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MEMOIRS:

On the Development of the Segments of the Head in Scyllium.

By

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With Plates 1 and 2 and 1 Text-figure.

THE object with which this work was undertaken was to describe the development of the skull in Scyllium and the relation of its elements to the general segmentation of the head, more especially in the occipital region. But it soon became evident that our knowledge of the segmental composition of the head in Elasmobranchs is still in a very unsatisfactory condition, and that a re-investigation of the whole question was necessary. In addition, then, to an account of the development of the skeletal segments, a short history of the mesoblastic somites is given, and incidentally of certain points in the development of the nerves and other structures in the head region.

In embryological investigations it is most essential to have as complete a series of stages as possible; most of the results recorded below have been reached with the help of careful graphic reconstructions of longitudinal sections from 10 to 15 μ thick in my possession; but I have also, through the kindness of Mrs. Jenkinson, had the privilege of making use of many series, especially of the early stages, in the collection of the late Capt. J. W. Jenkinson; and to Prof. J. P. Hill I am indebted for the loan of certain stages.

The foundation of our knowledge of the segmentation of the head of the Elasmobranch on embryological evidence was laid by Balfour in his epoch-making researches published in the years 1876–7–8 (**2**). Some of his results had already appeared in a preliminary note in 1874 (**1**), and in the later work he described the subdivision of the mesoblast of the head and its cœlom by the visceral clefts, and the development of cranial nerves 5, 7, 8, 9 and 10, which "all develop precisely as do the posterior roots of the spinal nerves." He further showed that each mesoblastic segment is related to a nerve running behind it, that its splanchnopleure gives rise to visceral muscles, and suggested that the pre-mandibular somite gives rise to some of the muscles of the eye. "The morphological importance of the sections of the body-cavity in the head," says Balfour, "cannot be overestimated and the fact that the walls become developed into the muscular system of the head renders it almost certain that we must regard them as equivalent to the muscle plates of the body, which originally contain, equally with those of the head, sections of the body-cavity." They therefore "serve as valuable guides to the number of segments which have coalesced to form the head," and there are "a pair of these head-cavities in front of the mandibular arch, a pair in the mandibular arch, and a pair in each succeeding arch. In all there are eight pairs of these cavities representing eight segments, the first of them preoral." No better or more convincing statement of the embryological evidence of the segmentation of the head could be wished, and the quotations are given in full because in many recent reviews of the literature the importance of the work of Balfour seems to me to have been somewhat underestimated. His tabular statement is given below, and it may be said at once that the best and most recent work has fully confirmed his main conclusions. All the many attempts made to prove that there are more or fewer segments embodied in the region of the head there dealt with may be said to have failed.

Table of the Cephalic Segments as determined by the Nerves, Visceral Arches, and Head-cavities (Balfour, **2**).

Segments.	Nerves.	Visceral arches.	Head-cavities or cranial muscle-plates.
Preoral 1 .	3rd and 4th and ? 6th nerves (perhaps representing more than one segment)	?	1st head-cavity
Postoral 2 .	5th nerve . . .	Mandibular . .	2nd head-cavity
„ 3 .	7th nerve . . .	Hyoid . . .	3rd „ „
„ 4 .	Glosso-pharyngeal nerve	1st branchial arch	4th „ „
„ 5 .	1st branch of vagus .	2nd „ „	5th „ „
„ 6 .	2nd „ „	3rd „ „	6th „ „
„ 7 .	3rd „ „	4th „ „	7th „ „
„ 8 .	4th „ „	5th „ „	8th „ „

Shortly afterwards appeared the work of Marshall (**18**) who emphasised the comparison between the more dorsal truly segmented somites with their head-cavities and the more ventral region in the arches with the somites and lateral plate in the trunk. The segmentation of the "head-cavities" dorsally is really independent of the visceral clefts. He further traced the origin from the premandibular somite of the four muscles supplied by the oculo-motor nerve and the origin of the rectus externus from a more posterior segment which he rightly supposed to be the third head-cavity supplied by the abducens nerve. Moreover, he identified this nerve as the ventral root of the facial.

The next important contribution came from van Wijhe in 1882 (**26**). He described in detail the development and fate of the eight head segments discovered by Balfour. A typical head-segment contains on each side, according to van Wijhe, a somite (myotome and sclerotome) below which extends the cavity of a visceral arch, and a visceral cleft. Related to each such segment is a dorsal and a ventral nerve root; these remain separate from each other, just as they have been shown by Balfour to be in early stages in the trunk, and as

they remain permanently in Amphioxus. The dorsal ganglionated root supplies the musculature derived from the lateral plate mesoblast, while the ventral root supplies the muscles developed from the segmental myotome. The "ciliary ganglion" of Marshall was identified by van Wijhe as belonging to the ophthalmicus profundus, the dorsal root of the first or premandibular segment; the third, fourth, and sixth cranial nerves as the ventral roots of the first three or pro-otic segments. Further, he definitely traced the development of the eye-muscles from the corresponding three myotomes, and the origin of the hypoglossal roots from the hinder meta-otic segments of which the vagus represents the dorsal roots only.

Van Wijhe, however, attributed nine segments to the head; the tenth segment, in which a typical spinal ganglion and mixed nerve develops, he considered to belong to the trunk. There is, however, an unfortunate discrepancy between the results of Balfour and van Wijhe which, in spite of the great value of the latter's work, seems to have led to a deal of unnecessary confusion and controversy.[1] For while van Wijhe describes and figures somites 3 to 8 as lying one above each of the six gill-slits, with the seventh, ninth, and four branches of the tenth nerve corresponding in the same way to slits and arches, he assumes, for reasons which are by no means clear and on what seems to me quite insufficient evidence, that the fourth somite belongs to the hyoid arch in front of it and not to the first branchial below and behind it. Now since the third somite obviously belongs to the hyoid segment and is continued below into the mesoblast and cavity of the hyoid arch, van Wijhe's interpretation leaves the

We need not enter here into an account of the long controversies carried on by Kastschenko, Killian, Dohrn, Rabl, Froriep, and others, as to the segmental and somitic nature of the head-somites mentioned above. That these somites produce muscles from their inner walls and are serially homologous with the trunk myotomes seems to have been clearly established by the work of Killian, Platt, Hoffmann, and Neal. A good discussion with full references to the literature will be found in Neal's papers (**19**, **20**).

fourth somite without corresponding slit, arch, or nerve, either in the embryo or in the adult. His assumption that these have disappeared seems both unjustified and unnecessary; so far as I am aware, no serious evidence of their presence has ever been found in spite of the fact that many investigators have sought for them. This view of van Wijhe, which would upset the orderly arrangement of gill-slits, somites, and nerves as set forth in Balfour's scheme, has been adopted in a more or less modified form by various later authors, for instance, by Miss Platt (**21**), Neal (**19**), Sewertzoff (**25**), and Braus (**3**). But it is not supported by Ziegler's observations on Torpedo (**28**), and is totally at variance with the excellent work of Koltzoff on Petromyzon (**17**), according to whom a somite, a dorsal and ventral nerve root and a visceral arch are present in every segment of the head from the mandibular to the most posterior. Johnston, in his valuable paper on the "Morphology of the Vertebrate Head" (**16**), adopts Koltzoff's results.

The Relation of the Nerves to the Myotomes.—Before attempting to enumerate the segments of the head it is very important to determine, if possible, the exact relation of the nerves to the myotomes and scleromeres in the trunk.[1]

Neal, in his important paper on the "Development of the Nervous System of Squalus" (Acanthias) concludes that the segmental dorsal roots are originally intersomitic, thus agreeing with Balfour. Hatschek had pointed out that in Amphioxus and Petromyzon the dorsal roots are septal; that is to say, run out in the septa between the myotomes (**13**). Now, in all other Craniate Vertebrates the dorsal roots shift somewhat in position and join with the ventral roots to form mixed spinal nerves, and the question arises as to whether a dorsal root and its ganglion combine with the ventral root behind or with the ventral root in front. The ventral roots themselves

[1] While the terms "myotome" and "myomere" are practically synonymous, the word "scleromere" is here used to signify the axial skeletal element of a segment derived from an earlier sclerotome which may also give rise to connective tissue and other parts.

