparatus. Retzius and van Gehuchten unhesitatingly say that all the cells of the cochlear ganglion are bipolar cells from which the two fine nerve filaments take their rise, one going to the periphery as the hair-cell element the other going to centre as the brain element. At either end the nerve is supposed to end freely and to acquire relations to other nervous structures by mere contact at the

very most. Their views are not sustained by the facts of adult anatomy. It will however be impossible to throw satisfactory light upon this matter until the development has been worked out with completeness. It may not be a waste of words to record the following conclusions which have grown out of my study of the innervation of the ear. Although tentative they have the harmonious support of the facts of adult anatomy and involve at the same time an explanation of the histogenetic processes which is entirely probable on account of the fact that the nerve process is continuous with the hair-cell (this point I have just recently verified by the Methylen-blue method which gives the needed histological details of the continuity wanting in the Golgi preparations) and we may thus hold that the hair-cell is a genuine nerve cell and the cell of origin of the auditory nerve fibre. The ganglion cell which lies at a distance from the hair-cell in the *canalis ganglionaris* of the adult was at the very least, in contact with the hair-cell at an early stage of its development for the ganglion is continuous with the superficial layer of cells of the embryo which become the superficial hair-cells of the adult. As development proceeds we know that the ganglion recedes further and further into the head until it reaches its adult position. My assumption is this—during the multiplication of the sense organ rudiment in early stages, the ganglion cells are the product of the division of the superficial hair-cells, and as development proceeds the protoplasmic bridge left over from an incomplete cell division is drawn out into a fine thread—the fibre which crosses the lamina of the adult. Either before or soon after this bipartition of the primitive sense cell began the centrad process started for the brain from the proximal end of the primitive sense cell which of course, in the adult, remains the proximal end of the ganglion cell. In case of the division of the superficial hair-cell the impulse<sup>1</sup> generated in it would of course travel to the other end of the cell and cause a like division of the ganglion cell so that we should expect to find bipolar cells in the ganglion, and they are there in abundance. But we also find many multipolar cells whose presence is only

<sup>1</sup> Impulse to division in a plane at right angles to the first division.

to be accounted for by supposing that the impulses to division sent onward from the periphery have not influenced the ganglion for some cause not now conspicuous. And finally, some of the divisions do not reach the ganglion cell, and thus give rise to branching fibres between the ganglion and hair-cells. As a result we would get from this process exactly what we find in the ganglion and organ of Corti, *viz.*, a single ganglion cell with two or more hair-cells connected with it by nerve fibres while only one nerve fibre proceeds from the ganglion cell to the nerve centre.

Most of the complicated figures and groups formed by hair-cells and nerves in the organ of Corti lend themselves to this explanation while I have failed to find any other satisfactory view applicable to the sensory structures of the whole ear. Only a detailed knowledge of the histogenesis of this organ can determine the full story of this process.

The cochlear ganglion (Pl. I, Fig. 1), is an elongate cylindrical body, spirally twisted to fit the helical inclination of the proximal portion of the lamina ossea which is here channeled by a lack of ossification. This *canalis ganglionaris* or Rosenthal's<sup>1</sup> canal of the human ear, is marked by the successive enlargements and constrictions of the cord of ganglion cells and nerve fibres which it contains and which really represents a chain of ganglionic bodies. A radial section passing vertically through the helix and through one of these enlargements shows the maximum size of the ganglion. The cells are quite regularly distributed through the body of the ganglion and are separated by the passing nerve fibres and the network of blood capillaries which riddle the ganglionic body, and which unite with a more compact vascular network forming a cover to the mass of ganglion cells. Most of the ganglion cells are bipolar, but multipolar cells (Pl. II, Figs. 12 and 13), are not uncommon (3-6 processes). Most bipolar cells have the nerve fibre running through them radially as this is defined above, but others are so placed that the fibre passes not directly out of the ganglion, but follows along in among the cells in one of the two directions, to sooner or later emerge from the body and make

<sup>1</sup> By an inadvertence this was printed Rosenberg's canal in my recent memoir.

its other connections. These may be called the spiral nerves of the ganglion.

The multipolar cells receive several radial fibres and unite them into one body and give issue to only one central fibre which may in its turn receive a collateral which passed the ganglion cell or split off from the ganglion cell so as to appear to arise from the central fibre (Pl. II, Fig. 12, 13). Not all of the fibres running to the periphery arise from the cell body since some of them may form by the splitting of the cell process even at a considerable distance from the cell.

The whole question on both sides may be summed up in these words. Both sides claim that the bipolar (and multipolar, Ayers) ganglion cells have contact both with the periphery and with the central nervous system. European investigators claim that although the bipolar ganglion cell was originally the superficial hair bearing acoustic cell it has gone below the surface by elongating its body peripherally into a long nerve filament which enters into relations with other superficial cells which have become hair bearing acoustic cells, while I claim that these investigators have overlooked the continuity of the bipolar ganglion cell with the hair-cell and that in consequence of this continuity we must still look upon the surface cell as one-half of the primitive acoustic cell and the bipolar cell as the other half which by incomplete cell division has become spatially separated from its congener though still structurally continuous with it. And I further claim that only on this assumption can we explain the many peculiarities of the finer anatomy of the auditory sense organs which their theory fails to elucidate. Ontogeny alone can decide this question.

#### G. *The Sauropsid Organ.*

The evanescent auditory organ which I have described for mammalian embryos has a very interesting history with regard to its innervation. At a time when the organ of Corti is still very small the Sauropsid organ has reached its greatest development and most of the nerves which pass into the cochlear ganglion really arise from its epithelial cells. The cells having

continuity with the nerve fibres are in many points unlike the hair-cells of Corti's organ, but they are probably, in essentials, the same. A detailed account of them I shall not attempt here but reserve for a later publication. Before adult life is reached this entire organ is resorbed and its place is marked in the mammalian cochlea by the *sulcus spiralis internus*. Its peripheral hair cells and their central prolongations, with their connections being thus annihilated must leave important changes in the nervous constitution of the growing mammal the history of which has yet to find a student.

In Figs. 20 and 21, Pl. III and in Fig. 1, Pl. I are shown the peripheral nerve branchings in the Sauropsid organ. The continuity of the hair cells of this organ with the nerve fibres is given in Pl. I, Fig. 3, where the cells are seen to be different in shape from those of its offspring the Cortian organ. From all I have seen thus far, however, I am inclined to the view that the two apparently different cells are essentially alike, but no definite conclusions can be drawn from Golgi pictures alone. After leaving the hair-cell the nerve (Pl. III, Fig. 21) runs directly to the basement membrane of the organ — *membrana basilaris*, and after piercing it bends suddenly inward on its way to the ganglion. Varicosities occur all along the course of the fibres but they are more numerous peripherad of the ganglion than centrad of it. This statement is true for all classes of vertebrates. Here as in the Cortian organ they are found close up to the hair-cells and in case of the fibres from both organs they occur in contact with the ganglion cells. In describing this organ as an embryonic sense organ in my recent paper I had not been able to satisfactorily determine that its cells received nerve fibres in the mammal. I was able to do so however in the case of its homologue in the Alligator. Under the circumstances it is a pleasure to add this further point to our knowledge of the race history of the mammalian stock and another important problem to the endless list already before morphological workers for solution. From an examination of Retzius' figures and Van Gehuchten's account of cochlear innervation I have no doubt that a part of what they have described as nerves in the organ of Corti are really nerves in the Sauropsid organ, and until they distinguish clearly

between these structures (the latter of which as yet they do not appear to recognize at all) they are sure of perpetuating some of the errors they have already published.

H. *The Maculae and Cristae, especially the Macula sacculi.*

The hair-cells of the maculae and cristae are readily stained in continuity with their nerve fibres and the conditions are not fundamentally different from those described above for the hair-cells of the cochlea, though the relation of the parts in the maculae and cristae is simpler and more primitive, and deserves careful study on this account, since it enables us to better unravel the intricacies of cochlear anatomy. The acoustic cells Pl. II, Fig. 16; Pl. III, Fig. 29, reach only about half way from the epithelial surface to the basement membrane and the nerves from these cells pass, of course, interepithelially downwards and through the floor of the sensory structure and form into the nerve trunk of the same name as its respective organ. Ganglion cells have not been stained on any of the fibres from the maculae in my Golgi preparations and I notice Van Gehuchten has had the same experience. There is however no reason to doubt their occurrence here and Retzius records finding them by this method. In most sections (perpendicular to the surface) of the maculae acusticae (specifically in this instance Mac. ac. sacculi) the hairs from the tops of the hair-cells are more or less bent, oftentimes considerably so, by the contraction and consequent greater pressure of the otolithic mass upon the surface of these organs, as is illustrated in Pl. II, Fig. 16 and Pl. III, Fig. 30 b. In such cases the length of the hairs on the cells is *seen to be double that usually obtained by measurement after removal of the otoliths.*

In Pl. III, Fig. 29 I have sketched with aid of a camera some of the stained nerve fibres and bodies present in a thick section of the saccular sense organ of the pig of 14 cm length. There are three points here shown which are of frequent occurrence and therefore usual and normal and which appear to be of importance in comparison with cochlear anatomy. At the bases of the hair-cells among the nerve fibres relatively large bodies certainly cellular in nature occur. They have many fibres running from them. These same figures are seen

in cochlear preparations lying among the supporting cells at the bases of the hair cells. The fibres of the saccular nerve do not always leave the organ as simple fibres but frequently as several branches which soon unite into a single fibre, and finally there are numerous fibres in the simpler sense organs of the ear which take a course at right angles to the long axes of the sense cells and run through the epithelial tissue of the organ for considerable distances. They are varicose fibres showing occasionally collateral branches and they sooner or later bend upward to end in hair-cells or downwards to unite with some other fibre on its way out of the organ, or to make a separate exit by itself. To my mind they are the homologues of the "spiral fibres of the Cortian organ.

In view of the facts above recited we may fairly conclude:

1. That hair-cell and bi- or multipolar cells of the cochlear ganglion — are both parts of a single morphological unit — the *acoustic element* which mediates between points at the surface and points in the nervous centre.

2. That there is no fundamental difference between the acoustic and olfactive elements yet made known.

3. That all fibres of the auditory nerve proceed out of hair-cells alone so far as has yet been satisfactorily determined.

4. That the so-called spiral fibres are but parts of individual radial fibres bent to a right angle, more or less, out of their course. They are in some cases the product of the lateral branches of the cochlear nerves within the organ of Corti, the region of the lamina or the cochlear ganglion itself.

5. That in the mammalian embryo the auditory nerve is at one time mostly composed of nerve processes arising in the Sauropsid organ, and that as this organ fades away and the mammalian organ increases in size the nerve becomes more and more a bundle of fibres from the acoustic cells of this latter organ.

6. That so far as we yet know there is no special significance to be attached to the fact that the majority of the cells of the cochlear ganglion are "bipolar" though intermixed with an important minority of multipolar cells of from 3 to 6 processes.

## EXPLANATION OF THE FIGURES.

Unless otherwise stated, all figures are from preparations of foetuses of *Sus scrofa domestica*.

## PLATE XX.

FIG. 1. A vertical section of the cochlea of a 14 mm. foetus from the center of that organ. The course of the fibres in the modiolus is well shown. The characteristic shrinkage in the size of the nerve-bundle after leaving the ganglion is given, and the appearance of the ganglion in cross-section as stained with the silver method is that commonly found in these preparations, but does by no means convey a truthful idea of natural conditions. At *a*, one hair-cell, two supporting cells, and some of the nerve branchings in the Sauropsid organ are shown; *b* shows the branching nerve better, while *c* does the same for the supporting cells; in *d* and *e* the Cortian organ and the nerves running to it are shown; non-nervous parts in outline. Htnk. obj.  $\frac{1}{2}$  of 2 Obh. camera.

FIG. 2. A single cochlear canal and its ganglion and nerves from a section similar to the above, more highly magnified. At *h*, three outer hair-cells and their relations to the nerve fibres indicated. The continuity of one nerve fibre and hair-cell is shown.

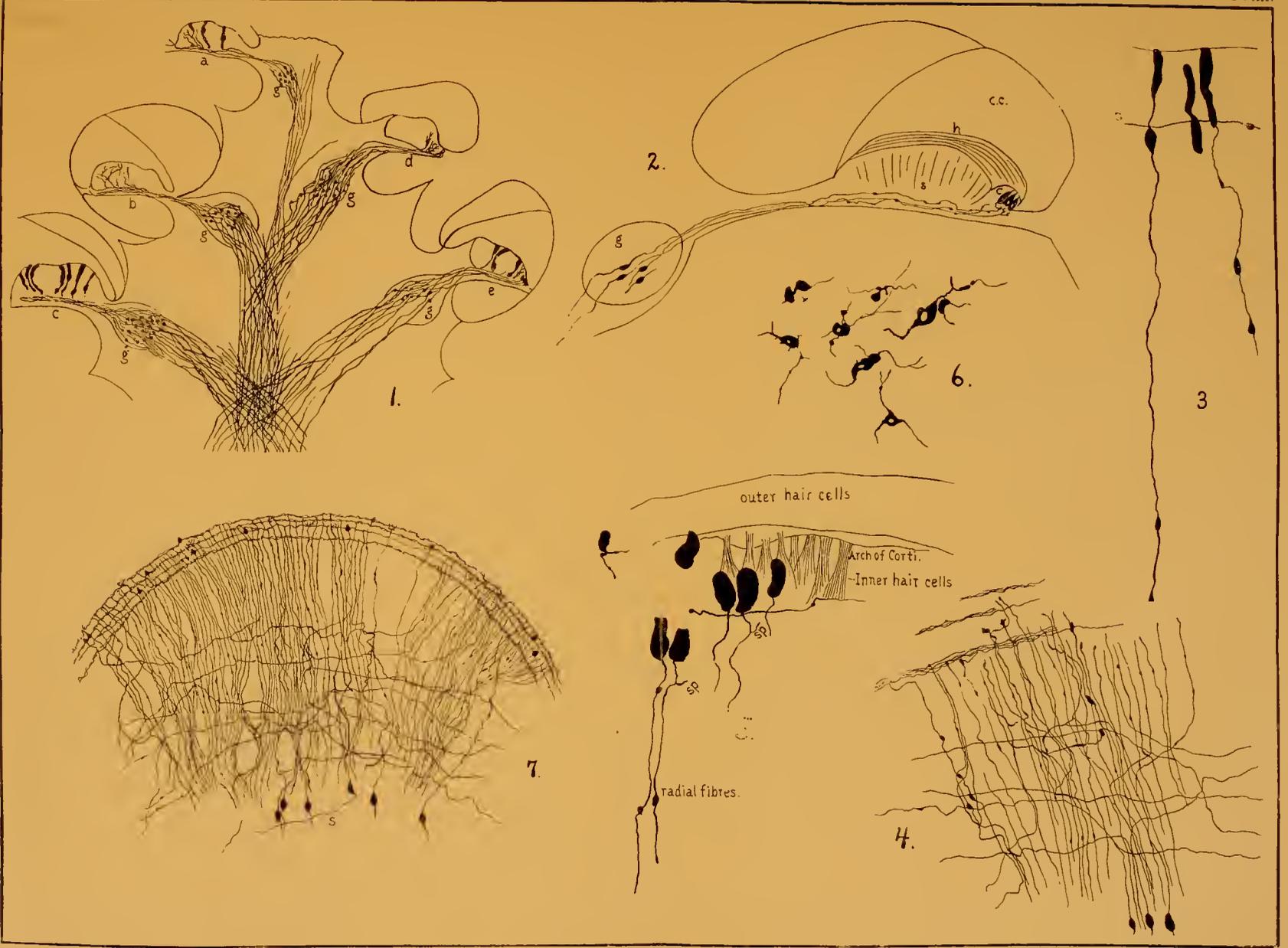
FIG. 3. Surface view of a portion of the Sauropsid organ showing the continuity of the nerve fibre and sensory cells.

FIG. 4. A portion of the nerves included between the cochlear ganglion and the outer hair-cells. With this magnification the connections of the radial nerves with the spiral bundle are not clearly defined. In the region of the lamina the aberrant fibres do not occur in bundles. Three ganglion cells and numerous sheath cells occurring as varicosities are shown. Htnk. obj. 2 Obh. cam.

FIG. 5. A portion of the arch of Corti with five inner hair-cells with their nerves. The elements of Corti's arch are stained a rusty brown, while cells and nerves are completely black. Htnk. obj. 5 Obh. cam.

FIG. 6. Six ganglion cells from the cochlear ganglion. These are all multipolar cells save one "X," which is a double ganglion cell. Htnk. obj. 5 Obh. cam.

FIG. 7. About one quarter of a complete turn of the cochlear lamina, showing the appearance and relations of the constituent parts of the nerve plate of the lamina. Near the ganglion one notices suboval spaces free from nerves; these spaces later on are occupied by bone. The nerve fibres run both ways from the ganglion. The spiral bundles of fibres appear as complete with this power. A few hair-cells are sketched in, but only such detail is put down as could be introduced into a drawing on this scale. Htnk. obj. 2 Obh. cam.







## PLATE XXI.

FIG. 8. A surface view of the cochlea of a 14 cm. foetus showing the nerves from the ganglion to the organ of Corti. The hair-cells are not stained and consequently the stain is incomplete. Htnk. obj. 5, Obh. cam.

FIG. 9. The peripheral portions of a few of the nerves from the last preparation more highly magnified, to show the manner of branching of the nerve end among the hair-cells.

FIG. 10. Two hair-cells and their nerves with a third fibre leaving unusually large varicosities. Htnk. obj. 5, Obh. cam.

FIG. 11. A surface view of a portion of the plexus of nerves in the Cortian organ. One fibre is seen to bend off and enter the spiral bundle.

FIG. 12. A single ganglion cell having three peripheral processes and one central process. Htnk. 5.

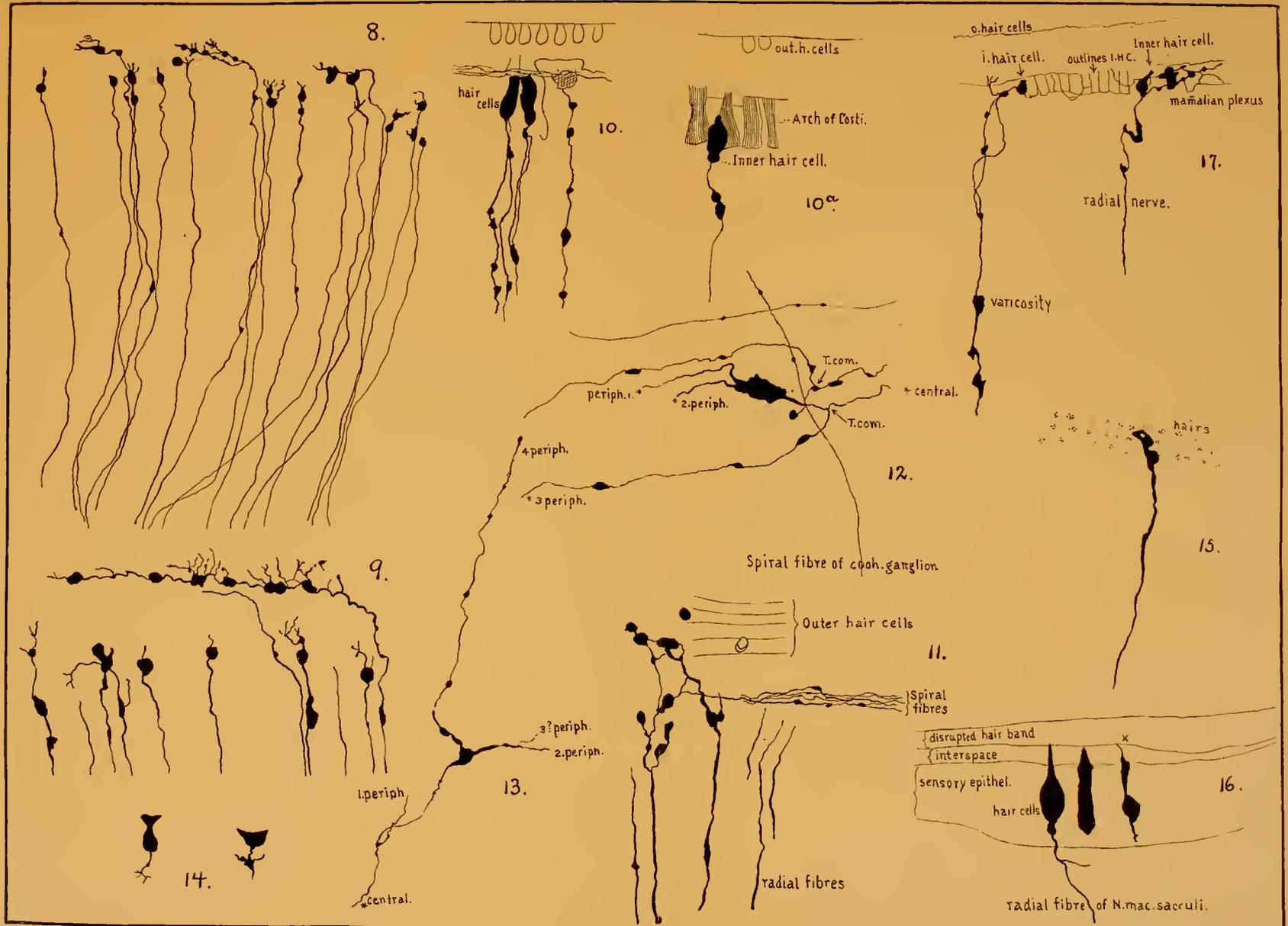
FIG. 13. A multipolar cell from a 14 cm. embryo, with four peripheral processes. Htnk. obj. 4, Obh. cam.

FIG. 14. Two cells from the Macula sacculi of a 14 cm. foetus with their nervous prolongations. Htnk. obj. 5, Obh. cam.

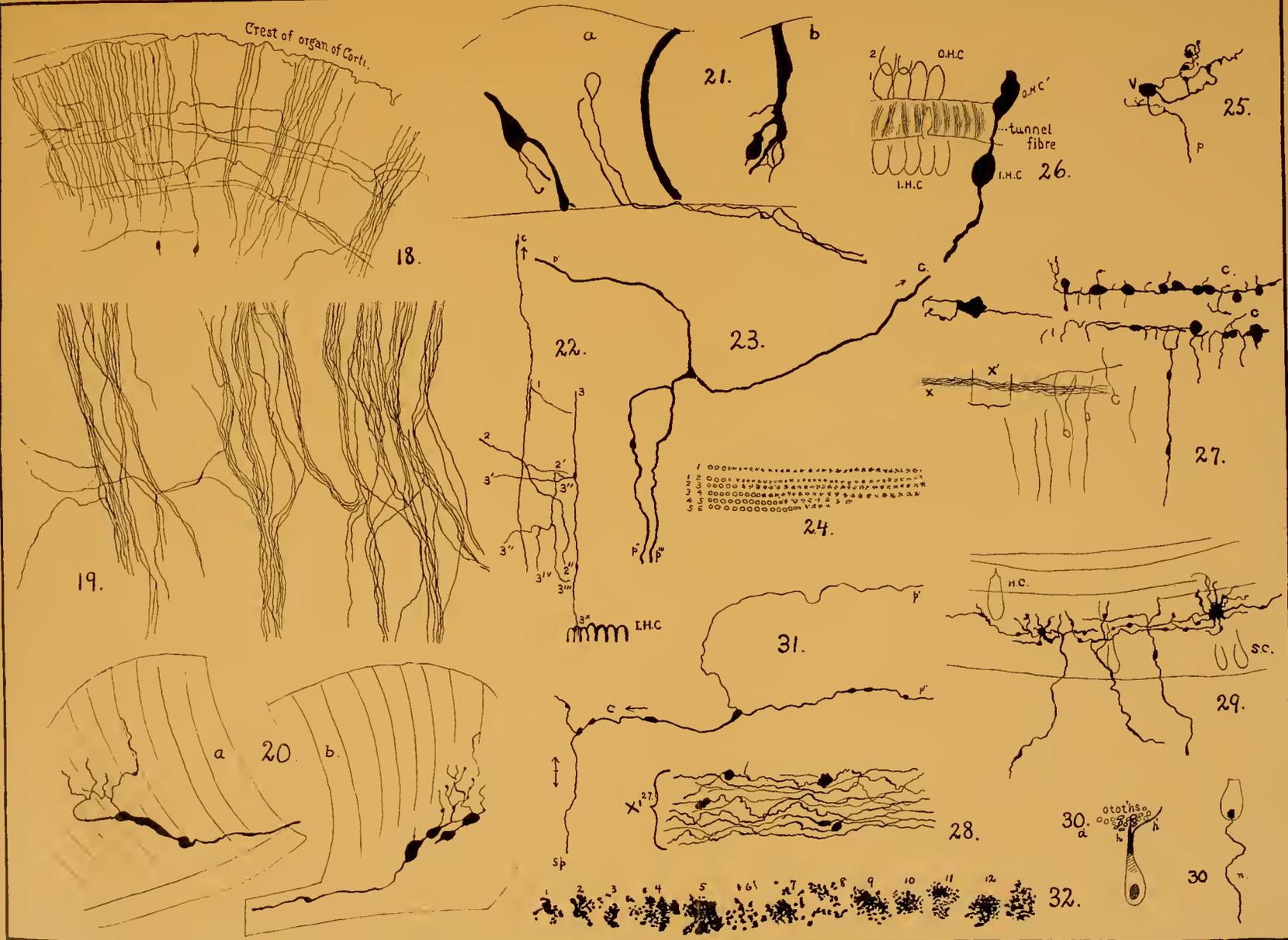
FIG. 15. A hair-cell from the second row of outer cells and its nerve. Three rows of stained hair butts indicating the position of the other hair-cells. Htnk. obj. 5, Obh. cam.

FIG. 16. Three hair-cells from the Macula sacculi of a 14 cm. foetus one of them showing the nerve continuity. Htnk. obj. 5, Obh. cam.

FIG. 17. Two nerve fibres and their hair-cell terminations some of which are not stained out to the cells themselves. Htnk. obj. 5, Obh. cam.









## PLATE XXII.

FIG. 18. Shows the character of the radial fibres between the ganglion and organ of Corti and illustrates the manner in which they may become for a greater or less distance spiral in course. Htnk. obj. 2, Obh. cam.

FIG. 19. A portion of the nerve plate from the lamina of a 10 cm. foetal ox to illustrate the mixing or anastomosing of the fibres of the nerve bundles between the ganglion and organ of Corti. Htnk. obj. 4, Obh. cam.

FIG. 20. Two end brushes of nerve fibres ending in the Sauropsid organ. The points of importance are the large varicosities (group of sheath cells) which the fibres possess just before breaking up into the terminal branches, Htnk. obj. 5, Obh. cam.

FIG. 21. Three supporting cells from the Sauropsid organ and two nerve fibres, which as stained, show in the one case an oval terminal body brown in color, and in the other case only a free end. Htnk. obj. 5, Obh. cam.

FIG. 22. Part of the nerve plate between ganglion and organ of Corti to show branching fibres. Fibre 3 has a varicosity from which is given off a collateral  $3^i$  which in turn gives off one of its own  $3^{ii}$  while the main trunk of 3 is continued to the hair-cell at 3. The collateral given off from  $3^i$  divides into three terminal branches or more correctly it gives off two other collaterals  $3^{iii}$   $3^{iv}$  while it is continued to a hair cell not shown in the preparation. Fibre 2 under goes one division as does fibre 1. Htnk. obj. 4, Obh. cam.

FIG. 23. A single radial nerve fibre which from a triangular enlargement gives off two processes one of which divides into two. These three processes extend to the peripheral organ. Htnk. obj. 5, Obh. cam.

FIG. 24. A surface view of the organ of Corti showing one row of inner hair-cells and five rows of outer hair-cells. The fifth row is not continuous, but suffers frequent interruptions. Htnk. obj. 4, Obh. cam.

FIG. 25. Surface view of a part of the network of nerve fibres in the Cortian organ at the base of the hair-cells.  $\rho$  the fibre which proceeds to the ganglion.

FIG. 26. A view from above of the upper surface of the Cortian organ the focus being on the arch of Corti. One outer hair-cell is stained and is connected with an inner hair-cell, equally well stained, by a nerve fibre which crosses the Cortian tunnel somewhat obliquely. Owing to the point of view the nerve appears to pass through the inner hair-cell, whereas it passes below it. Htnk. obj. 5, Obh. cam.

FIG. 27. Part of two commissures or connecting nerves lying just below, *i. e.*, in contact with the bases of the hair-cells of two inner rows of the outer series, showing the characteristic enlargements and the numerous delicate processes which are given off sometimes from the fibres, sometimes from the swellings. At  $x$  is shown the bundle of fibres lying below the inner row of hair-cells, which forms the most pronounced bundle of the so-called spiral nerves. A part of this structure is shown more highly magnified in the next figure. Htnk. obj. 5, Obh. cam.

FIG. 28. The bundle of nerve fibres associated with the inner hair-cells to show the nature of the fibres, which are extremely fine filaments. Leitz, obj. 12, Obh. cam.

FIG. 29. A portion of the nerve supply of the macula sacculi, with one of the peculiar ganglionic bodies found in the epithelial layer and the final branchings of the radial nerves, forming horizontal fibres, the homologues of the spiral fibres of the cochlea.

FIG. 30. (*a*) A hair-cell from the macula sacculi, with nerve end in the cell; (*b*) a hair-cell with its hair bent by the contraction of the tissues and of the otolithic mass. Htnk. obj. 5, Obh. cam.

FIG. 31. To show the branchings of a spiral fibre in the neighborhood of the ganglion. Htnk. obj. 5, Obh. cam.

FIG. 32. The habenula perforata of a young dog (16 days), showing the manner of entrance of the nerves into the cochlear canal and the absence of circumscribed foramina nervina. Surface view of the region after removal of the organ of Corti. Htnk. obj. 8, 160 mm. d. t. Obh. cam.

# A CONTRIBUTION TO THE MORPHOLOGY AND BIOLOGY OF THE STENTORS.

HERBERT P. JOHNSON.

## CONTENTS.

	PAGE
I. Introduction .....	468
Methods .....	469
II. Systematic and Faunistic .....	471
1. <i>Stentor igneus</i> .....	472
1a. <i>S. igneus</i> var. <i>nigricans</i> .....	473
2. <i>S. pyriformis</i> .....	474
3. <i>S. roeselii</i> .....	476
4. <i>S. polymorphus</i> .....	477
5. <i>S. cæruleus</i> .....	477
III. Morphology .....	478
A. Anatomy .....	479
1. Ecto- and Endoplasm .....	479
2. Ectoplasmic Structures .....	480
a. Pellicula .....	480
b. Stripes .....	481
c. Myonemes .....	483
d. Pigment .....	485
e. Cilia ; "Tactile spines" .....	486
3. Pharyngeal Funnel .....	488
4. Endoplasmic Structures .....	489
a. Membranellæ .....	489
b. Foot .....	492
5. Nuclei .....	493
a. Meganucleus .....	493
b. Micronuclei .....	498
c. Generalia .....	500
B. Fission .....	500
1. Cytoplasmic Phases .....	501
2. Nuclear Phases .....	512
a. Meganucleus .....	512
b. Micronuclei .....	517
3. Ontogeny and Phylogeny .....	518
C. Regeneration .....	521
1. Cytoplasmic Phases .....	522
2. Nuclear Phases .....	523
3. Generalia .....	525

D. Conjugation.....	527
1. External Phenomena .....	527
2. Nuclear Phenomena .....	528
E. Teratology .....	531
IV. Biology and Physiology .....	535
A. Rate of Multiplication .....	535
B. Relative Vitality of Large and Small Stentors .....	540
C. Nutrition ; Alimentary Vortex.....	542
D. Defecation ; Position of Anus .....	544
E. Contractile Vacuole .....	547
F. Merotomy .....	548
V. Literature cited .....	552

### I. INTRODUCTION.

OCCUPYING a place about midway between the lowest and the most highly-organized of the Ciliate Infusoria, and possessing in large measure the characteristics of both free-swimming and sedentary forms, the genus *Stentor* may well serve as type of its Class. Their abundance, large size, and strongly-marked morphological characters have made the Stentors favorite subjects for study with zoölogists ; and in recent years one species, *Stentor cæruleus*, has been repeatedly used in cytological research, particularly in experimental work undertaken to ascertain the physiological rôle of the nucleus.

In the present paper observations upon five species of American Stentors are recorded. All have been obtained in abundance except *Stentor roesclii*, which, not being gregarious, cannot be collected in large numbers.

The work began with a study of the nuclear changes of *S. cæruleus* at time of fission, undertaken at the suggestion of Prof. E. L. Mark, when I was under his instruction at the Zoölogical Laboratory of Harvard University. The scope of the work was afterwards extended, and the investigation of several phases in the life-history of the Stentors was carried on at Williams College, at the Marine Biological Laboratory, Wood's Holl, Mass., and at Clark University, Worcester, Mass.

Material has been obtained from four widely-separated localities in Massachusetts : Cambridge, Williamstown, Falmouth, and Worcester. Each locality has yielded in abundance some

species or variety found very sparingly or not at all at the others, and only one form, *roeselii*, has been collected at all four stations.

#### *Methods.*

The study of Infusoria requires a special technique; and, as the wonderful results recently obtained by Maupas ('88, '89) forcibly show, an accurate acquaintance with the life-history and habits of the species under investigation is of the utmost importance. One of the most valuable aids in all biological and morphological research upon these organisms is the isolation of individuals for continuous study and experiment,—a method exceedingly simple in conception, but, owing to the minuteness of most Infusoria, often far from easy to carry out. This difficulty, however, hardly exists with the Stentors, which rank among the largest of their Class, and are easily visible to the naked eye.

In collecting, it has been my custom to bring to the laboratory as much as convenient of the natural surroundings of the Stentors—vegetable *débris*, sticks, stones, water-weeds, etc.—and a considerable quantity of the water in which the Stentors live. The gathering is put into shallow glass dishes, which are replenished with tap-water from time to time to make up for evaporation. Under these conditions *S. cæruleus* will usually thrive and multiply for a week or two; but owing to the rapid exhaustion of food, and to less well-ascertained causes, one cannot in this way maintain a permanent colony. *S. polymorphus*, *S. igneus* and *S. pyriformis* will usually live in aquaria much longer than the Blue Stentor; they, however, merely survive as individuals and seem never to increase in number.

In order to select individuals in fission or other conditions desired for study, I have used a simple contrivance by means of which large numbers can be rapidly reviewed. A slide is prepared by affixing a piece of sheet-wax a millimeter or less in thickness and about an inch square, in which circular holes have been punched. The holes are conveniently made with a cork-borer, and measure 3 mm. in diameter. The wax plate is fused to the slide by very cautious heating of both slide and

wax. A drop of water containing one or more Stentors is placed in each cell, and the specimens examined in turn with a low power of the compound microscope.

As to killing reagents, no reliable method has yet been found for fixing, in a state of extension, these highly-contractile Infusoria. Hydrochlorate of hydroxylamine, used in .25 per cent solution, neutralized with sodic carbonate, has been recommended by B. Hofer<sup>1</sup> as a paralyzing reagent, but I have tried it in vain on *S. cæruleus*. On the other hand, it seems impossible to find an agent of sufficiently rapid action to fix the animal before it contracts. Fortunately, contracted or semi-contracted specimens answer very well for most purposes. I have used for fixation, absolute alcohol, Flemming's chrom-aceto-osmic mixture, 1 per cent to .25 per cent osmic acid, Merkel's fluid, saturated aqueous solution of corrosive sublimate, and picro-acetic. The last two have proved the most satisfactory. The picro-acetic mixture I make up as follows: saturated aqueous sol. picric acid, 6 parts; glacial acetic acid, 1 part. It gives excellent results used either hot or cold, and should invariably be washed out with 50 per cent to 70 per cent alcohol.

In staining, two methods have been followed. First, for immediate study, one may fix and stain with a single reagent, such as an aqueous solution of methyl-green, to which a little acetic acid has been added; or Schneider's aceto-carmin. This method is invaluable for study of the structure of the meganucleus. Neither are lasting stains. For fixed and hardened material, intended for permanent preparations, I find that almost any of the carmine stains are good, while haematoxylin tinges the cytoplasm too deeply. I have used mainly Czokor's alum cochineal and picro-carmin, for they stain the cytoplasm least. In making preparations for study of the nucleus, decolorizing with acidulated alcohol is important, and should be carried far enough to extract all stain from the cytoplasm.

I have found it perfectly feasible to imbed Stentors in paraffine, and cut serial sections  $5\mu$  to  $10\mu$  in thickness. Sec-

<sup>1</sup> Zeits. f. wiss. Mikr., vii, pp. 318-326, 1890.

tions mounted in balsam, damar, or glycerine are essential for study of the micronucleus, particularly at times of fission and conjugation.

## II. SYSTEMATIC AND FAUNISTIC.

The classification of the Stentors has always been difficult, on account of the great variability of most of the species. Consequently, different authorities have held very divergent views as to the number of species. Claparède and Lachmann ('59), for example, relegate the six forms described by Ehrenberg ('38) to two species, while Saville-Kent ('82) increases the number to nine. But the whole question of the specific distinctness of the different forms found in Europe has been so ably and fully discussed by Stein ('67) and his six species<sup>1</sup> have held their own so successfully that I see no occasion to alter his classification. Only one valid species, *Stentor auricula*, Sav. Kent, has been added to the European list since Stein; for I question very much whether *S. Barretti*, Barrett, will on further examination prove to be distinct from *S. roeselii*; and the various alleged "new species" of de Fromentel ('74) imperfectly described and too evidently based upon aberrant specimens, no longer hold a place in the system.

Our American Stentor fauna shows a surprising correspondence with that of Europe. With the exception of two marine forms, *S. multiformis* and *S. auricula*, every European species has been mentioned as occurring in this country.<sup>2</sup> In addition there seem to be two species (*S. globator*, Stokes ('85), and *S. pyriformis* sp. nov.) not found in Europe; but it must be admitted that neither of these species can be considered as fully established. *S. globator* is so aberrant that it might be regarded as based upon abnormal, dwarf specimens of one of the well-known Stentors, especially as the discoverer does not appear to have observed it more than once. While *S. pyriformis* is founded upon examination of a very large number of specimens, none of which showed transition to any known

<sup>1</sup> *S. polymorphus*, *cæruleus*, *roeselii*, *igneus*, *niger*, and *multiformis*.

<sup>2</sup> I have never myself seen *S. niger*; but it is given by Stokes ('88) in his list of American species.

species, it must be remembered that all the specimens came from a single locality; and I have found that Stentors, like many other animals of wide distribution, offer strongly-marked local varieties.

1. *Stentor igneus*, Ehrbg.

This species was found in abundance in two small ponds in Falmouth, Mass., during the summer of 1891. One of these ponds — a mere pool covering the bottom of a “kettle-hole” — is located near the village of Wood’s Holl. The pond is very shallow and choked with Sphagnum, among the fronds of which the Stentors live. The other pond was much larger, situated two miles from the first, near the village of Quisset. Here the Stentors were found among pond-lilies (*Nymphaea odorata* and *Brasenia peltata*) and other water-plants. They were not uniformly distributed, but colonized in certain places, apparently limited to the south side of the pond. This was probably owing to the fact that the other shores were wooded, and consequently shaded during a part of the day; for *S. igneus*, like all chlorophyll-bearing Stentors, is heliotropic.

This form agrees very accurately with Stein’s description and figures of *S. igneus*, and I have no hesitation in referring it to that species. The shape assumed by the animal in swimming varies from conical to pyriform. When attached and extended it assumes a trumpet-form very nearly like that of *S. polymorphus*, but not so attenuated. I have always found symbiotic green algæ (*Zoöchlorella*) present. The red pigment, however, is most frequently wanting. I have never found it in sufficient quantity to give a red tint to the animal as seen with the naked eye. The scantiness and frequent absence of pigment may be only a local or seasonal peculiarity, inasmuch as Leidy (’80) described a Stentor<sup>1</sup> from New Jersey in which the abundant pigment-granules imparted a crimson or lilac color to the animal.

As is well known, the meganucleus of *S. igneus* is a spherical or oval body, either single or multiple. Only small individuals,

<sup>1</sup> *S. amethystinus*, Leidy. I find nothing in his description, however, that would separate this form specifically from *S. igneus*.

so far as I have observed, have a single nucleus ; the majority have two, and many from three to five. Only once have I observed six. Stein ('67, p. 263) sometimes found three nuclei, but never a larger number.

1a. *Stentor igneus* var. *nigricans*, var. nov.

(Fig. 1; Pl. XXIII.)

In a little pond at Williamstown, Mass., I found countless numbers of a small Stentor, which seemed so different from all known species that for a long time I regarded it as new. The pond filled a slight depression in the open fields, covering about half-an-acre. It contained a scanty growth of aquatic grasses and rushes, but hardly any other vegetation. The Stentors were collected at various times during the fall of 1890 up to the time of freezing, and a few were kept in the laboratory through the winter. In the following April they were again obtained, at first in small numbers, but soon in the greatest abundance. By the middle of May they were so numerous as to give a dark coffee-color to the water of the pond, and completely cover all objects beneath its surface.

To the naked eye this form appears as a minute conical body, blackish or olive-green in color. Under the microscope the green tinge is seen to be due to symbiotic algae, while the blackish color is produced by a minute pigment in the ectoplasm. The meganucleus is conspicuous as a single, glistening body on the right side (Fig. 1, *mgn.*). When disturbed the animals swim rapidly with the usual rotatory motion for a long time ; but under quiet conditions attach themselves to fixed objects.

Later in the season I found at Wood's Holl the same Stentor in the small "kettle-hole" pond above mentioned, living in company with the typical *S. igneus*. The ordinary individuals of the two forms were so different that one could readily distinguish them by the naked eye. Not only do they differ in color, but also in shape and size. But after examining and closely comparing large numbers, I found individuals that could not be referred with certainty to either the type or its variety

*nigricans*. The new form must then be considered as specifically identical with *S. igneus*. But inasmuch as ordinary specimens of the variety differ so strikingly from the type, and even have in some cases a separate habitat, I consider it convenient to distinguish it by name.

Characters: *Small* (length, extended, .204 mm., diameter across frontal field, .136 mm.); form, in extension, broadly conical or top-shaped, broadest across frontal field; posteriorly, abruptly tapering, curved, acute; when swimming and semi-contracted, form conical, pear-shaped or nearly cylindrical; when fully contracted, almost spherical. Membranellæ small and weak; striation of body and frontal field obscure; minute, brownish-black pigment in the ectoplasm; pellicula thin, easily ruptured. Contractile vacuole (Fig. 1, c.v.) on ventral side. Meganucleus single, spherical, 30 $\mu$  in diameter, usually lying near right side (Fig. 1, mgn.). Symbiotic algæ (*Zoöchlorella*) always observed, usually abundant, indistinguishable from those of *S. igneus* and *S. polymorphus*.

## 2. *Stentor pyriformis*, sp. nov.

(Figs. 2 and 3; Pl. XXIII.)

In October, 1891, I found a colony of green Stentors covering vegetable débris and water-weeds at the north end of Lake Quinsigamond, Worcester, Mass. At first I mistook them for unusually large specimens of *S. polymorphus*. But after extracting the chlorophyll with alcohol and staining, the meganucleus was found to be not moniliform, but in form of two or more entirely separate, spherical bodies (Fig. 3). This fact led me to examine the animals with care, and various points of difference between them and *S. polymorphus* were speedily discovered. The new form could not fairly be identified with any described species. It ranks as one of the largest of Stentors, being scarcely inferior to *S. cæruleus* in size. *S. pyriformis* never assumes the slender trumpet-shape of the higher Stentors, and in fact changes its form very little from the pear or conical shape it has while swimming (Fig. 2).

This species does not endure confinement so well as *poly-*

*morphus*, and I have been unable to keep them more than a month. Although I have examined a large number, I have never found one in fission.

In April, 1892, I visited another colony of *S. pyriformis* in Lake Quinsigamond, for information in regard to which I am indebted to my friend Dr. Wm. S. Miller. This colony is located in a winding, shallow bay known as "the Sanctuary." About two acres of the bottom are literally covered with these Stentors, which give it the appearance of being overgrown with a minute green vegetation. The Stentors live at various depths down to four or five feet. The earliest specimens collected (April 18) contained much less chlorophyll than those obtained in the fall, and in many the very narrow and obscure stripes were visible, which is never the case when the zoöchlorellæ are abundant. The specimens, furthermore, were inert, remained constantly contracted, and in many cases I could not distinguish the adoral zone. I believe, therefore, that they had recently emerged from the cyst. Stentors collected at the same place April 23 were more active, and contained a much greater quantity of zoöchlorellæ.

Characters: *Large (length, extended, .5 mm., breadth across frontal field, .2 mm.); in extension, funnel-shaped, elongate conical, or pyriform; in semi-contraction, pyriform or nearly cylindrical; anterior third of body of nearly uniform diameter, same as frontal field; posterior extremity obtuse, rounded; stripes very obscure, narrow (3.5 $\mu$  in width) generally not visible in living animal; adoral zone narrow, membranelle weak, oral aperture placed considerably posterior to aboral extremity of zone; endoplasm whitish, opaque, crowded outwardly with zoöchlorellæ; no pigment; contractile vesicle in usual position, to left of mouth (Fig. 2, c.v.); a single meganucleus rarely observed, nearly always 2-4, spherical or oval, 40 $\mu$  in diameter. Hab., Lake Quinsigamond, Worcester, Mass.*

*S. pyriformis* obviously belongs to the group of small Stentors with simple, spherical meganuclei, and lowly-organized frontal field. Its nearest ally is evidently *S. igneus*, from which it differs in its much greater size, entire absence of pigment, thicker form when extended, and narrower stripes.

3. *Stentor roeselii*, Ehrbg.

(Fig. 4, Pl. XXIII.)

I find no essential difference between the American specimens of this form and the European *S. roeselii* so admirably figured by Stein ('67, Taf. VII, VIII). The meganucleus is often moniliform, it is true (Fig. 4, *mgn.*), but this condition has been found to obtain in the European form as well.

*S. roeselii*, like all its genus, is variable in size and shape. Specimens from Alewife Brook, Cambridge, are for the most part very small; while those found at Williamstown were unusually large, measuring when fully extended, 2 mm. in length and .19 mm. across the expanded frontal field.

This species frequents quiet waters where flocculent vegetable *débris* abounds, in the midst of which the so-called "sheath" is formed (Fig. 4, *sh.*). It is generally held that the sheath is due to a mucilaginous secretion. After observing carefully the habits of the animal, its surroundings, and the structure of the sheath, I am strongly inclined to the view that there is no secretion, but that the sheath is made up wholly of the slime and bacterial zoöglöea in the midst of which this *Stentor* delights to anchor itself. As I have observed it, the sheath is of the most indefinite form, and indistinguishable from the surrounding slime. As Stein long ago pointed out, the sheath has imbedded in its substance various minute foreign bodies (bacteria, diatoms, etc.), the presence of which is not favorable to the view that the sheath is secreted by the animal.

Although *S. roeselii* is perhaps the most sedentary of its genus, it is often seen swimming, when it assumes a clavate form, but slightly widened at the anterior end. On attaching itself, it expands into a graceful trumpet- or calla-like form (Fig. 4). In this condition *S. roeselii* is readily distinguishable from colorless specimens of *S. polymorphus* (the *S. Mülleri* of Ehrenberg) not only by its more slender form, but still more sharply by the characteristic shape of the frontal field. In fact, the frontal field of this species displays the highest development of any *Stentor*, and judged by this character alone, *S. roeselii* would rank the highest of the *Stentors*.

*S. roesclii* endures confinement better than the Blue Stentor, and I have kept specimens for months under seemingly very unfavorable conditions. They sometimes multiply to some extent in aquaria. The food consists largely of bacteria.

4. *Stentor polymorphus* (Müll.), Ehrbg.

The specimens of this species which I have studied do not differ in any important respect from the typical *S. polymorphus* of Europe. I found them in sparing numbers at Williamstown, and an immense colony at Worcester. This colony is established in a slow stream, from one-and-a-half to four feet in depth. The bottom is covered with large angular pebbles and small boulders. There is almost an entire absence of all except microscopic vegetation. For a distance of 100 yards the Stentors are attached to the stones in such numbers as to give to the whole bottom a mottled green appearance.

I have found not a single specimen of *S. polymorphus* entirely free of zoöchlorellæ, although some from Lake Quinsigamond contained very few, and appeared pure-white to the naked eye.

Although little inclined to multiply, this species bears confinement better than any other Stentor with which I am acquainted. One often finds them in very old gatherings that have stood in the laboratory for months.

5. *Stentor cæruleus*, Ehrbg.

This beautiful species is in all probability as widely diffused as the ubiquitous *S. polymorphus*, but seems to be more restricted to particular localities. With the exception of a very few found in Quisset Pond, Falmouth, and a few from Wolf Lake, South Chicago, Ill., my specimens have all come from Alewife Brook, Cambridge, where the species has multiplied exceedingly under rather peculiar conditions. Alewife Brook receives the sewage of a portion of North Cambridge, and also the escape-water from the engine of the pumping-station of the Cambridge Water-works. The water from the pumping-station warms the brook to such a degree that for a distance of 100 yards below its influx the brook freezes over only in the coldest

weather. This concurrence of favorable conditions causes the brook to swarm with all kinds of active aquatic life throughout the winter. I have obtained Blue Stentors at various points along the brook, and in every month from October to June. What becomes of them in summer I have never been able to determine. During July and August, 1890, I made numerous gatherings at many points along the stream, but without the least success.

The Blue Stentor of this country is undoubtedly identical with the *S. cæruleus* of Europe, but those I have obtained certainly much exceed the dimensions commonly given for the European form. Stein states ('67, p. 240) that the present species attains about the same size as *S. polymorphus*, the length of which in extension is given as half-a-line; *i. e.*, rather more than a millimeter. Maupas ('88, p. 230) gives as the "normal size," length 1.176 mm. and diameter .27 mm. In the semi-contracted condition of swimming the Alewife Brook specimens are commonly a millimeter long, while sessile and extended individuals generally attain a length of 2 mm. and a diameter of .476 mm. across the frontal field. Individuals in a state of extreme extension even reach a length of 4 mm. On the other hand, the largest measurement of *S. polymorphus* (fully extended) I have ever made was 1.466 mm. by .365 mm.

Stein remarks (p. 242) that the nodes of the meganucleus of *S. cæruleus* are usually spindle-shaped, while those of *S. polymorphus* are oval. I have frequently seen spindle-shaped nodes in the Blue Stentor, but oval nodes are much commoner.

### III. MORPHOLOGY.

For the investigation of nearly all subjects relating to the morphology and physiology of the Stentors, *S. cæruleus* is by far the most favorable form. It is of large size; all its structural features are strongly accentuated; internal parts are readily seen in the living animal; it is obtainable in large numbers, and multiplies freely in aquaria. These advantages have been recognized by the different workers upon the Infusoria, and the result is that our knowledge of the Stentor type is very largely based upon the study of *S. cæruleus*.

## A. ANATOMY.

The recent careful study of the external anatomy and the fission of the Blue Stentor by Schuberg ('90) added very much to our stock of information regarding this form. I have re-investigated every structure described by him, and our results coincide in nearly every particular. But, in regard to fission, our observations are at variance on one or two important points. I have been able, furthermore, to add a few details to our knowledge of this important process, especially in regard to the nuclear changes, which hitherto have received less attention than the cytoplasmic phenomena of fission.

1. *Ecto- and Endoplasm.*

Just as the fundamental Metazoan body has two cell-layers differing in function, so the unicellular body of a Protozoön is susceptible of division into two layers, ectoplasm and endoplasm. This division is by no means artificial, but is based upon a difference in structure and function. The differentiation of the two layers is not so conspicuous among the Infusoria as with the Rhizopods, mainly on account of the relatively extreme thinness of the ectoplasm, much the greater bulk of the cytosome being endoplasm. There is, besides, often an absence of delimitation between the ectoplasm and endoplasm. Such is the case with Stentor, where, however, the presence of ectoplasmic pigment, or of zoöchloræ limited strictly to the endoplasm, often helps to define the two. In *S. cæruleus* the ectoplasm contains abundant pigment, arranged in definite longitudinal bands, the "blue," or "granular" stripes ("Rippenstreifen" of Bütschli). Implanted in its substance are the contractile threads and the "roots" of the cilia. It is pierced by the basal plates of the membranellæ, which pass through it and some distance into the endoplasm. It is of denser structure and of a firmer consistence than the endoplasm.