are undoubtedly intrasomitic; that is to say, at first pass directly outwards from the nerve cord to the middle of the somite they supply, as was long ago shown by Balfour. Between successive myotomes pass out sclerotome cells to form the septum, and along the posterior face of this septum run vertically upwards the intersomitic segmental vessels, arteries and veins, from the dorsal aorta and cardinal veins. This disposition is constant throughout the Gnathostomes in the embryo, and is found to persist in the adult Petromyzon. Hatschek (13), when comparing the Ammocœte larva with Amphioxus, first concluded that a dorsal root really belongs to the ventral root in front of it. But soon after he changed his mind, and concluded that in Craniates the dorsal root becomes associated with the ventral root of the myotome lying behind it (14). The evidence on which he based his opinion is not clear, and it is obvious that if the roots combined according to the later suggestion they would embrace the segmental vessels between them.[1]

An examination of a complete series of stages of Scyllium embryos, cut in horizontal as well as vertical longitudinal sections, demonstrates conclusively that the rudiment of the spinal ganglion takes up a position from the first opposite the myotome, but near its hinder edge (Pl. 2, figs. 20–24). The sclerotome and scleromere tissue develops chiefly between adjacent myotomes, passing obliquely backwards and outwards to form the future septum and rib, while the main branch of the nerve coming from the ganglion also passes out to the skin behind the myotome (Pl. 2, fig. 45) (9). We are, therefore, justified in concluding that in the Gnathostomes the

[1] In Myxinoids (Myxine and Bdellostoma) I find that the segmental vessels pass up between the anterior ventral and posterior dorsal roots of each spinal nerve. Prof. F. J. Cole has very kindly provided me with a reconstruction from sections of Myxine which confirms my observation on dissections. This exceptional disposition would suggest that the roots have combined in some way differing from that which obtains in the Gnathostomes, and would thus support the view, put forward by Koltzoff, that the mixed spinal nerve of the Myxinoids has been formed independently of that of the Gnathostomes.

position taken up by the ganglion opposite the middle, or even the anterior region of the myotome, is secondary, and that originally the ganglion and the sensory nerves were intersomitic in position, that the main sensory nerve passed out behind the myotome of its segment, and that the dorsal root has combined with the ventral root of the myotome of its own segment (that is to say, the dorsal root joins the ventral root lying in front of it).

In the head region, then, where, as van Wijhe showed, cranial nerves represent dorsal or ventral roots retaining their primitive independence, we should consider the ventral roots as lying opposite their somites, and the dorsal roots as running behind the somites to which they belong.

The Three Pro-otic Segments.—In this paper it is not necessary to enter into a very detailed account of the development and fate of the three pro-otic or pre-auditory somites. They are the premandibular, the mandibular, and the hyoid somites of Balfour; their presence has been recognised by most authors not only in the Selachians, but also in Cyclostomes, Dipnoi, Amphibians, birds, and reptiles, and mammals. They are known to give rise to the eye muscles. The ophthalmicus profundus, trigeminal, and facial nerves are considered to represent the dorsal roots of these segments, the oculomotor, trochlear, and abducens the ventral roots. My own observations fully confirm these conclusions.

The reconstructions figured on plate (Pl. 1, figs. 1, 2, 3, 5, and 7) illustrate the development of the mesoblast in the pro-otic region. The lateral plate is seen becoming compressed and eventually subdivided by the outgrowing gill-pouches. The dorsal somites become differentiated dorsally above the hyoid and the mandibular arches. At first the cavity of the somite is continued into its corresponding arch; but very soon in the hyoid, and later in the mandibular arch, this cavity is obliterated. To the account of the development of the premandibular cavity given by previous authors, I have nothing of importance to add. It has been studied in minute detail by Dohrn (5). Beyond the anterior end of the gut

and notochordal plate the tissue is continued forwards as a flattened mass underlying the brain as far as the region of the neuropore, where, at the earliest stage figured, the neural tube is still continuous with the outer epiblast (Pl. 1, fig. 1), (*a*). This mass of tissue develops, according to Miss Platt, into transitory "anterior head-cavities," representing a segment in front of the premandibular (**22**)—a conclusion which is supported by Neal (**19**). Yet the evidence I find in Scyllium seems to me against this interpretation. No trace of such an anterior somite is found in Petromyzon by Koltzoff (**17**), and, like van Wijhe, I still consider that the premandibular is the first (Pl. 1, fig. 19). The plate of tissue in question soon disappears, may be considered rather as hypoblastic than mesoblastic, and might possibly represent the anterior prolongation of the notochord in Amphioxus. Moreover, I have elsewhere given strong evidence to support the more generally accepted view that the premandibular cavity corresponds to the first somite in Amphioxus (**12**).

A glance at Pl. 1, figs. 1 to 5, will show that from the mandibular somite backwards the regular correspondence of somites, dorsal nerve roots, and visceral pouches can be made out fairly easily. The second and third somites, however, become greatly modified, elongated, and irregularly subdivided. The cavities, which in the earlier stages may be in communication from segment to segment, may also become broken up into separate smaller spaces, some of which may disappear, while others swell up into the large head-cavities of later stages. It is these peculiarities which have led various authors (Dohrn, Killian, etc.) to hold that there are many more than three segments in front of the auditory sac. But there is much reason to believe that the appearances are due merely to the secondary subdivision of the mandibular and the hyoid somites, whose exceptional position and fate no doubt are responsible for their modification. The fact that they are stretched by the excessive cranial flexure, that they give rise not to large muscle segments but to eye muscles, and that they subsequently become for the most part drawn away into

the service of the optic capsule, would seem to sufficiently account for all the peculiarities of their development. With regard to the nerve supply of the three pro-otic somites, it is now generally agreed that the third and fourth cranial nerves represent the ventral roots of the premandibular and mandibular somites respectively. With this interpretation of van Wijhe my observations are in perfect harmony. As for the view that the sixth cranial nerve represents the ventral root of the facial segment supplying the hyoid somite, there is less unanimity. Neal (**20**) and Dorhn would have us believe that it is a compound nerve formed by the fusion of the ventral roots of several segments, some of which would necessarily belong originally to the post-auditory region. For this theory I can find no evidence in the development of Scyllium. Nor does Neal's contention that the abducens is a meta-otic nerve which has come to supply an eye muscle derived from a pro-otic somite seem to rest on convincing evidence. Pl. 1, fig. **7**, shows that at this stage it is not so very far from the third somite it supplies. It is true that the root of the facial is relatively far forward; but this seems to be due to the cranial flexure, and an anticipation, so to speak, of the great development of the auditory sac and capsule. If this explanation proves insufficient, the two following should be considered before adopting Neal's contention. On the one hand it is possible that the anomalous position of the abducens may be due simply to the shifting backwards of its root; on the other hand, if this nerve be really compound, the fourth myotome (first meta-otic) may have contributed to the formation of the external rectus muscle. But, whatever the final verdict may be about these debatable questions, the evidence seems to be overwhelmingly in favour of there being only three pro-otic segments as originally held by Balfour.

We next have to determine the fate of the fourth somite, and to examine van Wijhe's conclusion that it corresponds to the hyoid arch. This fourth somite we may call the first meta-otic, since it first appears behind the auditory thickening

or placode, Pl. 1, fig. 2. Later on, it becomes overgrown by the auditory sac, which, as it rapidly expands, not only crushes the hinder region of the third somite in front, but almost squashes the fourth somite out of existence, Pl. 1, figs. 5 and 7. The latter breaks up into mesenchymatous tissue without yielding any distinct myotome.

In his well-known paper (**26**) van Wijhe states that : "Das dritte Somit befindet sich mit seiner Hauptmasse über der ersten Kiementasche, nur sein hinterer Theil erstreckt sich ein wenig weiter caudalwärts und hängt noch gerade mit der soliden Zellmasse im Hyoidbogen zusammen." . . . "Das vierte Somit liegt über der Zweiten Kiementasche und unter der Ohreinstülpung." . . . "Das fünfte Somit, dessen vorderer Theil aussen von der Anlage des Glossopharyngeus gekreuzt wird, liegt über der dritten Kiementasche." But he finds the fourth somite to be connected with the mesoderm of the hyoid arch, and the fifth somite to be connected with the mesoderm of the first branchial arch. Thus, from the fourth segment backwards, he believes the somites to be related to the arches in front of them. Consequently, since the third somite is undoubtedly related to the hyoid arch, van Wijhe finds two somites (third and fourth) connected with this arch, and associates the ninth cranial nerve with the fifth instead of the fourth somite. This strange result quite dislocates the orderly scheme of the segments, as has been already pointed out above.