An alveolar layer of the ectoplasm has been demonstrated by Bütschli, Schuberg, Schewiakoff and others in many Infusoria, both holotrichous and heterotrichous. If such a layer exists in Stentor, as seems probable, it is extremely thin, and its

structure so obscure as to escape detection. In *S. cæruleus* the structure of the ectoplasm is further masked by the abundant pigment, which it is impossible to remove. Notwithstanding these observational difficulties, Bütschli ('89, p. 1298, Fig. 14 *d*) has figured an alveolar structure for the ectoplasm of *S. cæruleus*; but after repeated efforts, I am still unable to make it out.

The functions of the animal that have reference to the outside world — sensation, locomotion, contraction — are performed by the ectoplasm and its derivatives, cilia and myonemes; those that concern the internal economy of the organism — digestion, assimilation, excretion, and secretion — are subserved by the endoplasm. An important exception is presented by the membranellæ, whose two functions, capture of food and locomotion, certainly have reference to the outside world; and yet, contrary to the general opinion, I have found them to be derivatives of the endoplasm.

## 2. *Ectoplasmic Structures.*

*Pellicula*.<sup>1</sup>—In common with the Ciliata generally, the Stentors possess an external limiting membrane which corresponds morphologically to the cell-membrane of pluricellular animals. While the pellicula confines the soft cytoplasm and helps to give the cytosome a definite form, it is at the same time wonderfully elastic and flexible, and admits of the striking mobility characteristic of all species of the genus. In the living animal the pellicula is not very apparent as a distinct structure, although brought into evidence by certain folds or wrinkles which appear most frequently at the anterior end, and by numerous minute transverse crenulations along the blue stripes, often seen when the animal is contracted (Fig. 28).

After treatment with .25 per cent. osmic acid the pellicula becomes raised from the ectoplasm along the granular stripes, but remains adherent at the clear stripes (Fig. 5, *pl.*). It is then seen to be a thin, structureless membrane.

<sup>1</sup> This term was first used by Bütschli to replace the older and inappropriate name *cuticula*.

*Stripes.* — In all species of Stentor the body and frontal field are adorned with alternate bright (“Zwischenstreifen” of Bütschli) and granular (“Rippenstreifen,” Bütschli) stripes. Those of the body have a longitudinal course from the adoral zone to the foot; while the frontal stripes sweep in a left-handed spiral from the aboral portions of the zone, over the whole frontal field, and down the pharyngeal funnel to the oral aperture (Figs. 33, 37, 40). As we shall see later, the frontal stripes are the modified derivatives of stripes on the ventral surface.

The stripes are due to a difference in structure in the ectoplasm, the protoplasm of the bright stripes being more hyaline than that of the granular bands. The striation is naturally most conspicuous in species that possess pigment, for this is restricted to the granular stripes. Where pigment is absent and the endoplasm is rendered opaque by symbiotic algae, as in *S. polymorphus* and *S. pyriformis*, the stripes are generally not seen at all in the living animal, but may be demonstrated by treatment with aceto-methyl-green, aceto-gentian-violet, or osmic acid. If specimens of the same species, however, happen to be nearly free of chlorophyll, the stripes are visible in the living animal. In *S. roesellii*, again, the stripes are obscure, owing to the slight differentiation between hyaline and granular portions.

The only species I have found suitable for the study of the stripes is *S. cæruleus*. Here their course can be followed with comparative ease. The relative width of granular and bright stripes varies exceedingly in different parts of the body, and the frontal granular stripes are always much narrower than the granular stripes of the body. The relative width of granular and clear stripes at a point just under the adoral zone is seen in Fig. 6, *g. s.*, *c. s.* Here the granular bands measure  $22\mu$ , the clear bands about  $7\mu$  in width; but the average for the whole body would be considerably less. It is worthy of note that the gradual diminution in the width of stripes from the anterior to the posterior end of the animal takes place almost wholly in the granular stripes.

It was long ago pointed out by Stein ('67, p. 227), and has since been noted by Brauer ('85), Gruber ('86), and Schuberg

('90) that the course of the stripes in *S. cæruleus* is not always perfectly uniform from the anterior to the posterior extremity, but that forkings or breaks occur here and there. Brauer even mentions a particular clear stripe "auf der einen Seite gelegen, welche viele, bis 10, übereinanderstehende Seitenzweige abgab." But it remained for Schuberg ('90) to discover that a V-shaped area of branchings is present as a normal structure on the ventral aspect of *S. cæruleus*, and also to point out the interesting relation it bears to the anlage of the new adoral zone in fission. He thus describes this "ramifying zone" (p. 200): "Innerhalb zweier Streifen, welche kontinuierlich vom Hinterende nach vorne ziehen, findet man andere, welche zwar am vorderen Ende beginnen, das hintere jedoch nicht erreichen. Die ganze Zone, innerhalb welcher dies geschieht, sei 'Verästelungszone' genannt, der Streifen aber, welcher sie rechts begrenzt, sei als 'rechter,' der links als 'linker Grenzstreifen' bezeichnet. Beide Streifen beginnen am Hinterende nebeneinander; der rechte zieht von hier in ziemlich geradem Verlaufe bis zu der Stelle, wo die adorale Zone . . . dorsalwärts umbiegt, um in die Tiefe zu steigen, während der linke ungefähr in der Mitte der linken Körperseite auf die adorale Zone stösst, also etwa an der Stelle, wo After und contractile Vacuole liegen. . . . Die Streifen nun, welche innerhalb dieses Dreiecks verlaufen, entspringen sämtlich mit ihrem Hinterende mittelbar oder unmittelbar als rechtsseitige Abzweigungen des 'linken Grenzstreifens.'"

I have found the ramifying zone very constantly present in the Blue Stentor. In a few cases, however, careful examination has failed to reveal it; and in other species the striation is so obscure as almost to preclude its discovery. Branching of stripes is by no means confined to the ramifying zone, but its appearance on other parts of the body is adventitious and different in origin. As Gruber ('86) has remarked, these places are often the scars of former wounds, and can be artificially produced. It is doubtful, however, whether single forkings like those represented in Fig. 33 are so produced. I think it probable that these are formed in the same way that stripes

are multiplied in the new adoral zone at time of fission, *i.e.*, by the intercalation of a new clear stripe.

*Myonemes.*—Standing in close relation to the stripes are the contractile elements, called by Bütschli *myonemes*. One of these underlies each clear stripe, and thus subtends a row of cilia (Figs. 5, 7, 9, 12). In the living Stentor the myonemes are hyaline, flexible, and exceedingly contractile fibrils; in a Stentor killed with osmic acid or corrosive sublimate, they become rigid, highly-refractive rods (Fig. 11). Bütschli ('89, p. 1298) describes the myonemes of *S. cæruleus* as being oval in transverse optical section; to me, however, the section has always appeared to have a circular outline (Fig. 5). Neither have I been able to make out the transverse striations figured by Bütschli, even with the 2 mm. apochromatic homogeneous immersion of Zeiss. While I do not assert the absence of the striæ, owing to my inability to see them, I would nevertheless suggest that the appearance observed by Bütschli was of artificial origin.

Myonemes attain their largest size in *S. cæruleus*, where they are plainly visible in the living animal, if it be sufficiently compressed and examined with a power of 500 diameters. In other highly contractile Stentors, as *S. polymorphus* and *S. roeselii*, the myonemes are very distinct after application of reagents. In *S. pyriformis*, on the contrary, where the contractile power is much less, the myonemes are exceeding minute (Fig. 9, *mn.*).

The myonemes are to be regarded as highly-specialized differentiations of the ectoplasm, endowed with a contractile power not less than that of striped muscle. In both cases, the highly developed contractility undoubtedly has its origin in the comparatively feeble contractile power of protoplasm. Engelmann's ('75) beautiful researches on the contractile substances of the Protozoa have shown that the general ectoplasm of Stentor is anisotropic, and therefore probably contractile. He assigns to it the slow and gradual contraction one often sees in Stentor, so different from the lightning-like shortening effected by the myonemes.

It is exceedingly interesting to watch the action of the

myonemes in a strongly-compressed Stentor as they alternately extend and contract ; and no one who has once observed them under these conditions can doubt that they are responsible for the contractions of the animal. Lieberkühn ('57) was the first to observe the contraction of the myonemes, and it has since been very accurately and minutely studied by Engelmann ('75). Lieberkühn thus describes their action :

“Es sind scharf contourirte körnchenfreie Fasern etwa von der Breite der körnchenfreien Zwischenräume, unterhalb deren sie der Längsaxe des Körpers nach verlaufen ; sie setzen sich vorn unter dem grossen Wimperkreis und hinten am ‘Saugnapf’ an ; einige von ihnen vereinigen sich während ihres Verlaufs. Am deutlichsten sieht man die bei der Contraction eintretenden Veränderungen, wenn ein farbloser oder wenig farbiger Stentor gerade so liegt, dass man auf den kreisförmigen Saugnapf blickt ; man sieht alsdann von seinem Umfang im Zustand der Ruhe alle einzelnen Muskeln geschlängelt abgehen ; in demselben Moment aber, wo sich das Thier zusammenschnellt, also verkürzt, verschwindet die geschlängelte Form vollständig, die Muskeln strecken sich gerade.”

As Lieberkühn states, the myonemes extend from the adoral zone to the “sucking-disc,” or foot. When in the contracted state they are much thicker posteriorly than anteriorly (Fig. 11), probably because the most contraction takes place in the former portion. They always remain very indistinct towards the adoral zone, and although I have been able to trace them up to it, I have not seen the manner of their ending there. At the other extremity, however, the termination of the myonemes is evident enough (Fig. 12). They end abruptly just at the edge of the pellicula bordering the naked protoplasm of the foot.

In the ramifying zone not only the clear stripes branch, but also the myonemes underlying them, as I have sketched in Fig. 10. The myonemes are represented in the extended condition, and are therefore thrown into folds. The presence of these convolutions in the myonemes, entirely independent of any corresponding folds in the ectoplasm, indicate that there is no firm connection between the two. The convolutions are

so pronounced in the posterior part of the animal at the moment of extension that they completely underlie the granular stripes, as Stein has beautifully shown in one of his figures of *S. roeselii*. This would be impossible if the myonemes were bound firmly in place beneath the clear stripes. Bütschli ('89, p. 1298) has represented the myoneme of *S. cæruleus* as lodged in a "canal," lying in the "alveolarschicht" beneath the clear stripe. Although I have searched for it repeatedly, I have been unable to find the least evidence of such a structure, either in optical or actual sections.

*Pigment.* — Three distinct pigmentary substances are present in the Stentors: a blue pigment in *S. cæruleus* and *S. multiformis*, a brown pigment in *S. niger* and *S. igneus nigricans*, and a purple-red pigment in *S. igneus*. The pigment is, for the most part, lodged in the ectoplasm, and restricted (at least wherever its position can be accurately determined) to the granular stripes. It is thus placed in a position to receive the greatest amount of light.

The pigment of *S. cæruleus* is certainly one of the most remarkable of animal pigments. Under normal conditions it varies in tone from bright sky-blue to pale sea-green, and even to a dull bluish-gray. When individuals are kept in unfavorable environment, as under a cover-glass, the pigment becomes reduced in quantity, and changes to a yellowish-brown color; sometimes Stentors are almost wholly devoid of it, and appear nearly pure white. Schuberg ('90, p. 221) noted that the loss of pigment took place by excretion, and I have frequently observed the same fact. In a culture where Stentors are undergoing depigmentation, numerous bluish-gray clots are invariably found, composed largely of pigment granules. Schuberg often observed pigment in the excrement vacuole, and appears to be of the opinion that it is excreted in the same manner as the fæcal matter. I have frequently seen such pigment-containing vacuoles, but do not feel sure but that the pigment they hold often belongs to a small Stentor that has been swallowed as food. Furthermore, many of the clots of pigment in a culture may be due to the death and disintegration of the feebler members of the colony. But by starting a

small colony and keeping a rigid census of it, it is easy to ascertain that pigment is thrown out of the bodies of living Stentors. As Schuberg remarks, pigment accumulates about the foot of Stentors that remain long fixed in one place; it is evidently thrown out by the naked protoplasm of the foot.

The blue coloring-matter of *S. cæruleus* is perhaps the most indestructible of all animal pigments. Bütschli observed ('89, p. 1476) that neither alcohol, ether, nor chloroform dissolve it, and Lankester ('73) found it unchanged by dilute acetic, hydrochloric, and sulphuric acids, while an alkali (dilute potassic hydrate), "had the effect of intensifying the blue color." The only reagents I have used that attack it are osmic acid and platinic chloride, both of which change its color to brown.<sup>1</sup>

*Cilia.* — The whole surface of the Stentors, in common with all other Heterotricha,<sup>2</sup> is clothed with cilia. As previously stated, the cilia are inserted in rows along the clear stripes (Figs. 5, 7, *cl.*). The narrower the granular stripes, the nearer together the rows of cilia; consequently the ciliation is densest in the pharyngeal funnel, where the stripes are the narrowest of any on the body.<sup>3</sup> The cilia are not of equal length on all portions of the body. Those of the frontal field are shorter than those of other parts; and they are somewhat longer on the posterior than on the anterior regions of the body.

The structure of the cilia does not appear to differ from that of these organs among Metazoa, although I have not been able to detect a "root" within the pellicula. A thickened basal piece ("Fussstück") is, however, readily demonstrable by use of osmic acid. (Fig. 7). The absence of a "root" is not to be assumed because of failure to detect it; such a structure has been shown to exist in nearly all cilia and ciliary structures.

<sup>1</sup> The only recorded effort to determine the chemical composition of this remarkable substance is the spectroscopic examination made by Lankester ('73). Its spectrum indicated the presence of a peculiar substance, called by Lankester *Stentorin*. It is characterized by the presence of two strong absorption bands, one in the red, the other in the green. "Stentorin is interesting as being an addition to the very short list of animal substances which give banded and therefore characterizable absorption spectra."

<sup>2</sup> The aberrant *Cænomorpha* alone excepted.

<sup>3</sup> This fact is readily demonstrated by compressing a Stentor beneath a cover-glass sufficiently to cause eversion of the pharynx.

Its invisibility here is possibly due to the proximity of the underlying myoneme.

The occurrence "tactile spines" ("Tastborsten") in *S. cæruleus* and *S. roeselii* has been mentioned by Stein and others. To account for the suddenness of their appearance in places where none could be seen before, Stein expressed the view that they could be withdrawn into the body and again suddenly protruded. I have frequently observed these so-called "spines" in *S. cæruleus* and occasionally in *S. roeselii* (Fig. 4, right-hand outline). They are more frequently seen about the anterior end of the animal, when one is looking directly down upon the frontal field. I have often noted their sudden appearance and disappearance. It is my strong belief that these "spines" are simply cilia that have become momentarily rigid, and therefore visible, while their disappearance is due to the fact that they have resumed their rapid swinging motion, in which condition they are not individually discernable. I have not seen any "spines" so large as many figured by Stein, and their length was certainly not greater than that of cilia. In drawing such structures, however, it is an easy matter to exaggerate unconsciously their size, and this is probably the case with Stein's figures.

While it is perhaps idle to speculate on the function of temporarily rigid cilia, the view that they are improvised tactile organs seems the most reasonable. The anterior border of the animal, which is brought most frequently into contact with foreign objects, is naturally the most in need of tactile organs, and it is just here that I have most frequently observed the "spines." It is well-known that permanently rigid spines or bristles occur in many Infusoria, notably the Hypotricha (*e. g.*, the three caudal spines of *Stylonichia mytilus*), and there is every reason to believe that these possess a tactile function. All such spines are undoubtedly modified cilia. It would seem, then, that we have in the temporarily rigid cilium of Stentor an organ which is virtually a cilium, but having acquired enhanced tactile sense (probably possessed in some degree by all cilia), and the power of becoming rigid, is already on its way to become a specialized organ of touch.

### 3. Pharyngeal Funnel.

That portion of the frontal field that sinks spirally into the animal was regarded by Stein and later writers as the oesophagus, and its external aperture as the mouth. But Schuberg ('90, p. 202) has shown conclusively that the whole funnel is an in-turned portion of the frontal field, and that the real mouth lies at its innermost extremity (Figs. 35, 62, *o*). Schuberg has very accurately figured (Taf. XIV, Figs. 2, 3) the appearance of the pharyngeal funnel of *S. cæruleus* in the contracted state. With the extension of the animal, the funnel undergoes great enlargement, and the outer turn, or buccal pouch (Figs. I, 33, 62, *b.p.*) ("Peristomtasche" of Stein)

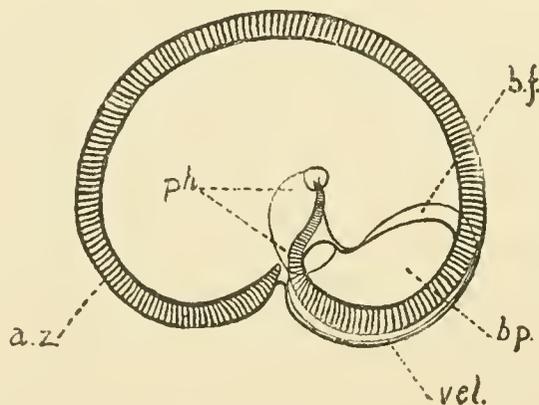


Fig. I.—Diagram of frontal field and pharynx of *S. cæruleus*, expanded. *a. z.*, adoral zone; *b. f.*, buccal fold; *b. p.*, buccal pouch; *ph.*, pharynx; *vel.*, velum.

sometimes attains nearly one-half the area of the whole frontal field. It serves as a very capacious "hopper" for whatever grist is brought by the "alimentary vortex." If one looks directly down upon the frontal field, the buccal pouch is seen to have an outline varying from almost circular in state of utmost expansion, to reniform and crescentic according to the amount of distension (Fig. I, *b.p.*). It also varies much in different individuals. The concave side of the crescent is always dorsal; the convex outline is formed by the "hypostom" of Stein. This hypostom is the thin raised edge of the ventral body-wall, crowned with the adoral zone as it passes towards the right to enter the funnel. Inasmuch as the name

“hypostom” is based upon the wrong assumption that the mouth is at the entrance of the funnel, it is desirable to give this structure a new designation, and I propose the term “velum” (Figs. I, 33, *vel.*). The velum is covered outwardly by the pellicula of the anterior portion of the body and internally by that lining the buccal pouch. It is probable that the ectoplasmic layers of the two surfaces are in contact, but when contraction takes place the endoplasm flows in between the surfaces, and the velum is almost or even wholly obliterated.

The descent from the higher and more aboral portion of the frontal field into the buccal pouch is very sharp and sudden. At the left the pouch curves dorsalwards and forms a pocket which is partially covered by a reduplication of the frontal field—the “buccal fold” (Fig. I, *b.f.*),—and by the adoral zone. This is the beginning of the “Rinne” of Schuberg ('90, p. 203), which he figures as a channel starting from this point and traversing the ventral side of the pouch towards the mouth. This channel, however, is almost entirely obliterated in the fully-expanded condition of the buccal pouch. At its right extremity the pouch narrows suddenly, and passes inward and dorsalward to form the inner turn of the pharyngeal spiral, or pharynx proper (Fig. I, *ph.*).

The foregoing description of buccal parts applies particularly to the Blue Stentor. These parts are, however, almost equally well-developed in *S. roeselii* and *S. polymorphus*; in both species the buccal pouch is especially prominent. In the lowly-organized forms, such as *S. igneus* and *S. pyriformis*, no buccal pouch is present. (Figs. I, 2.) Allied genera, as *Climacostomum* and *Folliculina*, also appear to be destitute of a buccal pouch, so that this structure may fairly be considered as developed among the Stentors, and only in the higher species of the group.

#### 4. *Endoplasmic Structures.*

*Membranellæ.*—It was first pointed out by Sterki ('78) that the adoral zone of the Heterotricha (Stentor) and Hypotricha is composed, not of large cilia as held by Stein ('67) and Simroth

(76), but of flat, transverse plates, called by Sterki "membranellæ." His observations have been confirmed by all subsequent writers upon these structures. Schuberg's careful description of the membranellæ of *S. cæruleus* relieves me from giving a detailed account of them. I have studied them as independently as I could, and my results agree with his at almost every point.

When the membranellæ are in rapid motion the impression is that of large, strong cilia, each inserted at the lower extremity of a glistening rod extending across the adoral zone. The same appearance is very strong when the membranellæ are moving slowly, and after a few observations of this sort it is almost impossible to avoid forming the conclusion that one has to do with large cilia. But in order to obtain a true conception of them, their motion must be arrested and a side-view obtained. By bringing sufficient cover-glass pressure upon the adoral zone, the membranellæ are flattened down and made to overlap one another, all motion being reduced to a slight tremor (Fig. 6, *m.*). Then the whole outline is seen.

. After fixing with corrosive sublimate or other suitable reagent, the membranellæ may be studied either in optical section or actual ones, and both extra- and intrapellicular portions of the membranellæ may then be observed. (Figs. 8, *m.* and 13, *a.z.*<sup>1</sup>) In side view, as in Fig. 8 (where the section cuts the newly-formed adoral zone transversely) the basal plate (*b.p.m.*) and its terminal filament (*t.f.*) are plainly seen. When the zone is examined in longitudinal optical section (Fig. 13), a third intrapellicular structure is visible, the connecting filament (*c.f.*), — the "Basalfibrille" of Schuberg. By focusing through the adoral zone, this fibre is also seen in the living animal. Another structure plainly seen, is the thickened and highly-refractive basal portion ("Basalsaum" of Schuberg?), Figs. 13, 30, just outside the pellicula. This I regard as homologous with the "basal piece" (Fussstück) of a cilium, and has in young membranellæ precisely the same appearance as in cilia. Although I have not traced it throughout the development of the membranellæ, there can be little

doubt that it becomes the highly-refractive "basal seam" that forms so conspicuous a portion of the membranella.<sup>1</sup>

The membranella is generally regarded as having originated by the fusion of cilia, and the ease with which it splits up longitudinally on application of reagents (see Fig. 8, *m.*) is held to be evidence of this. Besides, it is possible to homologize every part of the membranella, excepting the connecting filament, with parts already present in cilia. Furthermore, there are forms among the Holotricha (*e.g.* *Didinium balbianii* and *Dinophrya lieberkuehni*) in which Schewiakoff ('89) has figured ciliary organs which might fairly be considered as incipient membranellæ. He thus describes them in *Didinium balbianii* (p. 15): "Am Rande des abgestutzten Vorderendes befindet sich ein Kranz ziemlich langer Cilien, welche in kleinen Reihen sehr dicht angeordnet sind. Dieselben erscheinen auf den ersten Blick membranellenartig und an der Spitze zerfasert; es fällt aber nicht schwer sich zu überzeugen, dass es einzelne Cilien sind, gewöhnlich 6 an der Zahl, welche sehr nahe aneinander stehen und an der Basis wie verklebt erscheinen."

The function of the connecting filament, which binds together all the membranellæ of the adoral zone, is still very obscure. Brauer ('85), who appears to have been the first to mention the connecting filament, ascribed to it a contractile function, believing that it served to contract the frontal field; but Schuberg has very properly pointed out that this function is amply provided for by the spiral myonemes of the frontal field. On the other hand, the view that this intraplasmatic apparatus has a nervous function (first suggested by Engelmann, '80, for the inward prolongation of the cirri of *Stylonichia*) seems probable, but incapable of proof. The connecting filament in *Stentor*, as far as position goes, is admirably adapted to coördinate the action of the membranellæ, whose

<sup>1</sup> The "bilamellose" structure of membranellæ was first made out by Schuberg ('86) in the membranellæ of *Bursaria truncatella*, and was afterwards reported by Bütschli and Schewiakoff ('89, p. 1335), and by Schuberg himself ('90), to be present in those of *Stentor*. In transverse optical section the appearance is that of two parallel rows of dots—the optical expression of two fibrillated lamellæ. I have not been able to make out this structure in the membranellæ of *S. ceruleus*.

uniform vibration is essential to the production of an alimentary vortex.

*The Foot*, or organ of attachment, is beautifully adapted for anchoring the animal while it expands its frontal field. Attachment is accomplished, not by means of a "sucker" as believed by Stein and all the earlier writers, but by pseudopodia protruded from the naked disc of endoplasm (Figs. 14, 15, 16). The extent of protrusion and consequently the whole aspect of the foot depends upon the nature of the substance to which the animal is affixed. Thus Fig. 16 shows the foot of *S. polymorphus* attached to glass; pseudopodia are not protruded, but the whole foot is simply flattened against the glass, and sticks to it apparently by virtue of the intrinsic adhesiveness of the protoplasm. Fig. 15 is a sketch of the foot of *S. roeselii* fixed in loose and flacculent detritus. Spine-like pseudopodia are protruded in all directions; they are mostly unbranched. Fig. 14 represents the very beautiful, ramose pseudopodia of a Blue Stentor affixed to the surface film of water (in this case covered with a very thin bacterial zoöglœa). Although the figures are taken from three species, the aspect of the foot represented by each, together with an almost infinite series of intermediate forms, may be found in any one species.

A Stentor never becomes attached until the "stalk" is well protruded from the body. If the foot be carefully watched when the animal is preparing to affix itself, numerous minute processes in rapid vibration will be seen. These bear the closest resemblance to cilia; indeed, I have often been unable to distinguish, so far as the form of the structures is concerned, where the ciliary covering of the body ends, and the pseudocilia of the foot begin. They offer an interesting case of a ciliary organ on naked protoplasm. These minute processes are in all probability highly sensitive, and enable the animal to feel for a suitable place of attachment. Stentors appear to experience difficulty in fastening on clean glass. I have often watched one fully extended, dragging its foot over the under surface of a cover-glass before being able to fasten upon it. If a number of Stentors are put in a glass dish of water, very

few affix themselves to the sides of the dish until the glass has become coated with slime.

### *The Nuclei.*

*Meganucleus.* — The three principal forms in which the meganucleus of the Ciliata occurs — the spherical or oval, the vermiform, and the moniliform — are present among the Stentors. All the lower forms (*S. igneus*, *S. multiformis*, *S. niger* and *S. pyriformis*) have simple, rounded meganuclei. The higher forms (*S. auricula*, *S. cæruleus*, *S. polymorphus*, and sometimes *S. roeselii*) have moniliform nuclei. *S. roeselii* retains the vermiform nucleus for a long time after fission, but the moniliform condition is eventually attained.

The spherical meganucleus is evidently the primitive form, and the vermiform and moniliform shapes are derived from it. The stages of what must have been its phylogenetic development are repeated at every fission. The vermiform nucleus is not permanent among the Stentors. Even in *S. roeselii*, where it is of such frequent occurrence as to have been taken for a diagnostic feature by Ehrenberg ('38) and even by Stein ('67), it appears to be only a transient condition, readily passing into the moniliform state if a considerable time elapses between periods of fission (Fig. 4, *mgn.*).

The moniliform nuclei differ but slightly from one species to another. The meganucleus of *S. roeselii* is peculiar in that the anterior nodes are the thickest and largest, while the smaller posterior ones are long and spindle-shaped (Fig. 4). This results from the shape of the vermiform nucleus, which is always thickest anteriorly. Although the size and shape of the nodes vary considerably in *S. cæruleus*, and also the length and thickness of the commissures, I have but rarely noted any marked deviation from the typical form. One of these was an imperfectly-noded nucleus of a small individual (Fig. 17), which had but three well-defined constrictions, producing four elongated, unequal nodes, two of which showed indistinct constrictions indicating where commissures would normally be. The condition is evidently one of arrested development, for such imperfectly-noded nuclei are by no means rare at the

later stages of fission. The other instance of abnormal meganucleus is shown in Fig. 18. We here have the rare anomaly of a nucleus with a side-branch. The animal was killed at a late stage of fission, just as the nucleus was returning to the moniliform condition. If the process of nodulation had been completed, we should have had a noded branch such as Stein has figured ('67, Taf. V, Fig. 8) for *S. polymorphus*.

The number of nodes present in the meganucleus of one and the same species is well-known to be highly variable, but within definite limits. Thus in *S. polymorphus* I found only two meganuclei out of fifty which had fewer than 8 nodes (these had 6 and 7 respectively); and only two that had as many as 18. The average of the fifty examples was 12.42 nodes.<sup>1</sup> This agrees with Stein's ('67, p. 231) statement that the nucleus of *S. polymorphus* most frequently has 11–13 nodes, and that he only once found a nucleus that had as many as 20 nodes. The meganucleus of *S. cæruleus*, according to my experience, regularly has more nodes than that of *S. polymorphus*; but Stein (p. 242) found, on the contrary, a smaller number, and never counted in any meganucleus more than 13 nodes. Later observers, however, have frequently found 20 or more nodes in European specimens of *S. cæruleus*. I have counted the nodes in 75 stained specimens of this species, and have found the maximum number to be 20, which occurred but twice.<sup>2</sup> The minimum number was 9, found only once; but occasionally a smaller number is found in this species (see p. 524). The average number of nodes for the 75 examples was 14.16.

The finer structure of the infusorian meganucleus has been frequently described. It is well known that its appearance is strikingly different from that of the typical nucleus, and it has been appropriately termed "massive" in contradistinction to the "vesicular" nuclei of Metazoa and of plants. The nuclear substance of *Stentor* is dense, granular and viscous. It is enclosed by a firm membrane, very conspicuous in isolated

<sup>1</sup> The specimens in which the nodes were counted were taken at random and came from both Williamstown and Worcester.

<sup>2</sup> I have, however, seen as many as 22 nodes in this species (see p. 524).

nuclei killed and stained with aceto-methyl-green and examined in water or glycerine. The presence of a nuclear membrane in the meganucleus of Infusoria was denied by Jickeli ('84), but evidently on insufficient grounds. In the living condition the membrane fits closely over the nuclear substance.

In the meganucleus of all species of Stentor studied by me, I have observed, in addition to the granular chromatin, a minute, rather obscure chromatic network, which takes with all nuclear dyes a decidedly deeper stain than the granular chromatin (Figs. 8, 19-21, 60). This is evidently the structure seen and figured by Carnoy ('84) in *S. polymorphus*, and believed by him to be a much-involved chromatic filament. It has appeared to me, on the other hand, that the meshes are "closed," and that we have here to do with a chromatic "network." The observational difficulties of solving the problem are so great that it seems impossible to overcome them with our present means of investigation.

The chromatic network appears to be of wide occurrence in the meganuclei of the Ciliata. It has been figured by Schewiakoff ('89) for *Holophrya discolor*, *Didinium balbianii*, and *Dinophrya lieberkuchnii*. Carnoy found it in Stentor and in Vorticella. I have seen it very distinctly in *Spirostomum teres*, and less so in *Paramecium caudatum*. It is sometimes visible even in the living nucleus of *S. caeruleus*, so that it cannot be regarded as the effect of the reagent or of post-mortem change.

The chromatic network always presents the same aspect of delicate meshes composed of minute, wavy, granular-looking threads. It is not peripheral, but, as focusing and sections (Fig. 8, *mgn.*) show, runs all through the nucleus, and has the same appearance from whatever direction it is seen. It is evident, then, that the "network" is a web having three dimensions.

The network undergoes no visible change at time of division. Constricted nuclei (Fig. 21) and moniliform nuclei in a state of condensation (Fig. 8, *mgn.*) do not reveal any modification of it. I have never seen the least indication of a "striation" or linear arrangement of threads in any constricted nucleus of Stentor, such as Balbiani ('60), Bütschli ('76), Carnoy ('85),

Gruber ('84<sup>a</sup>), Jickeli ('84), and others have described as occurring in various Infusoria. In preparations of *Paramecium caudatum*, showing that form at many stages of fission, I have been equally unsuccessful in finding a longitudinal arrangement of chromatic threads. Furthermore, in R. Hertwig's ('89) beautiful memoir on the conjugation of *Paramecium aurelia*, a figure of that species in fission is given, but the strongly constricted meganucleus has no trace of striation. I believe, therefore, that any attempt to regard the longitudinal arrangement of chromatic threads a constant feature of meganuclear division, and adduce it as evidence of a disguised mitosis, is unwarranted.<sup>1</sup>

In moniliform nuclei both chromatic granules and network are restricted to the nodes, and do not extend into the commissures unless the latter are unusually thick (Figs. 19, 44, 45). In any case, the commissures doubtless contain a thread of the non-stainable, semi-fluid portion of the nucleus, the karyoplasm. The karyoplasm is so charged with granular chromatin that it is seldom distinguishable as a separate substance. The translucent oval bodies, looking like vacuoles, which are frequently present in the meganuclei of all Stentors (Figs. 19, 20), are probably to be regarded as segregations of karyoplasm free from chromatin. Each globule occasionally contains a minute, slightly-stainable, denser, and refractive body, the nature of which I am ignorant (Fig. 20).<sup>2</sup> It is not a micronucleus, for it frequently lies deep within the meganucleus.

An interesting fact in regard to the meganuclei of *S. igneus* and *S. pyriformis* is that they undergo division independently of that of the cytosome (Figs. 22-24). Indeed, the vast majority of individuals of both the above-named species have two or more entirely separate meganuclei. I have, besides, found nuclei in every stage of constriction. Owing to the frequent occurrence among the Ciliata of moniliform nuclei with very attenuate commissures, it has been held by some

<sup>1</sup> In a previous article ('92, p. 155) I have taken the ground that the appearance of longitudinal threads in a dividing nucleus is not *in itself* evidence of mitosis.

<sup>2</sup> This is evidently the structure figured by Stein in the nodes of *S. polymorphus* ('67, Taf. V, Figs. 7, 9, 12).

writers that, except in a very few instances (*e.g.*, *Opalina*), multiplicity of meganuclei does not obtain in this group. Even where the meganuclear substance has every appearance of being broken up into an immense number of separate elements, as in *Urostyla* (Bergh, '89), *Holosticha* (Gruber, '88), *Holophrya* (Maupas, '83), and other forms, it is maintained by Balbiani, Bütschli, and others that the apparently separate elements are all nodes of an immensely long and much-convoluted meganucleus, the commissures of which are so exceedingly fine as to escape observation. I am, however, of the opinion that complete meganuclear division does occur among the Ciliata independently of fission. Among the Stentors, commissures are comparatively thick and conspicuous, so that there is never the least difficulty in making them out. Furthermore, the node is tapered more or less where it is produced into the commissure (Fig. 19). But in *S. pyriformis* and *S. igneus* there is neither any trace of commissures nor of a tapering of the meganuclei (Figs. 3, 23). As long as the daughter-nuclei remain in connection, the constricted part is perfectly distinct, (Fig. 24) and absolutely no evidence has been found of its being drawn out into an exceedingly slender commissure.

Perhaps the strongest evidence that can be adduced in favor of the presence of invisible commissures joining the meganuclear elements, is the consolidation of all the nodes into a single mass at time of fission. It appears to me extremely doubtful whether this takes place when the nuclear elements are wholly separate. As all observations on the condensation of unquestionably moniliform nuclei show, the concentration takes place by a widening out of the commissures; and from watching the changes in the living meganucleus of *S. cæruleus*, I have been impressed by the fact that the nuclear membrane effectually prevents coalescence of separate nuclei, even when they are strongly pressed together (see p. 514). It would, therefore, be very interesting to observe the behavior of the meganuclei in *S. igneus* or *S. pyriformis* at time of fission. This unfortunately I have not been able to do, owing to the great rarity of fission in these forms when kept in confinement.

What purpose is subserved by the multiplication of meganuclei in the infusorian body? It seems to me that it is best referred to the same cause as that productive of branched, moniliform, and vermiform nuclei, both among Protozoa and Metazoa. I have elsewhere ('92, p. 138) expressed the view, in agreement with Chun, that one of the prime motives for amitotic division is the distribution of nuclear material through the cytoplasm, and the occasion is especially urgent where the mass of the nucleus (as is the case with *Stentor*) is small in comparison with the mass of the cytoplasm. A point of interest in this connection is the very frequent contemporaneity of meganuclear division when there are two or more meganuclei (Fig. 24). It is the usual thing to find both nuclei at nearly the same stage of division, but not infrequently one will be found to have outstripped the other, so that specimens occur with two separate nuclei and a third constricted one. This indicates a response on the part of both nuclei to the needs of the cytoplasm.<sup>1</sup>

*Micronuclei.*—The micronuclei of the *Stentors* have been among the most difficult to demonstrate of all the Ciliata. They escaped the scrutiny of all the earlier micrographers, and were first seen by Balbiani ('61) at time of conjugation, when they become greatly enlarged. But at that period Balbiani was no more successful than his predecessors in discovering them in the resting condition. A clear account of the presence of micronuclei in the quiescent state was first given by Maupas ('83, p. 661). More recently, Gruber ('86) has confirmed Maupas' announcement of their presence in *S. cæruleus*. Plate ('86), on the contrary, maintains that the minute bodies in question are "Assimilationsprodukte," basing his opinion on the fact that the "granules" are not visible in all specimens. As I have succeeded in finding them in mitotic division there is no longer reason for doubting their micronuclear nature.

The micronuclei of *Stentor* are difficult to find, not so much on account of their minuteness, as by reason of the vastly

<sup>1</sup> An interesting case of synchronous constriction of a large number of meganuclei is given by Gruber ('84b) for *Spirostomum lanceolatum*, n. s.

greater bulk of the deeply-staining meganucleus. It is useless to search for them until the Stentor has been cut into thin sections or the meganucleus isolated. The serial-section method is the most satisfactory, and the best stains I have tried are Czokor's alum cochineal and borax carmine. The micronuclei always lie close to the meganucleus, as usual among Infusoria (Figs. 19, *mcn.*, 25), but not always absolutely in contact with it. While sometimes seen with great distinctness, I have not been able to make them out in many specimens, although prepared in the same manner. They are peculiarly difficult to find in individuals that have entered upon fission, owing to the fact that they swell and become almost unstainable at time of their division.

The number of micronuclei in Stentor is larger than in any other Infusoria, so far as known, and has been greatly understated by Maupas ('83, p. 661), who mentions as the highest number, 28 for *S. roeselii*. In serial sections of a Blue Stentor in which the micronuclei were unusually distinct, I have counted 66, and in another instance, 54. The absence of numerical agreement which Maupas ('83, p. 661) found the micronuclei of *Spirostomum ambiguum* to possess, with reference to the nodes of the meganucleus, also prevails in Stentor. While often only one micronucleus may be found adherent to a node (Fig. 19, *mcn.*), there are sometimes as many as seven or eight.

The micronuclei of *S. cæruleus* do not differ materially from those of other Infusoria. They are spherical, highly-refractive, deeply-staining bodies measuring  $1.5\mu$ – $2\mu$  in diameter, apparently homogeneous and composed wholly of chromatin (Fig. 55). They are so minute and dense that I have been unable to discern a nuclear membrane in the resting state, but one is visible when the micronuclei are enlarged at time of fission or conjugation (Figs. 48, 56).

Hitherto, micronuclei have not been observed in the Stentors having simple meganuclei. I have found them in *S. igneus*, adherent in considerable numbers to the meganuclei (Fig. 25), and also in *S. pyriformis*. They are even smaller than the micronuclei of the Blue Stentor, measuring only  $1\mu$  in diameter.

The double nuclear apparatus of *Stentor* probably expresses the extreme of differentiation between micro- and meganuclei. The latter are highly modified as to structure and, as regards the higher *Stentors*, as to form; the former are simplified as much as possible, and reduced to a size so minute that, even with the highest powers, they appear as hardly more than mere specks lying on the surface of the immensely greater meganucleus.

*Generalia.*—There are few phenomena in cytology more interesting than the apportionment of nuclear functions in the Infusoria to two kinds of nuclei differing in size, structure, and mode of division. The meganucleus is comparable to certain somatic nuclei of the Metazoa that have become highly specialized to subserve some particular function; *e. g.*, the “giant nuclei” of gland- and excretory cells, found mainly among the Arthropods. The likeness between the two, as shown by the “massive,” granular structure, proneness to assume unusual shapes, and amitotic division, are very striking, and strongly suggest a similarity of function. The micronucleus corresponds to a metazoan germ-nucleus, and is the bearer of the “immortal” germ-plasm. The Infusorian, then, contains within the scope of a single cell both somatic and germinal elements. Through the exchange and copulation of micronuclei at time of conjugation, the perpetuity of the somatic part is assured in much the same way that the endless new generations of a Metazoan, each with its wonderful somatic development, are made possible by the periodic activity of the germ-plasm, likewise brought into play by the union of a male and female pronucleus.

#### B. FISSION.

The important process of self-division, or fission, has received more attention in the genus *Stentor* than in any other group of Infusoria. We have, in the first place, the surprisingly clear and accurate account by Abraham Trembley;<sup>1</sup> we have the descriptions and figures by Ehrenberg ('38), Balbiani ('60), Stein ('67), Moxon ('69), Cox ('76), and Schuberg ('90). The

<sup>1</sup> Phil. Trans. Roy. Soc. xliii, No. 474, p. 180, 1744.

external or cytoplasmic phenomena have been studied more frequently and are more accurately known than the nuclear changes, our knowledge of the latter being wholly due to the researches of Stein and Balbiani, made long before the discovery of modern cytological methods.

It was not until I had made considerable progress in the study of the fission of *S. cæruleus*, that I had access to Schuberg's account of it. I continued the work in order to test his results by personal observation, and perchance add something to his important contribution to the subject. I have, furthermore, thought it desirable to publish a series of drawings (Pl. XXIV, Figs. 26-37) illustrating the stages of fission of *S. cæruleus*; for hitherto no complete and accurate series has been given for any species of Stentor. These figures obviously do not represent successive stages in the bipartition of one individual; for that, by reason of the occasional change of position and contraction of the animal, I have found impracticable.

#### 1. *Cytoplasmic Phases.*

The first sign of fission is the formation of a rift (the anlage of the new adoral zone) in the pellicula and ectoplasm near to and almost parallel with the left boundary-stripe of the ramifying zone. (Fig. 26, *a.z.*<sup>1</sup>) This cleft has an early longitudinal direction; yet, as Schuberg has already shown, it cuts across the stripes of the ramifying zone, forming narrow angles with them (Fig. 26). Schuberg represents the anlage of the new zone as being very short at the outset, and gradually extending at the extremities, which curve more and more toward the right. This very early stage appears to be extremely transient, for out of the many specimens I have studied in the first stages of fission, I have seen but one or two that had a short rift.

The rift opens at the outset to the full width of the adoral zone. It opens entirely through the ectoplasm, as shown by the absence of pigment, so that the soft endoplasm is exposed and protrudes a little. From the start it exhibits a ciliary motion. At the very beginning this movement seems as undefined as that of the protruding cytoplasm of the foot;

but gradually, as the young membranellæ develop from the exposed endoplasmic ridge, the motion is restricted to undulations sweeping lengthwise along the new adoral zone. (Figs. 26, 27, *a.s.*<sup>1</sup>) The wave-like motion becomes more and more pronounced as the membranellæ increase in length and vigor of action (Figs. 30, 33, 35-37). It continues throughout all subsequent stages of fission, and even for a short time after division is accomplished. It is but rarely seen in old membranellæ, the action of which is more rapid and uniform.

The posterior extremity of the new zone, reaching about two-fifths of the way down the body when the Stentor is extended, becomes more sharply curved than the anterior end. Just within the curve the pharyngeal invagination appears (Figs. 27, 28, *o.*<sup>1</sup>). At this and the preceding stages, the aspect of the new zone is different according as the animal is in the extended or contracted state. When contracted the zone forms nearly a semicircle, and has strongly incurved extremities (Fig. 28). The new membranellæ are quiescent, and the whole zone is apt to stand out in strong relief upon the surface of the animal.

A structure of much morphological interest which makes its appearance very early, is a narrow light band running parallel with the zone along its right side (Figs. 27, 30, 32, *p*<sup>1</sup>), and having much the appearance of a clear stripe of unusual width. It persists throughout the phases of division, and is seen in the fully-formed frontal field, which it encircles just within the adoral zone (Figs. 6, 30, *p*), in the manner described by Schuberg. His interpretation of this band as a rudiment of the peristome of the lower Heterotricha, seems the most reasonable explanation. It is certain, as Schuberg (p. 234) maintains, that the frontal field ("Stirnfeld") of Stentor and its near allies, Climacostomum and Folliculina, cannot be regarded as homologous with the true peristome of the lower Heterotricha. Neither the structure of the frontal field nor its mode of formation are the same as those of the peristome. As Schuberg has pointed out, the frontal field of Stentor is a piece of the ventral body-wall, cut out, as it were, by the adoral zone during fission, finally shifted to a horizontal posi-

tion at the anterior end of the posterior zoöid, and almost encircled by the adoral zone. The peristome, if it persist at all, must remain adjacent to the adoral zone on its inner side (right side during the earlier stages of fission, in which it occupies exactly the same place as in *Spirostomum*), and, therefore, in the precise position of the light band.

The area of body-surface partially enclosed by the adoral zone and destined to become the frontal field of the posterior individual, undergoes little change up to the time of the formation of the pharynx (Fig. 27, *f*<sup>1</sup>). At this stage its stripes, already narrower than those outside the ramifying zone, become very much reduced in width, presumably by intercalation of new "clear stripes" (Fig. 28, *f*<sup>1</sup>); but this I have not been able to prove by direct observation. As fission advances, the stripes of the new frontal field become narrower and more numerous, thus approaching the condition of the old frontal field (Figs. 33, 35, 36, *f*<sup>1</sup>). The relative width of the frontal stripes to those at same plane is clearly shown in a transverse section (Fig. 8, *f*<sup>1</sup>).

The phases thus far described, although preliminary to the actual fission, are extremely important, including as they do the principal *formative* act of the process—the development of a new adoral zone. The gradual evolution of structures so complicated as membranellæ from a mass of indifferent protoplasm, is very striking. The fact that the membranellæ are derived from the outer layer of the endoplasm is not without interest, showing as it does an ontogenetic development different from the phylogenetic. For there is every reason to believe that the membranellæ were originally formed from cilia or ciliary structures belonging to the ectoplasm. But the ectoplasm is itself a modified layer, and hence not so well fitted to give rise to a new and complex structure as the undifferentiated endoplasm. It may further be noted that its thickness is hardly sufficient to furnish material enough to build up the membranellæ.

Another organ that is formed *de novo* very early in fission is the contractile vacuole (Fig. 26, *c.v.*<sup>1</sup>). At first it is always smaller than the old contractile vesicle. In *S. cæruleus* it

evidently originates from one of the vacuoles which commonly lie in a row posterior to the contractile vesicle and often extend into the peduncle. At a point always a little above the level of the posterior extremity of the new zone, one of these vesicles takes on the contractile function and at once acquires the excretory pores (Fig. 26, *ex. p.*) originally described by Moxon ('69). It contracts at regular intervals, but not synchronously with the old vacuole.

The formation of the new vacuole in *S. roeselii* is brought about by a local dilatation of the longitudinal canal. It seems to have been first observed by Balbiani ('81, p. 322), but was studied independently by me while still in ignorance of Balbiani's mention of it. The dilatation of the canal occurs early in fission, preceding the formation of the mouth, and occupies the same relative position as the new contractile vacuole in *S. cæruleus* (Fig. 29, *c.v.*<sup>1</sup>). The new vacuole retains its connection with the portion of the longitudinal canal posterior to it, and for a time with the anterior portion also.

The further history of the excretory system of *S. roeselii* at time of fission offers a sufficient explanation of the much-discussed "ring-canal" discovered by Lachmann and figured in Claparède and Lachmann's great work on the Protozoa ('59). The "ring-canal," encircling the anterior end of the animal, just under the adoral zone, has been looked for in vain by nearly all modern micrographers. But Maupas ('83, p. 641) claims to have seen it in *S. cæruleus*, and Bütschli states ('89, p. 1443) that unpublished drawings of Engelmann (1861) show the ring-canal in *S. cæruleus* and *S. roeselii*.

The study of *S. roeselii* in fission gave me the true history of the ring-canal, as the diagrams in Fig. II will show. It is seen that the ring-canal (*rc.*) is that portion of the longitudinal canal lying immediately anterior to the new contractile vacuole (*cv.*). During the earlier stages of fission (*a*) it runs straight forward to the old vacuole; but as the new zone curves round and takes a more nearly horizontal position, the adjacent portion of the longitudinal canal curves with it, and in doing so has to lengthen (*b*). The newly-formed ring-canal is finally severed from the more anterior portion of the longitu-

dinal canal by the separation of the daughter-stentors — or possibly somewhat earlier. Within a few hours after fission, the ring canal atrophies without leaving a trace. It is evident, then, that Lachmann saw the ring-canal in a young specimen of *S. roeselii*, and later observers have for the most part failed to find it because they did not obtain young specimens.

As soon as the new zone has reached its full length, has curved inward at the ends, and its membranellæ have attained

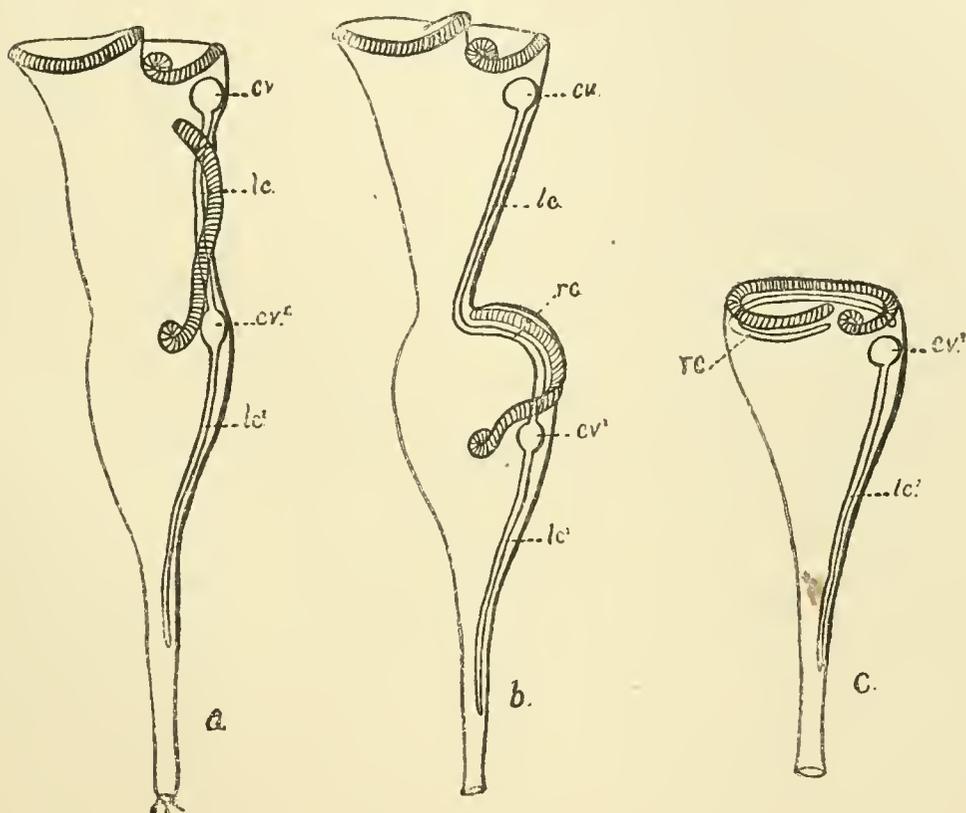


FIG. II. — Diagrams to illustrate the formation of the ring canal in *S. roeselii*. *cv.*, contractile vacuole; *cv.*,<sup>1</sup> new contractile vacuole; *lc.*, *lc.*,<sup>1</sup> longitudinal canal; *rc.*, ring-canal.

a definite shape, the new pharynx begins to develop (Figs. 27, 28, *o.*<sup>1</sup>). It starts as a slight depression in the body of the Stentor just at the posterior tip of the new zone. The first direction taken is obliquely inward towards the right. The invagination becomes deeper and larger, then turns spirally and pushes directly into the endoplasm (Fig. 13 *o.*<sup>1</sup> *inv.*). The tip of the new zone follows the invagination *pari passu*, so closely as to give the impression that the zone is pushing into

the cytoplasm. The new velum and buccal pouch (Figs. 33, 36, *vel.*<sup>1</sup>) become evident during the late stages of fission. The formation of the new pharynx produces no break in the integument, except at the inmost point where the mouth is located, and the pharynx is, therefore, lined with an integument that once covered the surface of the body; it is comparable to the stomodæum of a Vertebrate embryo.

As soon as the new pharynx begins to develop, the body of the Stentor becomes considerably elongated and more cylindrical than in the usual condition. A slight constriction appears about midway of its length (Fig. 27). This constriction is probably a mere result of the elongation, and has nothing to do with the fissional constriction which appears later. It seldom or never coincides with the fission-line (Fig. 31). Synchronous with the constriction above-mentioned, and perhaps a result of it, I have noticed a decided bend to the left in the new adoral zone (Fig. 30). This bend is important as indicating the point at which the fission-line starts (Fig. 31, *l*). The formation of this cleavage line was first observed by Moxon ('69), and has been accurately described by Schuberg (p. 228). I have little to add to his account. Its advent is so sudden, and it extends so rapidly around the animal, that it is difficult to ascertain where it first appears. Schuberg states that it starts on the left border of the new zone at the point where the latter reaches the left boundary-stripe of the ramifying zone. I am at least satisfied that this is the first point where the fission-line touches the zone. Thence it passes to the right around the body, cutting all the stripes at right angles (Fig. 31, *l*) until it reaches the right side, where it extends obliquely upward to meet the curved aboral end of the new zone. I do not find that it approaches the new mouth so closely as Schuberg has represented, but rather takes the direction shown in Fig. 33. At a slightly later stage the starting-point of the fission-line is found to have passed above the anterior curve of the new zone, now very pronounced, and to have joined the other extremity (Fig. 22, *l*). The process by which the transposition is brought about I have not been able to follow. The constriction which at length separates the two

zoöids now takes place in the path marked out by the fission-line. I cannot agree with Schuberg's view that the constriction is accompanied by a rupture of the pellicula. He says: "Bei Stentor nun muss . . . ein Durchreissen schon früher [than in other Infusoria] stattfinden, oder mit einem Wort *die ganze Durchschnürung ist von Anfang an mit einem Reissen der Pellicula* in bestimmter Richtung verbunden." Now, any rupture of the pellicula occasions a protrusion of the cytoplasm whenever the animal contracts, notwithstanding the strong tendency of the integument to close over every wound. Since no protrusion of cytoplasm is visible along the line of fission, it seems very doubtful whether the pellicula is actually ruptured. Longitudinal sections of Stentors in the middle and later stages of fission show no evidence of a rift in the pellicula.

Although I do not consider it a rupture of the pellicula, I have not come to a satisfactory conclusion in regard to the nature of the fission-line. It certainly divides the substance of the blue stripes (ectoplasm), and so produces a white line around the body, not unlike one of the clear stripes. Sections show that the line is superficial, and not the optical expression of a plane passing through the animal, as in ordinary cell-division. I have sometimes thought that a contractile fibre of extreme fineness was developed in close connection with the pellicula, which, by contracting, brought about the constriction in the manner of a string passed around a yielding body and tightened. The supposed fibre, however, may be merely the sharp fold in the integument, caused by constriction.

From the study of preparations I feel confident that the myonemes are not cut by the fission-line, but simply become bent sharply inward (Fig. 34). Until a late stage of fission the stripes on either side of the line remain very accurately matched (Figs. 31-35), notwithstanding the notable narrowing of the stripes at the posterior end of the distal individual.

At this point a series of remarkable changes in the position of the new frontal field and adoral zone begin, and proceed rapidly until the completion of fission. Hitherto the plane of the new frontal field has remained nearly parallel to the surface of the Stentor. It now begins to project more and

more, and gradually approach the horizontal position (Fig. 33, *f*<sup>1</sup>). This is accomplished by two distinct processes, — a shoving out of the cytoplasm towards the left, and a depression of the aboral end of the new zone, combined with a strong curvature dorsalward. The backward movement of the zone is brought about by a deep, narrow constriction between its projecting anterior end and the body of the Stentor (Figs. 32, 33). This is only a part of the general constriction which now obliquely encircles the animal coincident with the line of fission, but is much the deepest at this point. The contour of the new frontal field varies according as the animal is contracted or extended; when in the former condition it is concave (Figs. 31–33, the last being semi-contracted), whereas in the latter it is nearly flat.

Another factor in the process of fission is the elongation of the posterior portion of the distal zoöid. This starts at the beginning of constriction, and rapidly progresses *pari passu* with the constriction. Thus the distal zoöid speedily acquires the typical tapered form of Stentor (Figs. 35, 36). The elongation is to be referred to the same cytoplasmic action that produces ordinary extension. There are, then, three distinct factors in the middle and later stages of fission: (1) out-pushing of new zone into a horizontal position, (2) constriction, and (3) elongation of posterior part of distal zoöid. The shifting of the new zone into a horizontal position is accompanied by a shortening and broadening of the frontal field, and a more complete circumscription of it by the adoral zone (Figs. 35–37). Throughout the middle and later stages of fission the distance from the new pharynx to the fission-line remains nearly constant, but towards the end, when the constriction has become very great, this space is materially reduced, although, even in the newly-formed proximal individual, the unenclosed space on the ventral margin of the frontal field is considerably greater than in the ordinary condition. The reduction of the unenclosed space is brought about by the upward movement of the oral spiral, which is the last portion of the adoral zone to take a horizontal position.<sup>1</sup>

<sup>1</sup> This is more clearly seen in Fig. 46, *g*, Pl. XXV, than in Figs. 35, 36, because in the latter the zone is viewed obliquely.

The changes in the stripes during fission are an index to the extraordinary alterations in the contours of the animal. The most obvious modifications are in width and length. The granular stripes of the new frontal field are about half the breadth of those of the ramifying zone from which they are derived ; while the granular stripes at the posterior end of the distal zoïd become at length scarce a fifth of their former breadth. Their attenuation has kept pace with the constriction, and what they have lost in breadth they have gained in length. That the stripes are viscous like the rest of the cytoplasm, and have been drawn down to greater fineness by the elongation of the portion they cover, seems to me to explain sufficiently their attenuation. Another explanation must be found for the narrowing of stripes in the new frontal field ; for here certainly no lengthening of stripes takes place. I have postulated an increase in the number of striæ through the bisection of each granular stripe by a clear stripe. Schuberg (p. 227), although taking note of the increase in superficial extent of the new frontal field, nevertheless says : “Eine Vermehrung der Körperstreifen innerhalb derselben [*i. e.*, the frontal field] hat dabei ebensowenig wie bisher statt. . . . Die Zahl der Streifen, welche durch das Wachsthum der neuen Zone nach rechts und links im Ganzen durchquert wurden, beträgt zwischen 30–40, was denjenigen auf dem Peristom ausgebildeter Thiere entspricht.” Now, as the number of granular stripes in the ramifying zone varies, according to Schuberg, from 20 to 40, the number of stripes in the new frontal field must also be variable, and nothing short of counting in the living animal the number of stripes enclosed at the outset by the new zone, and again counting them in the fully-elaborated frontal field, and finding the numbers to agree, would warrant Schuberg's statement. I admit that my own view of the reduplication of stripes is based—not upon an actual count, for I have found that impossible—but upon their much greater fineness in the fully-formed frontal field.

The formation of the new ramifying zones is worthy of notice. That of the proximal zoïd is nothing more than the posterior portion of the original ramifying zone, as will be seen

by a comparison of Figs. 26-28, 33, 35-37, *r.s.* The ramifying zone of the distal zoöid is, on the contrary, formed anew. As soon as the constriction at the anterior end of the new zone has cut down an appreciable distance along the side of the animal, a very distinct derangement of stripes appears immediately above it, and lengthens with that portion of the distal zoöid (Figs. 35, 36, *r.s.*<sup>1</sup>). The newly formed ramifying zone is different from that of older individuals, inasmuch as it is not strictly a branching, but an inosculation of stripes, meeting at an angle of about  $45^\circ$  when the animal is extended. This condition of the ramifying zone persists for some hours after fission.

How is the new ramifying zone produced? Schuberg explains it as a result of a rupture of the pellicula in the fission-line, and apparent weight is lent to this view from the fact that lesions almost invariably lead to disorganization of the stripes as a result of the closure of the wound. A rupture of the pellicula, however, is not necessary to account for the ramifying zone. An inspection of Figs. 32 and 33 will show that the constriction-cleft at the aboral extremity of the new zone is the apex of the fission-line, which on both dorsal and ventral sides cuts the stripes obliquely. As the constriction becomes deeper, the ends of the stripes immediately posterior to the cleft in Fig. 33 must become pinched together as they come down to the constriction-line upon both dorsal and ventral sides of the anterior zoöid. This process continues until the end of fission. The result is a seam in the side of the distal zoöid, where the stripes meet and inosculate at their tips as above described.

After the daughter-stentors have assumed nearly their definitive shape, separation is effected by rupture of the connecting thread at the point where it joins the proximal individual. This is accomplished partly by the pulling and torsion of the distal individual, and in part by the sudden in-cutting of the now much reduced line of constriction (Figs. 36, 37). The latter process brings about a speedy separation (in most cases) of the twin zoöids, and also leaves an area of naked cytoplasm for the foot (Fig. 37, *ps.*<sup>1</sup>). Sometimes, especially if the animal is well-fed and vigorous, the incision separates the

twin Stentors at once, but more often they remain connected for some time by a thin cytoplasmic thread from the truncated tip of the anterior Stentor. (Fig. 37.) The point of attachment to the proximal zoöid is always at the aboral extremity (*d.az.*<sup>1</sup>) of the adoral zone. The body stripes of the posterior zoöid are strongly curved towards this point, and this character, combined with the notable roundness of the body-portion of this zoöid, as compared with the distal one (Fig. 36) serves to distinguish the daughter-stentors for an hour or two after fission is completed.

The time consumed by fission in Stentor doubtless varies in the different species, although I have found it approximately the same in *S. cæruleus* and *S. polymorphus*. It has been variously stated. Stein ('67) was unable to give its duration, but was correct in saying that the early stages require more time than the later. Cox ('76) stated the period as only two hours for *S. polymorphus* — an understatement due to the fact that he did not see the slow early development of the new zone. Schuberg ('90, p. 225) estimated it at six and one-half hours for *S. cæruleus*, four hours being consumed in the early formative stages, and two and one-half in the actual fission. I have found that the duration of the process in *S. cæruleus* varies somewhat according to the vigor of the animal, and doubtless also according to the temperature, but my observation of it at 17°–20° C. agrees very closely with Schuberg's. I have repeatedly observed a period of about seven hours, but it is sometimes an hour or more less.

It is seen from the foregoing description that the cytoplasmic phases of bipartition are divisible into two periods, the *formative period*, in which the anlagen of new organula are laid down, and the *constrictional period*, during which the actual fission takes place. As we shall see later in the consideration of Regeneration in Stentor, it is in all probability the constriction and not the neoformation of organula, that determines whether fission shall take place, or merely renewal of mouth and adoral zone. Our ignorance of the *primum movens* to a neoformation is complete. We can only say it lies in some peculiar molecular condition that incites the duplication of existing organs. And

the working-out of the impulse thus given is only partially dependent upon temperature, food, the size of the individual, or even, as Balbiani's ('92) and my own experiments in merotomy (see p. 550) show, upon the intact condition of the organism.

## 2. Nuclear Phases.

*Megamucleus.* — The best observations hitherto upon the meganucleus at time of fission are those of Balbiani ('60) and of Stein ('67), later observers of fission in *Stentor* having paid little or no attention to the nucleus.

The most satisfactory method of studying the meganuclear changes is to select a specimen at an early stage of fission in which the meganucleus is clearly visible, and then watch the nucleus throughout its changes until the two daughter-nuclei have attained their definitive shape. This method I have supplemented and controlled by the examination of stained preparations, but these can by no means supplant the study of the living meganucleus.

At the beginning of fission the meganucleus has its usual spiral disposition in the body. The first alteration, just previous to the formation of the new pharynx, is a straightening of the nucleus and disappearance of the commissures, the nodes becoming appressed. (Fig. 26, *mgn.*; Fig. 47 *a.*) The next step is coalescence of the nodes into a solid mass, which shortens rapidly until it assumes a nearly spherical form (Fig. 41 *a.*) The time required for coalescence of the nodes is about one hour, but it varies in different individuals. Usually the meganucleus has assumed the spherical shape when the pharyngeal funnel has begun to form. Its shape at this time is rarely a perfect sphere and often it is jagged and irregular. In fact, when observed in the living state it is seen to be a plastic, fluctuating mass, almost amœboid in its rapid change of form. (Figs. 46 *c.*, 47 *d.*, *e.*) These changes of form are the outward expression of an internal commotion that may be considered as a continuation of the forces that produced the coalescence of the nodes. In a few minutes the movements take a definite direction, producing an elongation of the

nucleus, always parallel to the long axis of the animal (Fig. 41 *d, e, f*). As soon as elongation has begun, a constriction appears about the nucleus, and persists for about half-an-hour (Fig. 41 *b-c*). In less than an hour the nucleus attains a slender, rod-like form (*f, g, h*), normally straight and cylindrical, but almost invariably bent or curved by the occasional contractions of the animal, as seen in Fig. 41 *g, h, i*. The meganucleus, however, always tends to straighten, and will do so if the intervals between contractions be sufficiently long.

When elongation has reached its limit, and sometimes perhaps even before, nodes appear at the tips of the meganucleus (Figs. 35, *mgn.*; 41 *i*). I have found no exception to the rule that the nodes develop first at the ends of the meganucleus. The nodulation advances rapidly and at nearly equal rate towards the middle of the nucleus, so that at any stage in the process one counts about as many nodes in one moiety of the nucleus as in the other; but there is usually a difference of one or two (Figs. 35, 41). The nodulation soon reaches the middle point of the nucleus, which normally lies in the plane of the constriction between the now nearly-divided daughter stentors. The newly-formed nodes are, as a rule, beautifully symmetrical and alike in size. As soon as the nucleus has become fully-noded, the middle commissure, lying just opposite the fission-line (Fig. 42) at once becomes longer and finer than any of the others. It differs from all the other commissures in being a commissure of division, comparable to the connective uniting the moieties of an amitotic nucleus. Rupture of the commissure takes place shortly before the separation of the daughter-stentors (Fig. 43), and the "tail-ends" at the adjoining tips of the meganuclei are speedily drawn into the terminal nodes.

The way in which nodulation takes place is worthy of note. The first thing observable is a series of constrictions, all of nearly equal depth and evenly spaced. At this early stage a stained preparation, such as Fig. 44 represents, shows that the chromatic substance has become aggregated in the incipient nodes, and the commissures are nearly destitute of it. Thus at the outset the commissures are relegated to the comparatively

unimportant duty of serving as connectives for the nodes. The rate of constriction at the different commissures is by no means uniform in the later stages of the process, and sometimes alternate commissures become developed sooner than the intermediate ones (Fig. 45), thus producing double nodes, which are of very frequent occurrence even in adult Stentors.

The mode of meganuclear division above described is undoubtedly typical for all Stentors having a moniliform or rod-shaped nucleus, and, indeed, for such meganuclei wherever they occur. It is, however, by no means essential that the actual division should be achieved in just this manner, although the process is always upon the same general plan: condensation, elongation, renodulation. The variation lies in the time at which division is interposed, and in this respect I have noted the following cases:

(1) Division may be deferred until the close of the phases, as in the foregoing description.

(2) It may occur at time of maximum coalescence, (Fig. 46 *a, b*).

(3) It may take place during the coalescence of the nodes. (Fig. 47, *a-d*.)

(4) The nucleus may be in two equal or nearly equal parts previous to fission, and no division whatever take place. But if the parts are very unequal (as in Fig. 35) division will occur as in (1) or (2).

Fig. 46, *a-g* represents a series drawn from the living nucleus, showing the phases when the nucleus divides at the moment of greatest condensation. The division was obviously unequal. Immediately after division both parts began to elongate, and soon came into contact (*d*). The larger, posterior piece lengthened more rapidly than the smaller, anterior portion. When the two daughter-nuclei pressed closely together at the point of contact, even bulging at that point owing to mutual pressure, I watched carefully to see whether fusion would take place. But it occurred neither in this instance nor any other in which I followed the process in the living meganucleus; they finally drew apart always at the very point where they had been in contact. Coalescence was evidently prevented by

the nuclear membranes. When both moieties had become moniliform they began to draw apart, the anterior remaining in the distal zoöid, the posterior lying mainly in the proximal, but about a fifth of its length extending into the distal offspring. (Fig. 46 *g*.) I have found it generally the case that when the meganucleus has divided somewhat unequally, the longer portion projects beyond the line of constriction into the zoöid having the shorter portion.

An interesting instance of division during coalescence is represented in the series of Fig. 47 *a-h*. The nucleus had originally 16 nodes. Fusion began at three different points: anterior, middle, and posterior (*a*), and proceeded slowly, requiring more than an hour to reach stage *d*. The result of the presence of three foci of coalescence was the attraction of material in opposite directions at two points, where consequently thin commissures were formed, which soon ruptured, thus breaking the nucleus into three pieces of unequal size (Fig. 47 *b, c, d*), of which the anterior contained the substance of 8 nodes, the middle of 6 nodes, the posterior of 2 nodes. Each piece soon attained its maximum coalescence, with repeated change of form (*d, e*), then the two anterior began to elongate, but the hindmost and smallest remained spherical until nodulation of the others had begun (*f, g, h*). The anterior piece, from which 12 nodes were formed, was apportioned to the distal individual; the other two, which together gave rise to 8 nodes (making 20 in all from a nucleus previously possessing 16), to the proximal. Thus the posterior zoöid started off in life with two separate meganuclei.

The fourth case, with absence of nuclear division, I have not actually observed, but that it occurs is evident from the considerable number of Stentors that possess two distinct meganuclei of about equal size.<sup>1</sup>

<sup>1</sup> It is not easy to find a satisfactory explanation of the variation in the time at which meganuclear division takes place. Such a variation has not, so far as I know, been observed in other species of Infusoria; but negative evidence on this point is of little value, considering the small number of recorded observations. The variation in *S. caruleus* is apparently limited to the three periods above stated; I have never seen division during the period of elongation (Fig. 41 *c-h*). I am inclined to regard the division at time of condensation (Fig. 46 *a, b*) as the

The remarkable metamorphosis undergone by moniliform and rod-shaped meganuclei at time of fission appears to be purely a change of form. As stated on a preceding page, I have not been able to detect the least structural alteration in the substance of the meganucleus at any stage. Perhaps the most obvious explanation of the changes is, that an equal division of the meganucleus in its moniliform, or cord-like, flexuous state is not easily brought about, owing to its spiral disposition in the body and the frequently unequal size of its nodes. The case is different when the nucleus divides after having returned to the moniliform condition, for the nucleus is then nearly straight, its middle point coincides with the plane of division, and the nodes, newly formed from a cylindrical rod of even diameter from end to end, are very nearly alike in size. That the division is fairly equal is evinced by the fact that the number of nodes in each daughter-stentor is usually about the same, as the following table will indicate:—

NO. OF PAIR.	NODES.	NO. OF PAIR.	NODES.
1	12—12	12	10—11
2	8—8	13	12—13
3	15—15	14	15—16
4	10—10	15	14—15
5	10—10	16	14—12
6	11—11	17	14—16
7	13—13	18	15—13
8	12—12	19	12—8
9	11—11	20	19—15
10	9—9	21	14—18
11	14—14	22	17—12

It is seen that in eleven instances out of the twenty-two given the number of nodes in each daughter-nucleus is exactly the same; in four other cases there is a difference of only one node. In 68 per cent, then, of the specimens examined, the division is fairly equal. But how shall we account for the four instances (pairs 19–22) of decidedly unequal nuclei? I believe

primitive type—a reminiscence of the time when the nucleus was always spherical. The transient appearance of a constriction about the nucleus (Fig. 41 *b-e*), whether it divides at this time or later, lends strength to this view; for on this basis we must regard it as an inherited tendency to division.

that they are due to premature, unequal divisions, such as I have described in detail (p. 515). This was certainly the case with pairs 19 and 20, the latter being represented at an early stage of nodulation in Fig. 35, and the former being shown in detail in Fig. 47.

Another reason for the reconstruction of the meganucleus is that it provides a means of increasing the number of nodes ; for increase does not, so far as my observation goes, take place while the nucleus is in its resting form. Increase of nodes is beautifully displayed in those species where the number of nodes is constant (*e.g.* in *Stylonichia*, *Onychodromus*, and many other Hypotricha), for in these forms the number is exactly doubled after condensation (Balbiani '60, Bütschli '76). In *S. cæruleus*, contrary to the statement made by Balbiani in 1860 (p. 77), and re-affirmed by him more recently ('81, p. 325), the number of nodes is not regularly increased to double the number in the parent ; yet an increase almost invariably takes place after condensation.<sup>1</sup> Thus the offspring are furnished with nuclei each having a number of nodes considerably greater than half the number possessed by the parent. The nodes are of course smaller.

*Micronuclei.* — The division of the micronuclei of Stentor at time of fission has not hitherto been described. By means of sections of *S. cæruleus* I have seen micronuclei in mitosis (Fig. 48), but in only a single specimen, although a large number were sectioned in all stages of fission. There is considerable difficulty in making out the micronuclei at all during fission, because they become very transparent and of nearly the same refractive index as the surrounding cytoplasm. Previous to formation of the spindle, the micronuclei enlarge from the normal size of  $2\mu$  to a diameter of  $4-5\mu$  ; at the same time they lose most of their stainability and their high refractive power. The spindles represented in Fig. 48 are evidently at different stages ; the one on the right shows the

<sup>1</sup> I have made only a few observations on this point. In one instance a Stentor in the first stages of fission had a nucleus of 17 nodes; the offspring had nuclei of 14 and 15 nodes respectively (pair 15, p. 516). Another case — increase from 16 nodes to 20 — has already been mentioned (p. 515).

"equatorial plate" distinctly, while that on the left is at the "spirem" stage figured by Maupas ('89, Pl. IX, Figs. 7-9). The meganucleus, of which only a peripheral section appears in the drawing, was at complete condensation, and in two distinct pieces. I made out 65 micronuclei adherent to the two, but none were found in the spindle stage except the two above-mentioned. It would be of interest to know whether all the micronuclei divide at every fission, and an even allotment of them to each daughter individual takes place.

A point worthy of note is the time at which micronuclear division takes place in different species of Infusoria, with reference to the phases of the meganucleus. In *Stentor* it is considerably earlier than in *Paramecium*, *Stylonichia*, or *Vorticella*, where the division of mega- and micronuclei are nearly simultaneous.

### 3. *Ontogeny and Phylogeny.*

The conception that the development of a new Infusorian by the process of fission is an ontogenetic development, comparable in some respects to the development of a Metazoön, has impressed itself strongly upon me in the study of fission in *Stentor*. I do not regard the formation of the proximal offspring as homologous with the building-up of a pluricellular animal from the egg. It is rather to be compared to the agamic reproduction of Metazoa by strobilation or segmentation, as in the *Scyphistoma* of *Acalephs*, in the *Turbellaria* (*Microstomum*), and in *Annelids* (*Autolytus*, *Nais*, etc.); for a somatic portion of the parent is converted by the regeneration of necessary organs into a new being, whereas the development from the egg has a single germ-cell as its starting-point.

Is it possible to discover in the stages of fission a repetition of the leading facts in the phylogeny of the species? When we consider how doubtfully and indistinctly phylogenetic history is generally expressed, even in the ontogeny of Metazoa, we shall not be surprised to find the evidences of such recapitulatory stages in the evolution of a Protozoön far from decisive. And while in most groups of the Metazoa we have two checks upon our embryological inferences regarding

phylogeny — comparative anatomy and palæontology — among the Infusoria we have only the former.

I regard the Heterotricha as showing more clearly than any other group of Infusoria a phylogenetic history. They possess one structure, the adoral zone, that we can consider absolutely homologous from the highest to the lowest of the group. Another structure, the peristome, which lies to the right of the zone and in the higher forms is partly or almost wholly enclosed by it, is also probably homologous throughout all the lower forms of the group. The fact has already been noted (p. 502) that in the Stentorina the peristome is replaced functionally by the frontal field. These three structures, adoral zone, peristome, and its successor, the frontal field, are more important than any others in studying the comparative anatomy of these forms. In passing from the lower to the higher Heterotricha, we find that the peristome and zone take a more and more terminal position, until in the Stentorina (*Climacostomum*, *Stentor*, *Folliculina*) it is completely apical (Fig. III, *d, e, f*). It is possible, then, to arrange a series (which we may regard as phylogenetic) based upon the position and development of the adoral zone and frontal field (Fig. III, *a-f*). In this series the forms in which these structures are most strictly lateral in position (*Spirostomum*, *Blepharisma*) will occupy the lowest place, and the others (*Condylostoma*, *Climacostomum*, *Stentor*) be arranged according as the zone becomes more and more terminal, and as the peristome (or its physiological equivalent, the frontal field) increases in breadth and becomes more and more circumscribed by the zone.

This much being attainable by comparative anatomy, does the ontogeny of one of the higher forms (*e.g.* *Stentor*) yield any evidence for or against the arrangement given in Fig. III? In comparing the various stages in the evolution of the zone and frontal field of the proximal zoöid of *S. cæruleus* (Pl. XXIV, Figs. 26–36) with the series of lower forms (Fig. III, *a-d*), we see that the successive positions taken by the new organula in question correspond quite accurately with the phylogenetic development of these structures. An exception is seen in the case of the peristome. This appears as a narrow band along

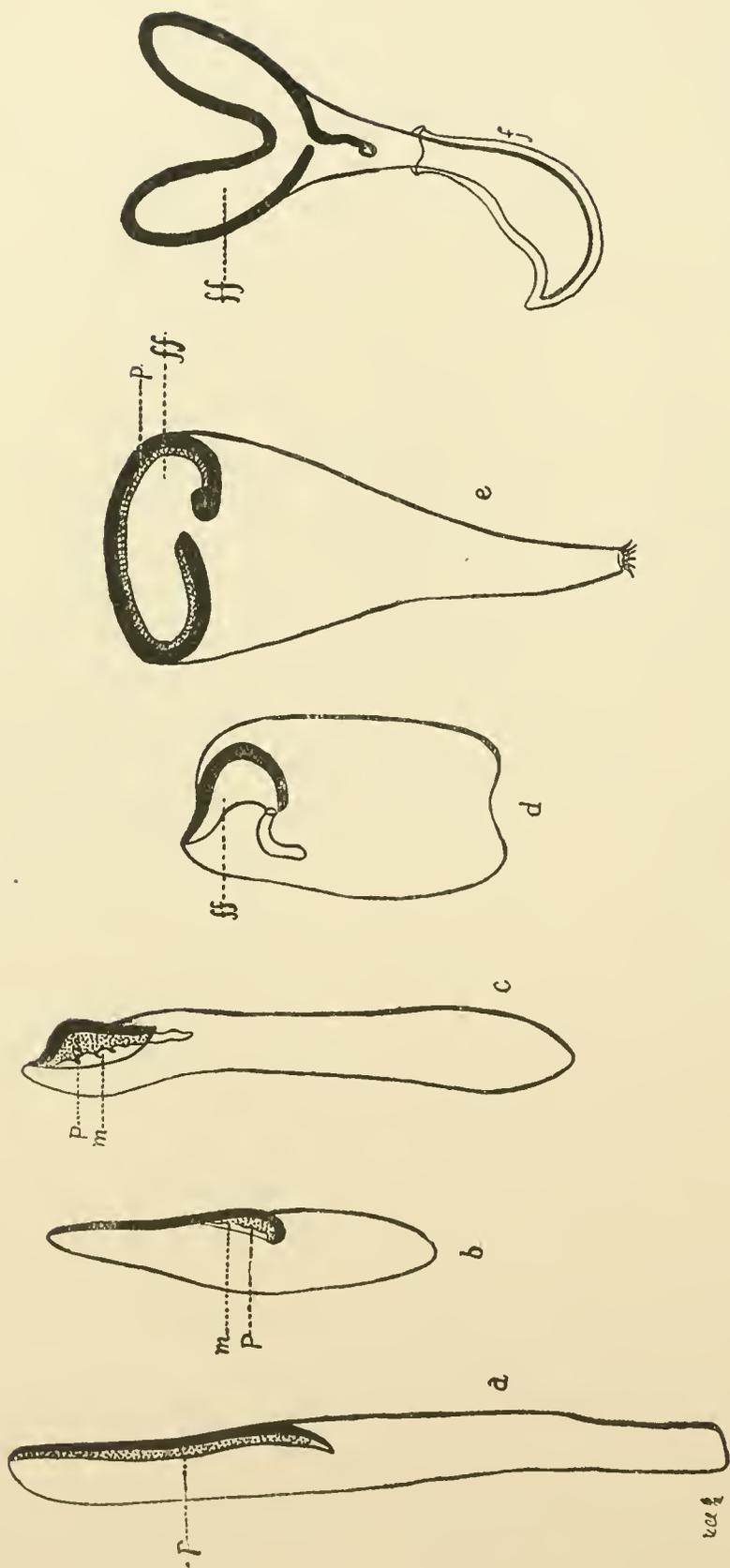


FIG. III.—Diagrams to illustrate the phylogeny of the Heterotricta. *a*, Spirostomum; *b*, Blepharisma; *c*, Condylostoma; *d*, Climacostomum; *e*, Stentor; *f*, Folliculina. *p*, Peristome; *m*, undulating membrane; *ff*, frontal field. The heavy black line indicates the adoral zone.

the right side of the zone, and a narrow band it remains throughout. It is a case of a rudimentary organ persisting after its function has been assumed by another structure.

The evolution of the zone and frontal field does not end with *Stentor*, although in this genus these structures are completely terminal. In *Folliculina* the field is produced into two lateral, ear-like appendages. (Fig. III, *f*.) Unfortunately, we lack sufficient information regarding the evolution of the new zone in *Folliculina* to determine whether it has a *Stentor*-like stage or not; but the brief account of the fission by Möbius ('86) shows that it is very aberrant, and obviously adjusted to the peculiar mode of life and highly-modified frontal field of this form. It is therefore improbable that it will reveal much in regard to the phylogeny of *Folliculina*.

### C. REGENERATION.

One of the most remarkable events in the life-history of *Stentor* is the periodic renewal of the mouth, pharynx, and adjacent parts of frontal field and adoral zone. That Infusoria are able to regenerate missing organs after encystment, conjugation, or merotomy, is well known. The peculiarity as regards *Stentor* is that regeneration also occurs when the parts to be renewed are already present and functional

Considering the frequency of the regenerative process in *S. cæruleus*, it is surprising that it so long escaped detection. Specimens in course of regeneration were indeed seen by Stein ('67) and Schuberg ('90). It remained, however, for Balbiani ('91<sup>b</sup>) to discover the real character of the process, and follow it through its stages. My own study of *S. cæruleus* had included the regenerative phenomena, and I had seen every stage of it previous to the publication of Balbiani's paper. I am able, therefore, to confirm his results from wholly independent observation, and add besides a few new facts. At the outset, I regarded the process as an abortive fission, believing that under certain circumstances the animal was unable to bring division to a successful issue, and its new frontal field then coalesced with the old, thereby inducing the atrophy of duplicated parts. But observation of the fact that successful

fission may follow closely the supposed abortive fission without change of the conditions of life, and the inevitable occurrence of the process when the adoral zone has become defective through atrophy or injury, have led me to adopt Balbiani's explanation as the true one.

### 1. *Cytoplasmic Phases.*

At the outset, the neoformation leading to regeneration is indistinguishable from that leading to fission. In both cases a new adoral zone is initiated in precisely the same manner and place. The first mark of distinction is the appearance of a new contractile vesicle if it is to be fission, and none if regeneration. But my observations of the process agree so well with Balbiani's description that there is no occasion to give them at length. Different stages of the regeneration are shown in Figs. 38, 39 A, and 39 B. It is interesting to see how perfectly the new membranellæ (*a.z.*<sup>1</sup>) become assimilated to those of the old zone. The *modus operandi* by which the new mouth (*o.*<sup>1</sup>) is gradually brought into the position of the old, and the slow advancement of the new zone to the anterior end of the animal, I have not been able to comprehend, nor does Balbiani offer any explanation. There is evidently a shortening of both the old and new zones.

During their formation, and until the new mouth has advanced nearly to the position of the old, the new membranellæ exhibit the same slow, undulating vibration as in fission. This is gradually quickened into the more vigorous vibration of the old membranellæ.

I have once seen two successive regenerations coming at a very short interval—a sort of hypertrophy of the process. (Figs. 39 A, 39 B.) When first observed the animal was at a slightly earlier stage than that represented in Fig. 39 A. The process, so far as the first-formed mouth (*o.*<sup>1</sup>) and zone (*a.z.*<sup>1</sup>) were concerned, was taking place normally. The old mouth (*o*) had nearly atrophied. To my surprise a second regeneration was also under way, the adoral zone being already visible. It developed rapidly, while its predecessor as rapidly disappeared (Fig. 39 B). The condition represented in Fig.

39 A was reached at 12.30 P.M., and that of Fig. 39 B at 2 P.M. A fact worthy of notice is that as soon as the second new mouth ( $o.^2$ ) was developed the first ( $o.^1$ ) began to atrophy, and also the newly-formed membranellæ of  $a.s.^1$  between the junction of  $a.s.^2$  with it and the mouth. Points of difference between this double regeneration and an ordinary one were the well-marked places of junction between older and newer adoral zones, and the slowness with which the process was completed. Twenty-four hours after the stage shown in Fig. 39 B, the new mouth had not quite reached its normal place.

As if to show the close connection existing between regeneration and fission, a Stentor that has started to renew its oral organs may undergo fission instead. I have only once observed it. When first seen, the new zone had already joined the old. I noticed, however, that a new contractile vacuole had appeared in the same place as in fission. Presently I noticed that the most anterior membranellæ of the new zone were becoming smaller. They soon atrophied entirely for about one-fourth the length of the new zone, and only a streak on the pellicula indicated where the zone had previously extended. The meganuclear phases were watched, and have already been described (Fig. 47). The subsequent cytoplasmic changes proceeded normally. Within the new ramifying zone of the distal zoïd a curious derangement of the stripes was observed (Fig. 40), undoubtedly due to the atrophy of the anterior portion of the adoral zone.

The time required by the process of regeneration varies in different individuals and doubtless at different temperatures. At 16°–20° C. I have found it requires 6½ to 8 hours. It is, therefore, a little slower than fission.

## 2. Nuclear Phases.

Balbiani ('91) calls attention to the interesting fact that the meganucleus goes through precisely the same changes in regeneration of the oral organs as in fission, with the exception that division normally occurs only in the latter instance. But I find him at error in the statement that the number of nodes is not increased at time of regeneration. I have made

repeated observations on this point, under conditions that admitted of accurate counting of the nodes both before and after regeneration, with what results the following table will show :—

STENTOR.	BEFORE REGENERATION.	AFTER.	STENTOR.	BEFORE REGENERATION.	AFTER.
1	9 nodes	15 nodes	10	15 nodes	13 nodes
2	11 “	13 “	11	12 “	13 “
3	16 “	16 “	12	12 “	16 “
4	7 “	16 “	13	19 “	14 “
5	13 “	13 “	14	9 “	17 “
6	20 “	22 “	15	13 “	19 “
7	12 “	13 “	16	10 “	15 “
8	19 “	20 “	17	11 “	16 “
9	18 “	20 “	18	15 “	17 “

Out of the 18 examples, 14, or nearly 78 per cent, show an increase of nodes. In two (Nos. 3 and 5) the number remains unaltered, and in two (Nos. 10 and 13) there is a decrease. Sometimes the nodes are more than doubled in number (No. 4). While the extent of the increase in the number of nodes is highly variable, it is as a rule greatest in meganuclei with a small number of nodes (Nos. 1, 4, 14, 16, 17), and smallest in meganuclei with an uncommonly large number (Nos. 6, 8, 9). The average number of nodes for the 18 regenerated Stentors is 16, while the average number for the 44 young Stentors (*i.e.* 22 pairs) given on p. 516 is only 12.6 nodes. It is possible, then, to see an important function of regeneration in the marked increase in the number of nodes over and above those formed at time of fission; for in this way the superficial extent of the nuclear substance is greatly enlarged. If the nodulation of the meganucleus has a physiological value—and we can hardly conceive of its being so carefully maintained unless it has—it is reasonable to suppose that an increase in the number of nodes, up to a certain limit, is for the advantage of the organism. We have seen that the process of renodulation brings about the same result, whether it occurs at time of fission or at time of regeneration. The increase of nodes in the former instance is for the advantage of the race, in the latter for the benefit of the individual.

The table shows that there is by no means always an increase of nodes; sometimes there is even a decrease. In regard to No. 13, there seems to be a reason for the great reduction in their number. The Stentor was very small, and the long nucleus, with its 19 nodes, almost filled the body. The frontal field and adoral zone were almost atrophied, and this circumstance was, unquestionably, the prime motive for regeneration. I believe that in this case it was an advantage to reduce the extent of the nuclear surface, and this was certainly accomplished by reducing the number of nodes.

Although I have sectioned many specimens in various stages of regeneration, I have not been so fortunate as to discover the behavior of the micronuclei. The same difficulty encountered in the study of them at time of fission was met with here — they become so transparent as almost always to escape observation. The question, Do they divide at time of regeneration? therefore remains unanswered. If such should prove to be the case, the extraordinary number of micronuclei in Stentor would be accounted for.

Doubtless the regeneration of buccal organs during the ordinary condition of life obtains in all species of Stentor, and may yet prove to be of wide prevalence among Infusoria. Single stages were seen by Stein ('67) in *S. cæruleus*, *S. polymorphus* and *S. niger*. Besides the Blue Stentor, I have studied it in *S. polymorphus*, where it is carried out in the same manner, and requires about the same length of time.

### 3. *Generalia.*

There are four — possibly five — occasions in the life-history of Stentor when a regeneration of the oral organs is imperative. They are:

1. Fission. New frontal field, adoral zone and pharynx formed *in toto* for proximal individual. No atrophy of existing organs.

2. After encystment. Not yet actually observed in Stentor, but undoubtedly occurs.

3. After amputation of, or serious mechanical injury to, the organs of nutrition. Often atrophy of defective parts, which are replaced by the newly-formed ones.

4. After enfeeblement or degeneration of organs of nutrition. Complete atrophy of old pharynx, and partial atrophy of old frontal field and zone.

5. After conjugation (?) Not yet observed in Stentor. Has been recorded in Spirostomum (Maupas, '89) and many other Infusoria (Maupas, R. Hertwig, '89).

On every occasion when regeneration is required, it is carried out in the same manner. In no instance have I followed the development of a new oral apparatus that did not entail meganuclear reconstitution. There is a subtle bond of union between the two phenomena, and the characteristic condensation of the meganucleus cannot be regarded as an intrinsic part of the process of fission alone. It is of interest to note, furthermore, that the meganucleus in no case takes the initiative; the cytoplasmic change invariably precedes the karyoplasmic, thus reversing the order of events prevailing in cell-division in Metazoan- and plant-cells.

I have made repeated experiments to ascertain whether membranellæ could be regenerated in any except the ordinary way. The adoral zones of Stentors were damaged with the needle in various ways and at different points, and portions of the zone were amputated with the scalpel. If the injury was slight, the wound closed up, bringing the cut ends of the zone into contact, so that the zone was, apparently, as good as ever. If enough of the zone was cut away to interfere seriously with its function, membranellæ were never formed in place of the lost ones; the loss was made good by regeneration in the usual way. The result of the experiments led me to the conclusion that the regeneration of the oral structures of Stentor does not take place *in situ*. As would be expected, injury to or removal of the *adoral* portions of the zone, especially if affecting the pharynx, were more likely to cause regeneration than injuries to the *aboral* portions.

Just as among pluricellular beings the regeneration of highly-specialized organs always takes place from the least differentiated cells available, so among unicellular organisms the more specialized portions ("organula") of the cell are regenerated

from the less specialized. Thus, in *Stentor*, the membranellæ are developed from the indifferent endoplasm, and in a place remote from their final position.

#### D. CONJUGATION.

My observations on the conjugation of *Stentor* are very fragmentary, for I have been unable to obtain sufficient material for a complete study of it. Conjugation in all species of *Stentor* is apparently a rare event. It was observed by Stein ('67, p. 217) in *S. niger* only; by Moxon ('69) in *S. cæruleus*; and by Balbiani ('61, '82, '92), who has been the only one heretofore to make any study of the nuclear phenomena of the process, in *S. cæruleus* and *S. roeselii*. I have found a few conjugated individuals of *S. cæruleus* and *S. igneus*. In spite of the fact that I have had many large colonies of the Blue *Stentor* for periods varying from two weeks to four months, I have never observed an "epidemic" of conjugation such as Balbiani ('82, p. 161) says he has seen repeatedly in case of *S. cæruleus* and *S. roeselii*.

*External Phenomena.*—The only conjugates of *S. cæruleus* that I have obtained were found May 12–15, 1891, in a small, ill-fed colony that had been in the laboratory for ten days. Every individual in the colony was examined repeatedly during the four days that conjugation prevailed, but out of two or three hundred *Stentors* only fifteen couples were found.

The *Stentors* in the culture were nearly all medium-sized or small, and, as food was scarce, were very free from food-vacuoles. Usually the gametes were unequal<sup>1</sup> in size—in one instance very strikingly so (Fig. 50, Pl. XXVI.). The mode of attachment I have found to be exactly as it was long ago described by Balbiani ('61), implicating only a small area in the left-hand portions of the frontal fields of the gametes, and thus compelling them to face in opposite directions, as it were. The line of suture appears as a dense, glistening vertical band (Fig. 49), and sections show that it is not merely a superficial line, but a plate covering the whole surface of contact, and

<sup>1</sup> Out of the 15 pairs, the gametes of 9 were notably unequal in size.

evidently formed from the ectoplasm, as it has the dense consistency of that layer and contains abundant pigment. At the time of interchange of the micronuclear spindles, an opening must, of course, be formed in the plate.

The behavior of the gametes shows little that is characteristic. They much prefer to remain fixed in one spot, when they become moderately extended, and stand at a wide angle to each other, the attitude being strongly suggestive of an effort to pull apart. If disturbed, the gametes swim easily, rotating about the long axis in precisely the same manner as the individual Stentor; if there is considerable difference in size, the larger gamete fairly carries the smaller about. The attachment of the gametes is very firm; not only is it maintained during their violent contractions, but they can be sucked up into a pipette without danger of separation.

As in case of *S. cæruleus*, I have only once observed the conjugation of *S. igneus*. In July, 1891, I found a few conjugated pairs at Wood's Holl, at different times from the 17th to the 25th. As usual, the gametes were of small size (in one instance of the variety *nigricans*), and nearly all showed a notable development of red pigment in the anterior part of the body. The manner of union and relative position of gametes does not differ from that of other Stentors, but the area of attachment is proportionally greater than in *S. cæruleus*.

*Nuclear Phenomena.* — Something of the internal processes of conjugation can be seen in the living specimen. Such observations are greatly facilitated by the absence of food-vacuoles.

One of the most noticeable changes of those observable in the living animal is the separation of the nodes of the meganucleus. This occurs very early in the process of conjugation. It is obviously brought about by rupture of the commissures, which, as in division, are promptly resorbed by the nodes. The latter then become perfectly spherical. Their structure undergoes no change at this time, and their affinity for stains is as strong as ever. After separation the nodes leave their place in the peripheral portion of the endo-

plasm, and, caught in the endoplasmic cyclosis, are carried to all parts of the animal. It is a striking fact that the protoplasmic circulatory movements are far more active at time of conjugation than at any other period in the life of Stentor that I have observed. The arrangement of cytoplasmic threads about the separated nodes of the meganucleus (Fig. 51) reminds one strongly of the streaming reticulum in *Noctiluca* or in the stamen-hairs of *Tradescantia*.

Micronuclei of different sizes and stages of development can be seen in the living gamete, and the movement of these is quite as active as that of the nodes. They have a glistening, refractive appearance. I was never able to see a micronuclear spindle in the living animal, the most of the micronuclei observed being probably already developing to become meganuclei (Fig. 50, *mgn.*<sup>1</sup>?).

By reason of the difficulty in obtaining material, and not less, perhaps, on account of the obscuration of the micronuclei by the comparatively great mass of cytoplasm, no adequate account of the complex details of conjugation in any species of Stentor has been given. The latter difficulty I have obviated by sectioning the gametes, which can be readily oriented. I have endeavored to secure material by the method used by Maupas ('89, p. 168), but without the least success.<sup>1</sup>

While it would be impossible to interpret the results I have obtained from the small amount of material at my command, if we had a less complete knowledge of conjugation in other forms, I believe it may be done in the light of the beautiful researches of Maupas ('89) and of R. Hertwig ('89) in this field.

Nearly all of my preparations show that the gametes were in the later stages of conjugation (Stage H of Maupas), and consequently I have not seen the exchange of micronuclear spindles. In only one preparation have I found spindles, two of different shape (from the same gamete) being represented in Figs. 53, 54. They are both in the "monaster" or "mother-star" stage, and have longitudinal, linear chromosomes which

<sup>1</sup> I have also tried Maupas' method with *Paramecium caudatum*, which multiplied enormously in hay infusion, and was afterwards subjected to long periods of fasting, but obtained no "epidemics" of conjugation.

coincide with the very distinct spindle-fibres. The spindle is enormously large (length,  $16\mu$ ) for the size of the resting micronucleus (Fig. 55). It is considerably larger than the spindle formed at time of fission.

In conjugates at a late stage and in ex-conjugates, spherical, translucent, unstainable bodies are found, varying in size from  $8\mu$  to  $12\mu$ . (Figs. 57, 58.) These are undoubtedly the anlagen of new meganuclei. Their behavior towards staining reagents is very characteristic, for I have not noticed the least tendency to take a stain with picro-carmin or Czokor's alum cochineal. This is in perfect accordance with the observations of Gruber ('87), Maupas ('89), and R. Hertwig ('89). The unstainability of the meganucleus during its evolution is due either to an absence of chromatin, or to a chemical change in it; the latter seems the more probable, for both in the micronuclear state and in the condition of a fully-developed meganucleus these bodies contain abundant, highly-stainable chromatin.

The general appearance presented by the nuclear structures of two gametes in Stage H is shown in Fig. 52. The separated nodes of the old meganucleus have not as yet undergone (in this particular example) any perceptible alteration of structure or loss of affinity for stains. Numerous micronuclei are present, distributed throughout the cytoplasm. They are much enlarged beyond the minute dimensions they have in the resting state (compare Figs. 55 and 56), and stain less deeply. They have a somewhat granular structure, with occasionally a suggestion of chromatic threads (Fig. 56). Besides micronuclei, the anlagen of new meganuclei are found (*mgn.*<sup>1</sup>)—in this instance as in some others, of very different size in the two gametes. Although, according to Balbiani's observations ('61, '92) only a single meganucleus develops in each gamete, I have observed that several often pass through the early stages of the evolution. We must, therefore, assume that the superfluous ones are absorbed by the cytoplasm, and so only one reaches the final stages of development.

Still other structures found at this stage are micronuclei within vesicles two or three times their diameter. (Fig. 52, lower gamete, Fig. 59.) These I agree with Maupas in

regarding as superfluous micronuclei undergoing digestion. The vesicle may be compared to a digestive vacuole.

In the later stages of conjugation, the separated nodes of the old meganucleus stain less deeply, and begin to lose their spherical contour. The example represented in Fig. 60 is from an ex-conjugate, being from the same specimen as Fig. 57. The chromatic reticulum is still existent. At two points clear vesicles, probably of karyoplasm, cause the nuclear membrane to bulge out. I have never seen such vesicles in the ordinary state of the meganucleus of Stentor.

#### E. TERATOLOGY.

As Balbiani has remarked in a recent paper ('91), the teratology of Protozoa is an almost untouched field of investigation, and is certainly a promising one. Balbiani's own contribution ('91) to the subject is based upon the study of two double monsters of *S. cæruleus*, one of them artificially produced by merotomy. In a still more recent and very suggestive paper ('92), the eminent French micrographer has shown how double monsters of Stentor may be produced at will by the merotomy of specimens in the earlier stages of fission. My own results are also derived from the study of a double monster of *S. cæruleus*, one which was not produced artificially.

When discovered, the specimen presented nearly the appearance shown in Fig. 61A, with the exception that the anlagen of new zones (*a.z.*,<sup>2</sup> *a.z.*3) had not appeared. The animal has the aspect of being in a stage of fission soon after the appearance of the constriction. The new adoral zone (*a.z.*1) had, however, no mouth at its lower extremity, although the place for it was marked by a deep fold, into which the posterior extremity of the new zone penetrated. Another peculiarity is the reversed position of the anterior zoöid. The twist is plainly indicated in the direction taken by the stripes, which apparently present a distinct system for each zoöid, meeting each other at a wide angle in the fission-line. As the ramifying zone<sup>1</sup> of the anterior zoöid is placed far to the right of its normal position, it is evident that the torsion has been from left to

<sup>1</sup> Its position is indicated by the new adoral zone *a.z.*3.

right. The meganucleus was already in the noded condition, and as nearly as could be made out in the living animal, was in two pieces, one of ten nodes lying in the proximal zoöid, the other of twelve nodes lying for the most part in the distal. A contractile vacuole was present in each zoöid.

The first change observed was the appearance of two new zones (Fig. 61A, *a.s.*<sup>2</sup>, *a.s.*<sup>3</sup>), one for each zoöid. That on the posterior zoöid (*a.s.*<sup>2</sup>) appeared a little before the other, but the evolution of the two went on very nearly *pari passu*. Three hours later a new mouth had appeared at the lower extremity of each, (*o.*<sup>2</sup>, *o.*<sup>3</sup>) and at the same time the meganucleus had undergone condensation into three or four masses. Six hours after the appearance of the new zones, *o.*<sup>2</sup> had been drawn up into the position of the absent mouth of the posterior zoöid (Fig. 61C), and at nearly the same time *o.*<sup>3</sup> had taken the place of *o.* Another change which had probably been in operation since the finding of the specimen, had by this time become very evident: viz., the forward movement of the whole posterior frontal field and zone to the plane of the anterior. Possibly it would be more accurate to describe the change as a *withdrawal* of the anterior zoöid into the posterior. Whichever movement took place, its important result was to bring the two frontal fields to the same level, and therefore the two parts of the double monster into closer union.

The next observations were made 15 hours later, and revealed a great change in the appearance of the monster (Fig. 61D). It now had one immensely large frontal field composed of the two preëxisting frontal fields, and encircled by an adoral zone made up of repeated neoformations arising at two different places on the body. The patchwork character of the zone is clearly marked by the numerous breaks in the series of membranellæ. The dualism of the specimen is still manifest in the arrangement of stripes in the frontal field in two distinct systems, in the development of two more new zones, and in the two contractile vacuoles (*c.v.*, *c.v.*<sup>1</sup>).

A point of much interest is the *contemporaneity* of the oral renovation, now observed for the second time in this specimen. In both instances the synchronism in the neoformation of oral

organs is the more striking, inasmuch as there seems to be no occasion for the regeneration of more than one of the zones. At the stage represented by Figs. 61A, B, it is the mouth on the left (*i.e.* posterior) that is wanting, while in the later stage (Fig. 61D) the mouth on the right is absent. The only explanation of this synchronism in the development of duplicate parts seems to be a close coördination of functions throughout the dual organism, which therefore *physiologically* is to be regarded as a single individual, but with duplicate organs and functions.