My own observations do not bear out this interpretation. On the contrary, as a comparison of Pl. 1, figs. 1 to 7 shows, in Scyllium the visceral pouches pierce the lateral plate mesoderm in such a way that the clefts alternate with the somites, and the latter come to lie over each arch, but extend forward over the pouch in front. The fourth somite is at first distinctly connected with the mesoblast of the first branchial arch, the fifth somite with the mesoblast of the second branchial arch, and so on. Very soon, however, the somites above become disconnected from the arches below, the mesenchymatous intermediate tissue becoming diffused. Then

somites 5 and 4, and part of somite 3 also, break up; so that in an embryo some 19 mm. long the exact relation of the parts can no longer be made out, Pl. 1, figs. 7 and 8. Moreover, it is somite 4 and not somite 5 which is crossed by the glosso-pharyngeal, and it is somite 5 and not somite 6, as stated by van Wijhe, which is the first of the series of meta-otic somites to develop muscle-fibres. This is clearly shown in Pl. 1, fig. 2. In fact, my results are in agreement with those of Ziegler working on Torpedo (**28**), and like that author I am inclined to think that van Wijhe has mistaken the hinder region of somite 3 for somite 4, and consequently somite 4 for somite 5, in his description. Such mistakes are extremely difficult to avoid, and it is only by the most careful comparison of a very complete series of stages that one can trace the fate of these segments with certainty. At all events, his figures seem to agree better with the order of the segments given above than with his own tabular statement.

The Development and Fate of the Meta-otic or Occipital Somites.—Much has been written on this subject by various authors since van Wijhe (**26**). One may mention the works of Sewertzoff (**24**, **25**), Froriep (**6**), Braus (**3**), and Dohrn (**4**).

It is important first of all to determine which is the first meta-otic segment to produce a myotome. Van Wijhe states that it is the sixth somite (overlying the fourth gill-slit). Now, my reconstructions prove beyond doubt that, as mentioned above, in Scyllium the fifth somite produces muscle-fibres, although its myotome never becomes fully developed, Pl. 1, figs. 2, 3, 6. Ziegler (**28**) likewise finds that the first myotome arises in Torpedo from the fifth somite, and apparently Braus (**3**) comes to the same result with Spinax.[1] We may take it, then, that the first myotome is vestigial, and develops from the second meta-otic somite in Scyllium and probably in other Selachians.

[1] Some confusion arises through certain authors calling the fifth somite the first and not the second meta-otic somite.

The next important point to determine is how many segments take part in the formation of the occipital region of the skull, meaning thereby the region behind the auditory sac. Van Wijhe considers that the ninth segment is the last of the head, and the tenth the first of the trunk; that the fourth and fifth form no myotomes, that the first vestigial myotome belongs to the sixth somite, for which he could find no corresponding ventral root; that the myotomes of somites 7, 8, and 9 are well developed, and each have a ventral hypoglossal root. Thus, according to our nomenclature, van Wijhe would ascribe six meta-otic segments or somites to the occipital region in Scyllium and Pristiurus. Various authors who have worked at Torpedo have described a larger number of meta-otic segments in this fish: Sewertzoff, 10; Froriep, 13; and Dohrn, 11. But, as already stated above, we believe these discordant results are due to the secondary breaking-up of the somites into pieces which have been reckoned as segmental. Ziegler, indeed, has brought Torpedo into conformity with other Elasmobranchs.

Fürbringer, in his monograph on the hypoglossal nerves (7) developing Gegenbaur's views, maintains that a large number of Neocranial segments have been added to the head behind the original Palæocranial region to which the vagus is supposed to belong. Eight such trunk segments, designated by the letters *s–z* from before backwards, are assumed to have thus become assimilated to the head, together with their nerves (of the same nature as the spinal nerves). These neocranial somites and their nerves are further supposed to become progressively reduced, so that in the adult only those representing the last three letters of the alphabet, *x*, *y*, *z*, remain in Scyllium. Braus (3), in an elaborate study of the development of the occipital region in Spinax and other Selachians, attempts to support this theory on embryological grounds. According to him, the first vestigial myotome is produced from the fifth somite and the first complete myotome from the sixth somite (= *u*); but all the myotomes in front of *x* are supposed to disappear in the course of develop-

ment. Braus describes a process of shifting forwards of the myotomes to a position below the vagus-root and behind the auditory capsule where they degenerate. But what definite evidence is there that such a procession of myotomes which plunge one after the other below the capsule and vanish in a cloud of mesenchyme really occurs? Neither Dohrn (**4**) nor myself can find any. On the contrary, there is good reason to believe that for the most part myotomes once laid down persist, and that the chief change that takes place in the course of ontogeny is the crushing of the anterior myotomes owing to the growth backwards of the auditory sac and capsule, of the vagus, and of the gill-sacs.

In his careful description of the development of the occipital somites in Acanthias, Hoffmann (**15**) follows van Wijhe, states that somites 4–8 lie each above the five branchial slits, that somite 6 produces the first muscle, which degenerates later, that somites 9 and 10 form the last occipital segments, that myotomes of somites 7–9 are cut in half by the vagus root growing backwards, and that the ventral roots of segments 7, 8, and 9 alone persist. He attributes ten segments to the head region, and assumes that the eleventh, with a complete spinal nerve, is the first segment of the trunk.

Turning now to our reconstructions of Scyllium, we find that the first few somites behind the auditory capsule undergo different changes and suffer different fates. The first (fourth somite), crossed by the rudiment of the glosso-pharyngeal nerve, forms no muscle, and soon breaks up into mesenchyme. For a long time its posterior upper extremity retains an epithelial structure, and can be recognised behind the glosso-pharyngeal (Pl. 1, figs. 4, 5, 6). The next meta-otic somite (*S*. 5) lies at first under the vagus root, and is crossed by the first vagus branchial nerve and ganglion (Pl. 1, figs. 4, 5, 6) later on it spreads out, acquires a lobed, irregular dorsal edge, and projects beyond the vagus root both in front and behind (Pl. 1, figs. 6, 8, 11). Muscle-fibres develop in its hinder region, forming the first meta-otic myotome. In early stages

no ventral root can be seen supplying this myotome. According to van Wijhe and later authors the first meta-otic myotome degenerates in the course of ontogeny; but, although I have devoted much time and the greatest care to the settlement of this point, I have never been able to make absolutely certain as to its fate. In stage J (Pl. 1, figs. 5, 6) *S* 5 can be clearly made out, and is still plainly related to the second branchial arch; owing to its position below the vagus it cannot form a complete myotome with a large dorsal process such as grows up from the sixth and succeeding somites. That the sixth somite forms a complete myotome passing up dorsally behind the vagus is clear from a comparison of Pl. 1, figs. 1–11, and Pl. 2, fig. 19. Although in later stages the upper dorsal region of this myotome becomes cut off by the vagus root from the lower ventral portion (Pl. 1, figs. 9 and 11), yet it persists throughout development, stretching farther and farther forward over the occipital region of the skull. The ventral root supplying this second meta-otic myotome (*S* 6) develops early (Pl. 1, fig. 10), and later, piercing the skull, passes into the vagus groove (Pl. 2, fig. 17). It is the nerve *y* of Fürbringer. The nerve *z* of Fürbringer passes through a foramen in the occipital region further back, and supplies the complete myotome of somite 7, dividing into a dorsal and a ventral branch (Pl. 2, fig. 17). The myotome of the next somite, 8, is supplied by the first spinal nerve, issuing between the occipital arch and the first neural arch (Pl. 2, figs. 15–18). If the enumeration of the segments given above is correct, it follows that there are only four meta-otic segments, of which the last three are represented by muscles and nerves in the full-grown fish. But the numbering all depends on the accurate determination of the small ventral slip of muscle lying entirely below the vagus root. Is this really in later stages the persistent remains of the myotome of the second meta-otic somite (*S* 5), or has this muscle degenerated, shifted forwards, and been replaced by that of the third meta-otic somite (*S* 6)? After a most careful consideration

of the facts as displayed in the series of reconstructions here figured, and of a large number of sections and whole preparations of intermediate stages not figured, I have come to the conclusion that the first interpretation is correct. During the earlier stages (Pl. 1, figs. 5, 6, 7), when the original relation of the second meta-otic somite to the first branch of the vagus is still easy to make out, it seems clear that the somite does not really alter its position fundamentally; its hinder upper corner always can be seen to pass just behind the vagus root, and sometimes forms here quite a considerable dorsal process (Pl. 1, fig. 8). Nor can any distinct signs of degeneration be detected in its muscle before cartilage is formed. In quite late stages the minute slip of epibranchial muscle it forms is either difficult to distinguish from that of the next segment (*S* 6), or has disappeared. The ventral nerve root of the first myotome (*S* 5) cannot be detected in quite early stages. It seems to develop late, and is sometimes clearly visible when cartilage has begun to form (Pl. 2, fig. 15). In quite late stages it is seen to issue through a foramen as a slender nerve which joins the next behind. It seems to me probable that its comparatively large size in some of these later stages is due to its contributing to supply the hypoglossal muscles, some of which have probably been derived from the fifth somite.