The double Stentor underwent little modification during the next nine hours, in the course of which it was frequently examined. The new mouths (*o.*<sup>4</sup> *o.*<sup>5</sup>) were gradually drawn into position, and *o.*<sup>2</sup> atrophied. By the following morning, 25 hours after Fig. 61 D was drawn, a great change had taken place (Fig. 61 E), amounting to a loss of dualism, and therefore a return almost to the normal condition. The right-hand mouth (*o.*<sup>4</sup>) had atrophied. The breaks in the zone had, with one exception, disappeared; the frontal field was much reduced in size, and its two systems of stripes had nearly melted into one. Only one contractile vacuole (*c.v.*<sup>1</sup>) was present. The regeneration (*a.z.*<sup>6</sup>) of the oral apparatus (*o.*<sup>4</sup>) was in progress, but no similar regeneration was under way for the atrophied *o.*<sup>5</sup>—strong evidence of the loss of physiological duality.

The subsequent modifications of our Stentor were unimportant, and consisted mainly in the obliteration of every trace of duality. After a day or two it differed from normal Stentors only in the slightly greater size and oval shape of the frontal field. No fission occurred during the four or five days I had the Stentor under observation.

One of the double monsters studied in detail by Balbiani ('91) differed from mine in that duality was present, not in the anterior, but in the posterior portion of the body. The monster was furthermore artificially produced by amputating the frontal field and dividing the posterior part of the body by a longitudinal incision, so as to give it a bipedal appearance. The regeneration of the frontal field was not observed. The interesting point of likeness between Balbiani's specimen and mine is the gradual obliteration of duality and return to the

normal form. A striking fact in the history of Balbiani's monster is its fission *previous* to the disappearance of its bifid structure, which it therefore transmits to its proximal offspring. There was, however, not the slightest development of duality in the distal offspring; even in the proximal it quickly disappeared, and the normal form was regained.

In the light of Balbiani's most recent experiments upon *Stentor* ('92), I am led to believe that double monsters of this species, and doubtless of Infusoria in general, owe their origin to the temporary dualism of the cytosome at time of fission. If through imperfect development or mutilation the normal progress of fission is interfered with, the dualism already set up in the cytoplasm does not fail to manifest itself in the production of a double monster. Some of Balbiani's figures (especially Fig. 7 b<sup>1</sup>-b<sup>5</sup>, Pl. II, '92), show the gradations between a nearly-divided merozoan (originally cut from a *Stentor* in fission), and a perfect double monster with two frontal fields.

The duplication of newly-formed organs in both zooids in fission is another interesting instance of dualism, comparable to the simultaneous appearance of new zones in a double monster. It was observed by Stein ('67) and carefully studied by Sterki ('78) in the neoformation of marginal cilia and of cirri in both daughter-individuals of *Stylonichia*.<sup>1</sup>

The position taken by the meganuclei in a double monster is worthy of notice. A nucleus is usually present in each moiety

<sup>1</sup> The regeneration of ciliary organs in the Oxytrichina is of especial interest. These organs, as is well-known, are highly differentiated, occurring in form of cirri, spines, etc., which are definite in number. In regard to their development, the work of Stein and of Sterki has shown that the frontal, ventral, and anal cirri by no means originate in their definitive form and position. Their earliest appearance as a group of six parallel rows of minute, similar processes, from which the cirri develop and are shifted to their final positions, is very suggestive of the primitive six rows of cilia of the lower Hypotricha (*e. g.*, *Urostyla*). The point of chief interest in this connection is, that new cirri are formed, not alone for the posterior zooid, but also for the anterior, where the old frontal and ventral cirri atrophy and are replaced by the new ones. In the posterior zooid, on the other hand, the caudal cirri of the parent are replaced by the newly-formed cirri. Just as in *Stentor*, the neoformation of a structure demands the atrophy of its preëxisting homologue.

of the body; or if there is but one, it extends into both (Fig. 61 A).

#### IV. BIOLOGY AND PHYSIOLOGY.

I have set apart this portion of the present paper for an account of certain experimental work and physiological observations. Many of the results are obviously far from complete; but are, I believe, not without interest, and will at least serve to show how well-adapted are the Stentors for various lines of biological and physiological experimentation.

##### A. *Rate of Multiplication.*

Most species of Stentor do not multiply readily in confinement. This is especially true of the chlorophyll-bearing forms, none of which have I seen in fission more than a very few times. But, considering the enormous colonies these species form in favorable localities, multiplication must be more rapid under natural conditions. The most prolific species that have come under my observation are *S. cæruleus* and *S. roeselii*. If food be abundant, a few individuals of *S. cæruleus* will in a week or so become the progenitors of thousands and fairly overstock the aquarium.

After reading Maupas' account of his highly-successful experiments upon the multiplication of Infusoria ('88) I determined to test the results he obtained from the propagation of the Blue Stentor (p. 229). I followed his method closely, using the excellent moist chamber that he recommends (p. 179), and making the same sort of slide-cultures. For these I used cover-glasses 18 mm. square, and elevated about a millimeter from the slide with wax feet. For food I raised immense numbers of *Glaucoma scintillans* in hay-infusion. These were fed to the Stentors at intervals of a day or two, and were devoured by them with the greatest avidity. The body of each Stentor soon became gorged with the Glaucomas. Under such generous feeding multiplication was rapid for a few days, often one bipartition to every 24 hours, at a temperature varying from 12° to 22° C. But that this rapid increase was

not, as Maupas supposes, a normal rate, was shown by the fact that it could never be maintained for any length of time.

I soon became convinced that the slide-culture method, however successful it might be with other species of Infusoria, was not adapted to Stentor. So I made cultures in large watch-glasses, each covered with another watch-glass to prevent evaporation. Most of the watch-glass cultures were supplied, in addition to the food-infusoria, with unicellular green algae, usually a species of *Scenedesmus*. The rapid growth of these algae kept the water pure, and to some extent furnished food for the Stentors. But the watch-glass cultures were only in a slight degree more successful than the slide-cultures; for while I was able to raise Stentors on the slide to the 7th or 8th generation, I did not succeed in getting any colony in a watch-glass beyond the 10th.

One of the most rapid multiplications that I have noted was of a culture started in a 2-inch watch-glass with a single Blue Stentor, Dec. 1, 1891. Following is the record of the culture:—

	INDIVIDUALS.				BIPARTITIONS.			
Dec. 2	.	.	.	2	.	.	.	1
	(One was isolated.)							
" 4	.	.	.	2	.	.	.	2
" 5	.	.	.	4	.	.	.	3
" 7	.	.	.	8	.	.	.	4
" 8	.	.	.	16	.	.	.	5
" 9	.	.	.	32	.	.	.	6
" 10	.	.	.	58	.	.	.	7
Temp. 15°–22° C.								

It is seen that there were two intervals when bipartitions were 48 hours apart, whereas the rest were at the minimum interval of 24 hours. After attaining the 7th generation the Stentors almost ceased to multiply and many died. The culture having received abundant feed from beginning to end, lack of nutriment could not be considered as the cause of its decadence.

What was the effect of such frequent bipartitions upon the individual? Neither in this nor in any other instance of

equally frequent bipartitions that I have observed accurately, has the size of the original progenitor been maintained. After only two or three consecutive fissions at 24-hours' interval, the reduced dimensions of the offspring become very noticeable. I believe the cause to be a lack of sufficient time between the bipartitions for the offspring to attain the size of the parent, and consequently after the next bipartition the grand-children, so to speak, are only a little over a fourth the size of the grand-parent, whereas they should normally be half its size. As the culture described above had undergone the most rapid multiplication, its members were reduced to the most minute dimensions. They became mere dwarfs, measuring at the 7th bipartition only .630 mm. (extended) by .285 mm.,<sup>1</sup> and were rapidly passing into a lethargic condition.

On Dec. 16 they were again examined. I had repeated the observation made by Maupas—that inability to take food did not altogether prevent fission; but such fission was of course not followed by any growth of the offspring, and therefore produced a rapid reduction in size. The few Stentors still living were in a moribund state. The movement of the membranellæ was hardly perceptible. In most instances the mouth parts were nearly or wholly atrophied. The average size was .450 mm. in length, and .120 mm. across the frontal field. On staining specimens with methyl-green, I found the nodes of the meganucleus only 5–10 in number, and much reduced in size, although still very large in proportion to the size of the animal, which consequently possessed an abnormally large amount of nuclear substance in proportion to the cytoplasm. The nuclein took as deep a stain as usual.

My most successful culture was made in a large watch-glass kept supplied with fresh water to make good that lost by evaporation, not over-supplied with food (*Glaucoma scintillans*) and furnished with minute green algae, which grew freely and kept the water well oxygenated. The temperature was variable, falling to 12°–15° C. at night, and rising to 22° during the day. The record is as follows:—

<sup>1</sup> The normal size of the species, it will be remembered, is 2 mm. by .5 mm.

## ONE BLUE STENTOR, ISOLATED DEC. 3, 1891.

	INDIVIDUALS.	BIPARTITIONS.
Dec. 4 . . . . .	2 . . . . .	1
" 6 . . . . .	4 . . . . .	2
" 8 . . . . .	8 . . . . .	3
" 9 . . . . .	7 (one died) . . . . .	3
" 10 . . . . .	14 . . . . .	4
" 11-15 . . . . .	(Increased rapidly; not counted.)	
" 16 . . . . .	220 . . . . .	8
	(One was isolated.)	
" 17 . . . . .	2 . . . . .	9
" 19 . . . . .	3 . . . . .	9½
" 22 . . . . .	4 . . . . .	10
" 23 . . . . .	4 . . . . .	10

My unavoidable departure from Worcester at this time brought the culture to an untimely end. It will be seen that the usual rate of increase was one bipartition in 48 hours; that all the individuals of a given generation (especially during the earlier bipartitions) divide at about the same time; that the rate of increase is accelerated from the 4th to the 9th bipartition; that, finally, discrepancies in the times of fission of the offspring of each bipartition appear, and increase at each subsequent fission. In passing from the 9th to the 10th bipartitions the interval between the fission of the two individuals amounts to nearly four days.

My culture experiments, though unsuccessful in producing a long series of generations like the well-known experiments of Maupas, at least demonstrate that it is possible to stimulate Infusoria to a rate of increase beyond the normal, leading to speedy deterioration of the race. It is probable that Stentors are somewhat exceptional in this respect, and that such species as *Stylonichia pustulata* and *Onychodromus grandis*, in which the time required for fission is much less than in Stentor, are less susceptible to the pathologic effects of over-fecundity; but these effects should, nevertheless, be taken into account.

Since under rapid multiplication a Stentor culture deteriorates, under what conditions does the individual Stentor attain its best development? I gained a hint in the right direction upon examining an old culture that had stood

undisturbed for weeks. I had originally placed in it a large number of Blue Stentors from an unusually rich gathering collected in Alewife Brook, Cambridge. The culture had not done well, apparently from lack of food, and I believed that it had become barren. Examination showed that it contained a few Stentors of unusually large size and splendid development. The cytoplasm was very free from food-vacuoles, and it was evident that they had had a meagre diet for a considerable time. This I believe to be the prime cause of their large size and fine development—a regimen sufficient to maintain life and admit of growth, but not generous enough to promote fission. Several of the Stentors were placed in a watch-glass which already contained an abundant growth of *Scenedesmus*, upon which they fed. Fission took place only at long intervals, and the large size of the progenitors was maintained in their descendents.

It will perhaps be claimed that the large size and fine condition of the Stentors above-described was due, not to meagre diet, but to the fact that they were the survivors of a much larger number, and according to the doctrine of natural selection, must have been the most vigorous and best able to cope with adverse conditions. There is certainly something to be said in favor of this view. But an experiment hereafter to be described (see p. 540) has shown that the largest Stentors are not necessarily the most vigorous or prolific, and another experiment has demonstrated that it is possible to take Stentors of average size or less, put them on a scanty vegetable diet, and obtain large and finely-developed specimens. The details of the latter experiment are as follows: On Jan. 15, 1892, I found a Stentor (of about average size) in fission, which showed the division of the meganucleus at time of condensation. I placed its offspring in water which contained a growth of minute algae. On the 16th another Stentor, also of average size, was found, and it likewise exhibited the early division of the nucleus. Its offspring were placed with the others, the object being to ascertain whether the early division of the nucleus would reappear at subsequent fissions. The original four Stentors had increased to six on the 18th and to eight on

the 20th, but no further fission took place. The Stentors fed upon the algae, but obtained very little else. On Feb. 3 the culture was examined, and the Stentors were found to be much increased in size and finely developed. In the interval of two weeks during which there had been no increase in number, a slow growth had taken place.

B. *Relative Vitality of Large and Small Stentors.*

It is a fact familiar to all who have observed Infusoria that there are many species individuals of which vary much in size. These differences are due to various causes, such as rapid multiplication without intermediate growth-periods, multiple fission within the cyst, bud-formation, scarcity of food, etc. Hardly any forms show such an astonishing variation in size as the Stentors, and as we have seen (p. 537), this is especially true of *S. cœruleus*.

It seemed to me probable that the dwarf Stentors were not necessarily enfeebled and dying members of the colony, and I surmised that under favorable conditions (which they certainly do not have in an aquarium inhabited by multitudes of their larger kindred, which overreach them and obtain most of the food), they would increase in size and prove as prolific as the larger ones. In order to test the truth of my supposition, I arranged the following simple experiment. On the 21st of March, 1891, I started eight colonies in small beakers, each containing 20 cc. of water, taken from a gathering made less than a week previously. Great care was taken not to introduce any Stentors with the water. Into each of four beakers ten of the largest Stentors obtainable were put, and into each of the remaining four, ten of the smallest. No Stentors in process of fission were introduced. The beakers were placed upon a table out of direct sunlight, and those containing Stentors of different sizes were set abreast, two and two. The relative positions of the pairs were changed at intervals, those towards the light being placed away from it, and *vice versa*. Thus nearly all the conditions—light, temperature, size and shape of dish, quantity, quality, and depth of water—were made as uniform as possible. But one most important

factor, that of food, was not uniform, and it seemed impossible to make it so. While at the outset all the cultures probably contained nearly the same amount of available nutriment, the more extensive growth of plant-life and the greater multiplication of minute organisms (*e.g.* Arcella) in some of the cultures, placed the inhabitants of those cultures at great advantage. Hence the wide difference in the number of Stentors in the different cultures.

A month later a census was taken of the colonies, with the following result :—

	NO. OF STENTORS.	REMARKS.
No. 1 (large),	30	Large to small ; well-fed.
2 (small),	31	Mostly small ; not well-fed.
3 (large),	5	Small ; moribund.
4 (small),	55	Large to small ; well-fed.
5 (large),	40	Average size ; well-fed.
6 (small),	5	Average.
7 (large),	43	Large to small ; mostly well-fed.
8 (small),	144	Large to very small ; well-fed.

The record shows an increase for all colonies except Nos. 3 and 6, which obviously were suffering from lack of food. The great variation seen in the increase of the other colonies is doubtless almost entirely due to the great difference in the amount of available food. But the experiment at least shows that the dwarf Stentors are not incapable of growth, and under favorable circumstances will multiply as rapidly as large ones ; for, comparing the cultures by twos (No. 1 with No. 2, No. 5 with No. 4, No. 7 with No. 8), we find in every instance a balance in favor of the small Stentors.

It is worthy of note that although the ten brood Stentors used in starting each culture were very nearly alike in size, their descendants soon came to show the usual marked difference in that respect.

Very interesting in this connection are the recently-published observations of Gruber ('92) upon dwarf Stentors. The occurrence of a natural colony of Blue Stentor dwarfs, all of which, as far as examined, possessed a simple nucleus the size and shape of a single node of the usual moniliform nucleus, was

certainly a remarkable fact, and strongly suggests the possibility that the supposed "dwarfs" were in reality a distinct species, especially since no transitions were found from the dwarf to the Stentor of normal size with moniliform nucleus.

### C. *Nutrition; Alimentary Vortex.*

I have made a few observations upon the beautiful method of capturing food prevailing among the Stentors—a method of wide occurrence among the Infusoria, which Maupas has fittingly termed the *alimentary vortex* ("tourbillon alimentaire"). The appearance of the alimentary vortex is shown in Fig. 62. The carmine-grains supplied in liberal quantity were eaten freely, as the drawing clearly shows. The little arrows indicate the direction of the currents created by the vibration of the membranellæ. It is impossible to detect the direction of the "strong beat" of the membranellæ by an inspection of these organs in action, but it is revealed indirectly by the course of the currents, which in turn are made manifest by the movement of the carmine-grains. Since, then, the particles move in an ascending curve to the left from positions of rest to the right of the zone (when viewed, of course, from the dorsal side, as in Fig. 62), and then sweep directly downward upon the frontal field, we must infer there is some sort of suction towards the frontal field, which could only be brought about by a strong *inward* stroke of the membranellæ. But this is combined with another stroke, the direction of which is *along* the zone towards the mouth. The result is that all particles coming within the sphere of influence of the alimentary vortex are drawn down to the frontal field, over the surface of which (probably propelled by the motion of the minute cilia), they glide towards the oral orifice. In *S. roeselii* I have seen the slow movement of nutrient particles along the marginal channel characteristic of that species; but in *S. cæruleus*, although the elevated adoral zone (Fig. 62) effectually prevents the food-particles from slipping over the edge of the frontal field, I have not noticed that the carmine-grains took any particular path on their way to the mouth.

Having reached the extreme left-hand portion of the frontal field the grains drop down into the buccal pouch (*b.p.*), within which they are rushed back and forth several times, until finally they slip into the inner turn of the spiral, thence through the mouth opening at its apex into the endoplasm (see arrows in Fig. 62). I have once or twice seen the grains take a definite path through the endoplasm, so as to suggest an œsophagus, but usually one sees nothing to indicate the presence of one. I have not seen the "Schlundstrang" described by Schuberg ('90), either in the living animal or in sections. Schuberg himself says it is in evidence only at the time fine food-particles are being taken in.

The grains of carmine speedily become distributed, either as single grains or little clusters, throughout the anterior part of the animal, but penetrate more slowly into the posterior portion. The dissemination is evidently brought about by the circulatory movements of the cytoplasm. On examining a specimen that had recently been fed with carmine, I was unable to detect that the carmine-masses lay within digestive vacuoles, and repeated scrutiny failed to reveal any vacuolar space around the food-masses. I do not think, however, that a digestive vacuole was absent, but believe it was either very small or wholly obscured by the dense cytoplasm.

The manner of capturing a living prey is different from the almost mechanical inception of non-motile food-particles. I have observed the capture of a small "skipping" Hypotrich of undetermined species. Notwithstanding the rapid and vigorous swimming powers of these little Infusoria, they are frequently entrapped in the powerful vortex of a Stentor, and thereby get drawn into the buccal pouch. Instantly the lips of the pouch (velum and buccal fold) close nearly together, making escape almost impossible. The captive then darts from end to end of the pouch. After a few seconds of this shuttle-like motion, the prey is driven into the inner spiral, thence through the oral opening, always accompanied by a bulk of water several times its size, into the endoplasm. The vacuole thus formed is speedily carried away from the oral aperture, but the Infusorian within still darts about for a minute or so before

death ensues. Almost immediately after death disintegration sets in.

It would be hardly possible for any Infusorian to have a more extensive bill-of-fare than the Blue Stentor. It devours not only Protozoa and Protophytes of almost every group, but small Rotifers as well. The latter, together with Paramecium, *Stentor igneus*, and dwarfs of its own species, are among the larger prey captured by this voracious animalcule. The booty sometimes equals nearly one-third the bulk of its captor. I have never observed the deglutition of these huge morsels; they must dilate the very distensible oral spiral to the utmost. The cannibalistic habit of *S. cæruleus* is not without interest. It shows how large and vigorous members of a colony may survive a period of scarcity, and at the same time reduce the number (always large at such times) of the dwarfs in the community.

#### D. Defecation; Position of Anus.

One of the favorite doctrines of micrographers is that of a localized place of defecation in the Infusoria. It is not commonly pretended that this so-called "anus" is a permanent structure, or visible at any time except at the evacuation of fœcal matter. An anus, then, among Infusoria is held to be not structurally but physiologically present.

But are we certain that there is a fixed place of defecation? Observations on this point are rather difficult to obtain, and often require long and patient watching. Consequently, the number of observations for any one form are generally very few; and, to make the matter still more doubtful, observers are by no means unanimous in their statements regarding the position of the anus in one and the same species. This is commonly held to be due to careless or incorrect observation. May it not rather be due to variation in the place of defecation?

My study of the defecatory process in the Stentors has led me to think that the temporary anus of the Infusoria is not so fixed in position as is commonly held. I have many times observed the evacuation of fœcal matter by *S. cæruleus* and *S. roeschii*, and have, as a rule, found that it took place in the

region indicated by Claparède and Lachmann ('59), Stein ('67), and Moxon ('69)—on the dorsal side at the same level with, and a little to the right of, the contractile vacuole. (Fig. 63, *a*.) But the evacuation of excreta is by no means always at this place; sometimes they are extruded at a spot but slightly removed; sometimes at places far distant, as at the lower part of the dorsal region, or on the right and left sides of the animal (Fig. 63, *fc*). These observations have been made upon animals not under cover-glass pressure, but living under free conditions. It cannot, therefore, be supposed, as Bütschli ('89, p. 1386) has done in case of a similar observation by Brauer upon *Bursaria truncatella*, that the evacuation of excreta in places other than the usual is due to pressure. But a statement should be made regarding the character of the substance discharged. It consisted in most instances of minute green algæ (in the example shown in Fig. 63, of *Scenedesmus*), which *S. cæruleus* sometimes devours in large quantities, but to all appearances does not digest; for the algæ are discharged in as bright and green a condition as they are taken in. Hence the evacuation of such ingesta is not the same thing as the discharge of the residue of digestion. But if undigested excreta can be extruded through the pellicula at various points, can we venture to assert that fæcal matter may not also be eliminated at more than one place?

Since in most Infusoria<sup>1</sup> no morphological structure indicates the place of the anus, why is defecation for the most part confined to a particular region of the body? It is unquestionably due to the fact that, after digestion is completed, fæcal matter is brought by the slow streaming movement of the endoplasm to one particular place, and there accumulates until the time of defecation. In *S. cæruleus* a large excrement-vacuole may be seen lying near the dorsal surface of the body, about at the level of the contractile vesicle. I have seen two excrement-vacuoles in this region come into contact and then

<sup>1</sup> Maupas ('83, p. 650) has expressed the view that the anus is permanently present as a cleft in the pellicula, which opens only at time of defecation. Bütschli ('89, p. 1387) adopts this view, and even finds in *Balantidium* a permanent anal pore. An anal tube was figured and described by Stein ('67) in *Nyctotherus*.

flow together into one ; and it seems highly probable that this is the mode by which fæcal matter distributed in vacuoles throughout the body is gradually collected into one large excrement-vacuole.

As to the process of defecation, Moxon ('69) says : " Not far from the opening in the contractile vesicle in *Stentor* is the point of surface which gives exit to the solid residues of the creature's food. . . . Its place is marked, not by any pore or opening, but by an irregularity or break in the course of one or two of the longitudinal blue bands before described. . . . The mass to be thrown out is brought to the surface and simply forced through it, sometimes carrying with it a thread of the glairy contents of the saccular body of the animal." I have observed no interruption in the course of the stripes at the usual place of defecation, and cannot regard it as a characteristic feature. The "break" in the stripes is rather to be looked upon as the cicatrix resulting from a previous rupture of the integument at that point.

Concerning the extrusion of fæcal matter, two things are to be noticed : (1) the contractile power of the cytoplasm surrounding the excreta, (2) the rupture and subsequent closure of the pellicula and ectoplasm. The contraction of the endoplasm around the excreta is comparable to the contraction of that about the contents of the pulsating vacuole, but its action is certainly slower and less vigorous. Yet it is sufficiently perfect to eliminate every particle of excreta in the vesicle, but usually, as Moxon states, with the loss of some of the cytoplasm.

Inasmuch as defecation can occur with equal success at many points it is evident that the contractile power of the cytoplasm is diffused, not restricted to one place, as we should probably think to be the case if we considered the contractile vacuole alone.

The rupture of the integument is an interesting process. There first appears over the excrement-vacuole a longitudinal slit through pellicle and ectoplasm, exposing the excreta. The lips of the slit rapidly spread apart, and in a moment lay bare the whole exterior surface of the mass of excreta, even though

it be half the breadth of the animal. While the integument has been opening, the contractile power of the endoplasm has been gently forcing the mass outward. (Fig. 63, *fc.*) The process often requires several minutes. As soon as the entire mass has passed beyond the periphery of the animal's body, the lips of the opening approach slowly, and completely cover the exposed place.<sup>1</sup>

### E. Contractile Vacuole.

I have made several observations upon the rate of pulsation of the contractile vacuole in *S. cæruleus* and *S. roeselii*, and find an unexpected amount of variation therein. This variation cannot be accounted for by difference in temperature, for it appears in consecutive pulsations of the same vacuole, when no sensible change in temperature has intervened.

The mode of discharge of the contents of the contractile vacuole of *S. cæruleus* was clearly and accurately stated by Moxon ('69); and the method in which the fluid is collected, and the changes in form of the vacuole during diastole have been elaborately described by Maupas ('83, p. 641).

In regard to the intervals between the systoles of the contractile vacuole, they have seemed to me to show variation characteristic of individuals rather than a variation due to change of temperature. The consecutive intervals, besides, are very unequal. Thus, one individual of *S. roeselii* observed by me had a vacuole which contracted five times at intervals of 50 seconds, the air-temperature being 20° C. Another, at 17° C. gave a record of four consecutive pulsations at intervals of 1 min. 20 sec. Still another contractile vesicle pulsated four times at unequal intervals: 1 min. 15 sec., 2 min., 1 min. 40 sec., 2 min. 10 sec.,—showing thereby a difference of 55 seconds between the first diastole and the last. Unfortunately,

<sup>1</sup> Recent observations have led me to think that the mode of defecation is influenced considerably by the nature of the excreta. When it is coarse and matted together, the integument must open widely, as above described. But when it is composed of minute, smooth, rounded bodies, a small opening is formed, and the excreta forced through it in a stream.

the temperature was not noted. Another, at temperature of 22° C. gave the following remarkable record :

1 min. 47 sec.	1 min. 43 sec.
1 " 43 "	1 " 47 "
1 " 43 "	1 " 43 "
2 " 0 "	2 " 40 "
1 " 50 "	

The irregularities in pulsation are quite as marked in *S. cæruleus* as in *S. roeselii*. At 19° C. I have noted an interval of 2 min. 20 sec. for four consecutive contractions. Another specimen yielded the following record (temp. 21° C.): 3 min. 30 sec., 3 min. 27 sec., 3 min. 35 sec., 3 min. 30 sec., 3 min. 25 sec., 3 min. 30 sec. We here detect what has been apparent in some of the foregoing records—a tendency to alternation of long and short diastoles, and constant recurrence of periods of same duration. It will be also noted that the rate of pulsation is much slower in *S. cæruleus* than in *S. roeselii*, whatever the temperature.

It is apparent that the differences in the rate of pulsation, however dependent upon temperature, are also matter of individual variation, just as we have seen to be the case with fission and regeneration. Hence observations undertaken to ascertain the variation in pulsation of the contractile vacuole coincident with changes in temperature must be made upon one and the same individual, and, furthermore, one in which the pulsations have been found to be isochronous.

#### F. Merotomy.<sup>1</sup>

The Stentors have been the subject of extensive merotomical experiments, having been used for this purpose by Gruber ('86), Balbiani ('88), Verworn ('89), and again very recently by Balbiani ('92). My own experiments in merotomy were made upon the Blue Stentor, which is, indeed, the most favorable

<sup>1</sup> The term *merotomy* ("mérotomie") was coined by Balbiani ('88) to designate the operation of artificial division upon unicellular organisms, with the purpose of observing the subsequent fate of the artificially-produced individuals, or "mérozoïtes." In place of this almost unpronounceable word, I propose the term *merozoön*, formed on the analogy of Protozoön, etc.

form for this work. While my experiments have been much less extensive and thorough-going than those of Gruber and Balbiani, the results have confirmed theirs with reference to the leading problem : viz., whether the presence of a portion of the nucleus is essential for the regeneration of lost parts and a continuance of the vital functions of a merozoön. Like my predecessors, I have uniformly found that enucleate fragments, even when of considerable size, soon perished without regenerating any of the lost parts. Merozoa containing a portion of the nucleus, on the contrary, were able to regenerate the adoral zone, mouth, and contractile vacuole (Figs. 64 A and 64 B). Such merozoa invariably lost their blue pigment after a day or two, and assumed a tawny color, such as often appears in specimens of this species that are kept under unfavorable conditions. I have never known the blue color to be regained after it is once lost.

Regeneration of the adoral zone takes place in a merozoön in the same manner as in the normal Stentor, and is accompanied by the same meganuclear changes (Fig. 64 A and B). Balbiani's figures (92) show that an increase in the number of nodes retained by the merozoön usually takes place at regeneration of the adoral zone, just as in ordinary regeneration. But I cannot agree with Balbiani's statement (p. 400) that the increase in the number of nodes is accomplished by the constriction of pre-existing nodes, — a method which he evidently believes to obtain in the normal condition of the Stentor ; for he says (p. 400) : “*Chez les individus normaux on n'observe point une multiplication aussi active des articles du noyau, dont le nombre augmente seulement de loin en loin par la division d'un quelconque des articles préexistants. Il faut en conclure que, pendant la régénération des mérozoïtes, le fragment de noyau qu'ils contiennent est le siège d'une excitation physiologique particulièrement active, excitation qui se manifeste par les régénérations organiques et la multiplication rapide de ses propres éléments.*”

I have already expressed the opinion (p. 517) that the nodes are increased only at time of renodulation ; and the “physiological excitement” of which Balbiani speaks is nothing more

nor less than the ordinary activity incident to regeneration, on whatever occasion it occurs.

As previously stated (p. 526), the regeneration of membranellæ, so far as observed, takes place in only one way—by formation of a new adoral zone on the ventral surface of the animal, normally along the left border of the ramifying zone. Although no definite experiments have been made to ascertain whether nucleate merozoa with no portion of the ramifying zone are able to regenerate the membranellæ, certain figures<sup>1</sup> and statements of Gruber ('86) and Balbiani ('92) make it very probable that such is the case. In his recent work upon the merotomy of *Stentor*, Balbiani ('92) implies that a new zone, or at any rate a rudiment of one, is sometimes developed *in situ* at the anterior end of the merozoön. It appears to me that in some of these cases the early stages of zone-formation were overlooked, while in others the structures taken for membranellæ are large cilia. I have sometimes noticed a crest of long cilia at the anterior extremity of a merozoön, long before the regeneration of membranellæ appeared.

An experiment, unfortunately a single one of the kind, gave the following interesting result : A *Stentor* was cut transversely at a point considerably nearer the posterior than the anterior end. A few hours later, the anlage of a new zone appeared on the anterior merozoön. No trace of it had been visible at time of bisection. Believing it to be simply a case of regeneration of the mouth, I paid little attention to it ; but, upon examining the animal two or three hours later I was astonished to find that the new zone was the forerunner of fission. The new mouth was formed in the same place as if the animal were entire, and, therefore, much nearer the posterior end of the merozoön than the anterior. Fission was carried through successfully, giving birth to offspring of very unequal size. An unequal bipartition in *S. cæruleus* is of very rare occurrence ; aside from the above, I have noted but one instance where the daughter-stentors were visibly unequal. The unequal division above noted may then be safely referred to the amputation of the posterior portion of the *Stentor*. The experiment seems

<sup>1</sup> *E.g.*, Gruber's Fig. 3, merozoa A, C, and Balbiani's Fig. 3, merozoön *b*.

to indicate that the plane of fission is determined before the slightest sign of bipartition is visible, and that the plane is not altered, nor division prevented by the removal of part of the cytosome.

In closing, I would express my sincere thanks to my former instructor, Prof. E. L. Mark, of Harvard, for many favors and valuable suggestions ; and to my present instructor, Prof. C. O. Whitman, through whose courtesy it was my privilege to occupy, during the summer of 1891, an investigator's room in the Marine Biological Laboratory at Wood's Holl. I would also acknowledge my indebtedness to Prof. S. F. Clarke, of Williams College, and to Mr. W. S. Nickerson, of Harvard, for sending me material.

MORPHOLOGICAL LABORATORY,  
UNIVERSITY OF CHICAGO, April, 1893.

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## ABBREVIATIONS.

<i>a.</i>	Anus.	<i>l.c.</i>	Longitudinal canal.
<i>a.z., a.z.,<sup>1</sup> a.z.,<sup>2</sup></i>	etc. Adoral zone.	<i>m.</i>	Membranella.
<i>b.p.</i>	Buccal pouch.	<i>mcn.</i>	Micronucleus.
<i>b.p.m.</i>	Basal plate of membranella.	<i>mgn.</i>	Meganucleus.
<i>c.f.</i>	Connecting filament uniting membranellæ.	<i>mgn.<sup>1</sup></i>	Anlage of meganucleus.
<i>cl.</i>	Cilium.	<i>mn.</i>	Myoneme.
<i>c.s.</i>	Clear stripe.	<i>o., o.,<sup>1</sup> o.,<sup>2</sup></i>	etc. Mouth.
<i>c.v., c.v.<sup>1</sup></i>	Contractile vacuole.	<i>o.<sup>1</sup> inv.</i>	Oral invagination.
<i>d.a.z.<sup>1</sup></i>	Distal extremity of adoral zone.	<i>p., p.<sup>1</sup></i>	Peristome band.
<i>ecp.</i>	Ectoplasm.	<i>pl.</i>	Pellicula.
<i>enp.</i>	Endoplasm.	<i>ps., ps.<sup>1</sup></i>	Foot.
<i>ex.p.</i>	Excretory pore of contractile vacuole.	<i>p.z.</i>	Pigmented zone of foot.
<i>f., f.,<sup>1</sup> f.<sup>2</sup></i>	Frontal field.	<i>r.b.s.</i>	Right boundary-stripe of rami- fying zone.
<i>fc.</i>	Fæcal matter.	<i>r.z., r.z.<sup>1</sup></i>	Ramifying zone.
<i>f.v.</i>	Food vacuole.	<i>sh.</i>	Sheath.
<i>g.s.</i>	Granular stripe.	<i>t.f.</i>	Terminal filament of mem- branella.
<i>l.</i>	Line of fission.	<i>v.</i>	Vacuole.
<i>l.b.s.</i>	Left boundary-stripe of rami- fying zone.	<i>vel., vel.<sup>1</sup></i>	Velum.

## EXPLANATION OF PLATE XXIII.

FIG. 1. *Stentor igneus* var. *nigricans*, var. nov. From Williamstown, Mass. Ventral aspect of a living, semi-contracted specimen, under slight cover-glass pressure.  $\times$  ca. 250.

FIG. 2. *Stentor pyriformis*, sp. nov. Ventral aspect of a living, fully-extended specimen (outlined with camera).  $\times$  230.

FIG. 3. *S. pyriformis*. Hardened and stained preparation to show meganuclei.  $\times$  110.

FIG. 4. *S. roeselii*. Living, extended specimen, containing numerous minute algae taken in as food. Dorsal aspect. Zeiss, obj. A. oc. 4.

FIG. 5. Transverse optical section of ectoplasm and pellicula of *S. caeruleus*. The pellicula is adherent only over the myonemes. 1% osmic acid preparation. Zeiss, 4 mm. apoc. obj., oc. 4 =  $\times$  433.

FIG. 6. Part of adoral zone of *S. caeruleus*. Living specimen under strong pressure. Zeiss, 4 mm., apoc. obj., oc. 12 = ca. 650.

FIG. 7. Two adjacent rows of cilia and myonemes, frontal field of *S. caeruleus*. Osmic acid preparation. Zeiss,  $\frac{\text{oc. } 18}{\text{obj. } 4 \text{ mm.}}$  (cam.) =  $\times$  1950.

FIG. 8. Posterior face of transverse section of *S. caeruleus*, taken at time of greatest concentration of meganucleus in fission. Zeiss,  $\frac{4}{8 \text{ mm.}}$  =  $\times$  230.

FIG. 9. Stripes of *S. pyriformis*. 1% osmic preparation. Zeiss,  $\frac{4}{4 \text{ mm.}}$  =  $\times$  433.

FIG. 10. Myonemes of ramifying zone, *S. caeruleus*. In relaxed condition. Living specimen under strong pressure. Zeiss,  $\frac{8}{4 \text{ mm.}}$ .

FIG. 11. Myonemes of *S. caeruleus*. From specimen fixed with picro-acetic, stained with borax-carmin. Zeiss, Homog. immers., 2 mm., oc. 2 =  $\times$  428.

FIG. 12. Posterior extremity of *S. caeruleus*. 1% osmic preparation, flattened by cover-glass.  $\frac{4}{4 \text{ mm.}}$  =  $\times$  433.

FIG. 13. Longitudinal optical section of new adoral zone of *S. caeruleus*. Corrosive sublimate preparation,  $\frac{2}{D}$  =  $\times$  380.

FIG. 14. Foot of *S. caeruleus*, attached to surface film of water. Zeiss,  $\frac{12}{16 \text{ mm.}}$ .

FIG. 15. Foot of *S. roeselii*, attached in slime. Zeiss,  $\frac{4}{D}$ .

FIG. 16. Foot of *S. polymorphus*, attached to glass. Zeiss,  $\frac{4}{D}$ .

FIGS. 17, 18. Abnormal forms of meganucleus, *S. caeruleus*. (17,  $\frac{4}{16 \text{ mm.}}$  =  $\times$  112; 18,  $\frac{4}{8}$  =  $\times$  230.)

FIG. 19. Two nodes of meganucleus, *S. caeruleus*, with adherent micronuclei (*mcn.*). The nodes contain vacuolar spaces. Aceto-methyl-green preparation, in  $\text{H}_2\text{O}$ .  $\frac{4}{4 \text{ mm.}}$  =  $\times$  433.

FIG. 20. Node of meganucleus, *S. caeruleus*, with several vacuolar spaces of different size, containing each a highly-refractive body. Aceto-methyl-green, glycerin. Zeiss,  $\frac{3}{D}$  =  $\times$  550.

FIG. 21. Meganucleus of *S. igneus*, elongated for division. Median optical section, showing chromatic network. Aceto-methyl-green,  $\text{H}_2\text{O}$ . Zeiss,  $\frac{4}{D}$  =  $\times$  630.

FIGS. 22-24. Nuclear multiplication in *S. igneus*. Zeiss,  $\frac{4}{8}$  =  $\times$  230.

FIG. 25. Meganucleus, *S. igneus*, with adherent micronuclei. Corrosive sublimate, borax-carmin, benzole-balsam. Zeiss, 2 mm. homog. immers., comp. oc. 4 =  $\times$  857.







## EXPLANATION OF PLATE XXIV.

All figures on this plate, except Fig. 29, illustrate the fission of *S. caruleus*.

FIG. 26. Early stage, previous to formation of new mouth, and at beginning of coalescence of meganucleus. Latero-dorsal aspect of extended specimen. From life. Zeiss,  $\frac{8}{16}$ .

FIG. 27. Later stage. New mouth ( $\rho^1$ ) has formed, and meganucleus (*mgn.*) has divided and begun to elongate. Ventral aspect. The new contractile vacuole, *c. v.*<sup>1</sup>, has become abnormally enlarged. From life. Zeiss,  $\frac{8}{16}$ .

FIG. 28. Same stage as Fig. 27, animal contracted. New adoral zone strongly curved. Blue stripes crenated. Ventro-lateral aspect. From life. Zeiss,  $\frac{8}{16}$ .

FIG. 29. Newly-formed contractile vacuole of *S. roeselii*. Longitudinal optical section, drawn from living animal. Zeiss,  $\frac{1.2}{8}$ .

FIG. 30. Stage of fission a little later than Figs. 27 and 28. The new zone is bent sharply towards the left, at the point where the fission-line would afterwards appear. Animal extended, latero-dorsal aspect. Drawn from life. Zeiss,  $\frac{1.2}{16}$ .

FIG. 31. Mid-dorsal portion of semi-contracted specimen, showing fission-line (*l*) running from dorsal border of new zone (*a. z.*<sup>1</sup>) around body to right. From life. Zeiss,  $\frac{1.2}{16}$ .

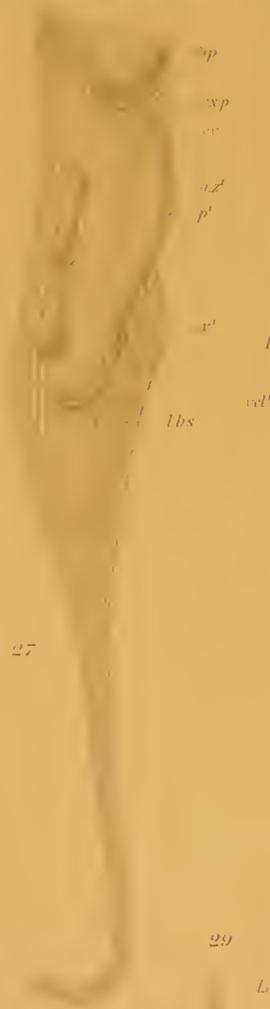
FIG. 32. Fission-line at a little later stage than in Fig. 31. It runs above the upper curve of the new zone, forming a conspicuous cleft. Hardened and fully-contracted specimen, dorsal aspect. Zeiss,  $\frac{4}{8} = \times 230$ .

FIGS. 33, 35-37. Later stages of fission, showing the process of constriction and shaping of frontal field of proximal zoöid. Ventral aspect. Fig. 33 in semi-contracted state; the others fully extended. Drawn from life. Zeiss,  $\frac{8}{16}$ .

FIG. 34. Five stripes and portion of fission-line, showing that, in contracted state, the stripes form an angle at the constriction-line. Picro-acetic, Delafield's haematoxylin, clove-oil. Zeiss,  $\frac{4}{8 \text{ mm.}} = \times 230$ .



26



27



35



55



56



57



28



29



50



51



52

Labels for Figure 29: *lc*, *cr*, *p'*, *cr'*, *lc*

Labels for Figure 50: *cr*, *p'*, *cr'*

Labels for Figure 51: *p'*, *az'*

Labels for Figure 52: *p'*, *cr'*

Labels for Figure 27: *bp*, *xp*, *cr*, *az'*, *p'*, *r'*, *l*, *d'*, *lbs*, *vel'*

Labels for Figure 35: *bp*, *vel*, *o*, *da z'*, *p'*, *cr'*, *l*, *rz*, *ugn*

Labels for Figure 55: *cr*, *rz'*, *p'*, *cr'*, *l*, *rz*, *ugn*

Labels for Figure 56: *cr*, *rz'*, *da z'*, *cr'*, *vel'*, *cr'*, *rz*

Labels for Figure 57: *l*, *ps'*





## EXPLANATION OF PLATE XXV.

Regeneration of frontal field and nuclear changes of *S. caeruleus*.

FIG. 38. Specimen in process of regeneration of mouth and frontal field. The buccal portion of adoral zone has already atrophied, and the buccal pouch is much reduced; the meganucleus (*mgn.*) has elongated after condensation. Zeiss,  $\frac{8}{16}$ .

FIG. 39, A and B. Two successive regenerations of same individual. From life; 39 B one-and-a-half hour later than 39 A.  $\frac{2}{8}$ .

FIG. 40. Distal individual just after a fission which, in its earlier stages, proceeded like a regeneration. Disorganization of stripes in ramifying zone, due to abnormal mode of fission. From life. Zeiss,  $\frac{12}{16}$ .

FIG. 41 *a-i*. Series showing changes in living meganucleus from time of greatest condensation to beginning of nodulation. Period of observation, 1.17 P. M. to 2.20 P. M. Zeiss,  $\frac{12}{16} \times$  ca. 160.

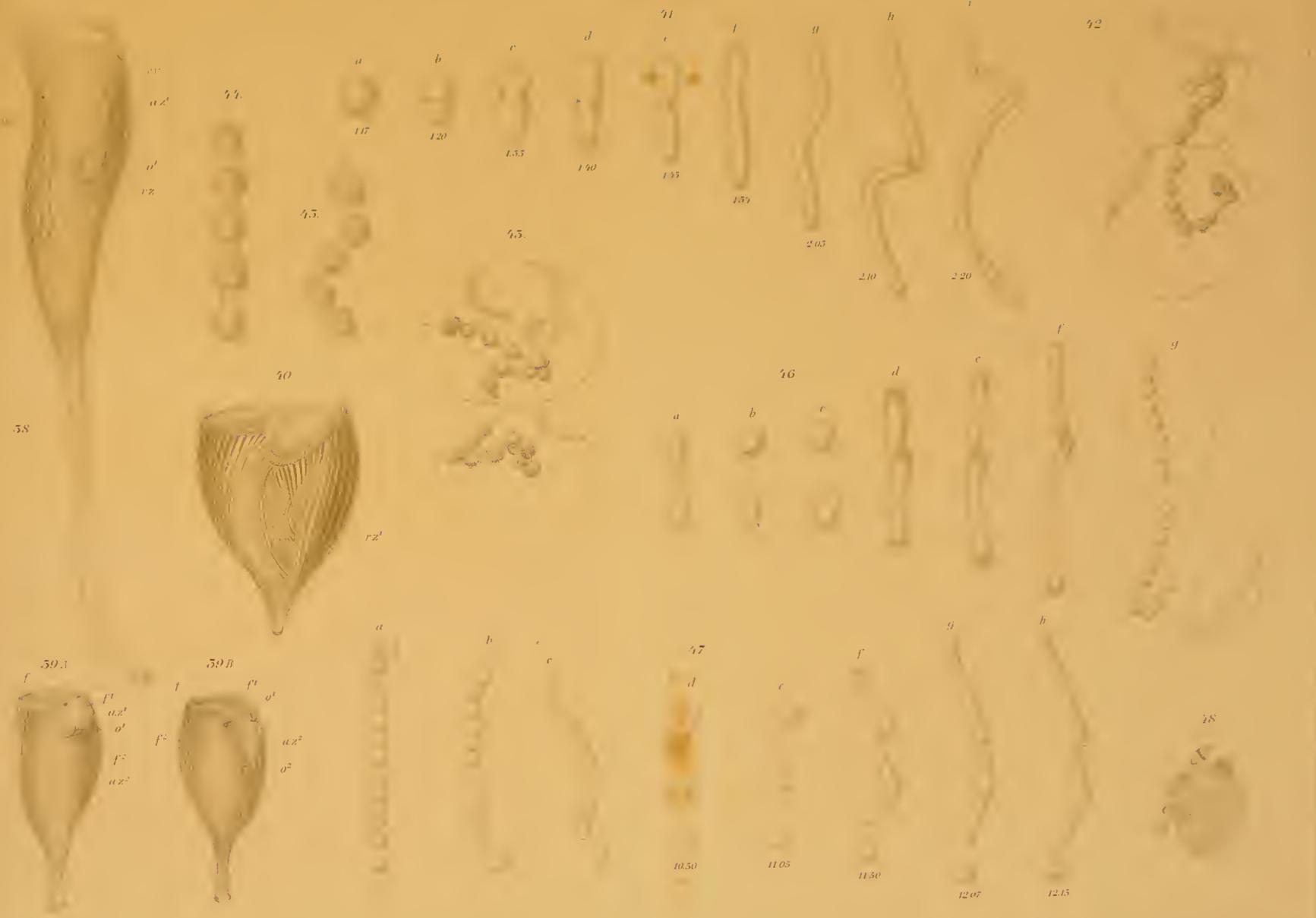
FIGS. 42, 43. Two consecutive stages in division of meganucleus, after completion of nodulation. In Fig. 43 division has just taken place. From preparations. Zeiss,  $\frac{4}{A}$  (*cam.*) =  $\times 150$ .

FIGS. 44, 45. Two modes of nodulation of meganucleus; 44 somewhat earlier than 45. Zeiss,  $\frac{2}{D}$  (*cam.*) =  $\times 380$ .

FIG. 46 *a-g*. Series showing changes in living meganucleus from division (in condensed stage) to complete nodulation. Zeiss,  $\frac{8}{16} =$  ca.  $\times 100$ .

FIG. 47 *a-h*. Changes in living meganucleus from beginning of condensation to beginning of renodulation. Zeiss,  $\frac{8}{16} =$  ca.  $\times 100$ .

FIG. 48. Tangential section of meganucleus in condensed state, with six micronuclei, two of them in mitotic division. Corr. sublimate, picro-carmin. Zeiss, homog. immers. 2 mm. and comp. oc. 4 =  $\times 857$ .







## EXPLANATION OF PLATE XXVI.

*Stentor caruleus*: conjugation, teratology, nutrition, defecation, and merotomy.

FIG. 49. To show line of junction of a conjugated pair. The suture is a dense, refractive plane passing through the whole surface of contact. Zeiss,  $\frac{4}{D}$ .

FIG. 50. A conjugated pair of very unequal size. Zeiss,  $\frac{2}{A} = \times 90$ .

FIG. 51. Two separated nodes of meganucleus of conjugated specimen, surrounded and connected by a reticulum of cytoplasm. *In situ*, from living specimen. Zeiss,  $\frac{4}{D}$ .

FIG. 52. A conjugated pair in Stage H of Maupas. The fragmented meganucleus yet retains its normal stainability. A new meganucleus (*mgn.*<sup>1</sup>) is developing in each gamete. In the lower gamete two of the micronuclei are undergoing atrophy within vacuoles. Composite of four transverse sections,  $5\mu$  thick. Corrosive sublimate, picro-carmin, xylol balsam. Zeiss,  $\frac{4}{8} = \times 240$ .

FIGS. 53-59 all studied with Zeiss' Hom. immers., 2 mm. and magnified 1400 diameters.

FIGS. 53, 54. Two micronuclear spindles of different form from same gamete, at "monaster" stage. Corrosive sublimate, picro-carmin, xylol balsam.

FIG. 55. Micronucleus in its normal or resting state. Corrosive sublimate, Czokor's alum cochineal, balsam.

FIG. 56. Micronucleus enlarged at time of conjugation (Stage H, same prep. as Fig. 52).

FIGS. 57, 58. Two stages in enlargement of anlage of new meganucleus. Unstainable. Example in Fig. 58 is accompanied by a micronucleus. Corrosive sublimate, balsam.

FIG. 59. Micronucleus within a digestive vacuole. Unstainable. Corrosive sublimate; Czokor's alum cochineal.

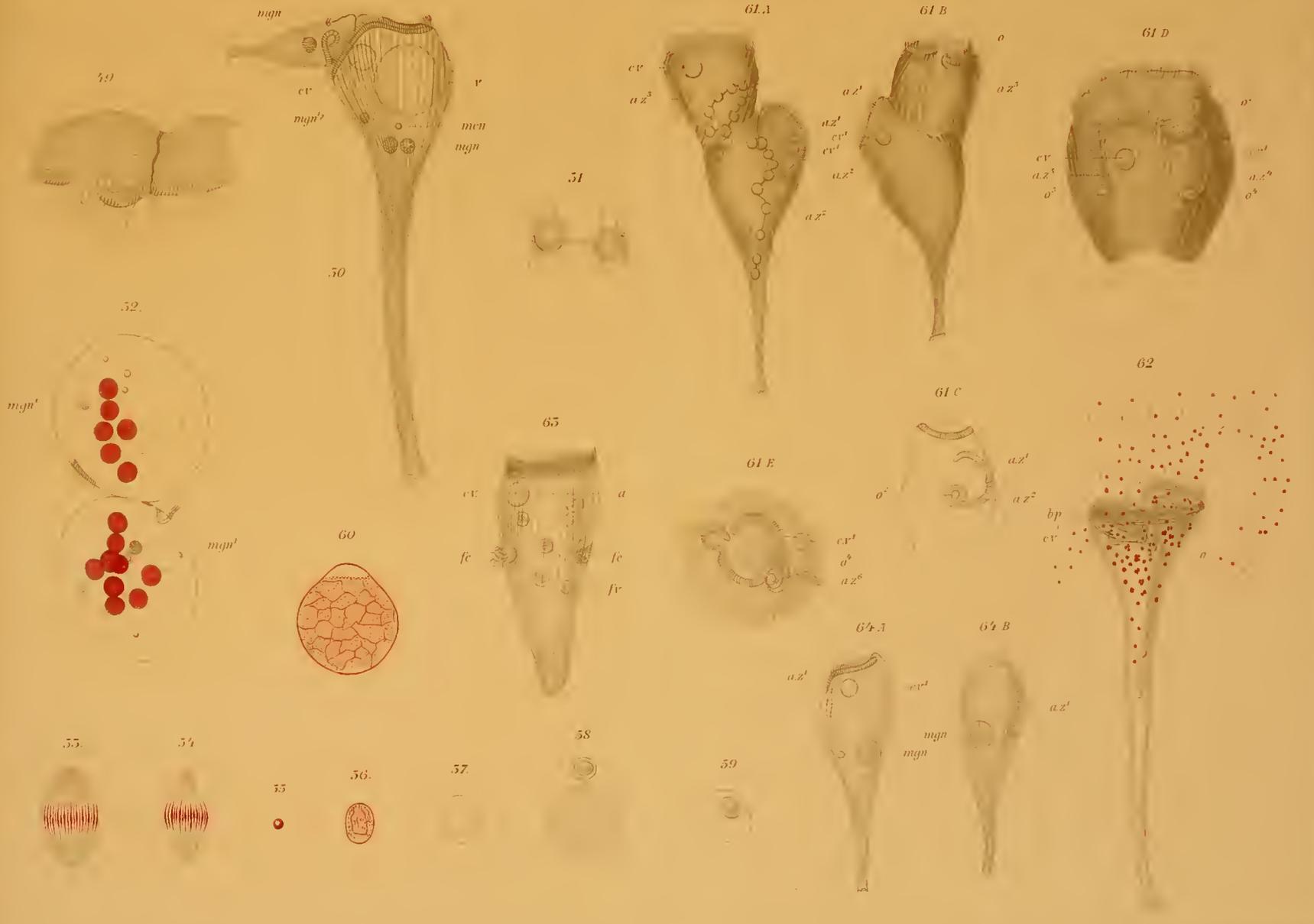
FIG. 60. Node of meganucleus, from an ex-conjugate. (Same gamete as Fig. 57.) Stainability much reduced. Two vesicles of clear karyoplasm under nuclear membrane. Zeiss, Homog. immers., 2 mm. comp. oc. 4 =  $\times 950$ .