Since one cannot follow the development of a given segment through successive stages in the same individual, it is impossible to remove all doubt as to the identification of a segment. But if the interpretation given above is wrong, and if the first meta-otic myotome really disappears in the course of ontogeny, as other authors have asserted, then this disappearance must take place late or very early. (It would seem that the belief in the early degeneration of the first myotome is partly due to the miscalculation of the segments made by van Wijhe and already discussed above.) In that case the somites numbered 5, 6, 7 in Pl. 1, figs. 7–11 should be numbered 6, 7, 8. There can, I think, be no doubt whatever that such a process of degeneration of myotomes

goes no further, if it takes place at all; for there is every reason to believe that these three somites are the same as the three numbered 5, 6, 7 in figures of later stages (Pl. 2, figs. 15–18). They can be followed step by step with comparative ease. In the latest stages studied, when cartilage has developed and the occipital region has practically acquired the adult structure, the spinal nerve of the second trunk segment is found provided with normal dorsal and ventral roots and a well-developed ganglion (Pl. 2, figs. 17, 18), while the first spinal nerve has a large ventral root, but only a vestige of a ganglion, and usually no distinct dorsal root. From this point forwards no trace of dorsal ganglia or roots can be found in late stages. Turning to earlier stages, we find that although transitory rudiments of ganglia are formed in all the anterior segments, the eighth somite never at any time has a fully-developed ganglionic rudiment. The history of the ganglia, then, affords evidence that the somite numbered 8 in my figures is the first trunk segment. The evidence, however, is not absolutely conclusive, since the rudiments are subject to much individual variation and there is a gradation in size from before backwards.

But in embryos 26 mm. long (Pl. 2, fig. 12), where the first traces of procartilage can be distinguished, the identity of the segments can be clearly made out. From that time onwards the fate of the myotomes can be traced with certainty, and there is neither a degeneration of muscles in front nor an assimilation of new myotomes behind.

To sum up the foregoing observations on the development of the meta-otic somites and nerves: In the adult Scyllium canicula the second trunk segment has a complete myotome and a complete spinal nerve, with dorsal and ventral root and a ganglion. In quite late stages the first trunk segment has a complete myotome, but a spinal nerve in which the dorsal root and its ganglion have been reduced to a mere vestige, if present at all (Pl. 2, fig. 17). The fully developed ventral root of this first spinal passes out between the occipital arch of the skull and the first neural arch of the vertebral column.

Two occipital nerves are always found piercing the hinder region of the skull. The larger and more posterior issues through a foramen lying on the inner aspect of the skull about halfway between the occipital margin and the vagus foramen. This nerve (*z* of Fürbringer) supplies the last occipital myotome (*S* 7). The foramen for the more anterior nerve lies below the vagus foramen; the nerve supplies the penultimate occipital myotome, complete, but subdivided by the vagus root into dorsal and ventral portions (Pl. 2, fig .16). According to Fürbringer (7), a third nerve passes out still further forward. I find that it occurs in some but not in all adults. It seems to develop late, and a mere trace of it can be detected in a stage 33 mm. long, while it is clearly seen in the later stage shown in Pl. 2, fig. 17. Since the last two occipital nerves can be identified for certain from the adult to the 26 mm. stage, when procartilage is only just coming into evidence, it may be concluded with practical certainty that this slender and inconstant nerve root is that of the first of the three occipital myotomes, which is never completed dorsally, being placed below the vagus root and crossed by the first branchial branch of the vagus. According to my observations this first meta-otic myotome, which may or may not persist in the adult, develops from the fifth somite (second meta-otic), and never moves much from its place of origin. No muscle at all is developed in the fourth somite, which is crossed by the rudiment of the glosso-pharyngeal and crushed by the enlarging auditory sac. The series of meta-otic somites is regularly related to the gill-slits, one being placed originally above each branchial slit from the first to the fifth, and connected with the following branchial arch. There are thus five somites in the branchial region. The last of these, situated above the fifth branchial slit (sixth gill-slit), and related to the fifth branchial bar (seventh visceral bar), has a myotome supplied by the first spinal nerve and therefore belonging to the trunk, if we draw the distinction between the head and the trunk at the occipital joint. Excepting for the first meta-otic myotome (*S* 5), which seems to disappear

in many individuals, there appears to be no further degeneration of myotomes at any stage, nor is there any evidence of the shifting forwards of myotomes or disappearance of successive segments such as has been described by many authors.

Concerning the nerves of the occipital region, it should be noticed, in addition to what has been mentioned above, that no ventral root ever appears belonging to the glosso-pharyngeal (*S* 4). The four branchial branches of the vagus with their epibranchial placodes represent the dorsal roots of meta-otic segments 5, 6, 7, 8. Therefore to the first of these belongs the vestigial and inconstant ventral root described above (p. 15), while to the second and third correspond the two posterior occipital nerves which pierce the skull. The ventral root of the eighth segment containing the fourth vagus branch is the first spinal nerve. Only incomplete dorsal roots and ganglia are developed in these segments, but they are all quite obvious at certain stages in ontogeny (Pl. 1, figs. 6, 11), disappearing later completely in segments 5, 6, and 7, and remaining only as a mere vestige in segment 8 (Pl. 2, fig. 17). Without entering into a detailed discussion of the structure and origin of the vagus nerve, so well dealt with by Johnston (16), it may here be pointed out that, while the embryological evidence in Scyllium (and especially in Petromyzon — see Koltzoff (17)) is definitely against the view of Gegenbaur that the vagus has been formed by the gathering together of a number of complete segmental nerves, yet it is in favour of the view that the vagus is a complex nerve, formed, not so much with the help of a longitudinal collector, as held by Koltzoff and Johnston, as by the gathering together of only certain portions of segmental nerves (four in Scyllium), leaving behind other portions or components, which remain as the incomplete and more or less transitional roots and ganglia of the vagus region. This theory seems to be the only one which will account for the facts, and at the same time explain the formation of the vagus without the disturbance either of the central or of the peripheral connections of the nerves; for the gathering

and sorting out of the components probably takes place at an early stage when the neural crest is still, in this region, continuous.

The Development of the Cartilaginous Elements.—My observations on the development of the cartilages of the skull in Scyllium differ in no very important respect from those of Sewertzoff on the skull of Acanthias and Pristiurus (**25**). The first sign of the appearance of the skull is in the form of a sheet of dense mesenchyme extending on either side of the notochord. From the level of somite 4 it thins out forwards, reaching to the infundibular region (Pl. 1, fig. 9). No distinct signs of segmentation are any longer visible at this stage in this tissue which, however, is doubtless derived from the sclerotomes of segments 4 and 3, and perhaps also of segments 2 and 1. Two outgrowths seem to mark the original position of sclerotome 4 below the glosso-pharyngeal nerves (Pl. 1, figs. 10, 12). The scleromeres are formed further back in segments 5, 6 and 7, just as they are in the trunk (p. 6) by a condensation of mesenchyme in the hinder region of each segment and stretching outwards behind the corresponding nerve and myotome (Pl. 1, figs. 10, 11). The thickened posterior edge of the parachordal sheet doubtless represents the scleromere of segment 4 (first meta-otic). In later stages the parachordal plate and occipital scleromeres become more and more developed, until the latter fuse with each other and with the plate. In an embryo 26 mm. long the first signs of procartilage are visible. Staining with thyonin brings out behind the wide parachordal plate (Pl. 2, fig. 12) two occipital arches rising from the floor of less dense tissue, and a more posterior accumulation of cells near the notochord representing the centrum. This element, probably derived from the sclerotome of the eighth segment, gives rise to the occipital condyles, if we may designate by this term the paired processes projecting backwards towards the centrum of the first vertebra in the adult. Very soon all these occipital elements become indistinguishably fused to the parachordal plate (Pl. 2, figs. 13, 14).

The two rudiments of occipital arches mentioned above arch over the last occipital nerve in the 26 mm. embryo (Pl. 2, fig. 12). Later on they together form on each side the large cartilaginous arch which grows upwards surrounding the foramen magnum, completes the side walls of the occipital region, abuts against the auditory capsule in front, and finally fuses with it (Pl. 2, figs. 13-18). Van Wijhe (**27**) considers that this large arch represents a single neural arch of the vertebral column (pierced by a ventral root in Acanthias). But the early relation of the two pillars of the arch to the enclosed nerve and to the septa seems to prove that the cartilaginous occipital arch is composed of two elements each equivalent to a neural arch and belonging to segments 6 and 7 (Pl. 2, figs. 12, 14). Further forward similar arches are indicated (Pl. 2, fig. 14) by uprisings of the parachordal plate, which eventually surround the anterior occipital nerves and complete the sides of the cranium behind and below the auditory capsule (Pl. 2, fig. 17).