FIGS. 61 A-E. Stages in the formation of a double monster, and its return to the normal condition. All drawn from living animal. Fig. 61 B (dorsal aspect) three hours later than 61 A (ventral aspect). Fig. 61 C (ventral) drawn six hours later than 61 A. Zeiss,  $\frac{2}{8}$ . Fig. 61 D drawn fifteen hours later than 61 C. Zeiss,  $\frac{4}{8}$ . Fig. 61 E represents the specimen thirteen hours later than 61 D. Zeiss,  $\frac{8}{16}$ .

FIG. 62. A *Stentor* feeding upon carmine grains, dorsal aspect. Arrows show the direction taken by the carmine-particles. The stripes are omitted for sake of clearness. Zeiss,  $\frac{4}{16}$ . From life.

FIG. 63. A specimen of *S. caruleus* that has fed upon minute green algae (*Scenedesmus*). Defecation taking place at two different points simultaneously, and a few minutes later at the normal place of defecation (*a*). From life. Zeiss,  $\frac{8}{16}$ .

FIGS. 64 A and B. Posterior merozoön of *S. caruleus*; A, dorsal, B, ventral aspect; regenerating adoral zone and mouth. A contractile vacuole present, and meganucleus in condensed state (*mgn.*). From life. Zeiss,  $\frac{8}{16}$ .





# THE STRUCTURE AND DEVELOPMENT OF THE AXILLARY GLAND OF BATRACHUS.

LOUISE B. WALLACE.

A STRIKING resemblance has long been known to exist between some of the Cat-fishes and the Toad-fish in the possession of a naked skin, powerful protective spines, and a sac, with a more or less wide opening, behind and in the axil of the pectoral fin. It has been thought not improbable that this peculiar sac bore a close relation to the spines and supplied them with poison to inflict dangerous wounds, although no cases of poisonous wounds produced by these fishes are on record.

At the suggestion of Dr. Whitman, I undertook to work out the morphology and development of the gland and, if possible, to ascertain its function.

The spinous dorsal in the Toad-fish is formed by three stout spines and the gill-covers are also armed with spines, all of which are erect when the animal is irritated and can give a slightly painful sting. At the same time the pectoral fins are thrown outward and forward until the large foramen of the axillary gland is opened to its full extent, but whether or not the gland is especially active at such a time has not been determined. When dissected out from the loose, underlying connective tissue, this gland is found to be a pouch-shaped sac with a tough, fibrous wall lined by a layer of epidermal tissue which is thrown into minute folds, giving a wrinkled appearance to the surface. The cavity is divided into chambers varying from two to five in number, each chamber representing a separate invagination of the skin. (Fig. I.)

In Fig. II, an enlarged view of one of the folds of Fig. I, are shown the six different kinds of cells found in the epidermal lining of the sac. (a) The superficial layer is characterized by small, deeply-staining nuclei closely crowded together and is extremely delicate, often torn off in sections, leaving (b), the

polygonal cells on the surface. These are much larger than the preceding, the nuclei measuring  $5\mu$  in diameter and the entire cell having an average diameter of  $12\mu$ . The nucleus takes a faint stain and has a distinct but not conspicuous nucleolus. (c) Beneath these, and differing from them only in form, are the elongated cells which are sometimes ovate, sometimes spindle-shaped, and as the cell increases in length the nucleus is seen nearer the upper end rather than at the center. The cells are  $35\mu$  in length and the round nucleus has a diameter of  $7\mu$ . (d) Resting on the basement-membrane and arranged with comparative regularity, are the columnar cells,  $20\mu$  in length and containing a nucleus  $7\mu$  in diameter. Neither these nor the elongated cells have a distinct nucleolus. The outline of the cell-wall is sometimes rounded at the top, but more often sends a prolongation into the interstices of the elongated cells lying above. (e) At regular intervals in the polygonal layer of cells and projecting through the superficial layer are the mucous cells, usually so full of well-stained mucin that little idea can be had of their general character beyond their shape and striated contents. If, however, they are carefully taken from living tissue and freshly stained with methyl green, the structure becomes clear. Globate or oval in form, they attain a length of  $30\mu$  and have a large, bright nucleus at the base  $10\mu$  in diameter. The protoplasm is vacuolated and sends protoplasmic strands through an aperture at the top, the vacuoles, no doubt, being filled with transparent mucin. (Fig. III, *a*.) In dead cells found in the secretion of the gland, the exact form of the aperture can be distinctly made out and is usually oval with a perfectly smooth edge. (f) The huge clavate cells vary somewhat in shape according to their position, the majority being rounded at the top with a tapering base. In sections the contents of the cell seems to be an evenly stained, homogeneous substance while in specimens macerated for twenty-four hours in nitric acid or Haller's Fluid distinct spherules are abundant, which are probably dissolved or broken up by long treatment. In Fig. II, the secretion of the cell is shown shrunken away from the cell-wall, leaving behind a thin layer of protoplasm in which is suspended,

toward the upper or free end of the cell, a large nucleus, not always round,  $10\mu$  in diameter, with a very conspicuous nucleolus  $3\mu$  in diameter. The fact that most of the clavate cells have no aperture, leads to the query : How does the secretion escape ?

In a paper "On the Skin and Cutaneous Sense Organs of *Amiurus*," Prof. Ramsay Wright, in speaking of similar cells, says — "Perhaps Pfitzner's suggestion that the secretion may be poured out into the inter-epithelial spaces, so as to prevent the entry of water, may not be very far from the truth. It is certain, at least in *Amiurus*, that there is no aperture to the clavate cell, such as the mucous cell possesses, and their position indicates that lubrication of the surface is not their function. Occasionally a clavate cell may be seen in sections protruding from the surface, but such appearances are probably due to a defect in the superficial layers of the epidermis and to the action of hardening reagents."

While it is certain in the Toad-fish also, that there is no aperture in the clavate cell such as the mucous cell possesses, it is not so certain that the secretion does not escape to the surface by another method. In sections many of the cells have a rounded top while others are constricted, often abruptly, into a little neck piercing the superficial layer of cells, the length of the neck depending greatly upon the size or maturity of the cell. In rare cases this neck is found ruptured, with the contents streaming out. Now, if the cell is much distended in a flask-shape, it follows that more sections would pass through the sides of the flask than through the neck itself ; hence the greater number of cells with rounded top. In maceration, isolated cells which are fully grown are found sending out prolongations, and as these neck-like protuberances are constant when the gland is macerated in various ways and also in sections, they do not appear to be due to the warping or shrinking effect of reagents. (Fig. III, *b*, *c*, *d*.) If a pipette be introduced into the foramen of the gland, and some of the clear, viscid secretion thus drawn out, be examined, it will be found to contain quantities of shrivelled, empty, clavate cells, each with a roughly torn neck and a distinct nucleus at the

upper end. (Fig. III, *c.*) While it is true that ruptured cells are not abundant in sections, this may be owing to the fact that they are much shrunken when emptied of contents and soon cast out, their places being filled by the distension of fresh cells. The great number of shrunken cells in the secretion make this more certain and in many cases they have been found in sections closely pressed between the rapidly filling fresh cells. The bursting of the cell seems to be a simple mechanical necessity, though why it should always occur at the upper end of the cell is not so clear, unless it be that this point alone is free, the delicate superficial layer offering little resistance.

A few points in the development of the axillary gland were studied, and it was found to make its first appearance in larvae about two-fifths of an inch in length, originating as a simple, epidermal thickening which sinks below the surface as an open pit. A little later the lining of the pit becomes differentiated into the cells shown in Fig. II. In larvae four-fifths of an inch in length, the mucous cells, which take their origin from the polygonal cells in which they lie embedded, are already active, while the clavate cells, still immature, are seen to be merely elongated cells which have grown and taken on the character of a secreting cell, the protoplasm being crowded to the wall to make room for the fluid which it secretes. (Fig. IV.) In Fig. V, representing a longitudinal section of the gland taken from a fish three inches long, the simple pit has begun to be thrown into folds, this process continuing with the multiplication of cells until we have the more complex structure of the adult shown in cross section in Fig. VI.

Some other structures on different parts of the body seem to bear such a close relation to the axillary gland that they must not be passed by without a word. One of the peculiarities of the operculum is that it lies close to the body-wall excepting at the upper point of attachment where it hangs out, making a large, foramen-like opening for the passage of water. Underneath this free border of the operculum, and near the base of the pectoral fin, is a patch of glands identical in structure with the one described above, but the clavate cells are larger and the whole structure is well developed at a time

when the former is at an early stage of development (Fig. VII). The glandular patch is perforated by numerous foramina, each opening into a separate chamber or invagination homologous with those of the axillary gland, so that if the opercular glands were depressed and had a common opening we would have a many-chambered axillary sac. It is possible that the pouch-shape of the latter is due to its position in the axil of the fin. On the under side of the pectoral fin, and following the general course of the rays, is a third set of similar glands and yet no connection between the three has been traced. Their relative position is shown by dotted lines in Fig. VIII.

Sections of the skin in different parts of the body prove it to be of essentially the same structure as the glands, the cells having a similar origin and apparently a similar method of emptying secretions. The large cell which corresponds to the clavate is rounded,  $70\mu$  in diameter, and it also pushes a projection through the superficial layer of the epidermis, emptying the contents on the surface. (Fig. IX.) The slime found in abundance over the whole body of the fish contains quantities of large, empty, rounded cells just as the secretion in the gland contains the empty flask-shaped cells, and secretion of the glands or of the skin turns blue litmus-paper red.

It is a significant fact that the three different sets of glands are all found in the region of the pectoral fin, though just what part they play in the life of the animal is an obscure point. The structure of the gland, the want of any close connection with the spines, and the results gained from a few simple experiments made with the living fish, do not seem to favor the idea of a poisonous secretion. My work, however, has not been sufficient to determine the nature of the secretion which still offers an interesting problem to the physiologist.

MARINE BIOLOGICAL LABORATORY,  
WOODS HOLL, MASS., Aug., 1892.

## DESCRIPTION OF PLATE.

- FIG. I. Cross section of axillary gland in adult.  $\times 18$ .  
FIG. II. Part of Fig. I, enlarged.  $\times 90$ .  
    (a) Superficial cells.  
    (b) Polygonal.  
    (c) Elongated.  
    (d) Columnar.  
    (e) Mucous.  
    (f) Clavate.  
FIG. III. Macerated cells.  
FIG. IV. Longitudinal section of gland in larva  $\frac{4}{5}$  inch in length.  $\times 80$ .  
FIG. V. Longitudinal section of gland in larva 3 inches in length.  $\times 70$ .  
FIG. VI. Enlarged view of Fig. I.  $\times 100$ .  
FIG. VII. Longitudinal section of opercular glands in larva  $\frac{4}{5}$  inch long.  $\times 50$ .  
FIG. VIII. Outline, showing position of glands.  
FIG. IX. Longitudinal section of skin.  $\times 120$ .





## PRELIMINARY ACCOUNT OF THE EMBRYOLOGY OF UNIO COMPLANATA.

F. R. LILLIE.

THE following paper is preliminary to a more extended one, which I hope to publish soon. In the complete paper I shall advance the proof of the statements made here.

In carrying out the work two main objects have been kept in mind:—

*Firstly* to settle definitely the question of the origin of the germ layers in the Unionidae, and

*Secondly* to extend the cytogenetic method in embryological research to the class of the Lamellibranchs; a thing which has not hitherto been attempted in a very minute fashion. In the attempt to attain to these two main objects of the work, other problems of greater or less interest have presented themselves.

Rabl<sup>1</sup> made a fundamental error in the interpretation of the shell-gland as the invagination of the entoderm. Balfour, while provisionally accepting Rabl's account, nevertheless calls attention in his text-book to the strong inherent improbability of the dorsal position of the blastopore. In 1891 Goette<sup>2</sup> published an account of his observations on the formation of the entoderm in Anodonta, and figured its invagination at the very region to which Rabl described it as wandering, after its supposed formation dorsally. In *Unio* the invagination takes place in the same region as described for *Anodonta* by Goette, but in an earlier stage of development.

Although Goette cleared up to a certain extent the question of the origin of the entoderm, he did not do so for the mesoderm; and for want of any contradiction, based on observation of the facts, Rabl's account of the origin of the mesoderm

<sup>1</sup> Rabl, Ueber Entw. der Malermuschel. Jen. Zeitschr. f. Naturwiss. Bd. X. pp. 310-393.

<sup>2</sup> Goette, Bemerkungen über die embr. Entw. von *A. piscinalis*. Zeitschr. f. wiss. Zool. lii.

teloblasts from the cells of the shell-gland, remains as the status of our present knowledge on the subject. I shall show that the origin of the mesoderm teloblasts in *Unio* is exactly the same as in *Nereis*, according to Wilson's<sup>1</sup> description. In addition to this yet another source of origin exists in *Unio*, *viz.*, from a cell on the left side of the young embryo of forty-six cells (Fig. 4, *Y*). I shall call the mesoblast derived from this source the *larval mesoblast*, inasmuch as it gives rise to structures which are *purely larval*.

#### SEGMENTATION.

The first plane of division is inclined at an angle of  $45^\circ$  to the future sagittal and transverse axes of the larva. It runs from the animal to the vegetative pole, and divides the egg into two unequal parts, of which the smaller *AB* is anterior to the larger *CD*. Both cells contain entoderm as well as ectoderm, and, therefore, Rabl's designation of animal and vegetative does not correspond with fact. The second plane of division runs likewise from the animal to the vegetative pole, and is practically at right angles to the first. It does not divide both cells at the same time. *CD* is the first to divide; the two resulting cells are of unequal size, and the smaller *C* lies on the right side of the future embryo, while *D* occupies the median posterior region (Fig. 1). *AB* divides shortly afterwards and into parts approximately equal, one of which *A* (*slightly the larger*) lies on the left side and the other *B* occupies the median anterior region. The median longitudinal axis of the future embryo runs through the centres of *B* and *D* (Fig. 1 *m. l. p.*).

So far my description of the segmentation agrees completely with Rabl's and Flemming's<sup>2</sup>; but my interpretation of the value of these first four blastomeres, and their axial relations, is entirely different. Rabl held that the large posterior cell, *D* (1 of Rabl), was chiefly entodermic, while, as a matter of fact, it contains only a very minute portion of entoderm; that

<sup>1</sup> Wilson, Cell-Lineage of *Nereis*. Journal of Morphology. Vol. VI, No. 3.

<sup>2</sup> Flemming, Studien in der Entw. der Najaden. Sitzungber. der Wiener Akad. 1875.

the three other cells were purely ectodermic, while, in fact, all contain entoderm. Rabl was right so far that all four cells contain ectoderm, but so do all contain entoderm; while *D* contains the posterior mesoblast, and *A* the larval mesoblast.

The next stage to which I will direct special attention is the eight-cell stage (Fig. 2), which has the typical form of four apical micromeres lying on four macromeres, and alternating with them (being given off in a right-handed spiral). Here I must differ from Rabl's account. The four micromeres do not, indeed, arise simultaneously, but one after the other, and generally in the following order,  $d^1$ ,  $c^1$ ,  $a^1$ ,  $b^1$  from *D*, *C*, *A*, and *B*, respectively. The last three arise some time after  $d^1$  and very nearly together. These four cells form the first generation of micromeres, and are purely ectodermic. According to Rabl  $d^1$  (5 of Rabl) divides at the same time as *C* (3 of Rabl). Whether the difference observed is due to the difference of species or not, I am unable to say.

The sixteen-cell stage does not actually occur as such, but the stage with seventeen cells corresponds to the sixteen-cell stage of Annelids, with one further division, which, in the Annelid ovum, occurs later. This stage does not arise at once from the eight-cell stage, but almost all the cells of the eight-cell stage divide at different times. The four macromeres are the first to divide, and of these *D* takes the lead, dividing by an equatorial plane into two unequal parts, of which the smaller *D* lies on the vegetative pole, and the larger  $d^2$  between *D* and  $c^1$  and  $d^1$  of the apical pole. *C*, *A*, and *B* divide next in the same plane as *D*, and in this general order. In each case the smaller product lies on the vegetative pole. I have, therefore, reserved the term macromere, and the designation by capital letters for these cells. The three larger cells are  $c^2$ ,  $a^2$  and  $b^2$ ; together with  $d^2$  these cells form the second generation of micromeres. It is interesting to note that the second generation of micromeres is given off from the macromeres in a left-handed spiral; thus the reverse of the first generation; and that the third generation is given off in a right-handed spiral again (Figs. 3 and 4). The spiral arrange-

ment of the cells is caused not so much by a rotation of the cells when formed, as by the direction of the spindles during the division. The same thing has been noted by Wilson.

After the divisions of the macromeres are completed,  $a^1$ ,  $b^1$ ,  $c^1$ , and  $d^1$  divide at different times, giving rise to eight apical cells, alternating with one another in the typical way. During the division of the apical cells a spindle forms in  $d^2$  and a small cell is segmented off on the right side of the vegetative pole ( $x^1$  Fig. 3).

I must remark here on the unsuitability, in this case and possibly in others, of the terms macromere and micromere. If we use the terms according to their etymological significance,  $a^2$ ,  $b^2$ ,  $c^2$  and  $d^2$  are the macromeres, and  $A$ ,  $B$ ,  $C$ , and  $D$  the micromeres; functionally the reverse is the case, inasmuch as  $A$ ,  $B$ ,  $C$ , and  $D$ , like the macromeres of *Nereis*, contain the entoderm.

The cell  $d^2$  corresponds exactly to the cell  $d^2$  or  $X$  of Wilson, which is the "first somatoblast." In the case of *Nereis* this cell forms the ventral plate and the mid-dorsal region as far as the prototroch.<sup>2</sup> In *Unio* it forms the shell-gland, and I think, that I can show, that in addition it forms the foot.

In general the apical pole lags far behind the vegetative pole in the further divisions, so that in the fifty-cell stage there are still only sixteen apical cells. This fact has made it impossible for me to follow the divisions of the apical pole cells to the late stage to which Wilson has followed them in *Nereis*, and Conklin<sup>1</sup> in *Crepidula*.

Let us now fix our attention upon the vegetative pole. Another division of  $A$ ,  $B$ ,  $C$ , and  $D$  takes place, giving rise in a right-handed spiral to the third generation of micromeres,  $a^3$ ,  $b^3$ ,  $c^3$  and  $d^3$  respectively. Shortly afterwards a fourth division of  $D$  takes place. This division is very unequal, the vegetative part  $D$  being much smaller than the part posterior

<sup>1</sup> Conklin, Preliminary Note on the Embryology of *Crepidula fornicata* and of *Urosalpinx cinerea*. Johns Hopkins Univ. Circulars, X, no. 88.

<sup>2</sup> This is true of the younger stages only; later the products of the first somatoblast become separated from the prototroch.

to it, *M* (Fig. 4). This cell lies between *X* and *D*, and corresponds exactly to the second somatoblast or mesoderm proteloblast of *Nereis*. Without further discussion I will simply say, that it is the proteloblast of the mesoderm in *Unio*, which is thus in every way identical with the mesoderm of Annelids (Wilson), and of Gasteropods (*vide* Conklin on *Crepidula*, and Erlanger<sup>1</sup> on *Bithynia*). Shortly afterwards *M* divides equally in a sagittal plane.

If so far the minute agreement of the segmentation with that of *Nereis* is surprising, I must confess that it seems little less than astounding that the next divisions should agree. To quote Wilson (Cell-lineage of *Nereis*, p. 411): "At the second division each of the primary mesoblasts buds forth a small cell at the surface near its anterior margin." Exactly the same thing occurs in *Unio*, though I cannot follow Wilson further and say, "the budding of the mesoblasts is continued in the same way for a considerable period." As a matter of fact I have observed only this one superficial cleavage of the mesoblasts, which are shortly afterwards, at the time of gastrulation, taken within the segmentation cavity, where each forms a "band" of cells.

Let us return to the cell *d*<sup>2</sup> or *X*. Here again we notice an extremely close resemblance to the same cell in *Nereis*. There is, however, one difference. In *Nereis* *X* buds off three small cells before its division into two equal parts. In *Unio* four such cells arise from *X*. These four divisions are as follows:—*x*<sup>1</sup> segmented off on the right side of the vegetative pole, *x*<sup>2</sup> on the left side of the same pole, *x*<sup>3</sup> in the median line towards the apical pole (and were a prototroch present in *Unio*, such as is present in *Nereis*, it would cause a similar median dorsal interruption of it), and *x*<sup>4</sup> in the median plane of the vegetative pole. (Not present in *Nereis*.) (Fig. 4.) *X* then divides sagittally and equally, and each half as in *Nereis* soon buds forth a small cell towards the vegetative pole. By continued division of the two large cells *Xl* and *Xr*, a plate of

<sup>1</sup> Erlanger, Beiträge zur Entw. der Gasteropoden. Mitth. aus der Zool. Stat. zu Neapel, Bd. X, Heft. 3.

large cells is produced dorsally, which invaginates *after gastrulation* to form the shell-gland (Figs. 5 and 6).<sup>1</sup>

I have already described the origin of  $a^2$ ; it is from this cell that the larval mesoblast arises. As this is a point of some importance, and is open to doubt on *a priori* grounds, I shall describe the process in some detail.  $a^2$  divides by an obliquely equatorial plane into  $a^{2.1}$  and  $a^{2.2}$  or  $Y$ .  $Y$  is larger than  $a^{2.1}$  and lies nearer to the vegetative pole.  $Y$  next buds off a small cell  $y^1$  to the right, which lies a little anterior to, and to the left of  $M$  (Fig. 4). Shortly after it buds off another small cell  $y^2$  to the left. Again it divides and buds off  $y^3$  posteriorly.  $Y$  is then gradually overgrown, and comes to lie in the segmentation cavity *anterior* to the entomeres. In the stage in which six large dorsal cells of the shell-gland are present, it divides sagittally into two equal parts. At about the time of the invagination of the entoderm each cell  $Yl$  and  $Yr$  divides into two parts, the dorsal product of which is in each case somewhat the larger (Fig. 5). But there is this distinction between the divisions of  $M$  and the divisions of  $Y$ : the divisions of  $M$  are typically teloblastic; those of  $Y$  are not (Figs. 5 and 6). The larval adductor muscle and some of the "Strangzellen" are formed from products of  $Y$ . These structures are purely larval, and take no part in the formation of adult tissues.

We have then in the formation and setting apart of this cell  $Y$  for this particular function an exceedingly instructive example of the precocious segregation of tissue elements. It seems as if in no other way could the adductor muscle, so important for the existence of the glochidium, be formed so early. Some light is thus thrown on the significance of the blastomeres of segmentation stages. It indicates that the mosaic arrangement is a derived condition, and has been acquired as the best means for the early separation of tissues.

The same cell in *Nereis* is the left stomatoblast (Wilson), which functions very differently. It may be interesting to

<sup>1</sup> It is interesting to note that in no other Mollusc has any cell been described comparable to the "first somatoblast" of Wilson. In the Gasteropods  $d^2$  does not differ from the other micromeres of the same generation.

note that, according to Lang,<sup>1</sup> all of the second generation of micromeres in the Polyclad *Discocelis* contribute to the mesoblast, and that the larval mesoblast of *Unio* is part of one of the second generation.

#### GASTRULATION.

I cannot state exactly how many cells enter into the formation of the entoderm, but whatever their number they are the products of *A*, *B*, *C*, and *D*. The third generation of micromeres is purely ectodermic. Gastrulation takes place at a stage when about twenty shell-gland cells are present. The invagination is never very deep, and in a superficial examination of whole embryos might easily escape observation altogether. That it has escaped observation hitherto is attributable to this fact. In section it is, however, very obvious (Fig. 5). Posterior to the blastopore in the segmentation cavity lie four cells, products of *M*, and anterior to it four cells, products of *Y* (Fig. 5). The blastopore occupies the posterior region of the body, and is afterwards occluded by products of *X* (Fig. 6). The anus arises later in the same area.

The oesophagus arises in the area described as oral plate ("Mundschild") by Flemming (Fig. 6, *oes.*). In some glochidia of Anodonta it is already in communication with the digestive tract, but as a rule the communication is not effected till post-embryonic stages, during the parasitic attachment of the glochidium to a fish.

#### SHELL AND FOOT.

Interpreting the entoderm invagination of Rabl as shell-gland, I can only corroborate his account of the formation of the shell.

The cells which lie between the blastopore and the shell-gland are derivatives of *X* (Figs. 5 and 6). These cells grow past the blastopore in later stages and anterior to it as far as the mouth. When the whole ventral region of the

<sup>1</sup> Lang, *Die Polycladen*, p. 332. Monographie 11. Herausgegeben von der Zool. Stat. zu Neapel.

larva undergoes a longitudinal invagination to form the mantle, they form the bottom of the invagination as far forward as the mouth and become the anlage of the foot and pedal structures. The lateral pits are derived from the same source.

*Thread-gland.*

The larval byssus gland, for which I will adopt the better term, "thread-gland," proposed by Schierholz,<sup>1</sup> is a *unicellular* structure, which opens between five large cells placed just ventral to the anterior end of the shell-gland. Its duct is, therefore, *intracellular*. The cell from which it is derived is ectodermic, and in earlier stages lies in the centre of the five large cells just mentioned.

The complete paper will contain an account of the further development up to the glochidium stage.

UNIVERSITY OF CHICAGO,  
Feb. 3, 1893.

<sup>1</sup> Schierholz, Ueber Entw. der Unioniden. Denkschr. der Math.-Naturwiss. Klasse Akad. Wien, LV. 1888.



## EXPLANATION OF PLATE.

FIG. 1. Four-cell stage from the apical pole. 1-1 = First plane of segmentation. 2-2 = Second plane of segmentation. *m. l. p.* = Median longitudinal plane of future larva. *Post.* = posterior; *Ant.* = Anterior.

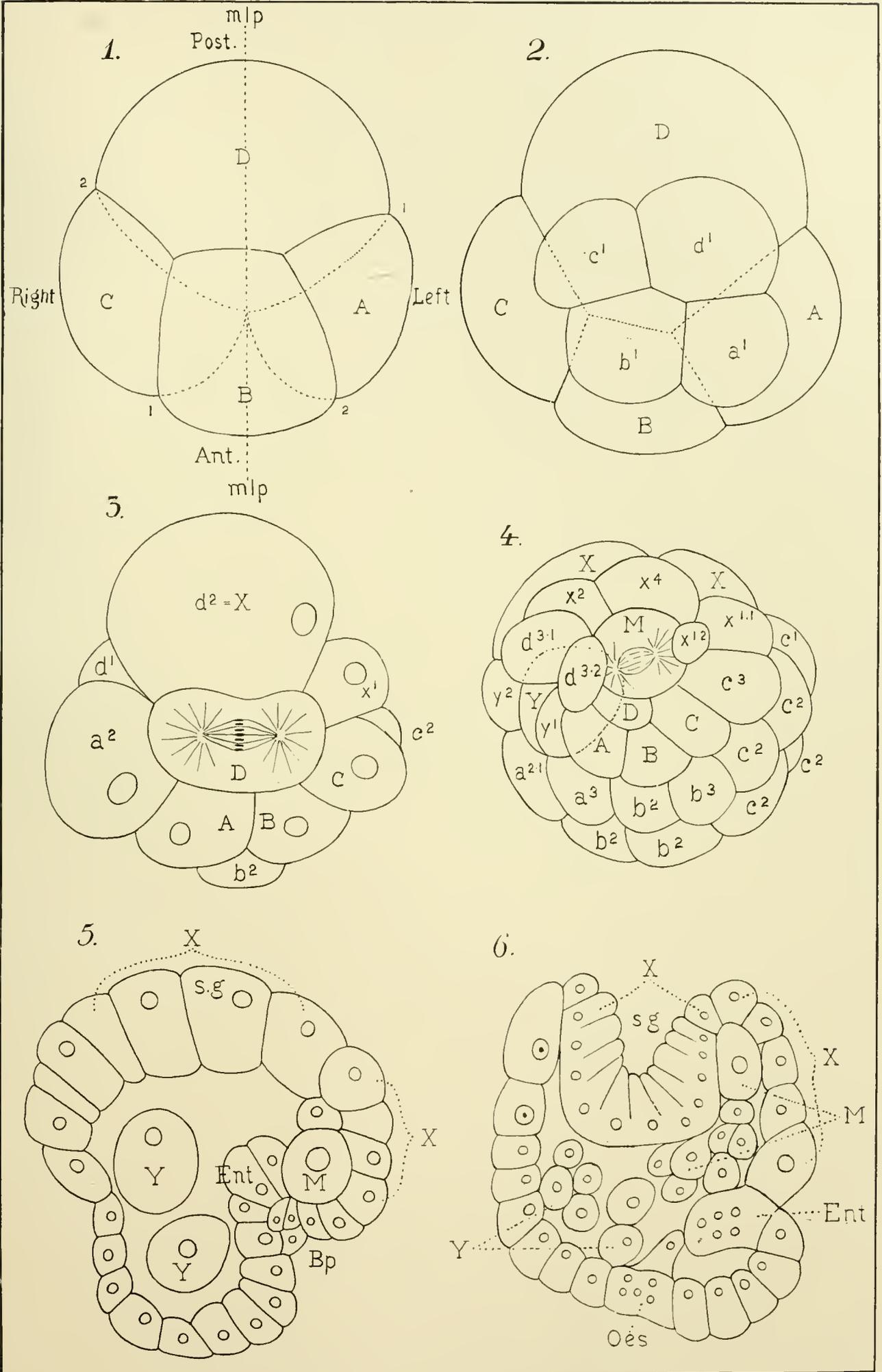
FIG. 2. Eight-cell stage from the apical pole.  $a^1, b^1, c^1, d^1$  the four apical micromeres derived from *A, B, C,* and *D,* respectively.

FIG. 3. Seventeen-cell stage from the vegetative pole. *A, B, C,* and *D* the four macromeres.  $a^2, b^2, c^2, d^2$  the second generation of micromeres.  $d^2$  or *X* is the first somatoblast.  $x^1$  is derived from *X* as indicated by the position of the nuclei. The spindle in *D* later separates  $d^3$  to the left.

FIG. 4. Vegetative pole of forty-six-cell stage.  $X, x^{1.1}, x^{1.2}, x^2, x^4$  = products of first somatoblast *X*. *M* = second somatoblast or mesoderm proteloblast, derived from *D*. *Y* = larval mesoblast, derived from  $a^2$ .  $y^1$  and  $y^2$  are products of *Y*. *A, B, C, D* = Entomeres.

FIG. 5. Sagittal section of young gastrula. *Bp.* = blastopore; *Ent.* = entoderm; *s. g.* = cells of shell-gland; *X* = products of the first somatoblast *X*; *M* = mesoderm teloblast of one side which has budded off one cell anteriorly. *Y* = larval mesoblast of one side.

FIG. 6. Sagittal section of an older stage. *Ent.* = entoderm; *s. g.* = shell-gland; *X, M,* and *Y* as above. *Oes.* = first indications of oesophageal invagination.





## AMPHIOXUS, AND THE MOSAIC THEORY OF DEVELOPMENT.

EDMUND B. WILSON.

IN view of certain current discussions regarding the nature of embryological development, the cleavage of the ovum in *Amphioxus* is of more than ordinary interest on account of its remarkably plastic character, which is shown in two directions. First, it normally exhibits a protean variability, and a study of its various forms yields new data bearing on the origin of cleavage-forms in general. Second, the development is capable in a very high degree of artificial modification through mechanical disturbances operating on the early stages. The descriptive portion of this paper is accordingly divided into two parts, the first treating of the natural forms of cleavage, and the second of induced forms caused by the isolation or mechanical displacement of the blastomeres. In a third and a fourth part the bearing of these facts on the origin of cleavage-forms and on some of the problems of embryological dynamics is briefly discussed.

In studying the natural forms of cleavage I have sought, especially, to find a basis, first, for the accurate comparison of the cleavage of the chordates with that of lower forms, and second, for the experimental studies described in Part II. To those who have followed recent inquiries into the significance of the first stages of cleavage I need not apologize for the description of many apparently trifling details, especially since my observations on the early development differ very materially from Hatschek's account.<sup>1</sup>

<sup>1</sup> The material for this study was procured at Faro during the months of June and July, 1892, by the following method: The animals, on being removed from the sand of the Pantano, were placed in large shallow glass vessels filled with clean water; here they lie perfectly quiescent and, if mature, discharge the reproductive elements within a few minutes. The eggs (which are invariably passed out through the atrial opening) were drawn up, during or immediately after their discharge, into a pipette and transferred to a smaller vessel of clean water to

## PART I. — NORMAL DEVELOPMENT.

As is well-known, the cleavage of *Amphioxus* after the 4-celled stage is distinctly unequal, and I shall in general use the terms macromeres and micromeres to designate the larger and smaller blastomeres, respectively. These terms are, however, used *solely for the sake of convenience* and imply nothing as to the ultimate fate of the blastomeres.

1. The first and second cleavages take place precisely in the manner described by Hatschek, dividing the egg into four equal parts (Plate XXIX, Figs. 1, 2, 3). Even in the 4-celled stage slight variations exist in the position of the blastomeres.

which a small quantity of freshly discharged spermatozoa had been added. By this method the eggs may be obtained perfectly clean and in any desired quantity. The embryos were preserved by a great variety of reagents, the best results being given by Lang's fluid (sublimate-acetic), various combinations of picric acid (picro-sulphuric, picro-acetic, picro-sublimate), and the Fol-Flemming chrom-osmium-acetic fluid. Perfect temporary preparation of the early stages is afforded by the use of the strong glycerine-acetic mixture ( $\frac{1}{3}$  glycerine,  $\frac{1}{3}$  water,  $\frac{1}{3}$  glacial acetic) that I have found of such essential service in the study of annelid development. Careful comparisons of preparations thus made with living embryos and with balsam preparations of picro-sulphuric, sublimate-acetic, etc., specimens show that with proper precautions the only perceptible alteration is a very slight swelling, and even this does not take place if the ingredients of the mixture are used in exactly the right proportion.

The eggs are always laid in the late afternoon, as a rule between five and six o'clock. There is a marked periodicity in the discharge of the reproductive elements, spawning-periods of two or three days alternating irregularly with quiescent periods of about the same length, during which no reproductive elements are discharged even in favorable weather. The beginning of a spawning-period is indicated by the accumulation of masses of ova or spermatozoa lying free in the anterior median ventral portion of the atrial chamber, where they can easily be seen in the living animal through the transparent atrial walls. Almost invariably the males are the first to discharge, and the clouds of spermatozoa set free in the water seem to stimulate the females to spawn. The reproductive elements are very rapidly discharged, passing in a steady narrow stream along the median ventral line of the atrial chamber and out to the exterior *through the atrial pore*. I have observed this process in hundreds of animals and have never in a single case seen a discharge through the mouth. My observations on this point, which were made with particular attention and care, agree with the original account of Quatrefages (22), afterwards confirmed by Willey (34), and differ from those of Kowalevsky (17) and Hatschek (10).

It is difficult to believe that Hatschek's very explicit statements (*Amphioxus*, p. 141) rest upon erroneous observation, but I can suggest no other explanation of the contradiction unless the animals vary in habits from year to year.

At first the four blastomeres are grouped about the vertical egg-axis with perfect radial symmetry, leaving a symmetrical rhomboidal space between them, as in Hatschek's Fig. 6. This space disappears completely as the embryo settles down into the succeeding quiescent period, and it is then that the variations first become apparent. Slight as they are, they deserve attentive consideration; for they give, I believe, a key to the more considerable deviations of later stages. In some cases the four blastomeres meet precisely along the egg-axis. More commonly, their mutual pressure gives rise to slight displacements, this way or that, through which "cross-furrows," or "Brechungslinien," are produced. The cross-furrows are, however, usually very short, and are not constant in direction. Occasionally they have the arrangement shown in the typical spiral cleavage of annelids, mollusks, etc., — *i. e.*, they are equal and at right angles to each other on the opposite poles of the embryo. More commonly, they are parallel to one another; that is, two of the blastomeres are in contact along the entire length of the egg-axis. In other cases the cross-furrow exists only at one pole, and disappears at the other. A careful comparison of all these cases leaves no doubt, in my opinion, that the arrangement of the blastomeres in the 4-celled stage is typically radial, and that the departures from this arrangement are purely accidental, probably depending on slight variations in the activity of the individual blastomeres as they settle down into the quiescent period.

2. The *third cleavage* (equatorial) is of considerable interest, for it shows distinctly three different forms (connected by every imaginable transition) each of which is a fixed type of cleavage elsewhere in the animal kingdom. These forms may be designated, respectively, as (1) radial (as in some echinoderms), (2) bilateral (as in tunicates and cephalopods), and (3) spiral (as in polyclades and annelids). (For more precise definitions of these forms see p. 599 and the accompanying diagrams.)

*a.* In the *radial form* the spindles are regularly arranged around the egg-axis and somewhat inclined inwards at their upper ends, as shown in Figs. 4, 5. The divisions result in

the formation of four smaller cells ( $a^1-d^1$ ,<sup>1</sup> first group of micromeres), lying exactly above four larger cells, which I shall call the primary macromeres ( $A-D$ , Figs. 6, 7). This agrees precisely with Hatschek's description and with the cleavage of *Antedon* as described by Seeliger (28). Cross-furrows may or may not be present, but in the former case are inconstant in position. In Fig. 7 they are equal and parallel. This form of division occurred in at least three-fourths of all the eggs observed.

*b.* In the *spiral form* (Figs. 8, 9, 10) the spindles are obliquely placed so that the upper quaterfoil of cells is rotated upon the lower, and invariably in the same direction, with the hands of a watch (right-handed spiral in Lang's sense), as in annelids, mollusks, and polyclades. All degrees of rotation exist from  $45^\circ$  (strict alternation of the blastomeres) down to a scarcely noticeable displacement. Cross-furrows almost invariably present. This form of division occurred in about one-fifth of the eggs observed.

*c.* The *bilateral form* (Figs. 12 and 13) differs at this stage from the radial only in the fact that two (sometimes all four) of the macromeres become slightly separated from each other along the line of the first cleavage. In the 8-celled stage, therefore, the embryo can be divided into corresponding halves only in the plane of the first cleavage. This form is rare in the 8-celled stage, though very common in the succeeding stage. I have followed it in the living egg in only a single case. (Figs. 13 to 18.) In this individual the gap between the two lower cells first arose *during* the third cleavage.

Various transitional forms exist between the three types described. Fig. 11 represents such a form, viewed from the lower pole. The four lower cells are distinctly bilateral in arrangement, but the upper quaterfoil is rotated nearly  $45$  degrees upon the lower.

3. The *fourth cleavage* exhibits an interesting series of forms, which may be arranged under the same three types as those of the third, with numerous transitional forms.

*a. Radial form.* This was the only form observed by Hatschek, whose description is as follows: "Es zerfällt jede

der Zellen durch eine meridionale Furche in zwei gleiche Theile. . . . Man kann nach der Beschaffenheit und Anordnung der Zellen nur eine Hauptachse, die vom animalen zum vegetativen Pole geht, unterscheiden." (10, p. 24.) This description applies to only a very small proportion of the embryos examined by me. Two of these, which represent the nearest approach to a true radial type I have seen, and agree closely with the 16-celled stage of *Antedon*, are shown in Figs. 19-21.

Even in these, however, the radial symmetry is not quite perfect, on account of the bilaterality suggested by the slightly smaller size of four of the lower cells ( $A^2-D^2$ ).

*b. Bilateral form.* This, which is much the most frequent of all the forms, occurs in two distinct varieties, of which the more usual is shown in Figs. 25 to 28. In this form each of the four upper cells divides equally in a plane parallel to that of the first cleavage. Each of the lower cells divides unequally in an oblique plane, thus giving rise to an anterior and a posterior pair of smaller cells (the secondary macromeres,  $A^2-B^2$ ,  $C^2-D^2$ ) lying at the respective ends of the embryo, and to two corresponding pairs of larger cells (the primary macromeres,  $A-B$ ,  $C-D$ ) that meet at the lower pole. In typical cases the bilaterality is complete and is strikingly apparent when the embryo is viewed from either pole. This form of cleavage occurred in a large percentage of the embryos examined, frequently modified, however, by variations in the cleavage-planes of the upper four cells, a series of which are shown in Figs. 22 to 24. In almost all cases an opening (cleavage-pore) exists at the lower pole, which varies in size from a mere pore (Fig. 27) to a very large opening (Fig. 30). It gradually closes in later stages, but I have seen it persist up to the gastrula stage (see p. 586).

The second variety of the bilateral form (Figs. 13 to 18) differs from the first in the fact that two of the secondary macromeres ( $C^2$ ,  $D^2$ ) are formed not at the end but at the sides of the embryo (Fig. 15). This form closely approximates to the cleavage of the tunicate *Clavelina*, as may be seen by a comparison of my Figs. 14, 15, with Figs. 7, 7<sup>a</sup>, 7<sup>b</sup> of Van Beneden's and Julin's paper (No. 31) (*cf.* Diagram,

p. 599) though the bilaterality of the tunicate is still more striking owing to the inequality of the second cleavage. It is much rarer than the first variety, and only in a single instance was I fortunate enough to follow it continuously in the living embryo from the undivided ovum up to a comparatively late stage (Figs. 13 to 18, which show the development of the same embryo from the 8-celled to the 128-celled stages).

*c. Spiral form.* (Figs. 31, 32.) In this type, which in its pure form is very rare, the blastomeres have precisely the arrangement found in the 16-celled stage of the annelids (Diagram, p. 599) I have not seen the origin of the blastomeres in the living embryo, and the connections of the blastomeres in the figures are hypothetical. The preparation figured leaves no doubt, however, that each of the primary macromeres must have divided unequally in the true spiral form to form the four secondary macromeres. The arrangement of the eight products of the first group of micromeres is exactly that which would result from a spiral division taking place in the reverse direction from that of the macromeres.

The three forms described are connected by a perfectly graduated series of intermediate forms, all of which I believe are capable of complete and normal development.

Beyond the 16-celled stage I have not endeavored to trace out in detail the variations of the cleavage, since they are even more numerous than those of the earlier stages.

In the 16-celled stage the embryo consists of eight equal micromeres, (products of  $a^1-d^1$ ), and eight macromeres, *viz.*, the primary larger central group of four ( $A-D$ ) and the secondary smaller peripheral group ( $A^2-D^2$ ). As development advances from the 16-celled to the 256-celled stage the general history is as follows: A second, third, fourth and perhaps a fifth group of micromeres, each of them eight in number, are formed by four successive unequal divisions of the macromeres. These micromeres, as well as the products of the first group ( $a^1, b^1, c^1, d^1$ ) divide equally, and thus a blastula is produced in which the size of the cells increases pretty regularly from the upper towards the lower pole. Throughout these stages, and even as late as the 512-celled stage (Fig. 48) most of the

specimens still show a more or less distinctly marked bilaterality when viewed from the lower pole. On the upper half of the embryo the bilaterality soon disappears; the planes of division in the micromeres conform to no general law and appear to be determined by individual mechanical conditions of environment.

The *fifth cleavage* (16-32) is shown in Figs. 33 to 40, and in Figs. 15 and 16. The eight new micromeres of the second group ( $a^3-d,3$   $a^{2.1}-d^{2.1}$ ) are formed by horizontal divisions. Those already present divide in most cases in planes approximately parallel to the equator, as Hatschek describes, but there are many individual exceptions. The bilateral symmetry is often very striking still.

The *sixth cleavage* (32-64, Figs. 41 to 44, and Figs. 16, 17) resembles the last in the origin of the eight new micromeres of the third group ( $a^4-d,4$   $a^{2.2}-d^{2.2}$ ). Most of the existing micromeres divide in meridional planes (Fig. 42), but there are many exceptions. It is noteworthy that one or more of the primary macromeres may divide *equally* at this cleavage (*e.g.*  $D-d,4$  in Fig. 17), but this may be followed by an unequal division in the following stage (Fig. 18).

The *seventh cleavage* (64-128, Figs. 47, 45 and Fig. 18) gives rise to eight micromeres of the fourth group ( $a^5-d,5$   $a^{2.3}-d^{2.3}$ ), while the remaining micromeres divide equally in the most diverse planes.

The *eighth cleavage* (128-256) still shows in many cases unequal divisions of the macromeres, a distinct group of which surround the lower pole in the 256-celled stage (Fig. 46). There are, however, many variations, and I have not been able to trace the history in detail beyond this point.

In the succeeding stage (approximately 512-celled, Figs. 48, 49) the blastula in some cases shows the first flattening of the lower hemisphere preparatory to the invagination. In some cases the cells still show traces of the bilateral arrangement (Fig. 48) and occasionally the cleavage-pore is present.

I shall not give in this paper a detailed account of the gastrulation, but will briefly call attention to a few leading points, bearing on the mode of invagination. The cleavage-pore, which

marks the lower pole of the blastula, sometimes persists up to a stage as late as the gastrula shown in Hatschek's Figs. 26, 27. In all such cases I examined *it lay exactly at the central point of the dome*—a fact that seems to show that the invagination is primarily symmetrical, as originally described by Kowalevsky. Whether this is true of later stages I have not yet certainly determined, but my observations do not accord with those of Lwoff (20). An extensive series of preparations (most of them fixed with picro-sulphuric acid, or with Flemming's fluid, stained with Mayer's hæma-calcium and mounted in balsam) show in the clearest manner that throughout the whole period of gastrulation, up to a stage later than Lwoff's Fig. 1, both macromeres and micromeres are undergoing active division. The macromeres like the micromeres show numerous conspicuous mitoses, and in every part of the entoblast plate. Lwoff's assertion, that during the invagination mitoses in the entoblast cells "so gut wie ganz fehlen," must rest upon very incomplete evidence; for in my preparations there is no perceptible difference between the two layers in this regard—though of course mitoses are absolutely more numerous in the ectoblast since the actual number of cells is greater.

#### PART II. — INDUCED FORMS OF DEVELOPMENT.

The blastomeres of the early segmentation-stages of *Amphioxus* may very easily be more or less completely separated from one another by Driesch's ingenious shaking-method, and in their subsequent development give rise to modified forms that are of the highest interest for the general problems of cleavage. The separation of the blastomeres is much more readily effected than in *Echinus*, and the induced forms of development may be obtained in great numbers; but this is offset by the fact that the eggs cannot be fertilized artificially, and the available time for work is, therefore, very limited. My stay in Faro could not be extended beyond six weeks, and it seemed best to devote most of it to the study of the earlier stages and to the preservation of material. For these reasons my work shows large and obvious gaps, and I have been unable

to reach conclusive results on a number of important points which it is to be hoped may be cleared up by other investigators.<sup>1</sup>

### A. General Summary.

1. An isolated  $\frac{1}{2}$  blastomere undergoes a cleavage identical with, or approximating to, that of a normal embryo. It produces a normally-formed blastula and gastrula of half the normal size, and finally may give rise to a half-sized dwarf

<sup>1</sup> The eggs should be *gently* shaken, in a small glass tube about half filled with water, and then poured into a larger vessel of fresh water. Violent shaking, such as is necessary in the case of *Echinus*, completely disintegrates the blastomeres of *Amphioxus*. The blastomeres can easily be isolated and develop well in the hanging drop for about twelve hours. For later stages they should be placed in a small covered vessel not more than half filled with water.

I have experimented with 2-, 4-, 8-, and 16-celled stages, giving most attention to the 2-celled and 8-celled, with the main object of determining first, the *limit* of regenerative power, and second, its *form of action* as shown in the mode of cleavage of the isolated blastomeres. In weighing the results certain sources of error should be carefully borne in mind. First, normal eggs and embryos vary considerably in size. Second, they do not develop precisely at the same rate, so that it is impossible to experiment with any stage absolutely unmixed (*e.g.*, a batch of 8-celled embryos almost invariably contains a certain number of 4-celled). Third, a certain number of the eggs always develop abnormally, whether shaken or not. Fourth, — perhaps most important of all — the rude operation of shaking *very often causes the isolated blastomeres, as well as the unsegmented ova, to break into still smaller fragments*. Some of these (those containing a nucleus no doubt) continue to develop and may thus give deceptive results — a blastomere of the 2-celled stage, for example, producing a gastrula of only  $\frac{1}{3}$  or  $\frac{1}{4}$  the normal size, or (?) even less. The only absolutely certain results, therefore, are in cases where the egg-membrane remains unbroken; and results derived from naked blastomeres, as well as those based on the study of preserved material, must be judged with a certain reserve.

It is necessary also to define clearly the terms to be employed. For the sake of brevity one of the blastomeres of the 2-celled stage may be called a  $\frac{1}{2}$  blastomere, one of the 4-celled stage a  $\frac{1}{4}$  blastomere, *etc.*, and the later stages may be correspondingly formed,  $\frac{1}{2}$  embryos,  $\frac{1}{2}$  blastulas,  $\frac{1}{2}$  gastrulas, *etc.*,  $\frac{1}{4}$  embryos, *etc.* For comparison with the normal (total) development, we may distinguish the  $\frac{1}{2}$  embryo (derived from a  $\frac{1}{2}$  blastomere) from the embryo-half (*i.e.*, half of a normal embryo derived from an unmodified egg), the  $\frac{1}{4}$  embryo from an embryo-fourth, and so on. By a double, triple, quadruple or multiple embryo, we designate one consisting of two, three, four, or several incompletely separated bodies. Multiple development is the origin from a single egg of more than one body, whether separate or partially united. Partial development gives rise to a partial embryo — that is, one which (as in the case of  $\frac{1}{8}$  or  $\frac{1}{16}$  embryo) is not a dwarf entire body but represents a fragment of a normal body.

larva exactly agreeing, except in size, with the normal larva up to the period when the first gill-slit is formed (Plate XXXVIII, Figs. 136, 138). In many cases, however, the tail is abnormally modified, posterior to the anus.

2. Displacement, without actual separation of the blastomeres of the 2-celled stage, may give rise to double embryos of many varieties, which may live up to the period of the first gill-slit. In the gastrula stage almost every possible transition occurs between forms slightly expanded laterally (Plate XXXIV, Fig. 64) and those in which two bodies are joined by only a narrow bridge of tissue (Fig. 68). The axes of the two bodies may form any angle. The blastopores, if separate, may be turned in any direction, and a great variety exists in the relation of the germ-layers of the two bodies (Plate XXXIV).