Since the last occipital segment corresponds to the seventh somite lying over the fourth branchial slit and fourth branchial bar supplied by the third branch of the vagus, it is clear that the last slit and vagus branch belong morphologically to a segment behind the posterior limit of the occipital arch in Scyllium (the condyles, however, probably belong to the eighth segment). This discrepancy between the skull and the other organs of the head is not unusual among Vertebrates. I have elsewhere shown that in Urodeles the vagus and gill-slits extend behind the occipital segments (**10** and **11**), and in Petromyzon the discrepancy is, of course, still more pronounced. The fact is that the process of cephalisation has to some extent been independently carried out in the visceral and in the cranial elements. As Koltzoff (**17**) points out, a different limit may be assigned to the head according as we take one system or the other as our criterion. To avoid the somewhat paradoxical conclusion that the head region extends into the trunk, it would be advisable for practical purposes to use the term "cranial

region" for the segments as far as the hind limit of the rigid skull at the occipital joint, and "visceral region" for the segments reaching back to the last gill-slit and vagus branchial nerve. Thus in Scyllium there would be seven cranial and eight visceral segments, in Siredon six cranial and seven visceral segments, while in Petromyzon there would be ten visceral but only four cranial segments. According to Braus (3) the last occipital and the first trunk spinal nerves are always the same in the Selachians, but Rosenberg (23) in Carcharias and van Wijhe in Acanthias and Heptanchus (27) believe a late addition is made to the skull by the assimilation of one or more vertebral segments. In Scyllium no such addition takes place, for the centra of the first two trunk segments are always separate from their neural arches (Pl. 2, fig. 17), differ in this respect from those behind, and can be detected in consequence even in embryos only 26 mm. long (Pl. 2, fig. 12).

Although this paper deals chiefly with the occipital region, a few words may be added about the development of the rest of the skull. The essential facts have already been described by Sewertzoff (24), and in a valuable preliminary note without figures by van Wijhe (27). The trabeculæ in an embryo 26 mm. long are scarcely discernible except as a slightly denser region of mesenchyme on either side of the infundibulum. In the 33 mm. stage figured (Pl. 2, figs. 13–16) they appear as distinct cartilage rods expanding in front into a procartilaginous sheet, which spreads out between the orbit and the nasal capsule—the first indication of Sewertzoff's ethmoid cartilage. At no stage in development do I find the trabeculæ bent down at right angles to the parachordal plate as figured by Sewertzoff, but always from the first more nearly in the same plane (Pl. 2, fig. 14). They soon join and fuse with the extreme anterior corner of the parachordal plate, below the ring which grows out from the plate to surround the anterior carotid (Pl. 2, figs. 14, 18). In front the trabeculæ join in the middle line (Pl. 2, fig. 18), and are continued forward and upward into the nasal septum. A film

of procartilage continuous with the upper edge of the septum extends over the nasal sac on either side, and develops into the overhanging nasal capsule completed behind by the expanding ethmoid wing. Above, in the inner wall of the orbit, arise the alisphenoid cartilages of Sewertzoff (sphenolateral of Gaupp (**8**), lamina antotica of van Wijhe). At first separate, they soon join the parachordals, spread out into a thin sheet of procartilage dorsally, and eventually become continuous with the auditory capsule behind and the ethmoid cartilage in front. Originally situated between the oculomotor and the trigeminal nerve, the lamina antotica forms the greater part of the wall of the orbit and surrounds the nerve exits in this region.

The development of the auditory capsule is of some importance. It is formed in the layer of tissue immediately surrounding the sac, faithfully following the folding of the sac when the semicircular canals begin to appear (Pl. 2, figs. 13–16). Cartilage develops much later in the capsule than in the parachordal plate or occipital arch. From the very first

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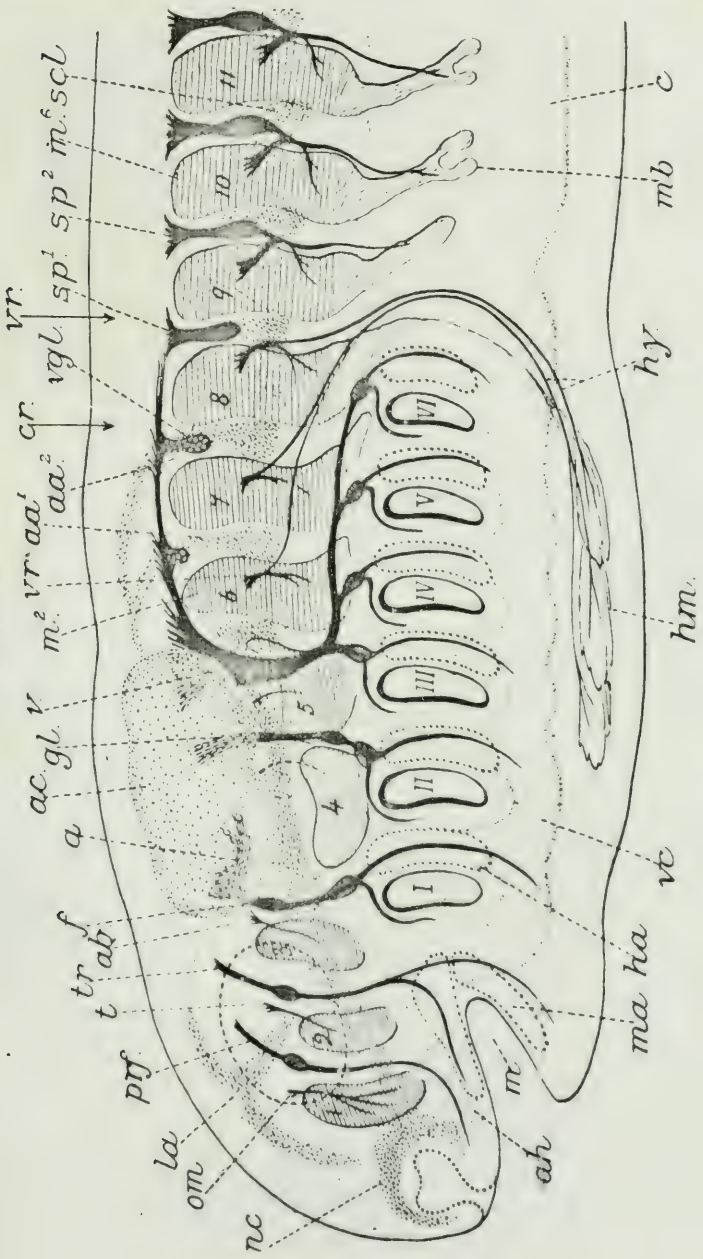
Diagram of the segmentation of the head in Scyllium canicula: *C.R.* Limit of cranial region. *V.R.* Limit of visceral region. I–VI. Gill-slits. 1–11. Somites, prootic from 3 forwards, and metaotic from 4 backwards. *a.* Auditory nerve. *ab.* Abducens nerve. *ac.* Auditory capsule. *ah.* Anterior head-cavity. *c.* Cœlom in lateral plate mesoblast. *f.* Facial nerve. *gl.* Glosso-pharyngeal nerve. *ha.* Hyoid cartilaginous arch. *hm.* Hypoglossal muscles from myotomes of somites 6, 7, 8. *hy.* Hypoglossal complex nerve. *la.* Lamina antotica. *M.* Mouth. m^2. Second metaotic myotome. m^6. Sixth metaotic myotome. *ma.* Mandibular cartilaginous arch. *mb.* Muscle-bud to pectoral fin. *nc.* Nasal capsule, continuous with trabecula behind. $aa.^1$ and $aa.^2$ First and second occipital arches of segments 6 and 7. *om.* Oculomotor nerve. *prf.* Profundus nerve. *scl.* Sclerotome of segment 10. $sp.^1$ Vestigial dorsal root and ganglion of first spinal nerve. $sp.^2$ Second spinal. *t.* Trochlear nerve. *tr.* Trigeminal nerve. *v.* Complex root of vagus nerve. *vgl.* Vestigial dorsal root and ganglion of segment 7. *vc.* Ventral cœlom extending up each visceral bar. *vr.* Ventral nerve-root of segment 6, supplying second metaotic myotome and hypoglossal muscle. The myotomes are longitudinally striated, the nerves black, and the scleromeres dotted. The cartilaginous visceral arches are represented by dotted outlines, also the optic capsule and the nasal sac.

TEXT-FIG. 1.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(Pl. 2, fig. 12) the slightly denser layer of tissue from which the capsule arises seems to be continuous ventrally with the parachordal plate, as described by Sewertzoff. This continuity is between the facial and the glosso-pharyngeal nerves (Pl. 2, fig. 12); but as the capsule expands backwards it passes above the glosso-pharyngeal and vagus, leaving a considerable gap through which these nerves pass out between the capsule and the plate. Later this gap forms the vagus groove (Pl. 2, figs. 15, 17). Cartilage spreads from two pillars rising up from the parachordal plate, one passing up the anterior outer corner of the capsule, and the other up its inner wall (Pl. 2, figs. 14, 18, *po.*, *p.*).

Summary.

Although the observations recorded above bring out no striking novelty, they will, I think, be useful in completing our knowledge of the development of the head region in Elasmobranchs, in clearing up some obscure points, and in settling certain questions about which there has been much uncertainty and controversy.

In a trunk segment of Scyllium the ventral root of the spinal nerve is mid-segmental or somitic, and the dorsal root intersegmental or intersomitic in morphological position. To form a mixed spinal nerve, the ganglionated dorsal root joins the ventral root in front, and the main branch passes outwards in the septum behind its myotome. In the head region, where the roots retain their original independence, the dorsal roots, therefore, are also morphologically situated behind the somites to which they belong.

There are three pro-otic segments, corresponding to the profundus, trigeminal, and facial nerves. Somite 1 is preoral, somite 2 lies above the mouth and is related to the mandibular bar. Somites 3 to 8 lie above each of the six gill-slits, and are related to the hyoid and five branchial bars, The three pro-otic somites are supplied by the oculomotor trochlear, and abducens nerves. The first meta-otic segment, with the glosso-pharyngeal nerve, contains somite 4, which

produces no myotome and has no ventral root. Three more meta-otic somites, supplied by the occipital ventral roots, and corresponding to the first three branchial branches of the vagus, complete the cranial region. The eighth somite belongs to the first spinal nerve, of which the dorsal root is absent or vestigial in later stages, and to the fourth branch of the vagus.

The vagus nerve has been formed by a partial gathering forward of components of four dorsal roots, without breaking either their central or their peripheral connections. The visceral region of the head extends one segment farther back than the cranial region, and the hind limit of the head differs according as we choose to determine it by the extent of the cranial or the visceral cephalisation. There is little or no degeneration or shifting forwards of myotomes behind the auditory capsule.

Segments 3 and 4, and possibly also 1 and 2, contribute to the formation of a basal mesenchymatous sheet below the hind brain, from which develops the parachordal cartilaginous plate on either side of the notochord. Scleromeres from segments 5, 6, and 7 become added to these plates behind, and the "condyles" seem to be formed from segment 8. The lateral and dorsal walls of the occipital region are formed by the upgrowth of elements corresponding to the neural arches. The two posterior of these elements, belonging to segments 6 and 7, combine to form the large occipital arch, which fuses with the auditory capsule. The neural arches develop in the denser posterior region of the sclerotomes, and in the first two segments of the vertebral column they are separate from the centra. The auditory capsule from its first origin is continuous with the parachordal plate between the facial and the glosso-pharyngeal nerves. It grows backwards, covering the latter, the vagus, and the occipital nerves. The trabeculæ develop later than the parachordals, with which they soon fuse; they meet in front to form the median nasal septum, and develop large ethmoid wings which contribute to the nasal capsule together with the septum. On either side a

lamina antotica arises separately in front of the trigeminal nerve, soon fuses with the parachordal, expands upwards, and eventually forms the greater part of the wall of the orbit and upper roof of the skull.

July 24th, 1917.

Bibliography.

1. Balfour, F. M.—"Preliminary Account of the Development of Elasmobranch Fishes,' 'Quart. Journ. Micr. Sci.,' vol. 14, 1874.
2. ——— "Monograph on the Development of Elasmobranch Fishes," 'Journ. Anat. and Phys.,' 1876–7–8.
3. Braus, H.—"Die metotischen Urwirbel," 'Morph. Jahrb.,' vol. xxvii, 1899.
4. Dohrn, A.—"Studien z. Urgesch. d. Wirbelthierkörpero," No. 18, "Die Occipitalsomite," 'Mitth. Geol. Sta. Neapel,' vol. xv, 1901.
5. ——— "Studien," No. 23, "Die Mandibularhöhle," and No. 24, "Die Prämandibularhöhle," ibid., vol. xvii, 1904.
6. Froriep, A.—"Genèse de la partie occipitale du crâne," 'C. R. Ass. Anat.' (Genève), vol. vii, 1905.
7. Fürbringer, M.—"Ueber die spino-occipitalen Nerven," 'Festschr. v. C. Gegenbaur,' vol. iii, Leipzig, 1897.
8. Gaupp, E.—"Allg. Entwickl. des Kopfskelettes," Hertwig's 'Handbuch d. Entw. d. Wirbeltiere,' vol. iii. 1906.
9. Goodrich, E. S.—"Vertebrata Craniata, 1st fasc, Cyclostomes and Fishes," 'Treatise on Zoology,' ed. by Lankester, London, 1909.
10. ——— "Occipital Region of the Head in the Batrachia urodela," 'Proc. Zool. Soc.,' 1911.
11. ——— "Metameric Segmentation and Homology," 'Quart. Journ. Micr. Sci.,' vol. 59, 1913.
12. ——— "Proboscis Pores in Craniate Vertebrates," 'ibid,' vol. lxii. 1917.
13. Hatschek, B.—"Die Metamerie des Amphioxus u. des Ammocœtes," 'Verh. Anat. Ges. Wien,' 1892.
14. ——— "Ueber Amphioxus," 'Anat. Anz.,' vol. viii, 1893.
15. Hoffmann, C. K.—"Beitr. zur Entwickl. der Selachii," 'Morph. Jahrb.,' vol. xxv, 1897.
16. Johnston, J. B.—"Morphology of the Vertebrate Head," 'Journ. Comp. Neur. and Psych,' vol. xv, 1905.

17. Koltzoff, N. K.—"Entwickl. d. Kopfes von Petromyzon planeri," 'Bull. Soc. Imp. Nat. Moscou,' vol. xv, 1902.

18. Marshall, A. M.—"On the Head-cavities and Associated Nerves in Elasmobranchs," 'Quart. Journ. Micr. Sci.,' vol. 21, 1881.

19. Neal, H. V.—"Segmentation of Nervous System in Squalus acanthias," 'Bull. Mus. Comp. Zool. Harvard,' vol. xxxi, 1898.

20. ——— "Morphology of the Eye-muscle Nerves," 'Journ. Morph.,' vol. xxv, 1914.

21. Platt, Julia B.—"Contribution to the Morphology of Vertebrate Head," 'Journ. Morph.,' vol. v, 1891.

22. ——— "Further Contributions, etc.," 'Anat. Anz.,' vol. vi, 1891.

23. Rosenberg, E.—"Die occipitalregion d. Cranium e. Selachier," 'Dorpat,' 1884.

24. Sewertzoff, A. N.—"Studien z. Entwickl. d. Wirbelthierkopfes," 'Bull. Soc. Imp. Nat. Moscou,' 1898.

25. ——— "Die Entwickl. des Selachierschädels," 'Festschr. 1 v. Kupffer,' Jena, 1899.

26. Wijhe, J. W. van.—'Ueber d. Mesodermsegmente u. die Entw. der Nerven des Selachierkopfes,' Amsterdam, 1882. Reprinted, 1915.

27. Wijhe, J. W. van.—'Ueber die Entwickl. des Kopfskeletts bei Selachiern," 'C. R., 6e Congrès Intern. Zool.,' Berne,' 1904.

28. Ziegler, H. E.—"Die phylog. Enstehung des Kopfes." 'Jena Zeitschr. Naturw.,' vol. xliii, 1908.

EXPLANATION OF PLATES 1 AND 2,

Illustrating Mr. Edwin S. Goodrich's paper "On the Development of the Segments of the Head in Scyllium."