3. An isolated  $\frac{1}{4}$  blastomere may undergo a cleavage nearly or quite identical with that of a normal ovum, but often varies more or less widely from it. It may give rise to a  $\frac{1}{4}$  blastula and  $\frac{1}{4}$  gastrula, differing from the normal only in size. The segmented stage, with a notochord, is rarely attained and no normally constituted ones were observed. In a single, isolated instance a  $\frac{1}{4}$  larva was obtained (Fig. 139), at a stage nearly corresponding with that of the first gill-slit. This larva possessed a nearly normal notochord, neural tube and neuropore, mesoblastic somites and præ-oral pit, but had no mouth, no gill-slit, no anus, and the posterior region of the intestine was aborted.

4. A 4-celled stage, if separated into two pairs of cells, as often happens, may give rise to two perfect, half-sized dwarfs ( $\frac{2}{4}$  embryos).

5. Incomplete separation of the blastomeres of a 4-celled stage gives rise to (a) double embryos, (b) triple-embryos, one body being twice the size of the other two (Figs. 61, 62), and (c) very rarely to quadruple embryos. The double and triple embryos may attain the gastrula stage, but I have never brought the quadruple forms beyond the blastula. In the case of triple embryos one body is obviously a  $\frac{2}{4}$  embryo, produced from one pair of cells, while the others are fourths.

6. In three instances gastrulas about  $\frac{1}{8}$  the normal size were produced from 4-celled stages (two of the three are shown in Fig. 53). Since there is reason to believe (see below) that the  $\frac{1}{8}$  blastomere never gives rise to a gastrula, I believe the  $\frac{1}{4}$  blastomere must have been broken by the operation into fragments, from which these minute forms arose. The gastrulas were nearly typical in form, with nearly the normal ratio of size between the cells of the two layers.

7. The  $\frac{1}{8}$  blastomeres are of two sizes (micromeres and macromeres) which, as far as could be determined, do not differ essentially in mode of development. The isolated blastomere segments in a form (Figs. 121–129) approaching that of a complete ovum but *never identical with it*. In rare cases a  $\frac{1}{8}$  blastula is formed, either closed or with a pore at the lower pole; but the gastrula-stage is never attained. As a rule, however, the  $\frac{1}{8}$  blastomeres produces a flat or curved plate of cells, of which those at one margin are at first larger (Figs. 129, 130–134). All gradations exist between blastulas having a cleavage-pore and the curved plates. In later stages the curved plates often appear at the same age in two varieties, in one of which the cells are much larger than in the other (Figs. 132, 133). These two forms are similar to fragments of ectoblast or entoblast of the normal gastrula at the corresponding age, and suggest the view that the corresponding blastomeres have undergone a partial development — *i.e.*, as if they still formed part of a complete normal embryo. *This subject requires further investigation.* All the forms of  $\frac{1}{8}$  embryos acquire cilia and swim actively about. The plates invariably died after 12 to 18 hours; the closed blastulas lived in some cases for two or three days. (*Cf.* Driesch, No. 8, p. 9, who has observed  $\frac{1}{4}$  blastulas of the same type, which likewise lived for several days without forming a gastrula.)

8. Incomplete separation of the blastomeres of the 8-celled stage gives rise to a great variety of forms which, however, I have not been able to study satisfactorily. Among them were typical  $\frac{1}{2}$  and  $\frac{1}{4}$  gastrulas (Figs. 54, 56) as well as double gastrulas; but these may have arisen from 4-celled stages mixed with the 8-celled.

9. The 16-celled stages when shaken give rise to an immense variety of forms which I have not carefully studied. The isolated blastomeres continue to divide for some time, but as far as observed always give rise merely to flattened plates or shapeless masses of cells. Small closed ciliated blastulas were also produced of about the same size as those derived from the  $\frac{1}{4}$  blastomeres.

#### B. *Cleavage of the Isolated Blastomeres.*

The isolated blastomere retains for a short time its original, flattened form (Figs. 77, 87). It soon becomes more rounded (Figs. 78, 88) but not perfectly spherical, and the first division (invariably equal) was in every case observed *transverse to the longest diameter*. It is an interesting fact that soon after their isolation the blastomeres frequently explode with such violence as to reduce them to minute granules, so that I have often found scarcely a trace of them in the hanging drop where they had carefully been isolated. The explosion, which I have witnessed several times, is sudden and complete, and shows that the substance of the blastomere is in a state of high tension.

The isolated blastomeres undergo a cleavage that approximates more or less nearly to that of a normal ovum, but *the extent of divergence is nearly proportional to the age of the initial form*.

*a.* The  $\frac{1}{2}$  blastomere in many cases agrees exactly with the normal ovum up to the 8-celled stage (Figs. 77-81). In later stages, likewise, the general history is the same (Figs. 97-100) though the bilaterality is usually lost, and a cleavage-pore is more usually present. In many cases, however, the second cleavage is distinctly unequal, so that in the 4- and 8-celled stages (Figs. 90, 104, 105) two of the macromeres are smaller than the others. The four micromeres appear to be always equal. In later stages these embryos agree with the others, except in the somewhat greater size of the macromeres towards one side of the entoblast plate.

*b.* The  $\frac{1}{4}$  blastomere may likewise segment quite like the entire ovum (Figs. 87-91), but the unequal type of the 4-celled

stage (Fig. 92) is more frequent than in the  $\frac{1}{2}$  embryo, and the blastulas show correspondingly more frequent variations from the typical forms.

*c.* The  $\frac{1}{8}$  blastomeres, whether large or small, divided unequally at the second cleavage, in every case observed (Figs. 121, 126). Four micromeres are formed as usual by an equatorial cleavage, but these may be equal (Fig. 127) or unequal (Fig. 122). At the ensuing division (Figs. 123, 128) only the two larger macromeres divide unequally, all the other cells dividing equally; and at the same time a large cleavage-pore appears at the lower pole, where only two macromeres can be distinguished (Fig. 124). These continue to divide unequally for some time (Figs. 128–130) while the others divide equally. Meanwhile the cleavage-pore undergoes a varied fate. In a very large proportion of cases it enlarges at each division until the embryo consists of a plate of cells (Figs. 129, 130) sometimes perfectly flat, more usually curved. In cases of only moderate enlargement of the pore, half-closed blastulas are produced (Fig. 131). In a few cases, however, the pore closes completely, and then perfect closed  $\frac{1}{8}$  blastulas arise (Figs. 125, 135).

All of these forms may live 18 hours or more, acquire cilia and swim actively about. None, however, invaginate to form a gastrula. (I have isolated about a score in the hanging drop, and have examined many hundreds more, both living and in preparations.) It is, therefore, highly probable that the  $\frac{1}{8}$  blastomeres are unable to advance beyond the blastula stage—a result in agreement with those of Roux and Driesch. The  $\frac{1}{8}$  blastulas (like the exceptional  $\frac{1}{4}$  blastulas observed by Driesch) swim with great activity for several days. The wall consists of uniform columnar ciliated cells (Fig. 135) enclosing a large cavity, in which, after 24–26 hours, often appear numerous clear rounded bodies that resemble the mesenchyme cells of echinoderm embryos.

### *C. Cleavage of Double Embryos.*

I have examined the early stages of double embryos with especial interest to determine first the relation between the

cleavage of an isolated  $\frac{1}{2}$  blastomere and that of a normal cleavage-half, and second the period at which the axes of the twin bodies are determined. The results demonstrate (1) *that the blastomeres of the half-sized embryos cannot be identified individually with those of half a normal embryo; and (2) that the axes of the twin bodies are determined with the first division after the operation.*

A series of forms exists among the double cleavage-stages that shows as obvious relation to a corresponding series among the double gastrulas, though I have never in a single instance succeeded in tracing the connection in the living state. In the simplest case (Fig. 112) the 16-celled stage shows but a slight lateral extension, corresponding with the laterally expanded, but still undivided gastrulas (Figs. 64, 66). From this case every transition exists to an obviously double 8-celled stage (Fig. 114) in which the embryo clearly consists of two 8-celled stages, adherent along the line of first cleavage and with parallel axes. Such forms probably give rise to gastrulas like that shown in Fig. 71, in which the axes are parallel and the blastopores turned in the same direction. In still more highly modified cases (Figs. 111, 118, etc.) the axes of the two halves form various angles, clearly foreshadowing such conditions in the gastrula stage as are shown in Figs. 70-76.

A study of the earlier stages shows that the axial relations are already definitely established in the double 2-celled and 4-celled stages. This is clearly shown in Figs. 106-110. In Fig. 110 (double 4-celled stage) the axes are vertical and parallel, while in Fig. 109 they form an angle. In Fig. 107 (double 2-celled stage division) they are nearly at right angles; in Fig. 106 they form an acute angle. A study of these various forms leaves no doubt that *the direction of the axes is determined with the first division of the displaced blastomeres*, which (as I have shown at p. 590) stands in definite relation to their longest diameter, and this in turn is determined by the shape of the cells *before displacement*. These facts seem to justify the conclusion that the axial relations of the twin bodies is purely fortuitous. *The first cleavage of each blastomere is in the same plane it would have followed in the normal*

cleavage; its ultimate direction is determined by the position accidentally assumed by the blastomere after its displacement. Thus in Fig. 106 the two blastomeres must have been slightly rotated on one another in a vertical plane nearly parallel to that of the first cleavage. In Fig. 107 the left blastomere was rotated in a vertical plane, the right in a horizontal plane at right angles to the first.

Let us now consider the form of cleavage. In this regard *the double embryos show a series of forms intermediate between a half-cleavage (like that of an isolated  $\frac{1}{2}$  blastomere) and a cleavage-half (like one-half the normal cleavage)*. Several such forms are shown in Plate XXXVI. In Fig. 108 the left half consists of two macromeres with two micromeres lying above them; that is, it is a typical cleavage-half. The right half, on the contrary, consists of four equal cells, lying in a horizontal plane; the third cleavage has here become vertical instead of horizontal, and equal instead of unequal. In Fig. 110 the third cleavage is vertical and equal on both sides (typical double 4-celled stage). Fig. 109 is intermediate as regards the planes of cleavage, but all the cells are equal.

Analogous and still more interesting transitional forms occur in the following stage. The most striking of these forms is shown in Fig. 114 (which represents the individual that first called my attention to the importance of the double cleavage). The nuclear spindles (all distinctly visible in the preparation) leave no doubt as to the origin of the cells, and demonstrate the mode of transition. In each half four micromeres are in process of formation. Two of them (those in the upper half of each twin) agree with the normal cleavage, arising by the vertical fission of a parent micromere of the (common) 8-celled stage. The other two are in process of separate formation from the two corresponding macromeres. Thus, the upper half of the figure follows the normal mode of cleavage, while the lower half shows the regular double cleavage. Inspection of the figure will show that the disturbance in the form of cleavage showed itself at the third (common) division, which, instead of being as usual equatorial in all of the four blastomeres, became vertical in two of them. In the true double

cleavage the third division is vertical in all four, as shown in Fig. 110. A second somewhat similar case is shown in Fig. 113. On the left side of the middle line the division corresponds precisely with that of an isolated  $\frac{1}{2}$  blastomere. The right side, however, corresponds with one-half of Fig. 114 (allowing for a slight displacement of the cells).

These facts are of great importance, for they demonstrate that *even a slight displacement of the blastomeres in the 2-celled stage causes a change in the form of cleavage, such that the blastomeres of the half-embryo cannot be identified individually with those of a normal embryo half. The normal embryo develops as a unit; if it be disturbed in the 2-celled stage, this unity is destroyed and two new units are established.* In the transitional forms the new units show, as it were, reminiscences of their parentage.

It would be interesting to determine whether the isolated half-embryos ever show such reminiscences. The case illustrated by Figs. 104 and 105, where the second division of the isolated  $\frac{1}{2}$  blastomere is unequal, may perhaps be so regarded; but only in respect to the arrangement and relative size of the cells, not (in about a score of observed cases) in their mode of origin. The micromeres always arose separately by unequal divisions of the macromeres, as in the normal ovum.

#### D. *Cleavage of the $\frac{2}{4}$ Embryo.*

The early cleavage of the  $\frac{2}{4}$  embryo was observed in only three cases. In one of these, the first division of the isolated pair of cells was equal (Fig. 85). In the other two it was unequal (Fig. 84), and the ensuing 8-celled stage (Fig. 86) was similar to the unequal  $\frac{1}{2}$  embryos (Fig. 104). The later history was not followed. Several other isolated  $\frac{2}{4}$  embryos gave rise to normally formed half-sized gastrulas.

#### E. *Unilateral Development.*

It occasionally happens that only one of the blastomeres of the 2-celled stage develops, as Driesch has likewise observed in *Echinus* (*cf.* Roux and Chabry). I have obtained only a few such embryos, three of which are figured. Fig. 59 repre-

sents a  $\frac{1}{2}$  gastrula attached firmly by one side to an unsegmented  $\frac{1}{2}$  blastomere (both within one membrane). The gastrula is somewhat distorted, but is clearly of the normal type. Fig. 102 shows a unilateral  $\frac{1}{2}$  blastula, the lower pole of which is turned towards the unsegmented blastomere. Fig. 101 shows a unilateral 4-celled stage, the left  $\frac{1}{2}$  blastomere being unsegmented. The two halves are firmly united and enclosed within a single membrane. The segmented half shows the normal form of entire cleavage, being divided into four equal cells.

These facts, as well as those afforded by the double embryos, show that *the unity of the normal embryo is not caused by a mere juxtaposition of the cells.* They indicate that *this unity is not mechanical but physiological, and point toward the conclusion that there must be a structural continuity from cell to cell that is the medium of coördination, and that is broken by mechanical displacements of the blastomeres.*

#### F. *The Later Stages.*

The modified forms of development, whether isolated dwarfs or multiple monsters, steadily diminish in number as the development advances. Half-sized, quarter-sized, and double gastrulas are very common (6–8 hours.) Among the oval larvæ with notochord and 8–9 somites (Hatschek's, Figs. 49–52) half-sized dwarfs are not uncommon (Fig. 142), but I have seen only two or three  $\frac{1}{4}$  larvæ, and none of these were normally developed (Fig. 143).<sup>1</sup>

Double embryos at this stage are rare. In the individual represented in Fig. 144 the two bodies are united along the left side, the anterior part of each being free (*cf.* the corresponding double gastrula, Fig. 68). The two notochords are separate, but the archenteric cavities appear to communicate posteriorly.

<sup>1</sup> I may call attention to the fact that Hatschek's Fig. 49 ("Kleines Individuum") probably represents a  $\frac{1}{2}$  larva accidentally produced by manipulations of the early stages. Hatschek specially calls attention to the small size of this and a few other of his figures, which he says is due to individual variation. I found also a considerable variation in the size of the ova, but never sufficient to account for so great a difference in the larvæ.

In the stage of the first gill-slit (Figs. 136-138) half-sized dwarfs are rather rare, and most of them show a distinct tendency towards an abnormal development of the tail region, which is often thickened and distorted, and frequently bent downwards posterior to the anus. Some of the full-sized embryos, however, show a similar modification. A few of the  $\frac{1}{2}$  larvæ at this stage are perfectly developed in every respect; but, as Figs. 137, 138 show, the normal proportions of the body are not quite maintained, the regions behind the expanded anterior part being relatively shorter. The notochord is relatively thicker, its diameter being nearly or quite equal to that of the normal larva. The unique fourth-sized dwarf observed at this stage (Fig. 139) is well developed in front, showing a very distinct præ-oral pit and neuropore, but the mouth and first gill-slit have not broken through. Posteriorly it is distinctly segmented, but shows two modifications: (1) The intestine is wanting behind the enlargement, the stomach ending blindly as shown in the figure; (2) the tip of the tail, including a portion of the notochord, is sharply flexed toward the dorsal side and adherent to the body wall in that region.

Double embryos are extremely rare, and I have seen only two specimens, neither of which was normally developed. In the specimen shown (Fig. 140) the twins seem to be joined by the ectoblast alone, and all the internal parts are distinct. One of the individuals is nearly normal in structure. The other is defective in the head region, and shows no præ-enteric region.<sup>1</sup>

The foregoing facts leave no doubt that all the induced forms of development show in general a lack of developmental power that becomes more pronounced as the ontogeny advances. I have not determined whether the double embryos ever split apart to form two separate twins, as Driesch observed in *Echinus*.

<sup>1</sup> Mr. Willey, who has done so much to advance our knowledge of the later stages of *Amphioxus*, informs me that he has observed free-swimming double embryos at this stage in which the twins were joined back to back, and appeared to be normally formed.

## PART III.

A. *Cleavage and Germ-layers in Annelids and Chordates.*

A comparison of the cleavage of *Amphioxus* with that of annelids shows that they differ widely, not only in form but also in the mode of differentiation of the germ-layers. I was led to examine the cleavage of *Amphioxus* primarily in order to determine the origin of the mesoblastic pole-cells described by Hatschek, and thus to find a definite basis for comparison with the annelids, where they are known to exist in a large series of forms, always arising in the same way, having the same relation to the blastopore, and agreeing exactly with those of mollusks (35). I was hardly prepared for the result, although it was of extreme simplicity and beauty; *the pole-cells of Amphioxus are a myth.*

Not unmindful of the proverbial difficulty of proving a negative, I nevertheless speak unreservedly on this point, after a painstaking search, with complete conviction that the same result will be reached by any observer who will take the trouble to make a close study of the actual embryos. At no period during the entire cleavage and gastrulation can the pole-cells be distinguished from the other entoblast cells, either in living embryos or in perfectly fixed and stained preparations (picrosulphuric, Flemming's fluid, osmic-carmin, sublimate-acetic, etc.). Neither do they exist in later stages. In these the posterior region of the larva is rapidly growing and numerous mitoses may be observed in all the cells in the region of the neurenteric canal. The most careful study of this region in sections and total preparations, in various stages, fails to show any cells that can be identified with the pole-cells. Hatschek was, I believe, misled in the early stages by observing entoblast cells at the lip of the blastopore in the rounded form assumed during division. I have occasionally seen a pair of such cells in the position described by Hatschek, but exactly similar pairs of cells may be seen at the same time at other portions of the blastopore-lip and in various parts of the entoblast-plate. In later stages the error seems to have arisen by mistaking the lateral walls of the neurenteric canal as seen in optical section

(perhaps during the division of the cells in this region) for a definite pair of large cells.<sup>1</sup>

But apart from the origin of the mesoblast the differentiation of the germ-layers in *Amphioxus* differs widely in other respects from that of annelids. In the latter forms the cleavage is always unequal (even in *Polygordius*, as shown by my own unpublished observations), and after the formation of the first four cells several groups of micromeres, each consisting of four, are formed from them before the gastrulation takes place (there are three of these groups in *Nereis*, four in *Psygmorebranchus* and *Lumbriconereis*, five in *Aricia* and *Polymnia*; see No. 35, appendix). In all cases the first three groups give rise to ectoblast; of the fourth group one blastomere gives rise to mesoblast and three to entoblast; while the fifth group are pure entoblast.

*Amphioxus* diverges from the annelid at the fourth cleavage; for the smaller cells (the "secondary macromeres" A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub>, D<sub>2</sub>) separated at that time from the primary macromeres are not ectoblastic but mixed in character, giving rise both to entoblast and ectoblast and perhaps also to mesoblast. These cells, as I have shown, remain throughout the development in contiguity with the primary macromeres, forming with them the ento-mesoblast plate, and like them continue to bud forth micromeres until a late stage. Thus, in annelids, the fourth cleavage is qualitative (using the word in a purely prospective sense) while in *Amphioxus*, although unequal as in the annelid, it is quantitative only. Whether this difference may hereafter be found to be of any phyletic importance is an open question.

#### B. On Normal Cleavage-Types.

I have elsewhere pointed out (35) that irrespective of yolk-storage, or of the mode of gastrulation, the early stages of cleavage in the animal ovum exhibit three more or less

<sup>1</sup> These conclusions were laid before the American Morphological Society at the December meeting, 1892. The same result was published by Lwoff in a paper received soon afterwards, and I know of at least two other admirable observers who have sought in vain for the mythical pole-cells. They will no doubt long continue to haunt our text-books, where they are already quite at home; but it is to be hoped that the ghost may in time be laid.

clearly marked types, each of which is characteristic of a particular group or series of animals. Although these types have little phyletic importance, their origin and meaning is of great interest in the study of cell-dynamics, and their careful investigation is an indispensable accompaniment of experimental researches on cell division and differentiation. These types may be designated as (1) *bilateral*, (2) *radial*, and (3) *spiral*,

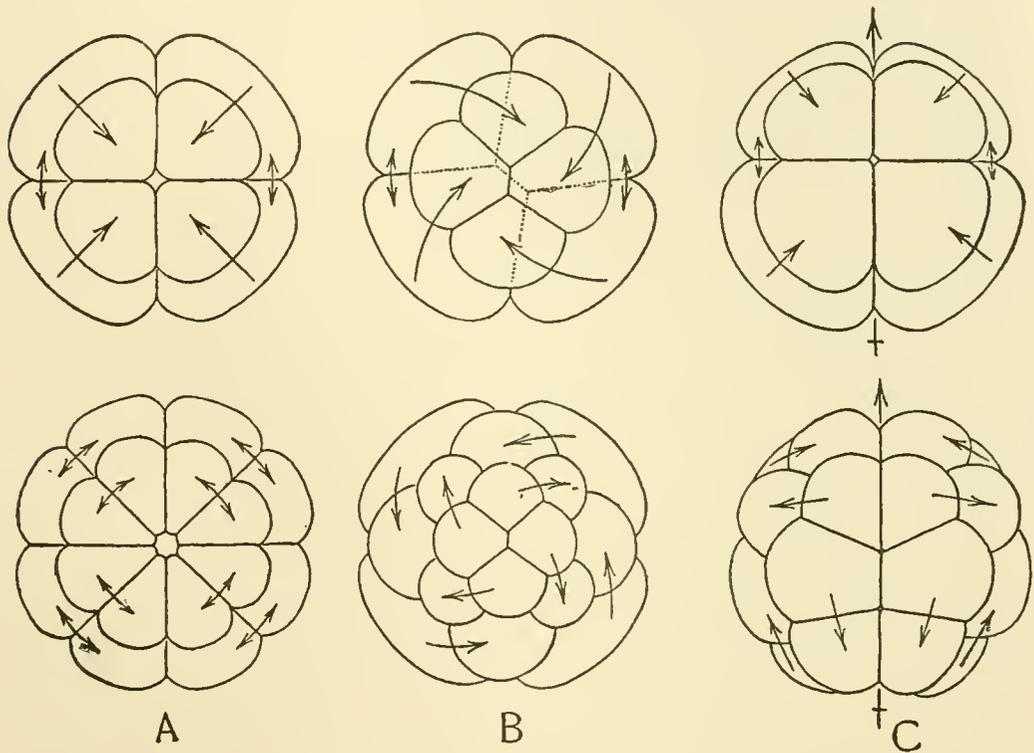


DIAGRAM OF CLEAVAGE-FORMS.

- A. Radial type (*Antedon*; modified from Seeliger).
- B. Spiral type (*Discocoelis*; after Lang).
- C. Bilateral type (*Clavelina*; after Van Beneden and Julin. The upper figure is considerably modified, the inequality between the macromeres and micromeres being exaggerated).

The upper figures represent 8-celled stages seen from the upper pole. The lower figures are the corresponding 16-celled stages in the same position. In the case of unequal division the arrow points towards the smaller cell.

and their definition at this point will facilitate the discussion of certain questions suggested by the fact that all three of them normally occur in *Amphioxus* (p. 581). It will be convenient to consider only the 8-celled and 16-celled stages which most clearly show the characters (Diagram above).

I. In the *bilateral type* (Diagram C) the cell-divisions are symmetrical with respect to a single plane—usually that of the first cleavage—which generally coincides with the median plane of the adult body. This appears in astonishing perfection in the tunicate (*Clavelina*, Van Beneden and Julin, No. 31) and in the cephalopod (*Loligo*, Watase, No. 32) in both of which the bilaterality persists up to a relatively late stage and conforms to the symmetry of the adult. It occurs also in the ctenophore (Agassiz 1, Chun 6), in many bryozoa (Barrois, 2) in *Amphioxus* and in some vertebrates.

II. In the *radial type* (Diagram A) there are two systems of cleavage-planes of which one set are meridional, and radially symmetrical to the egg-axis, while the other set intersect the meridians at right angles—*i.e.*, they pass through the equator or are parallel to it. This form occurs in the sponge *Sycandra* (Schultze, No. 27) and in great perfection in some echinoderms (*Antedon*, Seeliger, No. 28, *Echinus*, Selenka, No. 30).

III. The *spiral type* (Diagram B) arises from the radial through a twisting of the radii, as it were, the blastomeres being displaced or rotated, with respect to the egg-axis, either to the right, following the hands of a watch (right-handed spiral) or in the reverse direction (left-handed spiral) as the case may be. (The direction of rotation typically alternates in successive cleavages; cf. *Nereis*, No. 35.) The term “spiral,” refers to the fact that the curved radii, if prolonged, would form a spiral about the egg-axis.<sup>1</sup>

In this case the two sets of cleavage planes are both oblique to the egg-axis, though approximately rectangular to each other. The spiral type occurs in great perfection throughout the annelids, mollusks (excepting cephalopods) and polyclades, and it is a remarkable fact, that throughout all these forms the direction of rotation is constant. In all, namely, the first group of micromeres (formed at the third cleavage) is rotated to the

<sup>1</sup> The term spiral was first applied to this form of cleavage as far as I am aware by Selenka (29) in the case of Polyclades, and was afterwards adopted by Lang (18). (Barrois No. 3 had already used it, in a different sense, in the case of nemerteans.) It is perhaps not very happily chosen, and the term alternating or alternate type might be preferable.

right (with the hands of a watch), the second group to the left, the third to the right, and so on.

These types are connected by various intermediate forms and are obviously devoid of any great phyletic importance. In annelids, for example, the cleavage is strictly spiral up to the 32-celled stage, after which it becomes bilateral, though not strictly so. In the nemertean *Lincus* (Barrois, 3) it is perfectly radial up to the 8-celled stage, and then assumes the spiral form by a bodily rotation of the micromeres. In gasteropods the cleavage is at first strictly spiral; in cephalopods it is perfectly bilateral from the beginning. In *Amphioxus* the first two cleavages are perfectly radial, after which any of the three forms may be shown.

But although these forms of cleavage are thus seen to be without value in the investigation of general phyletic questions they are very important for an analysis of the factors that determine the form of cell-division; for they show, I believe, that *cleavage-forms are not determined by mechanical conditions alone*. In this regard it is necessary to distinguish clearly between the three types. The spiral type, as I have elsewhere pointed out (35) is a direct modification of the radial type, and is an effect of mutual pressure among the blastomeres (however caused) in accordance with Berthold's principle of minimal contact surfaces.<sup>1</sup>

This is clearly shown in the case of *Lincus*, cited above, where a typically radial cleavage passes over at the 8-celled stage, into the spiral form by an actual rotation of the blastomeres. The spiral type, therefore, owes its peculiarities entirely to mechanical conditions, the blastomeres assuming the position of greatest economy of space, precisely like soap-bubbles or other elastic spheres.

With the radial type the case is not so clear. This form is obviously an expression of Sachs's law of the rectangular intersection of division-planes operating on a spherical mass. Hertwig has pointed out the causal basis of this law (11 and

<sup>1</sup> I am indebted to Dr. Driesch for calling my attention to Berthold's fine treatise (No. 4), with which I was unacquainted at the time my paper on *Nereis* was written.

14) by showing that the axis of the spindle coincides with the direction of greatest elongation in the surrounding protoplasmic mass ; from which it follows at once that the first three cleavages of a spherical mass will be at right angles to one another and successively in the three dimensions of space, as in the radial type. Pflüger (21), and especially Driesch (8), have shown finally that the direction of protoplasmic elongation (and consequently that of the spindle-axis) *may* be determined by pressure, but it does not yet appear whether this is a general law. Roux's contradictory results on frog's eggs remain unexplained ; and there are cases (*e. g.*, in the cambium-cells of plant-stems) in which the plane of division is regularly parallel to the long axis of the cell and at right angles to the direction of pressure. Still, the fact remains that the radial type of cleavage is determined by causes, whether physical or physiological, that concern the embryonic stages alone, and have no direct connection with the adult state.

With the bilateral type the case is widely different, for the cleavage is more or less completely dominated by the symmetry of the adult body, which may be manifested from the very beginning. It does not seem possible, in the present state of our knowledge, to explain this bilaterality as the result of purely mechanical conditions such as pressure, distribution of yolk, and the like. It occurs in small ova with total cleavage (tunicate) as well as in large yolk-laden eggs with partial cleavage (cephalopod) ; it is in some cases actually opposed to mechanical conditions. In *Clavelina*, for example, as may clearly be seen from Van Benedin's and Julin's figures (*e. g.*, Figs. 7, 8, 10, etc.), the bilateral arrangement of the blastomeres causes the cell-group to depart widely from the arrangement demanded by the law of minimal surfaces. Thus, along the median line four cleavage-planes generally meet at a point, instead of three as the law requires ; and this is only gradually corrected by displacements of the blastomeres as the cleavage advances. The same is true of the early stages of the frog (*cf.* Rauber, No. 23, Figs. 34, 35). It seems necessary to conclude, therefore, that *bilaterality in cleavage is an inherited character* that has arisen by the remodelling of a non-bilateral

type. The fact which I have elsewhere described (35), that in some animals the bilaterality does not appear at the beginning (in annelids not until the 38-celled stage) indicates that this process has taken place by a gradual shifting backwards in the ontogeny of the adult bilaterality, which thus became projected, as it were, upon the cleavage-stages.

Accepting these conclusions, the variable cleavage of *Amphioxus* is a very interesting case; for we here observe, as it were, a conflict between an hereditary tendency not yet firmly established, and mechanical conditions that are, in a measure, opposed to it. It is impossible not to recognize a distinct tendency towards bilaterality in almost all forms of the cleavage, but it is often disturbed by displacements of the blastomeres. In some cases the displacements are very slight, and the bilateral form is scarcely modified, but every transition exists (Figs. 22, 23, 24) up to a condition in which the bilaterality is completely lost, and the cells are arranged precisely as in the true spiral cleavage (Figs. 31, 32). In the latter case the cells are arranged with greatest economy of space, in accordance with Berthold's principle, and a careful study of the various transitional forms shows that the displacements of the individual blastomeres show, in a large proportion of cases, a distinctly recognizable conformity to the same principle, as may be seen by a comparison of the dividing micromeres in Figs. 22, 23, 24, for example, with the corresponding cells in Fig. 19. These displacements, it should be noted, do not, in such cases, take place subsequent to the division of the blastomeres, but during the division itself, and are, probably, in many cases due to modifications of form caused by pressure. There can be no doubt that many details of egg-cleavage, in general, are directly determined by this influence, the cell elongating in the direction of least pressure, and the spindle placing itself, with its long axis, in the same direction. This may be clearly made out, for example, in many of the detailed figures given of the cleavage-stages of *Nereis* (No. 35), for example, in Figs. 13, 16, 19, 22, etc. It is to this cause, as I believe, that we must ascribe many, if not all, of the variations in *Amphioxus*. Their first origin is probably to be sought in slight, accidental

displacements in the 8-celled, or even in earlier stages, as the blastomeres settle down to the resting phase after division. *Amphioxus* differs from *Clavelina* in the fact that the bilaterality is less firmly established, and in this respect agrees very nearly with the frog-cleavage, as described by Roux (No. 24) and Rauber (No. 23). It would be interesting to examine a larger series of *Clavelina* embryos, for it seems, on *a priori* grounds, very probable that even in this case the bilaterality may undergo occasional modification without affecting the end result.

In view of these considerations I conclude that the bilateral cleavage of *Amphioxus* is an hereditary form, which is in process of development, or perhaps of disappearance. The primitive type, from which both the others arose, was probably the radial, and a comparison of Figs. 19–21 with 25–27 will show how easily the bilateral type may be derived from it.

I must finally call attention to the remarkable fact described at p. 582, that in the 8-celled stage the occasional rotation of the four micromeres is invariably in the same direction as in annelids, mollusks and polyclades — *i.e.*, with the hands of a watch. In annelids and mollusks, as I have pointed out (35, p. 454) the direction of the rotation stands in a definite relation to the unilateral origin of the mesoblast. In *Amphioxus* the mesoblast has a bilateral origin, and the direction of the rotation must be otherwise explained. The rotation itself might well be due to purely mechanical causes, but such causes leave its constancy of direction unexplained, and the possibility of its being an ancestral reminiscence must be held open.

#### PART IV.—REGENERATION AND THE MOSAIC THEORY OF DEVELOPMENT.

Two independent and nearly simultaneous attempts have recently been made to harmonize the facts of regeneration (or “postgeneration”) in animal embryos, with the so-called mosaic theory of development. The first is contained in Weismann’s remarkable work on the Germ-plasm (33); the second is by Roux (25), who has reached conclusions essentially in agreement with those of Weismann. Both these attempts are

worked out with extreme ingenuity, and especially Roux's admirably clear and pointed discussion has the merit of placing the whole question upon a well-defined basis for further investigation. Both, however, are based upon two leading assumptions, which are not only without foundation in observed fact, but are, as I believe, unnecessary for the interpretation of the known phenomena; and these assumptions I must briefly consider, since Roux has endeavored to interpret some of my own observations, as well as those of Driesch, in accordance with them.

The first assumption relates to the causes of histological differentiation. It is assumed that in normal development differentiation is primarily determined by the nature of cell-division, karyokinesis being conceived as qualitative in character in such wise that cells of different prospective value receive correspondingly different forms of idioplasm at the moment of their separation. Every cell, therefore, has an independent power of self-determination ("Selbstdifferenzierung") inherent in the structure of its idioplasm, and this in turn owes its character to the nature of the mitosis by which the cell-nucleus arose. The entire ontogeny is, therefore, compared by Roux to a mosaic-work; it is essentially a whole arising from a number of independent self-determining parts, though Roux admits that the self-determining power of the cell is capable in some measure of modification through interaction with its fellows.

The second of the Roux-Weismann assumptions is logically necessitated by the first in view of the phenomena of regeneration. Obviously these phenomena are inexplicable under a theory of strictly qualitative division. Both Weismann and Roux, therefore, assume that during cell-division each cell may receive, in addition to its specific form of idioplasm, a portion of unmodified idioplasm afforded by purely quantitative division. This unmodified idioplasm ("accessory idioplasm" of Weismann, or in some cases "germ-plasm"; "post-generation or regeneration idioplasm" of Roux), remains latent in normal development which is controlled by the active specific idioplasm. Injury to the ovum — *e. g.*, mechanical separation of the blastomeres — acts as a stimulus to the latent idioplasm, which

thereupon becomes active, and causes a repetition of the original development. By assuming a variable latent period following the stimulus, Roux is able to explain the fact that regeneration takes place at different periods in different animals.

Considered as a purely formal explanation, the Roux-Weismann hypothesis is perfectly logical and complete. Its weakness lies in its highly artificial character ; for both of its two fundamental postulates — viz : qualitative nuclear division, and accessory latent idioplasm — are purely imaginary. They are complicated assumptions in regard to phenomena of which we are really quite ignorant, and they lie at present beyond the reach not only of verification, but also of disproof. The “explanation” is, therefore, unreal ; it carries no conviction, and no real explanation will be possible until we possess more certain knowledge regarding the seat of the idioplasm (which is still an open question), and its internal composition and mode of action (which is wholly unknown). In the meantime we certainly are not bound to accept an artificial explanation like that of Roux, however logical and complete, unless it can be shown that the phenomena are not conceivable in any other way. I believe, however, that they are otherwise conceivable, and that by rejecting both of the Roux-Weismann postulates we can give, not indeed an explanation, but a simpler and more natural interpretation of the facts.

Among recent investigators Oscar Hertwig and Driesch have given the fullest and most explicit statement of such an interpretation, though essentially similar views have been held by a number of other writers. Hertwig (12, 14) like Kölliker argues with great force that karyokinetic division is not qualitative, but purely quantitative; that at every cell-division the daughter cells, whatever their prospective character, receive exactly equal kinds, as well as equal amounts, of nuclear material (regarded by both Hertwig and Roux as the seat of the idioplasm). With de Vries he regards differentiation as a result of *physiological* changes in the idioplasm, *subsequent to cell-division*, such that certain of the idioblastic units (variously known as idioblasts, pangens, biophores, etc.) remain latent,

while others become active and determine the specific form and activities of the cell. Finally, the physiological specialization of the idioplasm is brought about by the interaction of the cell with its fellows in the cell-complex, in accordance with the principle: "Die Theile eines Organismus entwickeln sich in Beziehung zu einander, oder die Entwicklung eines Theiles ist abhängig von der Entwicklung des Ganzen" (13, p. 480). Driesch is still more explicit (though he does not frame any hypothesis regarding the idioplasm), and his views form the opposite extreme to the mosaic theory. From his experiments on sea-urchin eggs, Driesch concludes that the ultimate fate of any particular blastomere is determined by its relative position in the mass; that is, to quote his own striking aphorism, "ihre prospective Bedeutung ist eine Funktion des Ortes" (*cf.* His. No. 16, p. 120). This conclusion, essentially in agreement with Hertwig's, rests upon a series of experiments so ingenious in conception, and brilliant in results, as to stand beside those of Roux as the mark of a new era in the history of embryology. The experiments on the alteration of cleavage-forms by pressure, temperature and other agents, taken in connection with the phenomena exhibited by isolated, or partially separated blastomeres, show that the embryo develops as a whole, as a unit, and demonstrate the truth of the principle urged by Whitman, Hertwig and others, that "the organism, as a whole, controls the formative processes going on in each part" (Whitman, 37, p. 48). I shall endeavor to interpret the leading facts in the development of *Amphioxus*, and some other forms, in accordance with this principle, and for the sake of drawing the issue clearly shall adopt the idioplasm hypothesis, leaving aside, however, as an open question, the problem of the exact seat of the idioplasm and its mode of specialization.

There is no escape from the conclusion that in *Amphioxus* as in *Echinus*, the prospective value of the blastomeres of the 2-celled or 4-celled stage is a "function of the location." It is a statement not of theory but of fact that the mode of development of each blastomere in these stages *is determined by its relation to its fellows*, for the mode of development

immediately changes if the blastomere be isolated. Thus at the third normal cleavage of *Amphioxus* (first unequal division) each blastomere divides unequally *because it is one of four that have a certain relation to one another*; for if one of them be separated from its fellows of the 4-celled stage it invariably divides equally and not unequally.<sup>1</sup> In this case, therefore, it must be the interaction of the cells that determines their specific form of development, whether the nature of the interaction be mechanical or physiological. In terms of the idioplasm hypothesis, the idioplasm of the first two or four blastomeres of *Amphioxus* must be fundamentally of the same character, and the nature of its ensuing activity in each blastomere depends on circumstances—that is, on the relation of the cell to its fellows. This, however, is only to say that *the activity of the idioplasm is determined by cellular interaction*, as Oscar Hertwig maintains. In this case the Weismann-Roux assumption of qualitative division does not harmonize with the observed facts, while that of accessory idioplasm is perfectly gratuitous and unnecessary. And if this is true of any one stage of the ontogeny a very strong presumption is created that it is true of all.

The most significant fact in the development of isolated blastomeres of *Amphioxus* is that up to the 8-celled stage *their power of development progressively diminishes as the cleavage advances*. (a) The  $\frac{1}{2}$  blastomere often segments like a normal ovum, but not seldom shows variations some of which are reminiscent of the normal cleavage-half. It gives rise to a normally formed blastula and gastrula, which may develop into a perfectly formed half sized dwarf larva having all of the essential characteristics of the adult. (b) The  $\frac{1}{4}$  blastomere may likewise segment nearly or quite like a normal egg, but in general varies more from the normal type than the  $\frac{1}{2}$  blastomere. A perfect gastrula may be formed, but the development rarely continues long beyond the closure of the blastopore and then gives rise only to abnormal or defective

<sup>1</sup> The inequality in the normal cleavage cannot be due to an unequal distribution of yolk caused by gravity, for, as in *Echinus*, the segmentation planes show no constant relation to the vertical.

larvæ. In only a single isolated case did one of these larva develop as far as the latest stage reached by the  $\frac{1}{2}$  larva, and this individual showed several defects. (c) The  $\frac{1}{8}$  blastomere segments in a form suggesting that of the normal ovum but never identical with it. The gastrula stage is never reached and only in comparatively rare cases is a blastula formed. Most individuals develop approximately as they would if still forming part of an entire embryo and give rise to partial embryos—*i.e.*, those that correspond in a measure to fragments of an entire embryo. The same is true of the  $\frac{1}{16}$  blastomere.

It might be supposed that these progressive limitations are due simply to the progressive diminution in the size of the individual blastomeres. There are, however, two considerations that seem to render this explanation inadequate. The first is the occasional production of  $\frac{1}{8}$ -sized gastrulas from (? fragments of) 2-celled or 4-celled stages (p. 589). The second is the progressive change in the *form* of cleavage (*e.g.*, the inequality of the second cleavage of isolated  $\frac{1}{8}$  blastomeres), which does not seem explicable as the result either of diminished size or of alteration in contour, since all of the isolated blastomeres become nearly spherical before their first division. It seems to me, therefore, that this gradual diminution of developmental and regenerative power in the individual blastomeres as the cleavage advances, together with their increasing departure from the forms of cleavage shown by the entire ovum, must be due to their progressive differentiation and the relation of this process to regeneration may be conceived as follows, in accordance with Hertwig's general view. As the ontogeny advances the idioplasm of the cells undergoes gradual and progressive *physiological* modifications (brought about by the interaction of the various parts of the embryo), without, however, anywhere losing any of its elements. The isolation of a blastomere restores it in a measure to the condition of the original ovum and the idioplasm, therefore, tends to return to the condition of the original germ-plasm and thus to cause a repetition of the development from the beginning. The manner in which the blastomere responds to this change of environment depends, however, upon two conditions; first,

upon the extent to which its idioplasm has become modified; second, (as Driesch has suggested) upon new mechanical conditions due to the diminution of size or change of form, such as greater surface-tensions caused by diminished radius of curvature, and the like. In the 2-celled stage the modification of the idioplasm is in *Amphioxus* very slight, and the isolated blastomere generally reverts at once to the condition of the original ovum, *though sometimes varying from it to some degree*. In the 4-celled stage the idioplasm is further modified, though not to such a degree as to prevent its return to the original condition. By the 8-celled stage it is incapable of returning to the original state, and the normal type of cleavage is no longer repeated; and so in a still greater degree in later stages. We can thus understand how it comes to pass that the differentiated germ-layers of later stages lose in general their power to regenerate the other germ-layers. The specialization of the idioplasm, like that of the cell as a whole, appears to be a cumulative process that results in a more and more fixed mode of action. Hence its gradual loss of power to return under changed conditions to the state of original germ-plasm, though it may contain all of the elements of germ-plasm. The independent, self-determining power of the cell ("Selbstdifferenzierung" of Roux), therefore, steadily increases as the cleavage advances. In other words: *the ontogeny assumes more and more of the character of a mosaic-work as it goes forwards. In the earlier stages the morphological value of a cell may be determined by its location. In later stages this is less strictly true and in the end the cell may become more or less completely independent of its location, its substance having become finally and permanently changed*. The power of regeneration, often existing in late stages or in the adult, may be regarded as a special adaptation such that the idioplasm in some of the cells retains in a greater or less degree the plasticity of earlier stages — why or how, it is at present impossible to say further than that the power to do so is in some way the outcome of a specific peculiarity of the original germ-plasm.

How, now, shall we apply this interpretation to the case of *Echinus*, in which the isolated  $\frac{1}{2}$  blastomere pursues for a

short time a half-development, although no other half exists; or to that of the frog and ctenophore, in which the half-development continues up to a very late period? Roux endeavors to explain these facts by assuming that the separation of the blastomeres acts as a stimulus to the inactive accessory idioplasm; that the latent period following the stimulus varies; and that upon the duration of the latent period depends the time at which regeneration takes place. Roux is logically compelled to assume the existence of accessory idioplasm by committing himself in advance to the theory of qualitative nuclear division. If, however, we discard this theory, the assumption of an accessory idioplasm is as unnecessary in the case of *Echinus* as in that of *Amphioxus*. It is only a statement of fact to say that in *Echinus* the blastomeres of the 2-celled stage have become in some way more highly modified than in *Amphioxus*, — this is proved by their behavior when isolated, — and this initial modification influences the subsequent divisions for some time. If we conceive this modification to affect the idioplasm, we may say that the stimulus of the operation is not in this case sufficient to cause an immediate return of the idioplasm to its original state, and its normal equilibrium is only gradually restored through subsequent cellular interaction. This is admittedly not an explanation but only a re-statement of the fact. But it is at present inexplicable under any theory why the reaction of the blastomeres of the 2-celled stage should differ in the two cases; just as it is inexplicable why the two animals should differ in any other respect. We can only say that the difference depends on a difference of organization which, in turn, ultimately depends on the nature of the original germ plasm. The character of any reaction is determined no less by the nature of the body stimulated than by the nature of the stimulus.<sup>1</sup>

<sup>1</sup> The fact (p. 593) that displaced  $\frac{1}{2}$  blastomeres of *Amphioxus* sometimes show in their mode of cleavage reminiscences of the normal (entire) development may be taken to indicate that the extent of modification in the 2-celled stage varies in different individuals. Whether the idioplasm shall at once completely revert to the original form or not may depend on the extent of this change, and this in turn on the time that has elapsed between the formation of the blastomeres and their

Let us attempt finally to apply this general conception to the highly differentiated types of cleavage, such as we find, for example, in the annelids. These forms have been practically ignored by Hertwig and Driesch. Neither of these authors has given sufficient attention to the fact that *the period of differentiation varies in different cases*, and both have been thus led, as I believe, to a one-sided and premature judgment of the mosaic theory.

In *Amphioxus* the first visible differentiation takes place at the third cleavage, in *Polygordius* and *Echinus* at the fourth, in *Synapta* at a much later period. In the tunicate *Clavelina* it occurs at the second cleavage, and in many annelids at the first. In *Nereis*, for example, which is the best-known form, the first division of the egg is unequal and the two resulting cells not only differ markedly in size, but have a wholly different prospective morphological value. The larger blastomere gives rise to the entire mesoblast, to the germ-cells, the head-kidneys, the ventral nerve-cord and the setæ; the smaller produces only a portion of the head and of the alimentary epithelium. Let us contrast *Amphioxus* and *Nereis* in this regard. In *Amphioxus*, as in the echinoderm, differentiation advances very slowly. Not until the actual invagination takes place can the limit between the primary germ-layers be fixed, and the mesoblast arises still later. In *Nereis* the median plane is marked out at the second cleavage; at the third the entire ectoblast of the trochal and præ-trochal regions is formed; at the fourth cleavage the material for the entire "ventral plate" (including the ventral nerve-cord and the seta-sacs) is segregated in a single cell, that for the stomodæum in three cells; the fifth cleavage completes the ectoblast, and by the 38-celled stage the germ-layers are completely segregated (the mesoblast in a single cell) and the architecture of the embryo is fully outlined in the arrangement of the parent blastomeres, or protoblasts.

In such a case as *Nereis* we cannot accept Oscar Hertwig's implication that the relation between the individual blastomeres

*artificial separation*. It would be very interesting to compare the cleavage of a blastomere isolated immediately upon completion of the first cleavage with that of one isolated just before the second cleavage. Much light might thus be thrown on the general question of cellular interaction.

and the adult structures to which they give rise is a purely casual one. ["In Folge der Kontinuität der Entwicklung muss ja natürlicherweise jede ältere Zellengruppe sich auf eine vorausgegangene jüngere Gruppe, und so schliesslich bestimmte Körpertheile auf bestimmte Furchungszellen zurückführen lassen"] (13, p. 479). For the blastomeres are differentiated, in size at least, from the first moment of their formation. The development is here a visible mosaic-work, not one ideally conceived by a mental projection of the adult characteristics back upon the cleavage stages. The principle of "organbildende Keimbezirke" has here a real meaning and value, and this would remain true even if it should hereafter be shown that both of the first two blastomeres of *Nereis*, if isolated, could produce a perfect embryo. How then shall we reconcile this case with that of *Amphioxus*? The answer to this question becomes comparatively simple if we regard the ontogeny as a connected series of interactions between the blastomeres in which each step conditions that which succeeds. The character of the whole series depends upon the first step, and this in turn upon the constitution of the original ovum. In *Nereis* the mosaic-like character appears from the beginning *because of the inequality of the first cleavage*, and this conditions the entire subsequent development through the peculiar inter-relations established by it. The cause of the inequality must lie in the undivided ovum, and it seems to me must in the last analysis be sought in the constitution of the original germ-plasm. It might be objected that the inequality may depend simply on an unequal horizontal distribution of yolk, and not upon the idioplasm. As a matter of fact no such inequality of distribution is visible in the actual ovum (an extraordinarily favorable object for examination); but waiving this rejoinder, the objection only shifts the question further back. If such inequalities exist they must be determined by a definite cause, since the size-ratio of the first two blastomeres does not perceptibly vary and we are in the end thrown back upon the germ-plasm in every attempt to find this cause. Very numerous facts support Roux's conclusion that the form of division in cells is controlled by internal as well as external factors; and,

if we accept the idioplasm hypothesis at all, we must accept the further conclusion that the internal factors are to be sought in the nature of the idioplasm. I conceive accordingly that the first cleavage of *Nereis* differs from that of *Amphioxus* because of an original difference of germ-plasm in the two ova, and the effects of this initial difference come more and more clearly into view as the cleavage proceeds.

So it is with any ontogeny. The entire series of events is primarily determined by the organization of the undivided ovum that forms its first term, and, as such, conditions every succeeding term. The morphological value of the individual blastomere at any particular stage is the product of two factors, one of which (the embryonic environment) is external, while the other (the nature of the idioplasm) is internal. Only under such a view can we find some understanding of the remarkable fact, on the importance of which I have elsewhere insisted (35, p. 441) that, in cleavage-forms that are identical up to a comparatively late stage, blastomeres may exactly correspond in position, mode of origin, and embryonic environment, and yet be of entirely different morphological value; and it is in this sense that we may regard cleavage-forms as controlled by a definite hereditary element apart from purely mechanical conditions (p. 602).

I cannot accept Driesch's conclusion that his experiments definitely overturn the principle of "organbildende Keimbezirke"; they prove only that this principle does not extend to the unsegmented ovum, or to the early stages of *Echinus*, not that the principle is devoid of reality or value. Roux, I believe, hits the mark when he says in his latest paper (published after my preliminary paper on *Amphioxus*): "Das Prinzip der organbildenden Keimbezirke beginnt somit erst mit der Furchung eine feste Bedeutung zu erhalten; und diese seine causale und topographische Bedeutung wird mit dem Fortschreiten der Furchung eine immer speziellere" (25, p. 310). My studies on *Nereis* and *Amphioxus* had led me independently to the same conclusion (*cf.* p. 610; see also No. 35), though under a fundamentally different conception of the nature of differentiation. To this, however, we must add the

further qualification, that the mosaic-like character of the ontogeny emerges from the indifferent condition of the early stages *at different periods in different animals*, and in many cases appears more or less distinctly from the beginning. We are thus enabled, in a measure, to reconcile the apparently conflicting results of Roux on the one hand, and those of Hertwig and Driesch on the other. It is true that no middle ground is possible in the question of qualitative *versus* quantitative division; but it is otherwise with the external phenomena of cleavage. I have endeavored to show that the phenomena of regeneration are not incompatible with a modified form of the mosaic theory, in which the hypothesis of qualitative division is repudiated. Thus modified, the mosaic theory is of the utmost importance, and is destined, I believe, to form the basis of all exact and thorough investigations on animal ontogeny.

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NEW YORK, April 10, 1893.

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## EXPLANATION OF PLATES.

[Unless otherwise indicated all of the segmentation stages are placed with the plane of first cleavage vertical to the page. Macromeres are designated throughout by capital letters, micromeres by small letters, cleavage planes by Roman numerals.]

*A, B, C, D.* The first four blastomeres and later the primary macromeres.

*A,<sup>2</sup> B,<sup>2</sup> C,<sup>2</sup> D.<sup>2</sup>* The secondary macromeres, which correspond in time of origin to the "second group of micromeres" in annelids and mollusks.