Explanation of Lettering.

a. Anterior tissue (ant. head-cavity of Platt). *ab.* Abducens nerve. *ac.* Auditory capsule. *al.* Alimentary canal. *als.* Alisphenoid cartilage or lamina antotica. *ant. c.* Anterior carotid. *ar.* Artery. *as.* Auditory sac. *Ba.* $^{1-5}$ Branchial bar 1–5. *bh.* Basihyal. *bp.* Basal parachordal plate. c^{1} Centrum of first trunk segment. *c. Ba.* Cartilaginous branchial arch. *cv.* Posterior cardinal vein. *da.* Dorsal aorta. *f.* Facial nerve and its rudiment. *eg.* External gill. *eth.* Ethmoid cartilage. *ggl.* Spinal ganglion. *gl.* Glosso-pharyngeal nerve and its rudiment. *gp.* $^{1-6}$ Gill-pouch 1–6. *H.* Hyoid bar. *hm.* Hyoman-

dibular cartilage. *ht.* Heart. *hy.* Hypophysis. *lp.* Lateral plate mesoblast. *m.* Myotome. *m.* [1-x] First and succeeding myotomes. *ma.* Mandibular artery. *mc.* Mandibular cœlomic canal. *md.* Mandibular bar. *mdc.* Mandibular cartilage. *na.* Neural arch. *nac.* Nasal capsule. *nc.* Nerve cord. *ns.* Nasal septum. *nt.* Notochord. *oc.* Occipital condyle. *oca.* Occipital arch; *oca*[1]. and *oca*[2]. its first and second pillars. *ocm.* Oculomotor nerve. *opn.* Optic nerve. *p.* Dorsal process of parachordal plate. *pc.* Pericardium. *pn.* Pronephros. *po.* Outer dorsal process of parachordal plate. *post. c.* Posterior carotid. *ppl.* Basal parachordal plate. *pqc.* Palatoquadrate cartilage. *pr.* Posterior denser region of sclerome. *S.* [1-10]. First to tenth somite. *sa.* Segmental artery. *sn.* Sensory nerve from dorsal ganglion. *sof.* Superior ophthalmic branch of facial nerve. *sotg.* Superior ophthalmic branch of trigeminal nerve. *sv.* Segmental vein. *tg.* Trigeminal nerve and its rudiment. *tra.* Trabecula cranii. *tro.* Trochlear nerve. *v.* Vagus nerve. *v.* [1-4] Its four branchial branches and ganglia. *vc.* Cut root of vagus. *vggl.* Vestigial ganglion of first spinal nerve. *vgr.* Vagus groove. *vn.* Vein. *vr.* Ventral root of spinal nerve.

[All the figures are of various stages of Scyllium canicula, L. Figs. 1, 2, 3, 5, 13–17 are reconstructed on median longitudinal vertical sections, and the outer epidermal covering is, for the most part, omitted. The somites and other mesoblastic structures are drawn in red on Plate 1; myotomes being indicated by horizontal strokes, mesenchyme and sclerotomal tissue by dots. Cartilage is coloured purple, and procartilage is represented in purple dots on Plate 2.]

PLATE 1.

Fig. 1.—Left side view of the anterior region of an embryo at stage F.

Fig. 2.—Similar view of stage G.

Fig. 3.—Stage G, rather later than Fig. 2. Inner view of right half of embryo.

Fig. 4.—Stage I (about 6 mm.). Dorsal view, the left half at the level of the middle of the notochord; the right half cut more dorsally. On the right the roots of the glosso-pharyngeal and vagus nerves have been completely reconstructed to show their position over the somites.

Fig. 5.—Stage J (about 7 mm.). Left side view, with the lateral wall of the gill-bars shaved off, exposing the five gill-pouches.

Fig. 6.—More enlarged inner view of somites 4–11, with the related nerve roots, etc., of the right side of the same embryo.

Fig. 7.—Left side view of the anterior region of an embryo 10 mm. long. The side of the body has been cut away more deeply than in Fig. 5.

Fig. 8.—Embryo 19 mm. long. Reconstruction of the nervous system, and myotomes of the auditory and occipital regions of the right side.

Fig. 9.—Embryo 26 mm. long. Reconstruction of the auditory sac, nervous system, myotomes, etc., of the left side of the auditory and occipital regions. The dense mesenchyme or blastema of the basal parachordal plate and more posterior scleromeres is indicated by dots.

Fig. 10.—Dorsal view of a slice of the left side of the occipital region reconstructed from horizontal sections of an embryo 20 mm. long.

Fig. 11.—Portion of the nervous system, somites, etc., of the left side of an embryo of stage J (of Balfour). The slice includes only the roots of the glosso-pharyngeal and vagus.

PLATE 2.

Fig. 12.—First appearance of the skeleton of the head, embryo 26 mm. long, in the form of procartilage indicated by dots. Dorsal view (slightly oblique) reconstructed from horizontal sections. On the left the nervous system is more completely shown. On the right the auditory capsule is indicated.

Fig. 13.—Embryo 33 mm. long. Right-side view of head region with nervous system and skeleton.

Fig. 14.—More enlarged view of the skull, showing the occipital arch, parachordal plate, trabecula, and alisphenoid cartilages. The procartilaginous extensions of the two latter are cut off.

Figs. 15 and 16.—Two views of the occipital region of the 26 mm. embryo of Fig. 13 more enlarged. Figs. 15 represents a slice showing the skeleton and nerve roots. Fig. 16 a thicker slice including more of the skull and nerves, and the myotomes. The near-cut surfaces of the cartilages are dotted.

Fig. 17.—Thick slice of the left occipital region of an advanced embryo with fully developed skeleton. The occipital region of the skull, the first three segments of the vertebral column, and the nerves are shown. The three occipital nerves are represented hanging down from the vagus groove and outside the cartilage.

Fig. 18.—Dorsal view (slightly oblique) of the head skeleton, nerves, etc., of an embryo about 33 mm. long, but more advanced than that shown in Fig. 13. On the left the nasal capsule, alisphenoid cartilage, auditory capsule, and vertebral column are completely reconstructed; the cranial and spinal nerves, and the myotomes are also shown. On the right side, while the alisphenoid cartilage and auditory capsule have been removed, the mandibular and hyoid arches are included, and also some arteries.

Fig. 19.—Left side view of the anterior region of an embryo of stage G. drawn from a specimen stained and mounted whole.

Figs. 20 and 21.—Reconstructions, somewhat diagrammatic of the right side of some trunk segments of stage J, Fig. 20, and stage N. Fig. 21; showing the relation of the parts to each other.

Figs. 22, 23, and 24.—Three horizontal sections showing the relation of the various parts of the segments on the left side. Fig. 22 represents the most dorsal, and Fig. 24 the most ventral section of the same segments.

[NOTE.—The embryonic stages of the Dog-fish indicated by capital letters in this memoir are those so indicated in the well-known classification of stages used by Balfour.]

On some new Phelliinæ from New Guinea.

By

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With Plates 3–5, and 2 Text-figures.