*a,<sup>1</sup> b,<sup>1</sup> c,<sup>1</sup> d.<sup>1</sup>* First group of micromeres.

*a,<sup>3</sup> b,<sup>3</sup> c,<sup>3</sup> d.<sup>3</sup>*  
*a,<sup>2.1</sup> b,<sup>2.1</sup> c,<sup>2.1</sup> d.<sup>2.1</sup>* } Second group of micromeres.

*a<sup>4</sup>-d.<sup>4</sup>*  
*a<sup>2.2</sup>-d.<sup>2.2</sup>* } Third group of micromeres.

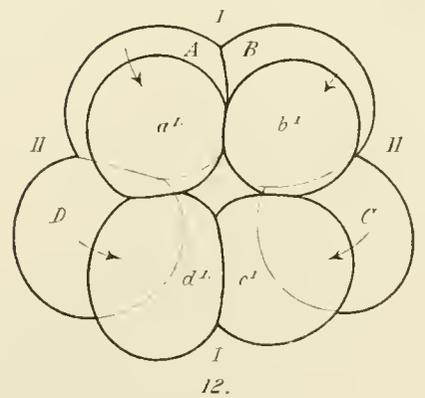
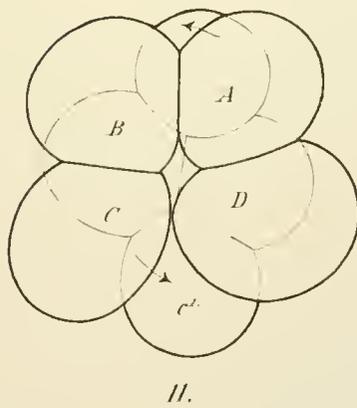
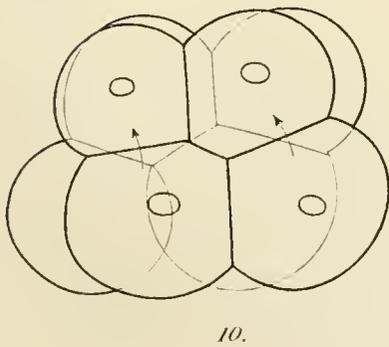
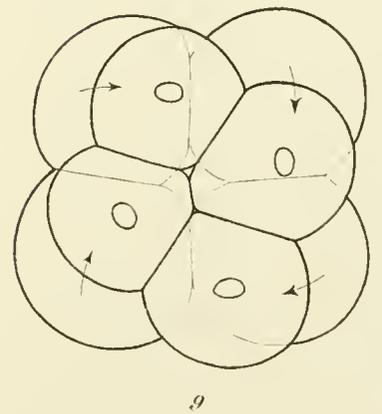
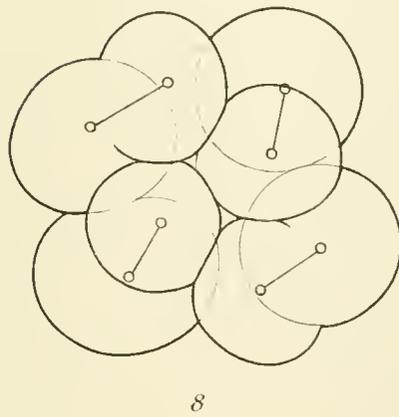
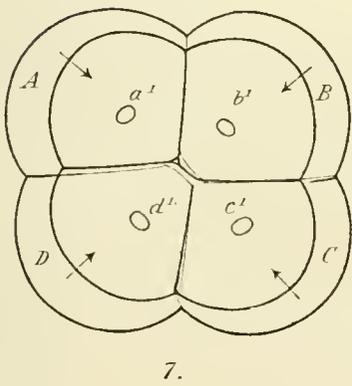
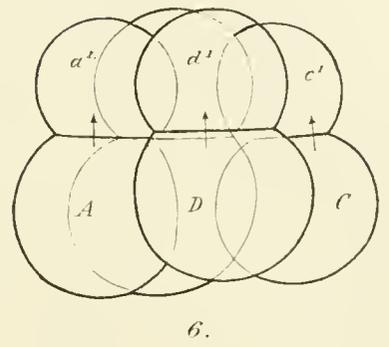
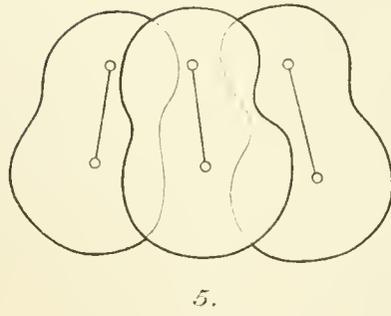
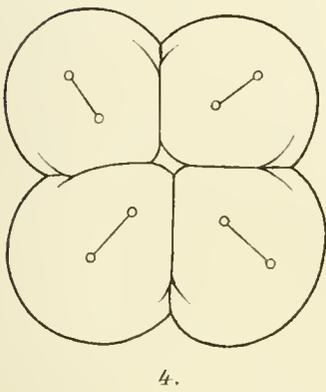
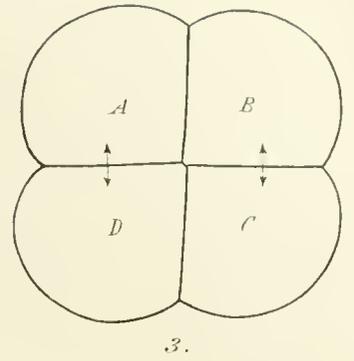
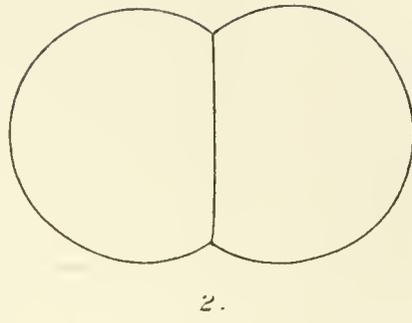
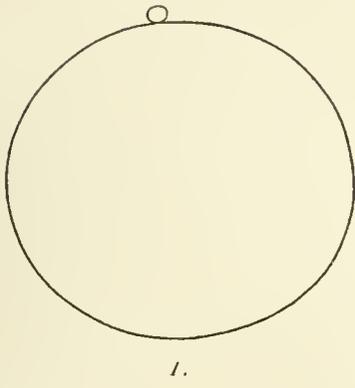
*a<sup>5</sup>-d.<sup>5</sup>*  
*a<sup>2.3</sup>-d.<sup>2.3</sup>* } Fourth group of micromeres.



## PLATE XXIX.

[Figs. 1-3, from living specimens ; the others from preparations ; 270 diameters.]

- FIGS. 1-3. First and second cleavages.  
FIGS. 4-7. Third cleavage ; pure radial form.  
FIGS. 8-10. The same ; spiral form.  
FIG. 11. Mixed form ; from lower pole.  
FIG. 12. Bilateral form.







## PLATE XXX.

[Figs. 13 to 18, from a living specimen; the others from preparations; 270 diameters.]

FIGS. 13 to 18. Successive stages of a living embryo, viewed from the lower pole, followed from the undivided egg up to the 128-celled stage. Strict bilateral form *II*. The straight lines represent as nearly as could be determined the axis of the spindles in the stage following each figure.

FIG. 13. 8-celled stage; 14, fourth cleavage; 15, 16-celled stage (cf. Figs. 21, 27); 16, 32-celled stage.

FIG. 17. 64-celled stage. Note the extreme lateral position of  $C^2$ ,  $D^2$  and the equality of  $D$  and  $d^4$ . First appearance of cross-furrow by displacement.

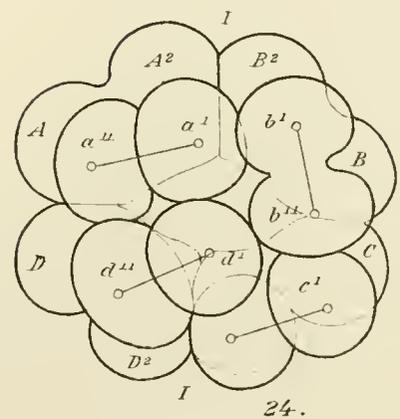
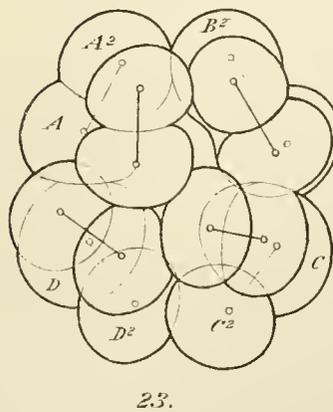
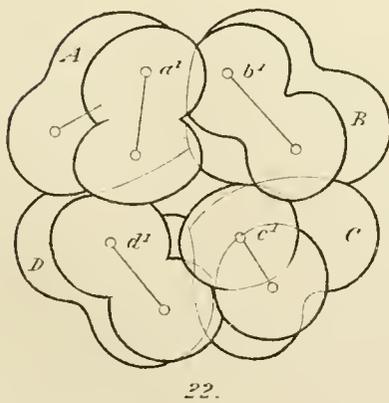
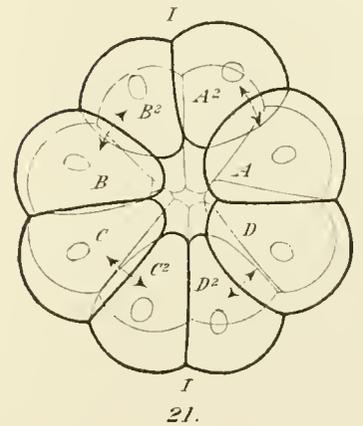
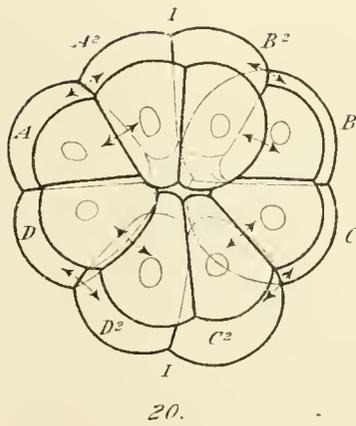
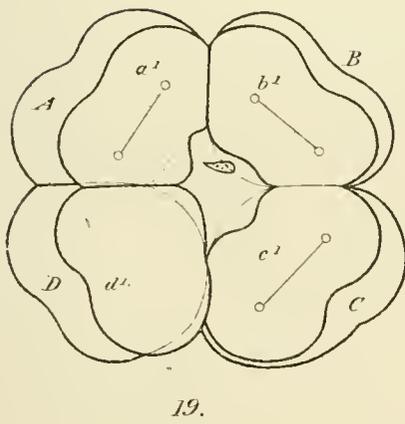
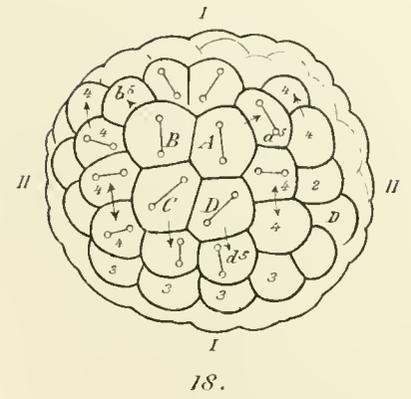
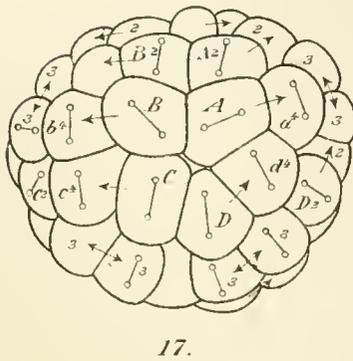
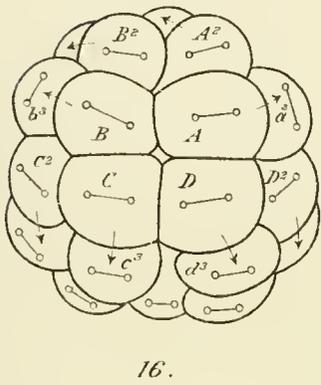
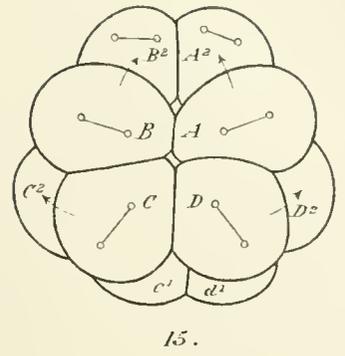
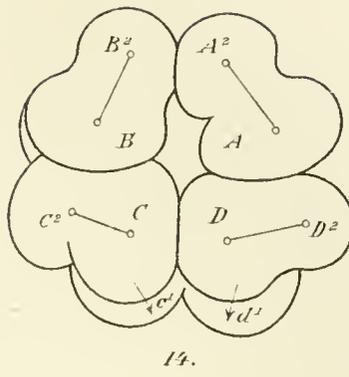
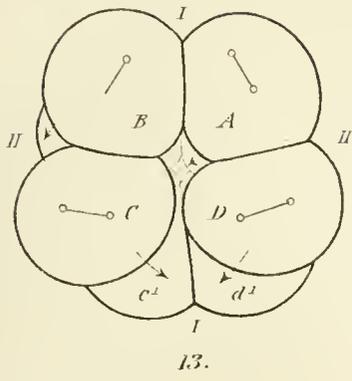
FIG. 18. 128-celled stage. Restoration of bilateral symmetry. Cross-furrow.

FIG. 19. Fourth cleavage, radial form, upper pole.

FIG. 20. 16-celled radial form, upper pole.

FIG. 21. The same embryo from the lower pole.

FIGS. 22 to 24. Three examples of modified bilateral forms, fourth cleavage.







## PLATE XXXI.

[All from preparations ; 270 diameters.]

FIG. 25. Fourth cleavage, strict bilateral form *I*, upper pole.

FIGS. 26-28. 16-celled stage, bilateral form *I*; the same specimen shown from upper pole, lower pole, and from the side.

FIG. 29. The same specimen as Fig. 22, from lower pole, showing cleavage-pore.

FIG. 30. Bilateral form *I*, from lower pole ; large cleavage-pore.

FIGS. 31-32. Strict spiral form, 16-celled stage ; the same specimen from opposite poles. Cell-connections hypothetical.

FIGS. 33-34. Fifth cleavage, nearly pure bilateral form *I*; 33 from lower pole, 34 from side. Origin of second group of micromeres.

FIGS. 35-36. Fifth cleavage, bilateral form *I*; 35 from side, 36 from upper pole. Note asymmetry of spindle in *x*.



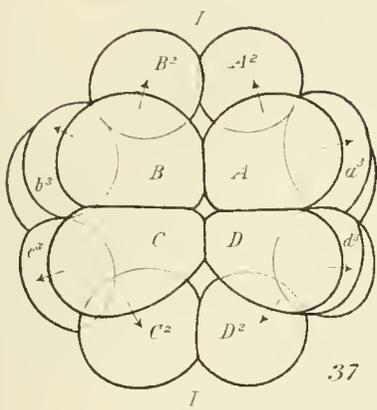




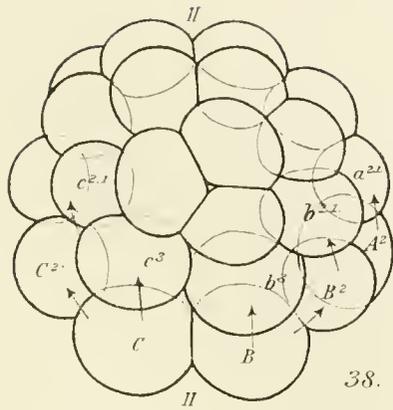
## PLATE XXXII.

[All from preparations ; 270 diameters.]

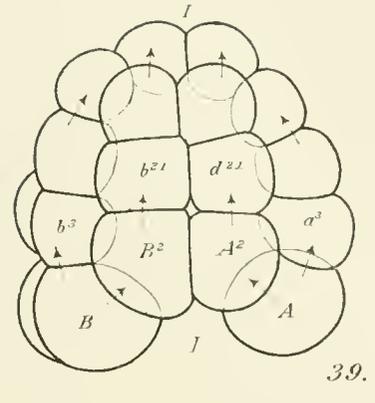
- FIG. 37. 32-celled stage, bilateral form *I*, lower pole.  
FIG. 38. The same specimen from the side.  
FIG. 39. 32-celled stage, strict bilateral form *I*, from one end. [Compare Hatschek, Taf. I, Fig. 10.]  
FIG. 40. 32-celled stage, slightly modified radial form, lower pole.  
FIG. 41. Sixth cleavage, bilateral form *I*, viewed obliquely from below. Origin of third group of micromeres.  
FIG. 42. The same specimen from the side. Note complete irregularity of the cleavage-planes in the micromeres.  
FIG. 43. 64-celled stage, from the side.  
FIG. 44. The same specimen from lower pole. Slightly modified bilateral form *I*.  
FIG. 45. 128-celled stage, bilateral form *I*, from lower pole, showing fourth group of micromeres.  
FIG. 46. 256-celled stage, from lower pole, mixed form. Designations of the macromeres arbitrary.  
FIG. 47. Seventh cleavage, bilateral form *I*, from the side, showing origin of fourth group of micromeres.



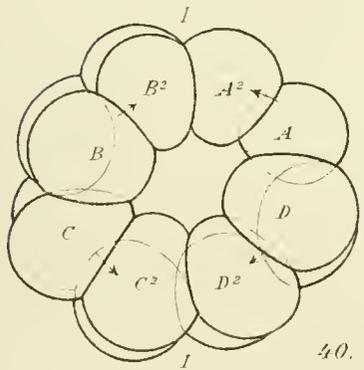
37



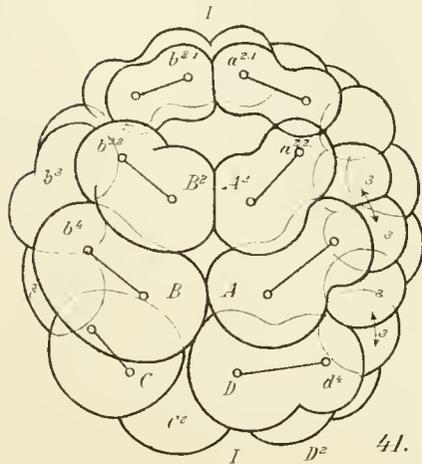
38.



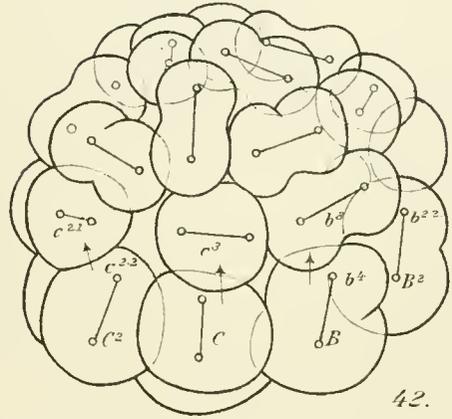
39.



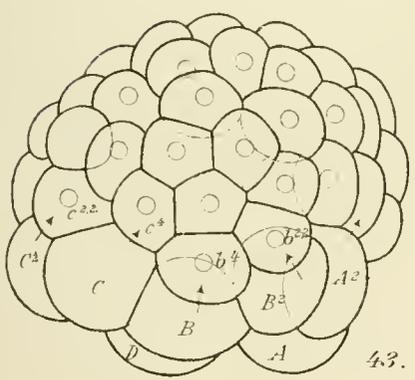
40.



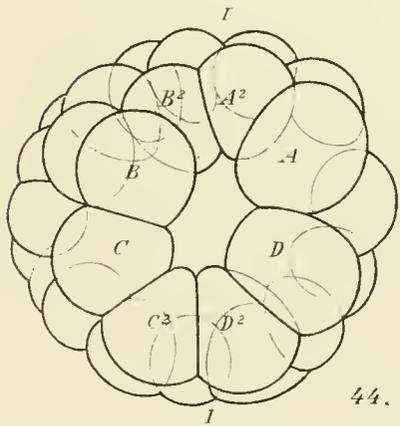
41.



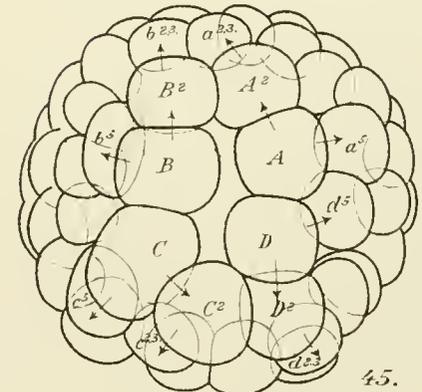
42.



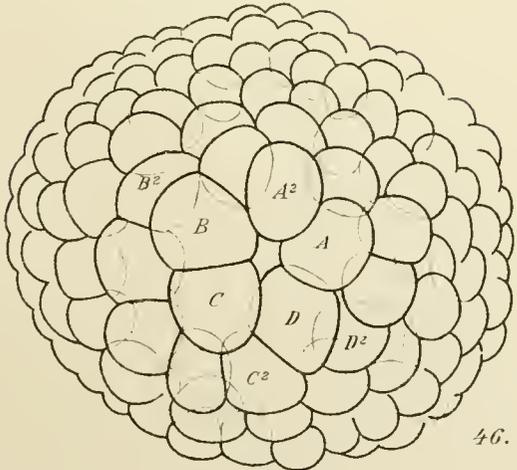
43.



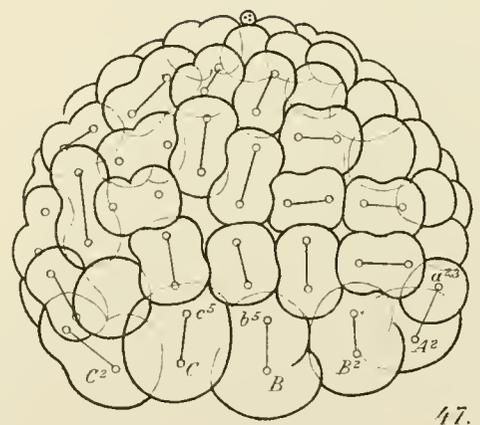
44.



45.



46.



47.





## PLATE XXXIII.

[Figs. 50-53 from life; the others from preparations. Figs. 48, 49  $\times 300$ ; the others  $\times 270$ .]

FIG. 48. Early flattened blastula ( $\pm 512$  cells) from the lower pole, showing the last trace of bilaterality. Lettering only for comparison with next figure.

FIG. 49. The same from the side, in optical section (showing also the lateral cells).

FIG. 50. Normal gastrula, 7 hours.

FIG. 51. From shaken 2- and 4-celled stages. One of two separate twins within the same membrane. From the same spawning as the last.

FIG. 52. Fourth-sized gastrula. With the last.

FIG. 53. Two  $\frac{1}{8}$  sized gastrulas. With the last.

FIG. 54. Fourth-sized gastrula ( $\frac{2}{8}$  embryo?). From shaken 8-celled stage. 8 hours.

FIG. 55. Half-sized gastrula ( $\frac{2}{4}$  embryo). From shaken 4-celled stage. 7 hours.

FIG. 56. Half-sized gastrula ( $\frac{4}{8}$  embryo?). From the same preparation as Fig. 54.

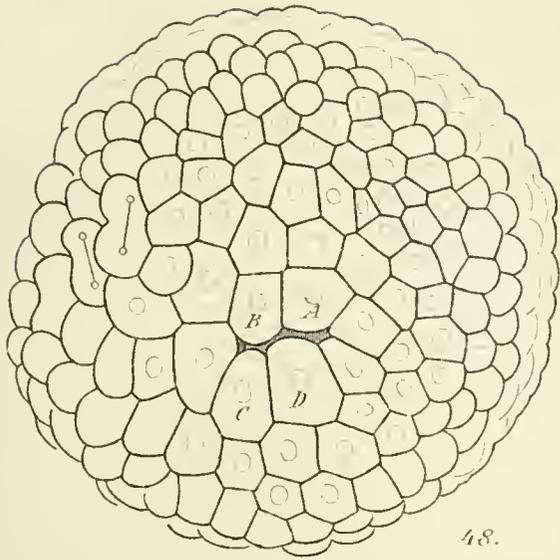
FIG. 57. Gastrula intermediate in size between the normal and the  $\frac{1}{2}$  gastrula (*cf.* Fig. 63 for normal size at this stage). With the last.

FIG. 58. Fourth-sized gastrula ( $\frac{1}{4}$  embryo). From same preparation as Fig. 55.

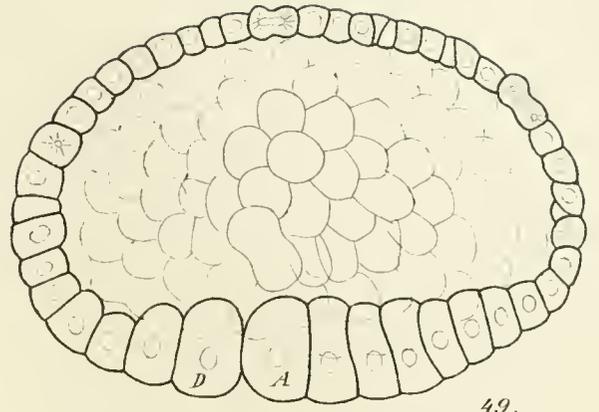
FIG. 59. Unilateral development. Half-sized gastrula, from shaken 2-celled stage, attached to the other undivided  $\frac{1}{2}$  blastomere. Membrane intact; 8 hours.

FIG. 60. From shaken 2- and 4-celled stages, 7 hours. Attached to a gastrula of rather more than  $\frac{1}{4}$  the normal size is a gastrula (?) about  $\frac{1}{8}$  the normal.

FIGS. 61-62. Two triple gastrulas (membranes intact), 7 hours. From shaken 4-celled stage.



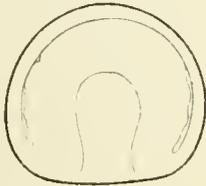
48.



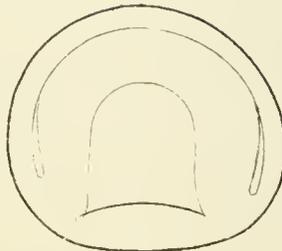
49.



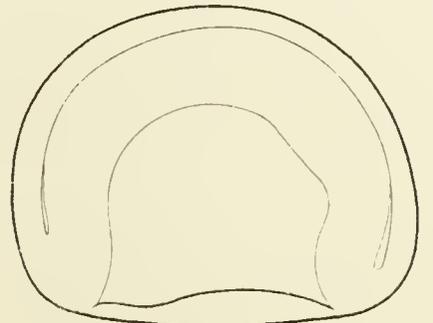
53.



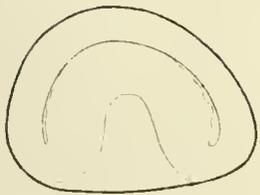
52.



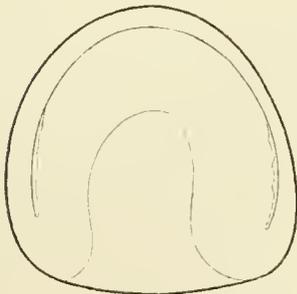
51.



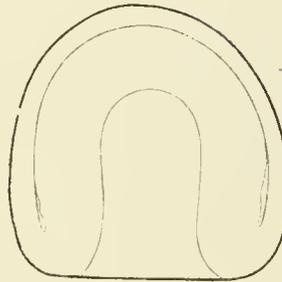
50.



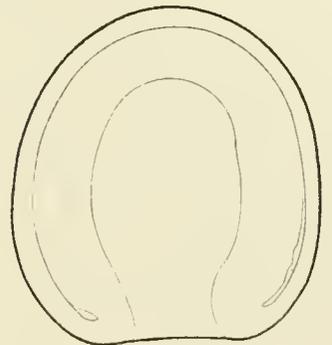
54.



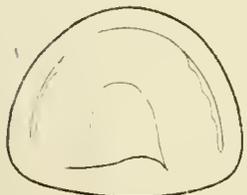
55.



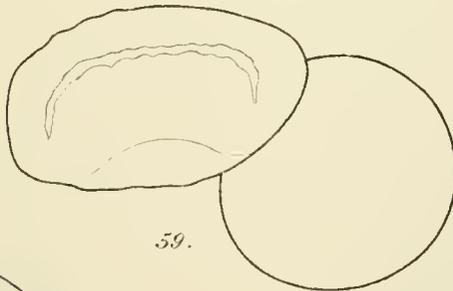
56.



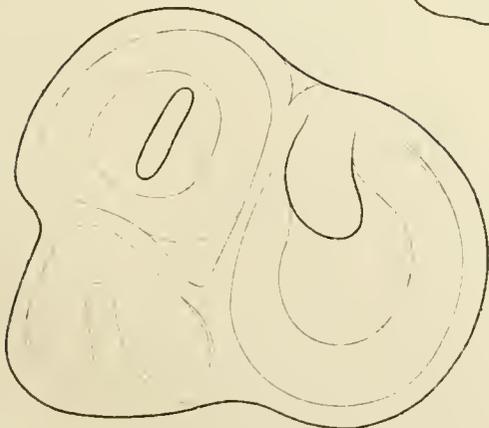
57.



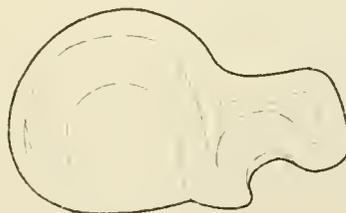
58.



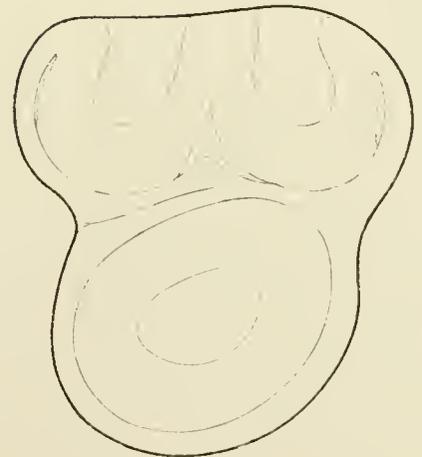
59.



61.



60.



62.





## PLATE XXXIV.

[All from preparations; 270 diameters.] A series of gastrulas, derived from shaken 2-celled stages, 7 hours. All from the same spawning.

FIG. 63. Normal gastrua.

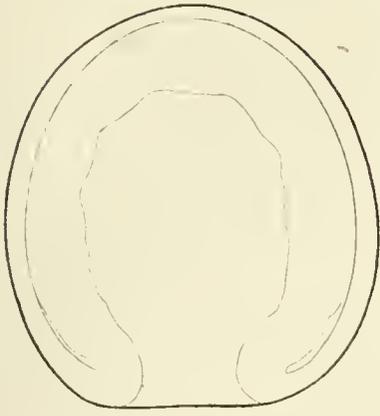
FIGS. 64 to 68. Series leading from slightly expanded form to true double gastrulas.

FIG. 69. Two separate twins, within one membrane.

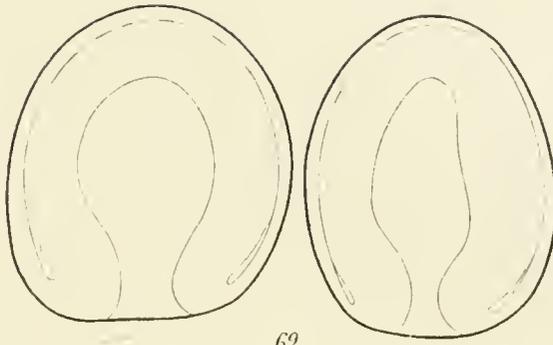
FIGS. 70 to 74. Show various relations of the axes and blastopores.

FIG. 75. In this case the two archentera are not separated by a layer of ectoblast (*cf.* Fig. 72).

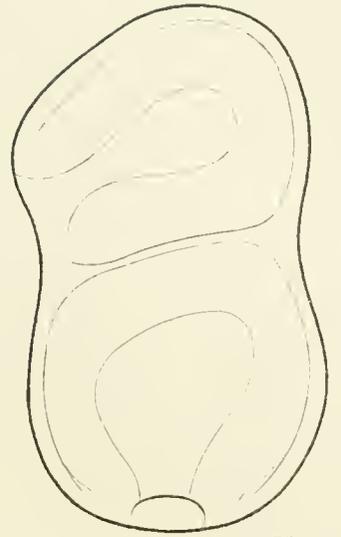
FIG. 76. One of the bodies fits like a cap upon one side of the other.



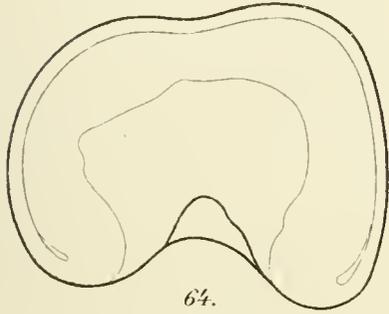
63.



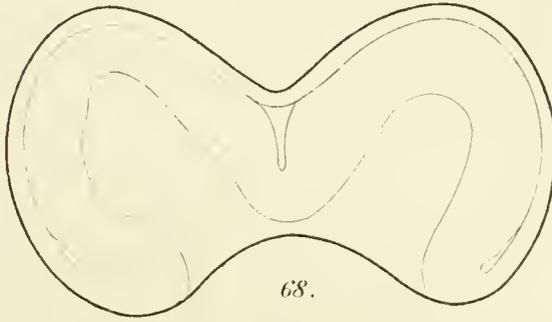
69.



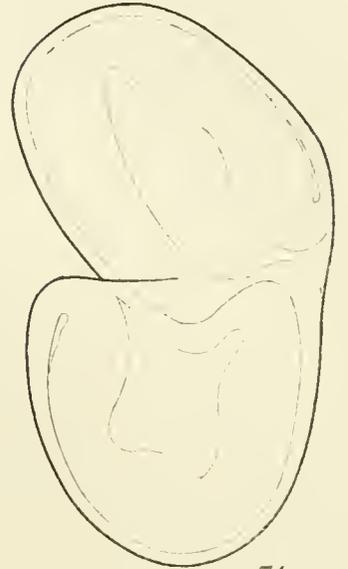
73.



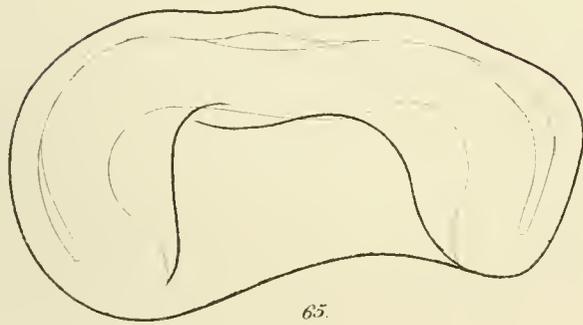
64.



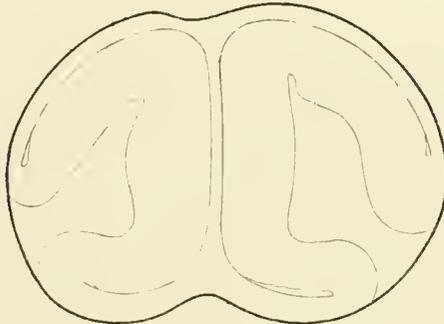
68.



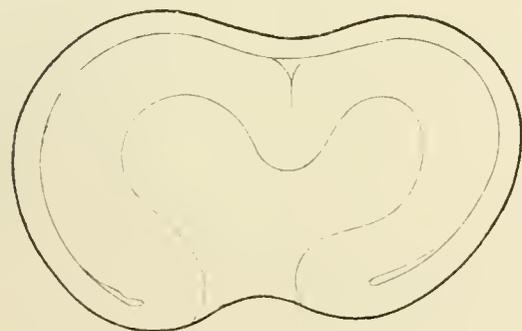
74.



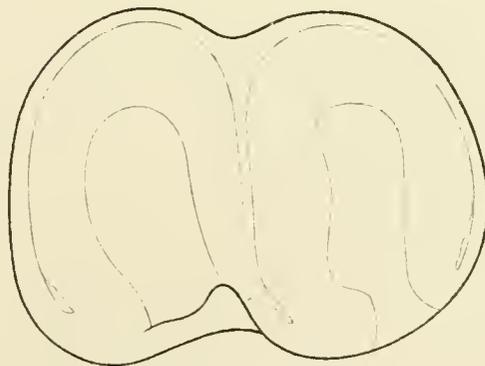
65.



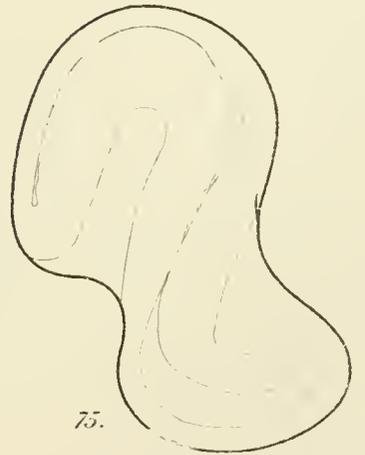
70.



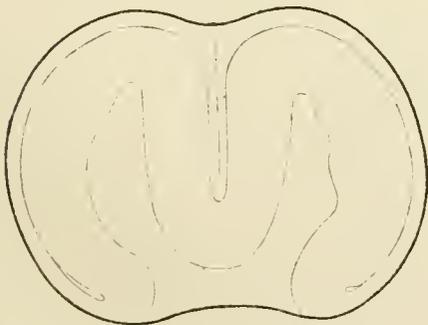
66.



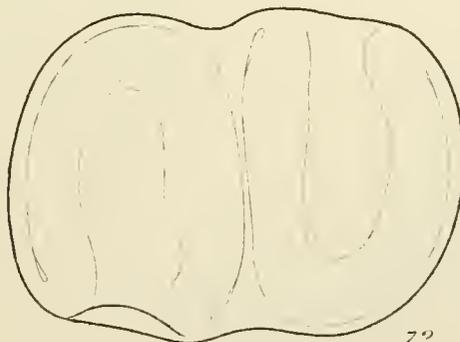
71.



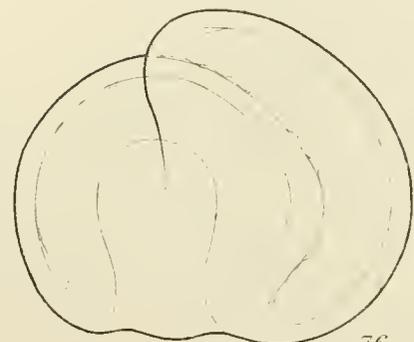
75.



67.



72.



76.





## PLATE XXXV.

[Fig. 77 to 95, from life ; the others from preparations ; 270 diameters.]

FIGS. 77 to 81. Cleavage of an isolated  $\frac{1}{2}$  blastomere up to the 8-celled stage, observed in one position. Fig. 78 shows the nearest approach to the spherical form. Identical with normal form of cleavage.

FIGS. 82 to 86. Cleavage of  $\frac{2}{4}$  embryo (a pair of blastomeres from 4-celled stage). Slight inequality in the 4-celled stage. Fig. 85 represents another specimen showing perfect equality in the 4-celled stage.

FIGS. 87 to 91. Cleavage of isolated  $\frac{1}{4}$  blastomere, exactly corresponding with that of the  $\frac{1}{2}$  blastomere shown in Figs. 77-81.

FIG. 92. Unequal 4-celled stage of  $\frac{1}{4}$  blastomere ; *cf.* Fig. 90.

FIG. 93. The same individual as Fig. 91 ; 16-celled stage. A large cleavage-pore on the lower side.

FIG. 94. Another specimen like the last (16-celled stage,  $\frac{1}{4}$  embryo), followed from the beginning ; the 4-celled stage was equal.

FIG. 95. The same specimen later ; 32-celled stage. Large cleavage-pore on the lower side.

FIG. 96. Another specimen (32-celled  $\frac{1}{4}$  embryo). Cleavage-pore very small.

FIG. 97. 16-celled stage from isolated  $\frac{1}{2}$  blastomere, from the side.

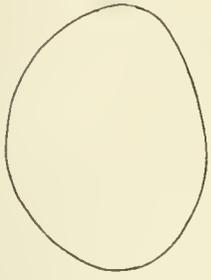
FIG. 98. The same, nearly from lower pole. No cleavage-pore.

FIG. 99. From isolated  $\frac{1}{2}$  blastomere. Transition from 16 to 32-celled stage. From lower pole. No pore. Bilaterality slightly indicated.

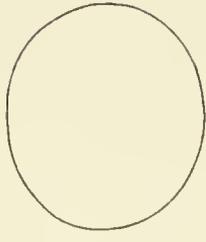
FIG. 100. From isolated  $\frac{1}{2}$  blastomere ; 32 to 64-celled stage. Attached to it is a blastula of about  $\frac{1}{8}$  the normal size ( $\pm 24$  cells). No membrane.

FIG. 101. Unilateral development. Half-sized 4-celled stage (equal), attached to the other undivided  $\frac{1}{2}$  blastomere. Membrane intact.

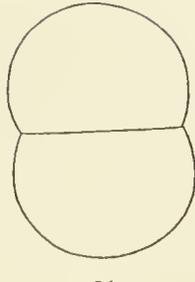
FIG. 102. Unilateral development. Half-sized blastula ( $\pm 128$  cells), with undivided  $\frac{1}{2}$  blastomere. Membrane intact.



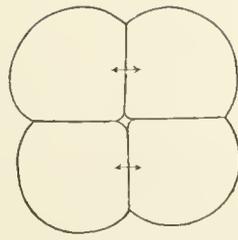
77.



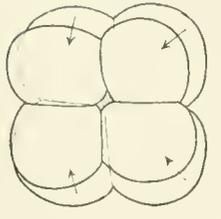
78.



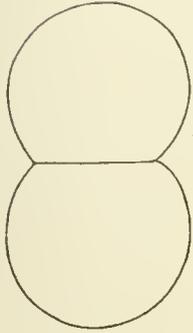
79.



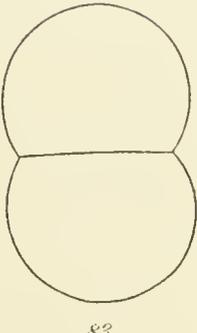
80.



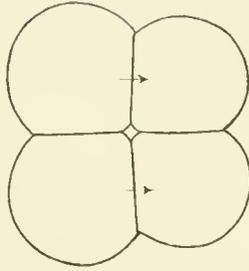
81.



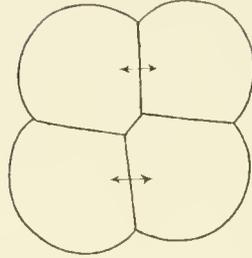
82.



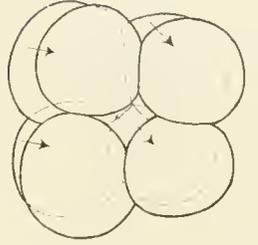
83.



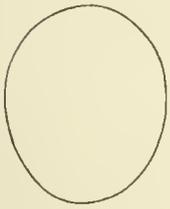
84.



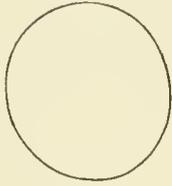
85.



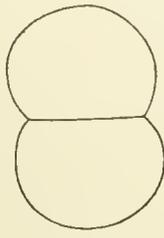
86.



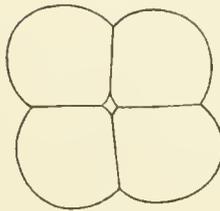
87.



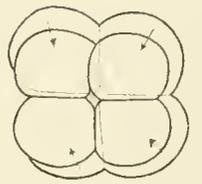
88.



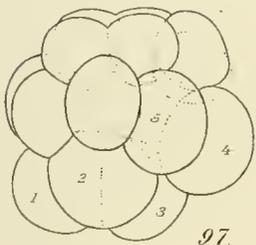
89.



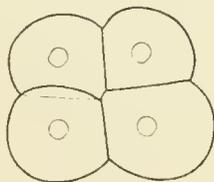
90.



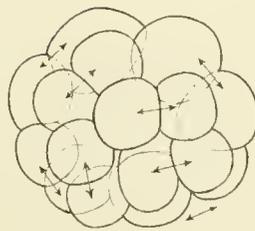
91.



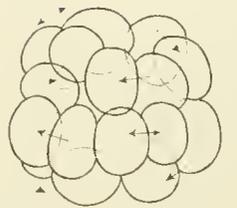
97.



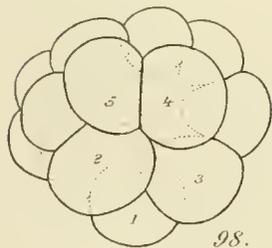
92.



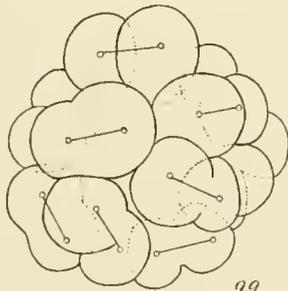
94.



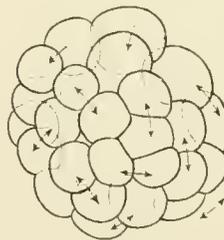
93.



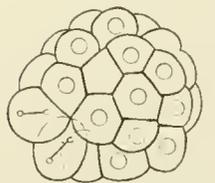
98.



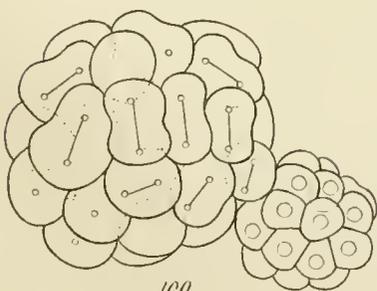
99.



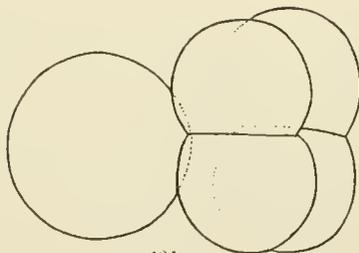
95.



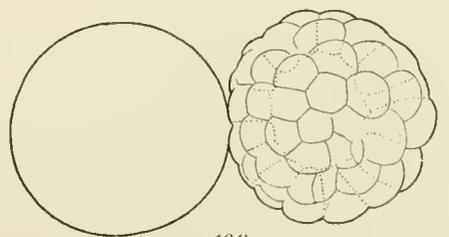
96.



100.



101.



102.





## PLATE XXXVI.

[All from preparations and the cell-connections hypothetical, except in Figs. 113, 114; 350 diameters.]

FIG. 103. Half-sized 8-celled stage, from isolated  $\frac{1}{2}$  blastomere, exactly equal type. The other twin (enclosed in the same membrane) is unequal, like Fig. 104.

FIGS. 104, 105. Half-sized 8-celled stages, with last. Unequal type. Fig. 105 shows spindles of following division.

FIG. 106. Double 2-celled stage, showing the effects of displacement in the single 2-celled stage.

FIG. 107. Double 2-celled stage (in division). Axes of the twin embryos at right angles.

FIG. 108. Double 4-celled stage, transitional form. The left side, as in the normal entire ovum; the right side shows the double form.

FIG. 109. Double 4-celled form with slight obliquity of the axes.

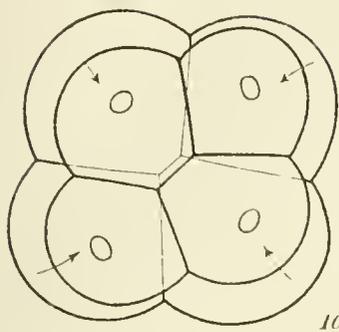
FIG. 110. Double 4-celled form with parallel axes.

FIG. 111. Double 8-celled form with oblique axes.

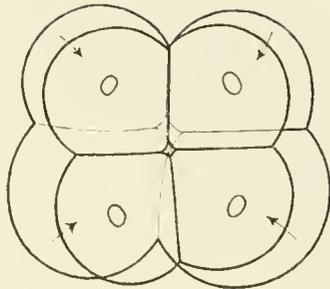
FIG. 112. Transition from normal 16-celled form to double 8-celled form.

FIG. 113. Double 4-celled form (transition to double 8-celled) showing reminiscence of the normal form of cleavage in the lower right hand fourth.

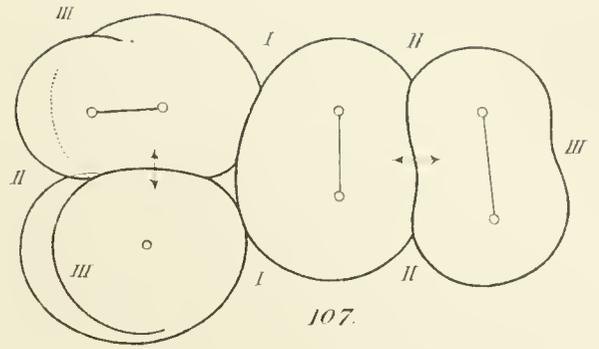
FIG. 114. Individual of similar type. Nearly normal form of cleavage in the upper half of each twin, double form in the lower half of each.



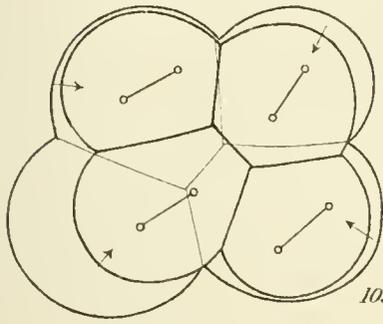
103.



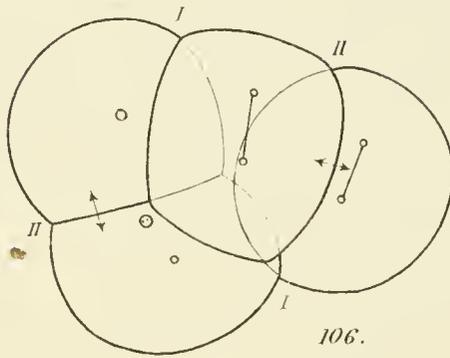
104.



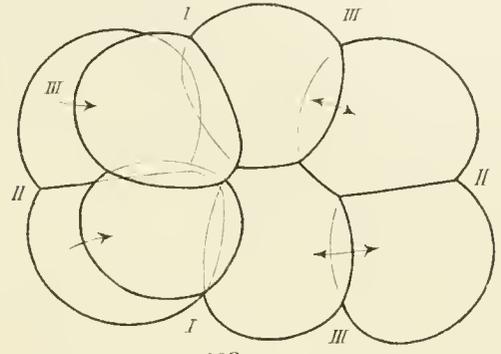
107.



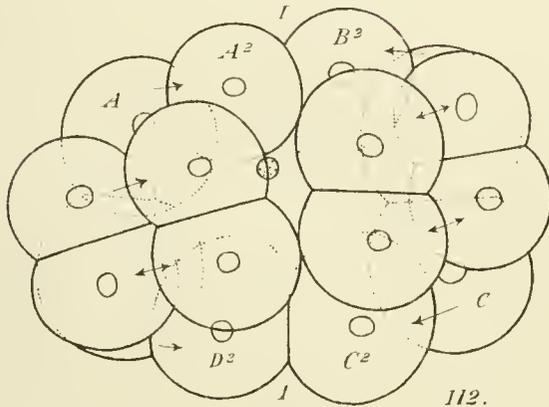
105.



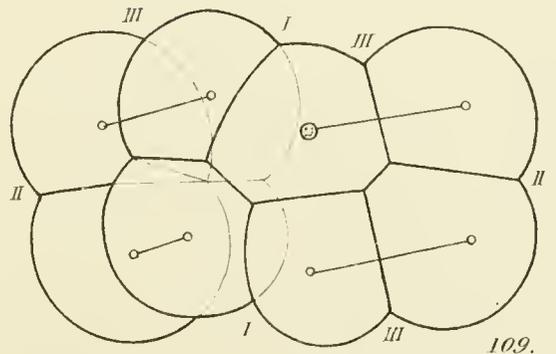
106.



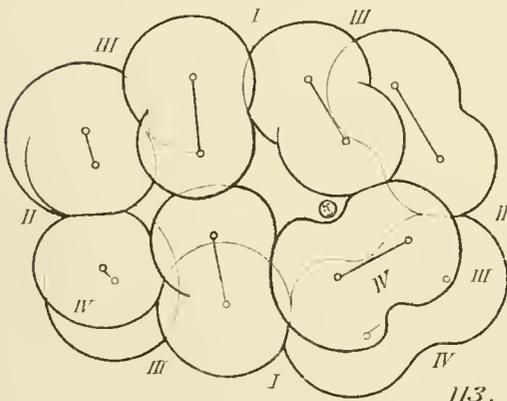
108.



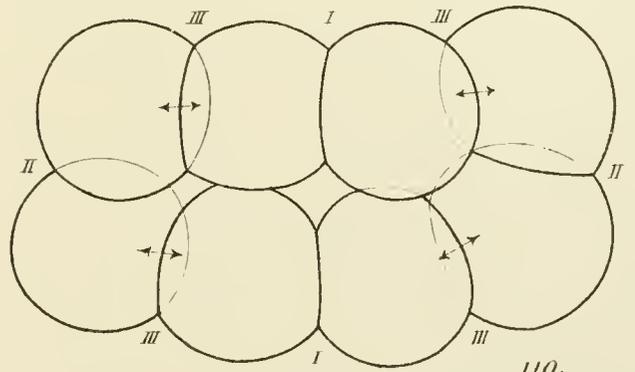
112.



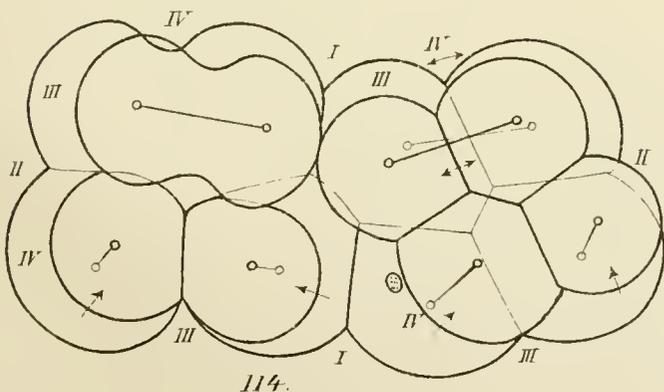
109.



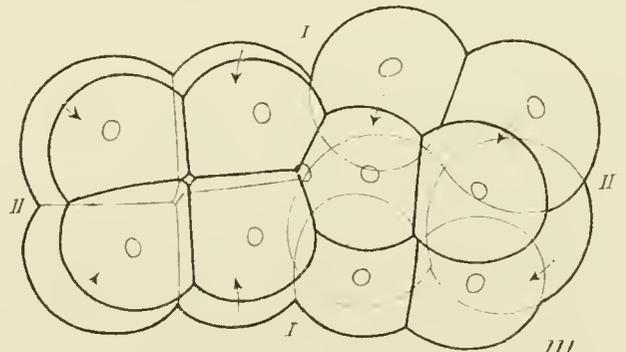
113.



110.



114.



111.

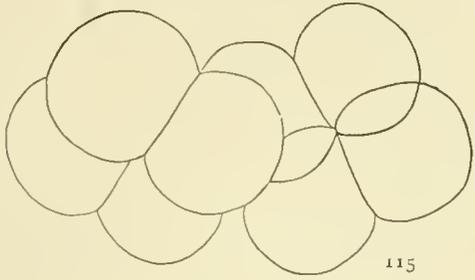




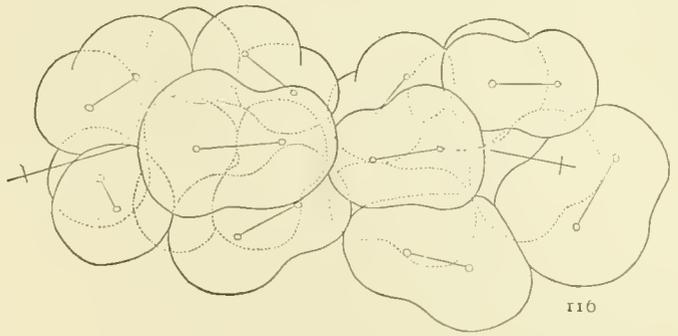
## PLATE XXXVII.

[Figs. 121 to 123, 126 to 130, from life ; the others from preparations. 270 diameters.]

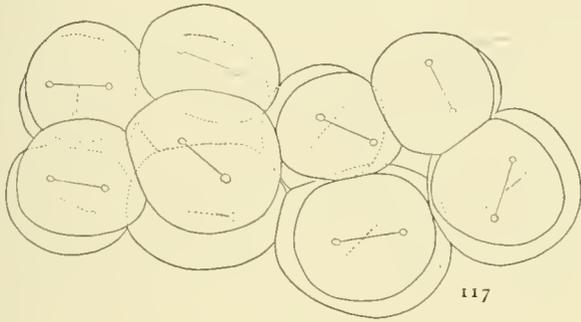
- FIG. 115. Double 4-celled stage. Nearly equal type.
- FIG. 116. Transition from double 8-celled to double 16-celled stages, obliquely from lower pole of each twin. Both of the unequal type. The axes form an acute angle.
- FIG. 117. Double 8-celled form, preparing for division. Both of the unequal type. The axes at right angles.
- FIG. 118. Double 8-celled stage.
- FIG. 119. Double 8-celled form, preparing for division ; unequal type.
- FIG. 120. Double blastula,  $3\frac{1}{2}$  hours.
- FIG. 121. Four-celled form, from isolated micromere of 8-celled stage.
- FIG. 122. Succeeding 8-celled stage (from another but similar specimen).
- FIG. 123. Succeeding 16-celled stage of the same individual ; upper pole.
- FIG. 124. A similar form from the lower pole.
- FIG. 125. Closed  $\frac{1}{8}$  blastula, from shaken 8-celled stage ; 4 hours.
- FIG. 126. Four-celled stage from isolated macromere of 8-celled stage.
- FIG. 127. Eight-celled stage of the same individual.
- FIG. 128. Sixteen-celled stage of the same. Large cleavage-pore.
- FIG. 129. Thirty-two-celled stage of the same ; plate-form.
- FIG. 130. Later stage (another specimen); flat plate; 4 hrs.
- FIG. 131. Half-closed  $\frac{1}{8}$  blastula, with the last.
- FIGS. 132, 133. Two curved plate-forms,  $\frac{1}{8}$  embryos, 6 hrs., closely corresponding with fragments of normal ectoblast and entoblast of the gastrulas at this stage.
- FIG. 134. Curved plate-form,  $\frac{1}{8}$  embryo; 16 hours, free-swimming in life.
- FIG. 135. Closed  $\frac{1}{8}$  blastula with last.



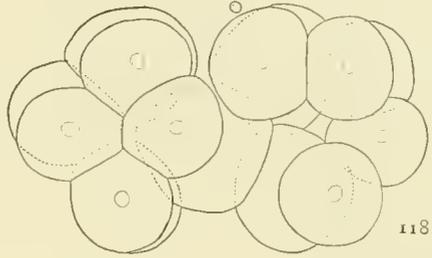
115



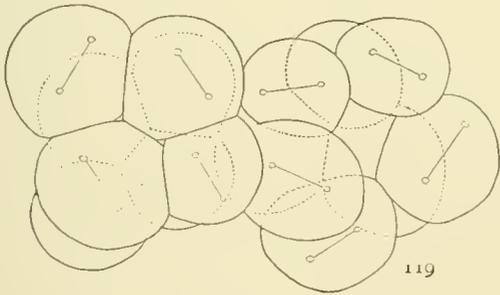
116



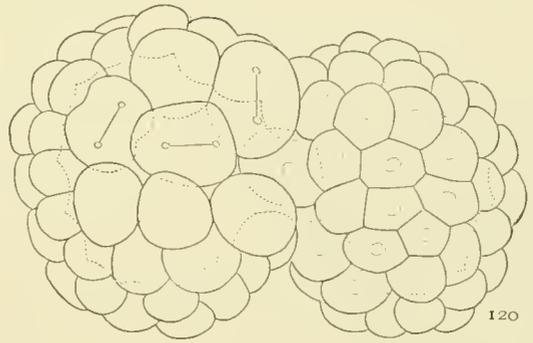
117



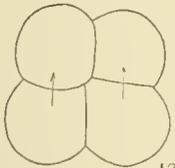
118



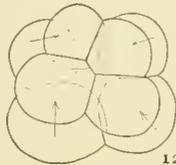
119



120



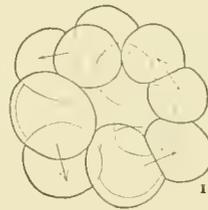
121



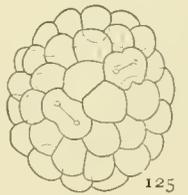
122



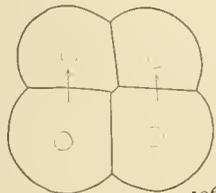
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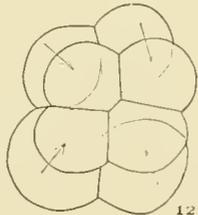
124



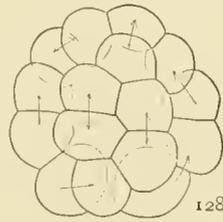
125



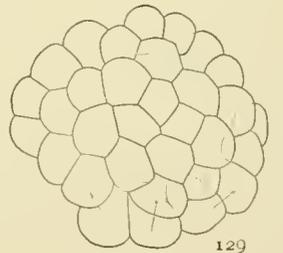
126



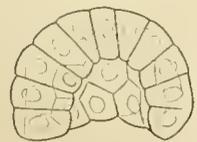
127



128



129



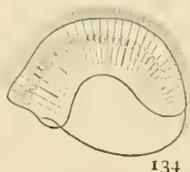
131



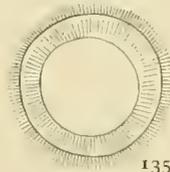
132



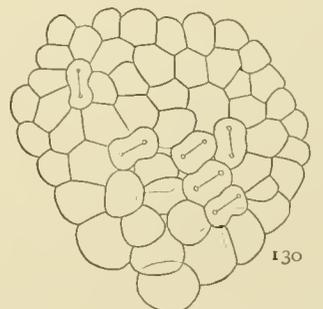
133



134



135



130





## PLATE XXXVIII.

[From preparations. Figs. 136 to 140 enlarged 225 diameters; the others 270 diameters.]

FIG. 136. Normal larva at the stage of the first gill-slit, 48 hrs.

FIG. 137. Half-sized dwarf (probably a  $\frac{2}{4}$  embryo) derived from shaken 4-celled stages (48 hrs.).

FIG. 138. Half-sized dwarf ( $\frac{1}{2}$  embryo) from isolated  $\frac{1}{2}$  blastomere. From the same spawning as Fig. 136 (48 hrs.).

FIG. 139. Fourth-sized dwarf ( $\frac{1}{4}$  embryo) from a blastomere of the 4-celled stage. From the same spawning as Fig. 137 (48 hrs.). The larva shows several defects.

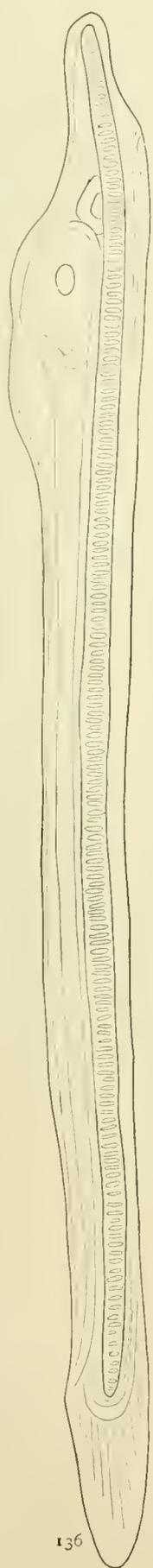
FIG. 140. Double monster, from shaken 2-celled stage (48 hrs.).

FIG. 141. Normal segmented larva (16 hrs.).

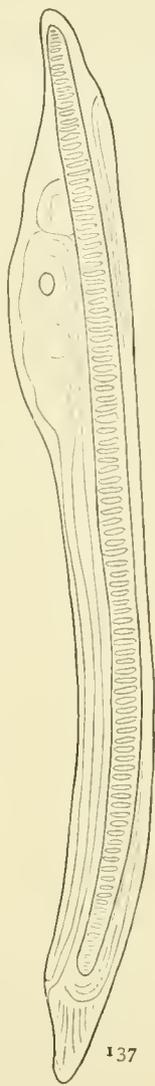
FIG. 142. Half-sized dwarf ( $\frac{1}{2}$  embryo) from a blastomere of the 2-celled stage. From the same set of eggs as the last (16 hrs.).

FIG. 143. Fourth-sized dwarf ( $\frac{1}{4}$  embryo) from a blastomere of the 4-celled stage. The notochord is shown, but the somites could not be distinguished.

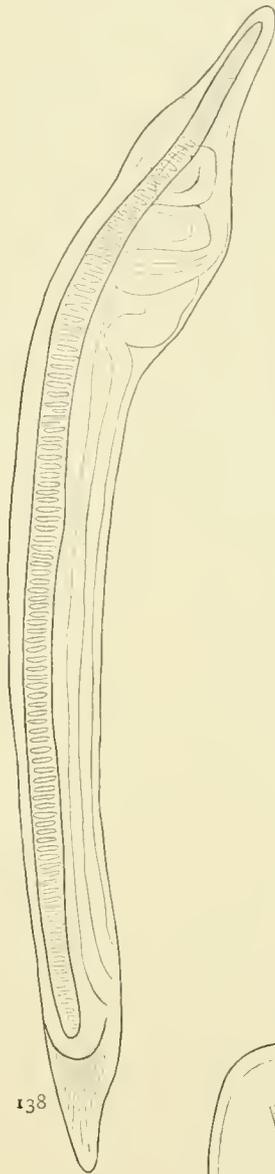
FIG. 144. Double monster, from the same set as Fig. 142. The anterior extremities free.



136



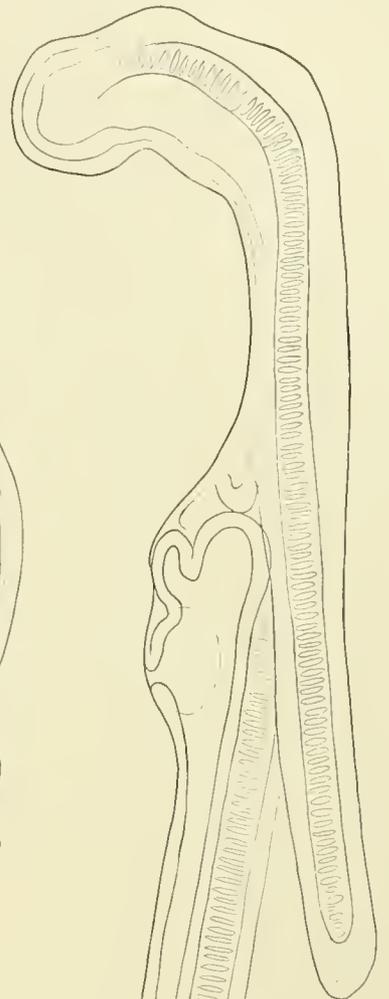
137



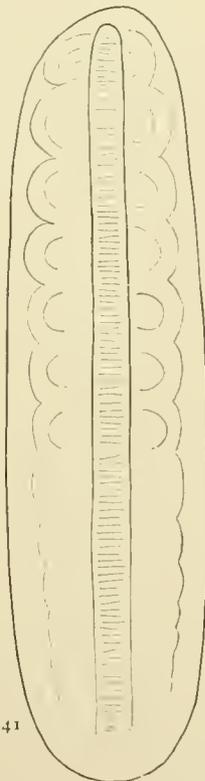
138



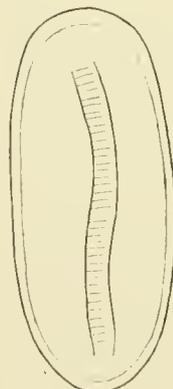
139



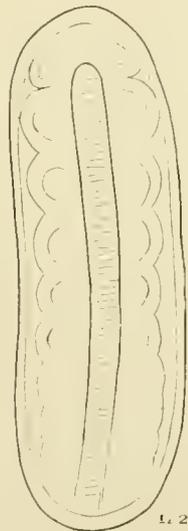
140



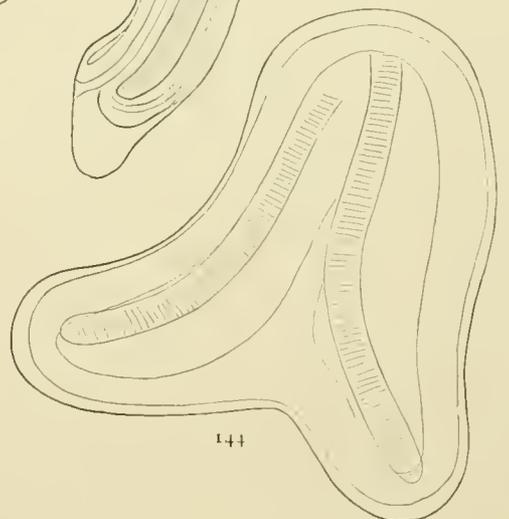
141



143



142



144



## THE INADEQUACY OF THE CELL-THEORY OF DEVELOPMENT.<sup>1</sup>

C. O. WHITMAN.

THE doctrine of Schleiden and Schwann that in *cell-formation* lies the whole secret of organic development, has held the place of a central axiom in biological work and speculation for over half a century. All this time the cell has been, as it were, the alpha and omega of both morphological and physiological research. Regarded as a primary element of structure, it has come to signify in the organic world what the atom and molecule signify in the physical world.

The traditional *cell-standpoint* has been most exactly defined by Schleiden and Schwann. In his celebrated "Beiträge zur Phytogenesis" (Müller's Archiv, 1838), Schleiden sets forth the cell-doctrine, which he limited to plants, in the following words: "*Each cell leads a double life; an independent one, pertaining to its own development alone; and another incidental, in so far as it has become an integral part of a plant.*"

"The entire plant appears to live only for and through the elementary organ" (cell).

Schwann, in his classical Researches of 1839, extends the same view to the entire organic world.

"*Each cell,*" he affirms, "*is, within certain limits, an individual, an independent whole. The vital phenomena of one are repeated, entirely or in part, in all the rest. These individuals, however, are not ranged side by side as a mere aggregate, but so operate together, in a manner unknown to us, as to produce an harmonious whole.*" (Introduction, p. 2.)

"*The whole organism subsists only by means of the reciprocal action of the single elementary parts.*" (Theory of cells, p. 191.)

The method of reasoning is precisely the same as we have seen in some of the latest experimental studies on cleavage. Witness the following: "If we find that some of these

<sup>1</sup> Read Aug. 31, at the Zoölogical Congress of the World's Columbian Exposition.

elementary parts, not differing from the others, *are capable of separating themselves from the organism*, and pursuing an independent growth, we may thence conclude that each of the other elementary parts, each cell, is already possessed of power to take up fresh molecules and grow; and that, therefore, *every elementary part possesses a power of its own, an independent life, by means of which it would be enabled to develop independently*, IF THE RELATIONS WHICH IT BORE TO EXTERNAL PARTS WERE BUT SIMILAR TO THOSE IN WHICH IT STANDS IN THE ORGANISM. The ova of animals afford us examples of such independent cells, growing apart from the organism." (*l. c.* p. 192).

In these words of Schleiden and Schwann we see no vague anticipation, but a clear statement, of the cell-standpoint of to-day. The organism consists, morphologically, of cells, and subsists, physiologically, by means of the "reciprocal action" of the cells. *Organization* means cellular structure, and *ontogeny* means cell-formation. "*Der gleiche Elementarorganismus ist es, der Thiere und Pflanzen zusammensetzt.*" (Schwann.)

In this "double life," this "harmonious whole," this "reciprocal action" of "elementary organisms," this "operating together in an unknown manner," we see the "cell-state" theory, the "unknown principle of correlation," the "correlative differentiation," the "cellular interaction" of current literature.

Much as we have enlarged our knowledge of the cell, we are still looking at the problems of life from the point of view occupied by the founder of the cell-doctrine. The most notable advances in cytology have but tended to define and emphasize the cell-standpoint. The discovery that *all cells arise by division of preëxisting cells*, neatly embodied in Virchow's maxim, "*omnis cellula e cellula*"; the extension and verification of this maxim furnished by Gegenbaur in 1861, in demonstrating *the vertebrate egg to be a single cell*; and the proof obtained during the last twenty years that the *internal processes of cell-division are fundamentally the same in both plants and animals*, — all these capital steps forward have tended to magnify the importance of the cell as a universal unit of structure.

All higher organization is supposed to begin with cell-formation, and to reach its fullest expression in the mutuality of the constituent cells. Whether the cytoplasm be regarded as isotropic or as definitely organized, whether the hereditary substance be identified with the egg as a whole, or with the nuclear chromosomes alone, the cell-dogma is still supreme.

Our microscopes resolve the organism into cells, and ontogeny shows that the many cells arise from one cell; hence, the organism seems to be the product of cell-formation, and the cleavage of the germ seems to be a *building* process. The cell-theory points us to very definite units, as the elements of organization, and thus offers what has for a long time appeared to be a rational basis for the investigation of life-phenomena. All the search-lights of the biological sciences have been turned upon the cell; it has been hunted up and down through every grade of organization; it has been searched inside and out, experimented upon, and studied in its manifold relations as a unit of form and function. It has been taken as the key to ontogeny and phylogeny, and on it theories of heredity and variation have been built. For a long time it has been regarded as a decisive test of homology in germ-layers, tissues, and organs. Fundamental distinctions have been made between *intra-cellular* and *inter-cellular* organization, between unicellular and multicellular organisms and organs, between cellular and acellular growth and development, between the processes of fission and regeneration in the protozoan and the metazoan, between differentiation *within* the cell and *among* cells, between the formative forces which shape the infusorian and those which act in a many-celled organism.

An organism of many cells is supposed to differ from one of one cell, somewhat as a complex molecule differs from a simple one. The complex unit bears not only the structure of its individual parts, but also a totally new structure formed by the union of these parts. In like manner the organism is fancied to carry at least two distinct organizations, the organization of the separate cells and that of the cells united. The higher organization thus differs, *qualitatively*, from the lower, so that

we may have analogies, but no homology of organs between unicellular and multicellular organisms.

How sharply the line is drawn in this regard is shown in the scrupulous care with which authors avoid the suggestion of anything comparable to muscle or nerve in the infusorian. The Ehrenberg view of infusorian organization demanded altogether too much, and we have swung to the opposite extreme of thinking that the very idea of such comparison is forbidden by the cell-doctrine. Any suggestion of a possible community of origin between an organ — say the *mouth* — of such an animal and the corresponding structure of a cellular organism, would be quickly relegated to the *limbus fatuorum*. Who dares question the proposition that there *can* be no morphological identity between an organ formed without cells and one formed with cells? No matter how complete the physiological correspondence, the two things must be assumed to differ *toto coclo*, as measured by the cell-rule. That is the cell-standpoint.

While the cell-doctrine has been carried steadily forward, confidence in its all-sufficiency has been somewhat shaken from time to time, and a few cautious protests have been ventured against the complete ascendancy of the cell as a unit of organization. Botanists, among whom in this particular the name of Sachs stands foremost, have led the way to another standpoint, which, in contradistinction to the prevailing one, may be called the *organism-standpoint*. Among zoölogists, Rauber has most boldly and ably defended this point of view; and more recently Wilson has expressed similar views, but with reservations that still uphold the cell-standpoint. Driesch, too, obtains experimental proof that “the *mode* of cleavage is something unessential to the future animal,” but still he feels compelled to explain the organism from the cell-standpoint, — that is, he supposes that the organism is determined by *correlative* differentiation of homodynamous (“omnipotent”) cells or nuclei. The position is altogether similar to that of Oscar Hertwig and Wilson. Wilson, however, holds that the cleavage may secondarily acquire a “mosaic” significance, and herein makes a decided advance towards a pre-organization

theory. *A certain grade of organization as the result of heredity* rather than of cleavage is conceded for annelid development, and for all forms, in so far as future characters are foreshadowed in cleavage stages. This is a limited application of the view which I believe holds true of all eggs, *even before cleavage begins*. It will be easy to show that the very facts generally relied upon to disprove the existence of organization in the egg furnish very strong evidence in support of it.

The question as to the presence of organization is not settled by the *form* of cleavage. Eggs that admit of complete orientation at the first or second cleavage, or even before cleavage begins, are commonly supposed to reflect *precociously* the later organization, while eggs, in which such early orientation is impossible, are supposed to be more or less completely isotropic and destitute of organization. When the region of apical growth is represented by conspicuous teloblasts, the fate of which is seen to be definitely fixed from the moment of their appearance, we find it impossible to doubt the evidence of organization, or "precocious differentiation," as it is conventionally called. When the same region is composed of more numerous cells, among which we are unable to distinguish special proliferating cells, we lapse into the irrational conviction that the absence of definitely orientable cells means just so much less organization.

Cell-orientation may enable us to infer organization, but to regard it as a measure of organization is a serious error. The organization of a vertebrate embryo cannot be said to be less advanced than that of an annelid embryo, because it lacks the unicellular teloblasts which the latter may possess. The regular holoblastic cleavage of the mammalian egg is evidently no index to its grade of organization. The more carefully we compare the cleavage in different eggs, the more clear it becomes that the test of organization in the egg does not lie in its mode of cleavage, but in subtile formative processes. We find the most unlike forms of cleavage issuing in the same remarkable form-phases; for example, the primitive streak of mammalian and avian eggs; and conversely, we find identical forms of cleavage leading to fundamentally different

results ; for example, in the egg of the polyclad as compared with that of the mollusc or the annelid, where "*cells having precisely the same origin in the cleavage, occupying the same position in the embryo, and placed under the same mechanical conditions, may nevertheless differ fundamentally in morphological significance.*" (Wilson.)

The most remarkable feature of avian development is the primitive streak. The presence of this feature in typical form, in such an egg as that of the mammal, is certainly one of the most significant facts in embryology. The conclusion is here forced upon us — and I see no escape from it — that the formation of the embryo is not controlled by the form of cleavage. The plastic forces heed no cell-boundaries, but mould the germ-mass regardless of the way it is cut up into cells. That the forms assumed by the embryo in successive stages are not dependent on cell-division, may be demonstrated in almost any egg. Watch the expansion of the blastoderm in the pelagic teleost egg, the formation of the germ-ring, and especially the *axial concentration of material*, which is so beautifully illustrated in these eggs. Such developmental processes are, if I mistake not, clearly indicative of some sort of organization.

The formation of the whole from a part, regarded by some as conclusive evidence of isotropy and correlative *cell*-differentiation, no more disproves the existence of definite organization in the case of the egg than in the case of hydra. A fragment of a hydra may reproduce the whole organism ; and in so doing act as a unit, not as a fraction of a unit. In the same way, one of the first two or four blastomeres, when severed from vital connection with its fellow or fellows, may develop *as a unit, not as a half-unit*, precisely as Wilson insists is the case in Amphioxus.

If the isolated blastomere continues for a while to form cells as if it were a half-unit or a quarter-unit, and only later manifests the whole unit-power of the organism, I see no reason to conclude that the case is fundamentally different. In either case the part has the power of reorganizing itself into the whole, and it makes no essential difference whether the reor-

ganization be accomplished at once, before cells are formed, or gradually, while cell-formation is going on.

If we no longer hesitate to accept Brücke's view that the functions of the cell are proof of organization, although our best microscopes fail to give us any idea of what it consists in, it certainly ought not to be difficult to regard the egg as a young organism, and the developmental phenomena as proof of organization. Such organization is, in fact, conceded when we speak of the egg as the rudiment of an organism ("Anlage eines Organismus," O. Hertwig), but, nevertheless, we go on insisting that cellular structure is the essence of a higher organization.

We are so captured with the personality of the cell that we habitually draw a boundary-line around it, and question the testimony of our microscopes when we fail to find such an indication of isolation. We have so long insisted on these boundary-lines as limiting homologies that we find it extremely difficult to ignore them. How difficult it is, for example to regard a multicellular nephridial funnel as the exact homologue of the unicellular funnel. If the organ consist of one cell, the tube is *intra*-cellular; if of many cells, then it is *inter*-cellular. But we have the "tube" and the "flame" just as perfect with one cell as with many, as Vejdovsky's studies make very certain. How idle, then, to deny homology between two such organs merely because one is *intra*- and the other *inter*-cellular. And yet that is precisely what we have been accustomed to do.

Now this one case illustrates, as I believe, a general truth of no little importance. *The nephrostome is a nephrostome all the same whether it consist of one cell, two cells, or many cells. Its form and function are both independent of the number of component cells. Cells multiply, but the organ remains the same throughout. So far as homology is concerned, the existence of cells may be ignored.*

May we not go further, and say that an organism is an organism from the egg onward, quite independently of the number of cells present? In that case *continuity of organization* would be the essential thing, while division into cell-territories might

be a matter of quite secondary importance. As the nephrostome is not the result of cell-formation, but exists as such before division into cells, so the organism exists before cleavage sets in, and persists throughout every stage of cell-multiplication. Continuity of organization does not of course mean preformed organs, it means only that a definite *structural* foundation must be taken as the starting-point of each organism, and that the organism is not multiplied by cell-division, but rather continued as an individuality through all stages of transformation and sub-division into cells.

We have long been aware that the cell could not be taken as the ultimate unit of life, and every notable effort to account for heredity has led to the postulation of primary elements in comparison with which the cells appear as complex organisms. Since Ernst Brücke first contended for the organization of the cell in 1861, and the existence of "smallest parts" as the basis of this organization, we have seen similar ideas reappear in the "physiological units" of Herbert Spencer, the "gemmules" of Darwin, the "micellae" of Nägeli, the "plastidules" of Elsberg and Haeckel, the "inotagmata" of Th. Engelmann, the "pangens" of de Vries, the "plasomes" of Wiesner, the "idioblasts" of Oscar Hertwig, and the "biophores" of Weismann.

After the discovery of cell-division as the law of cell-formation, and after the scheme of the cell set up by Schleiden and Schwann had been revised and reduced to essentialities by Leydig, Max Schultze, and others, the next great step forward in the cell-doctrine must be credited to Brücke, who, seeing that the phenomena of life could not be referred to a *structureless* substance, declared for the *organization* of the cell in words that were scarcely less than revolutionary.

"We must therefore," says Brücke, ascribe to living cells, in addition to the molecular structure of the organic compounds which they contain, still another, and otherwise complicated, structure; and this it is that we designate by the name organization."

Further, in his own words: "*Wir müssen in der Zelle immer einen kleinen Thierleib sehen, und dürfen die Analogien, welche zwischen ihr und den kleinsten Thierformen existiren, niemals aus den Augen lassen.*" (Elementarorganismen, p. 387.)

On the botanical side, Sachs has maintained since 1865 ("Experimental-Physiologie") that protoplasm is an "*organized body*" (cf. Lectures on Physiology, 1887, p. 206-7). While Brücke contended for organization *within* the cell, and remained true to the cell-theory of all higher organization, Sachs, Goebel and some other botanists early challenged the doctrine of cell-hegemony. Sachs briefly indicates his standpoint in the following words :

"To many, the cell is always an independent living being, which sometimes exists for itself alone, and sometimes 'becomes joined with' others—millions of its like, in order to form a cell-colony, or, as Haeckel has named it for the plant particularly, a cell-republic. To others again, to whom the author of this book also belongs, cell-formation is a phenomenon very general, it is true, in organic life, but still only of *secondary significance*; at all events, it is merely one of the numerous expressions of the formative forces which reside in all matter, in the highest degree, however, in organic substance." (Lectures, etc., p. 73.)

Brücke's great merit consists in this, that he taught us the necessity of assuming *structure* as the basis of vital phenomena, in spite of the negative testimony of our imperfect microscopes. That function presupposes structure is now an accepted axiom, and we need only extend Brücke's method of reasoning, from the tissue-cell to the egg-cell, in order to see that there is no escape from the conclusion that the whole course of developmental phenomena must be referred to organization of some sort. *Development, no less than other vital phenomena, is a function of organization.*

Nägeli followed the same method of reasoning when he concluded that the organism was, in a certain sense, "*vorgebildet*" in the germ-cell (Beiträge zur wiss. Botanik, Heft II. 1860). This point of view is well expressed in his classical work, the "*Theorie der Abstammungslehre*," where he says: "Organisms differ from one another as egg-cells no less than in the adult state. The species is contained in the egg of the hen as completely as in the hen, and the hen's egg differs from the frog's egg just as widely as the hen from the frog."

While all will admit that the organization of the egg is such as to predetermine the organism, few will be prepared to admit that *the adult organization is identical in its INDIVIDUALITY*

*with that of the egg.* The organism is regarded rather as a community of such individualities, bound together by interaction and mutual dependence. According to this view, development does not consist in carrying forward continuous changes in the same individual organization, but in multiplying individualities, the complex of which represents, at every stage, not *the* organism, but one of an ascending series of organisms, which is to terminate in the adult form.

In the egg-cell we are supposed to have an *elementary* organism; in the two-cell stage, two elementary organisms, forming together an organism of a totally different order, based on a new scheme of organization. In the four-cell stage we have another organism, in the eight-cell stage another, and so on.

“Physiological division of labor,” as Milne-Edwards first phrased it, is unquestionably a principle of wide application. Given the cells as morphological units and this physiological principle, the evolution of a cellular organism, may be conceived of as a most simple affair. From a simple colony of like cells, we pass to a commonwealth of differentiated and mutually dependent cells. A multitude of independent cell-organisms, adopting mutual service as the best economy, find themselves in the end incapable of independent life, and so firmly bound together in interdependence, that they constitute a complex individual. The usual conception of this division of labor is, as Herbert Spencer<sup>1</sup> has recently stated it, “an *exchange of services*, — an arrangement under which, while one part devotes itself to one kind of action, and yields benefits to all the rest, all the rest, jointly and severally performing their special actions, yield benefits to it in exchange. Otherwise described, it is a system of *mutual* dependence.”

We habitually apply this anthropomorphic conception to every grade of organization. The higher organism is regarded as a colony of cells; the cell as a colony of simpler units, nucleus, centrosome, and so on; the nucleus as a colony of chromosomes; the chromosome, according to Weismann's terminology, as a colony of “ids”; the “id” as a colony of

<sup>1</sup> The Contemporary Review, February, March, and May, 1893.

“determinants”; the “determinant” as a colony of “biophores,” and the “biophore” as a colony of molecules.

In proportion as division of labor is carried out, *interdependence* is increased, and the units become more and more intimately associated. The struggle for existence is supposed to extend to the cells, and even to the biophores. Symbiotic relations are fought out, refined, and confirmed by natural selection, and eventually reduced to a system of mutual adaptations which are fancied to be the basis of organic unity.

Whether organization is wholly a matter of acquisition, and whether it became possible only as a result of symbiotic advantages accidentally discovered in the struggle for existence, need not here be discussed. It is enough for present purposes to know that organization exists, and that *organic* unity depends on *intrinsic properties* no less than does *molecular* unity.

It is not division of labor and mutual dependence that control the union of the blastomeres. It is neither functional *economy* nor social instinct that binds the two halves of an egg together, but the constitutional bond of *individual organization*. It is not simple adhesion of independent cells, but integral structural cohesion.

That organization precedes cell-formation and regulates it, rather than the reverse, is a conclusion that forces itself upon us from many sides. In the infusoria we see most complex organizations worked out within the limits of a single cell. We often see the formative forces at work and structural features established before fission is accomplished. Cell-division is here plainly the result, not the cause, of structural duplication. The multicellular *Microstoma* behaves essentially in the same way as the unicellular *Stentor*, or the multinucleate *Opalinopsis* of *Sepia*. The *Microstoma* organization duplicates itself, and fission follows. The chain of buds thus formed bears a most striking resemblance to that of *Opalinopsis*, and the resemblance must lie deeper in the organization than cell-boundaries.

Compare the results obtained by artificial division in two such forms as *Stentor* and *Hydra*. The two courses of regen-

eration are so exactly parallel that one cannot fail to see at once that the formative forces operate in essentially the same manner with the one-celled as with the many-celled organism. Gruber's experiment, as described in his recent article, "*Microscopic Vivisection*" (Berichte der Naturforschenden Gesellschaft zu Freiburg, Vol. VII, Part 1, 1893), illustrates well this point.

A Stentor was cut into three pieces, *A*, *B*, *C*, each of which regenerated the missing parts within 24 hours. The anterior

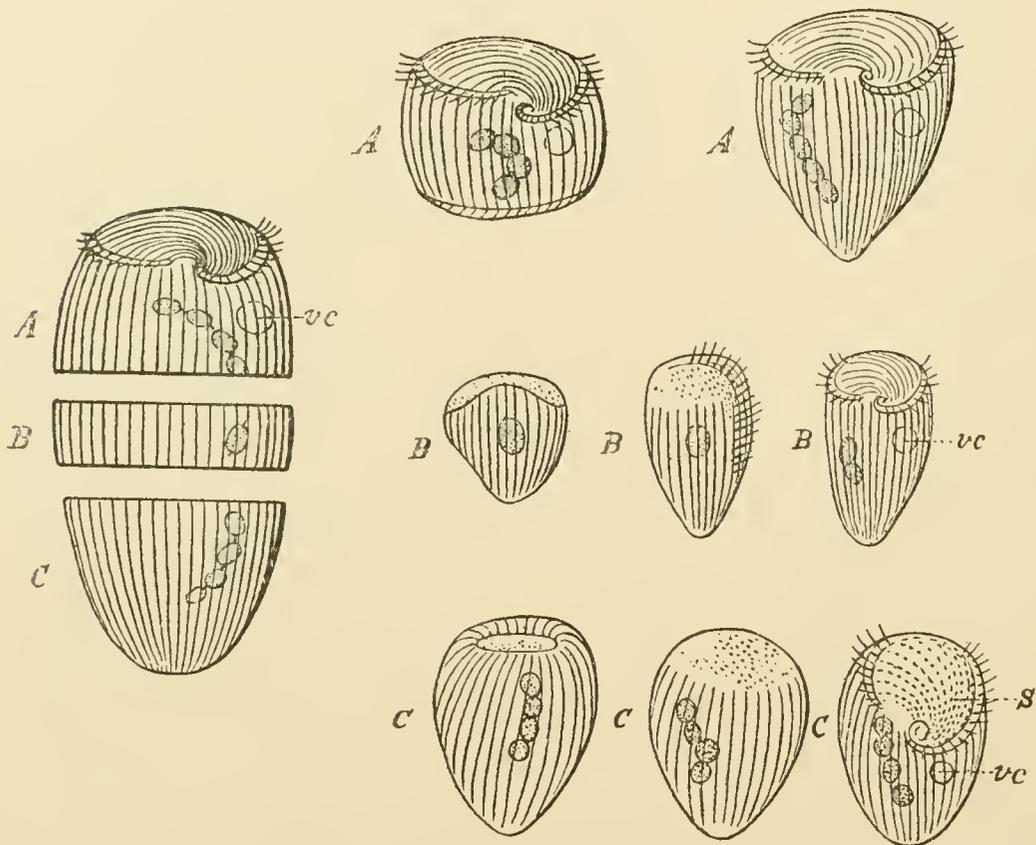


FIG. 1. — Regeneration of a Stentor cut into three parts, *A*, *B*, *C*. *vc* = pulsating vacuole. *S* = regenerating frontal field.

end regenerated posterior end, and *vice versa*. The middle piece regenerated both ends—the complicated frontal field with its mouth, pharynx, long cilia, pulsating vesicle, *etc.*, as well as the simpler posterior region.

Treat a Hydra in the same way and similar results will follow. In both cases the orientation of the parts will remain the same as that of the whole. Gruber repeated the division of Stentor four times in succession, getting perfect regeneration each

time, but *smaller* individuals, as no growth was possible. The experiment reminds one of the half- or quarter-sized embryos obtained by separating the first two or four blastomeres.

Gruber's highly interesting paper calls attention to the identity in form and structural detail of the "membranellae" of *Stentor* with the so-called "corner-cells" (*Eckzellen*) of molluscs (*Cyclas cornea*). The comparison is a most instructive one, illustrating in the most conclusive manner that differentiation of the parts of the *soma* depends, not on the interaction of cells, but upon the elementary structure of the protoplasm.

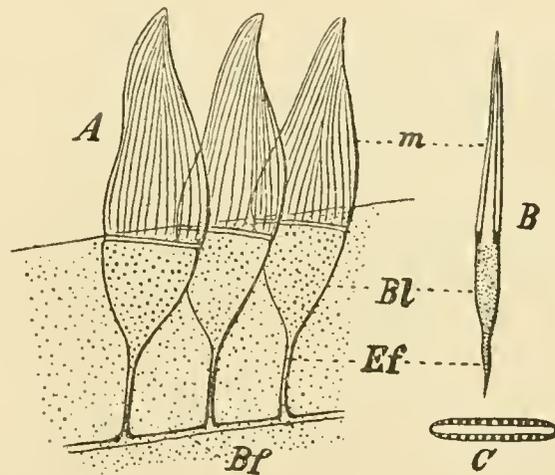


FIG. 2. — *A*, three membranellae of *Stentor*, *B*, membranella in section. *C*, Section at the base of the two plates. *Bl*, Basal lamella. *Ef*, Terminal fibre. *Bf*, Basal fibril. *K*, Nucleus.

The membranellae of the frontal field of *Stentor* consist of two thin, adherent plates, each of which represents a number of coalesced cilia. The structure has a basal seam or ridge<sup>1</sup> (*Leiste*), and a basal lamella which is continued into a terminal fibre. All these fibres are connected by the basal fibril, through which the movements of the membranellae are evidently regulated.

Now this highly differentiated organ, the membranella, is reproduced with most remarkable exactness in the "corner-cell" of *Cyclas*. But here the organ represents an individual

<sup>1</sup> This seam consists of a series of microsomes, as Dr. Watake has discovered.

cell, while in *Stentor* a whole crown of such organs is formed without any division into cells. Could one ask for a clearer demonstration? Are we not forced to conclude with Gruber that “*however great the difference between an infusorium and a highly organized animal, it cannot be a qualitative one. We can assume that the same vital elements serve in both as the foundation, only in ever new combinations. This kinship declares itself very clearly in the correspondence of many organs of the infusoria with those of the higher organisms*” (*l. c.* p. 16).

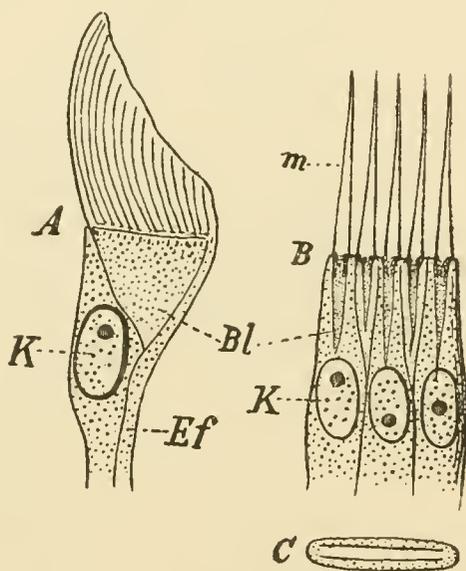


FIG. 3.—*A*, “Corner cell” of *Cyclos cornea*. *B*, Section of three cells. Other letters as in Fig. 2.

“So finden wir,” says Gruber, “in einem Thiere, das schon hoch auf der Stufenleiter der vielzelligen Organismen steht, dieselben Grundelemente wieder wie in dem einzelligen Infusionsthierchen. . . .”

“Wieder und wieder der Beweis von dem göttlich einfachen aber auch göttlich gewaltigen Gesetze der *Einheit der Natur*” (p. 18).

The entoderm of *Dicyema* illustrates one or two points of interest in this connection. We have here an organ in which, as often happens, in parasitic degradation, cell-formation has been dispensed with. The entoderm remains throughout life as a single cell, and the whole process of reproduction, for both kinds of embryos, is carried on *in the body of this cell* without any cellular organs whatever.

In one respect this unicellular organ, which was undoubtedly once multicellular,

What is the difference between an organization embracing one cell and one embracing two or many cells? Certainly the essential difference cannot lie in the *number* of cells. We must look entirely behind the cellular structure for the basis of organization. Even a highly differentiated organism may reach a relatively late stage of development just as well without cell-boundaries as with them, as we see so well illustrated in the insect egg. If we fall back on the number of nuclei as the essential thing, then we shall have to reckon with multinucleate infusoria. In these forms do we not see that it is always the *same* organism before us, as we follow its history through the whole cycle of nuclear phases?

The essence of organization can no more lie in the number of nuclei than in the number of cells. The structure which we see in a cell-mosaic is something superadded to organization, not itself the foundation of organization. Comparative embryology reminds us at every turn that the organism dominates cell-formation, using for the same purpose one, several, or many cells, massing its material and directing its movements, and shaping its organs, as if cells did not exist, or as if they existed only in complete subordination to its will, if I may so speak.

In the phenomena of regeneration and embryogenesis we find abundant evidence. For the present I must limit myself to a few features of development.

Perhaps the peculiar formation of the embryo Toad-fish (*Batrachus*) is as instructive a case as I am acquainted with.

is quite unique, for it may become the receptacle of nuclei belonging originally to other cells; in other words, *it becomes multinucleate, not by the multiplication of its own nucleus, but by the acquisition of exotic nuclei.*

The acquired nuclei are what I have called elsewhere the "residual" nuclei, which are left over when the formation of "infusoriform embryos" ceases. Each of these nuclei enters into vital relations with the cell, *and each undergoes the differentiations characteristic of the true entoderm nucleus*, so that in the end they can only be distinguished by their positions. This seems to show that the differentiation of nuclei may be controlled by the cell to which they are transplanted.

One of Boveri's observations\* shows that the same may be said of the chromosomes. One or more of the chromosomes, normally eliminated in the polar globules, are sometimes carried into the cleavage-nucleus. The supernumerary chromosomes here undergo the regular transformations, quite unlike those which they show when carried out in the polar globule.

\* Zellen-Studien. Heft 2, pp. 171-175.

If one will take the trouble to compare this formation with the ordinary type of teleostean development, he will not fail to see that the organizing forces, whatever they may be, operate to form an embryo under peculiar difficulties. It will be seen towards the end of embryogenesis that the material of the germ-ring, owing to the enormous size of the egg, has to travel over quite a long distance, in order to reach the embryo. A very thin bridge of cells connects the hind end of the embryo with the closing germ-ring, and this bridge is formed by the migrating cells of the germ-ring. What determines this wholly exceptional movement of the cell-material required to form the embryo? Is it possible that the cells move as so many independent individualities? But they do move, and no doubt in obedience to directing influences, acting, not in the cells as individuals, but in and through the entire formative material, irrespective of cells.

Whoever doubts this would do well to study more faithfully the *living* embryo during its formation. If the cell-ghost should still haunt his vision, I would suggest still another field for study. I would suggest first of all that he try to get as clear a notion as possible of the formation of the archenteron in *Amphioxus*, *Petromyzon*, and the Frog. The case of the reptile might then be studied with profit. Next the "chordacanal" of mammals, and finally Kupffer's vesicle in the teleost.

There is no longer any doubt in my mind—and here I am in accord with most authorities on this subject—that this little vesicle is a reminiscence of the archenteron. The development of *Gecco*, as traced by Ludwig Will, removes, as I think, the last doubt on this point.

If the development of Kupffer's vesicle be studied in the light of its phylogenetic significance, and studied in the *living* as well as the dead egg, I cannot help thinking that candid reflection on the facts will be sufficient to force conviction to the standpoint here taken.

Having learned the meaning of the vesicle, one should trace step by step its mode of origin in the pelagic fish-egg. Here one may see this remnant of an archenteric cavity arise, not

inside the embryonic tissues as in the eggs of fresh-water fishes, but actually outside the tissues on the inner face of the embryo, near its posterior end. Its whole ventral and lateral boundary is formed, not by archenteric cells, but by a periblastic layer often as thin as the wall of a soap-bubble, and completely free from all nuclei. It does not even arise as a single cavity, but as numerous minute cavities that look like a cluster of granules. These expand, flow together gradually, and finally form one bubble-like vesicle projecting almost wholly into the transparent yolk. Having attained a maximum size, its slightly concave roof becomes more and more deeply hollowed out, and thus it comes to inclose more and more the cavity, while the latter gradually shrinks in size, and finally vanishes as the true cell-walls close up. Such is briefly the history of this floor-less form—reminiscent of what is a more substantial rudiment in many other embryos.

This remarkable reproduction of a form-phase that is to last only for a few hours and then pass away without leaving a visible trace of its existence, cannot be explained as due to cell-formation nor as the result of *individual* action or interaction on the part of the cells. The embryonic mass acts rather as a *unit*, tending always to assume the form peculiar to the state of development reached by its “essential architectonic elements” (Brücke)—elements that are no less real because, like the atom and molecule, they are too minute to be seen by the aid of our present microscopes.

That cells as such do not participate in this formative act, is shown by the mode of development of the vesicle and by the absence of cells in its ventral and lateral walls. This fact, the absence of cells, has actually been urged recently against the identity of the structure with Kupffer’s vesicle,—an error which one is likely to fall into only while under the delusion that acellular walls cannot be homologous with cellular walls.

The evidence furnished by Kupffer’s vesicle will doubtless lose much of its force with those who have not had an opportunity to study the subject sufficiently to form an independent opinion about it. To some who are better acquainted with the structure, its meaning may still appear to be somewhat prob-

lematical, and the evidence drawn from it as therefore unsatisfactory. It would be useless in such a case to urge the point, and also wholly needless, as examples abound that are not open to such objections.

The form-changes by which the fish blastodisc passes into the germ-ring stage are examples of this kind. It is well known that the transformation of the blastodisc just before the appearance 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 concentration 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 embryonic plate ("head process"), which advances in an axial direction to the very centre 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-controlling. Cell-formation, to be sure, goes on, but it seems to me certain that it has no *directive* influence on the formative processes. 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.

In the axial concentration of the very broad embryonic plate we see a formative process that can have nothing whatever to do with cell-division. Again, in the establishment of the caudal end of the embryo, long before that part of the germ-ring which represents, historically at least, this end can be brought into place, we have another decisive test of formative power asserting itself, not only independently of cell-division, but also against all the obstructions interposed by the yolk. This prepotency of the "plastic power" (Schwann) is seen to great

advantage in the pelagic fish-egg, but still better in the Toad-fish-egg. It is needless to cite further examples of this sort, for the embryology of every animal is full of them, and no one can fail to find who looks for them.

If the formative processes cannot be referred to cell-division, 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 put us on the wrong track. Loeb's experiments in 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 distinctions 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 matter, for which *idiosomes* seems to me an appropriate name.

What these idiosomes are, and how they determine organization, form, and differentiation, is the problem of problems on which we must wait for more light. All growth, assimilation, reproduction, and regeneration may be supposed to have their seat in these fundamental elements. They make up all living matter, are the bearers of heredity, and the real builders of the organism. Their action and control are not limited by cell-boundaries. As Heitzmann and others have long insisted, the continuity of these elements is not broken by cell-walls. The organization of the egg is carried forward to the adult as an unbroken physiological unity, or individuality, through all modifications and transformations. The remarkable inversions of embryonic material in many eggs, all of which are *orderly* arranged in advance of cleavage,<sup>1</sup> and the interesting pressure experiments of Driesch by which a new distribution of nuclei is *forced* upon the egg, without any sensible modification of the

<sup>1</sup> As will be shown later.

embryo, furnish, as I believe, decisive proof of a definite organization in the egg, prior to any cell-formation. The opinion expressed by Huxley in his review of "The Cell-Theory," in 1853, forms a fitting conclusion to this introductory sketch.

"They [the cells] are no more the producers of the vital phenomena than the shells scattered along the sea-beach are the instruments by which the gravitative force of the moon acts upon the ocean. Like these, the cells mark only where the vital tides have been, and how they have acted."<sup>1</sup>

<sup>1</sup> British and Foreign Medico-chirurgical Review, Vol. XII, p. 314. Oct., 1853.













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