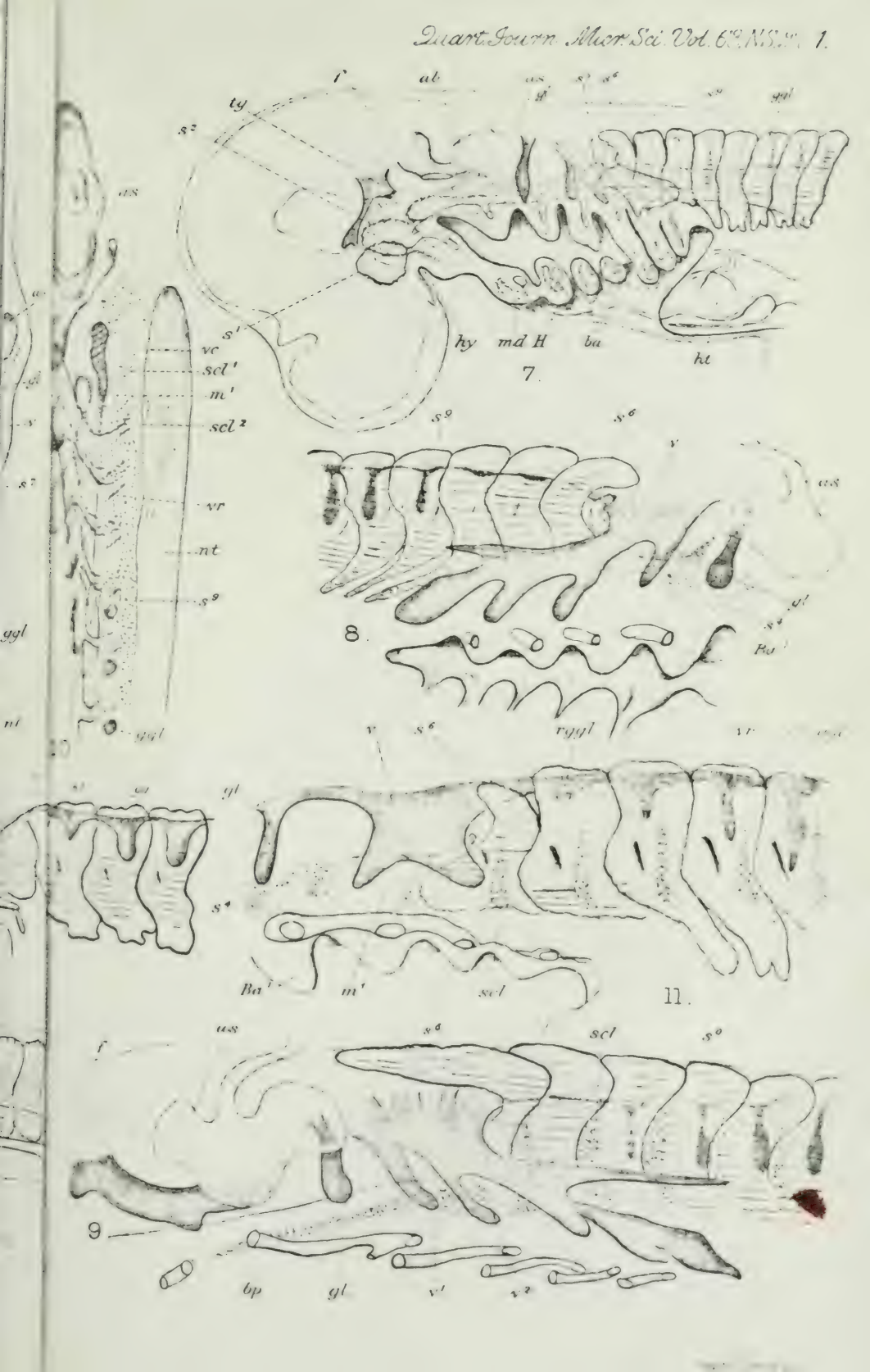
The genus Phellia, instituted by Gosse (**14**) and placed in the family Sagartiadæ, was originally defined by him as follows: "Base adhering to rocks; little exceeding the column. Column pillar-like in expansion; the margin tentaculate, without parapet or fosse. Surface smooth, pierced with loop-holes; partly clotted with a tough epidermis, which is rough externally, firmly adherent to the skin. Disc concave; the edge not undulate. Tentacles few, in more than one row; barred. Mouth not raised on a cone; lip thickened. Acontia discharged but reluctantly."

The type species is Phellia murocincta, Gosse, and to this the author of the 'Actinologia Britannica' added gausapata and picta, and included the genus in his family Sagartiadæ, one of the characters of which, as given by Gosse, was "integument pierced with loop-holes (cinclides) —special orifices, through which are emitted and retracted fleshy cords (acontia) which have their origin in the membranous partitions of the body-cavity."

In 1867 Verrill (**28**) erected the sub-family Phelliinæ of the family Actinidæ, defining it as follows: "Column elongated; covered with a persisent thickened epidermal deposit, except that, near the margin and sometimes close to the base, the

surface is naked and may be retracted within the thickened portion. Acontia very few and seldom emitted—perhaps entirely wanting in some species." In his definition of the genus Phellia Verrill says of the acontia that they are "sparingly emitted from the mouth and from pores near the base," and in his description of a large and handsome species, Phellia panamensis, he notes among other characters the following: "Column . . . capable of contracting into the form of a tall cone by involving the summit. . . Tentacles about 96 in number, the 12 inner ones large and stout. . . . In dissecting a large specimen it was found that the 12 septa corresponding to the 12 large inner tentacles, are much larger than the others, with the inner edges strongly thickened and muscular, and bear the large convoluted ovaries throughout nearly their whole length, while the intervening septa are very narrow, not thickened, and bear no sexual organs." This is the first recorded account of the internal anatomy of a number of the genus Phellia. It should be observed that Verrill keeps the Phellinæ distinct from his sub-family Sagartinæ.

In 1884 Andres (1) makes the Phellidæ a sub-family of the family Actininæ, keeping them separate from the Sagartiidæ, and includes in the sub-family the genera Octophellia, Phellia, Hyactis, Chitonactis and Ammonactis. Of these Octophellia does not appear to have been heard of again, and the three last have been removed from the sub-family on anatomical grounds. Andres' definition of the Phellinæ is founded on external characters, but in his account of the anatomy of Actinians he takes Phellia limicola as an illustration, and figures and describes six pairs of complete and fertile mesenteries with labial and parietal mesenterial stomata; eighteen pairs of infertile incomplete mesenteries. In the light of more recent work the accuracyof some of his anatomicaly statements is open to suspicion. Thus, he omits to figure or describe the characteristic parietal muscles of the mesenteries; he figures and describes, correctly enough, the muscle banners of the large longitudinal retractors of the twelve primary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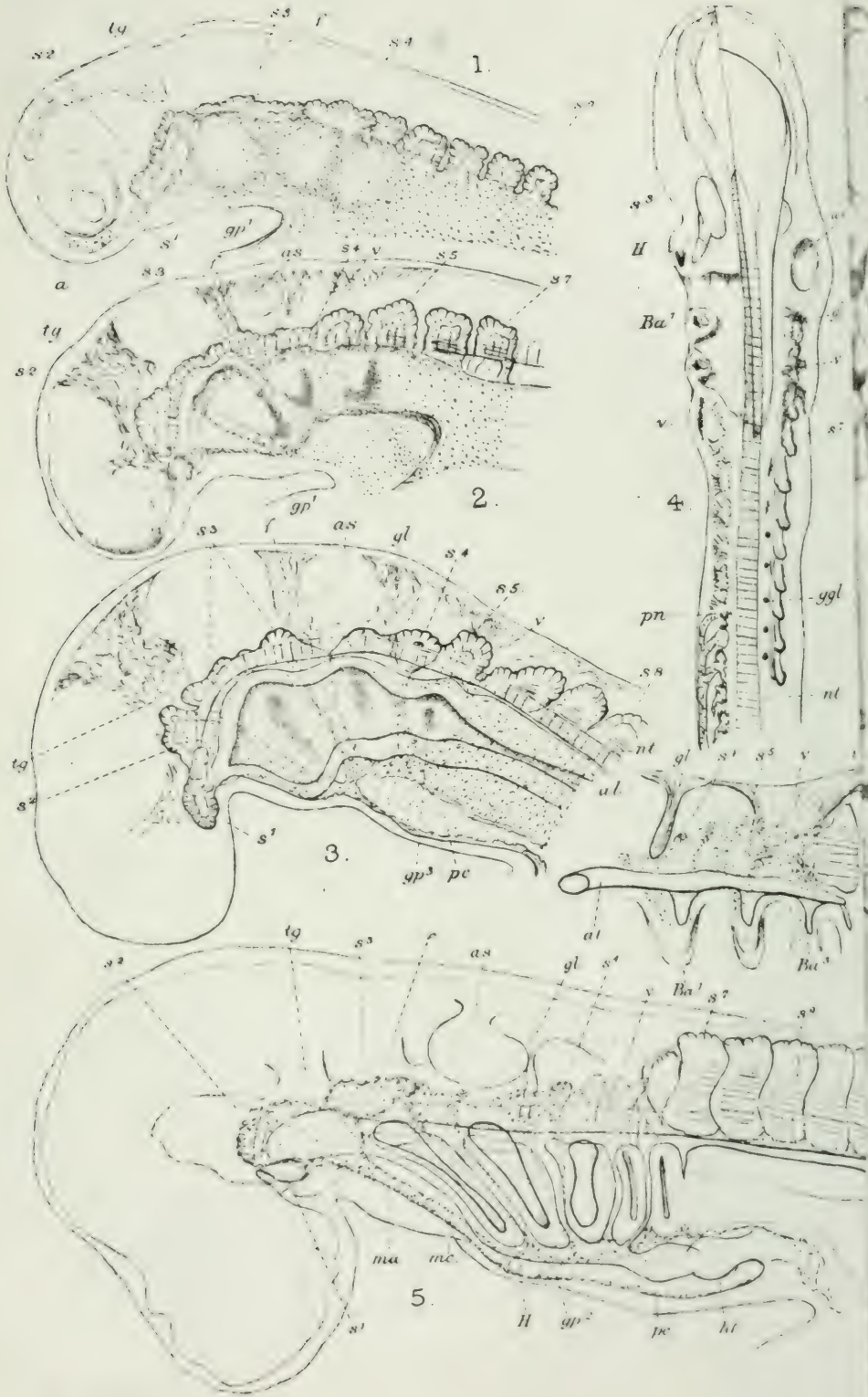

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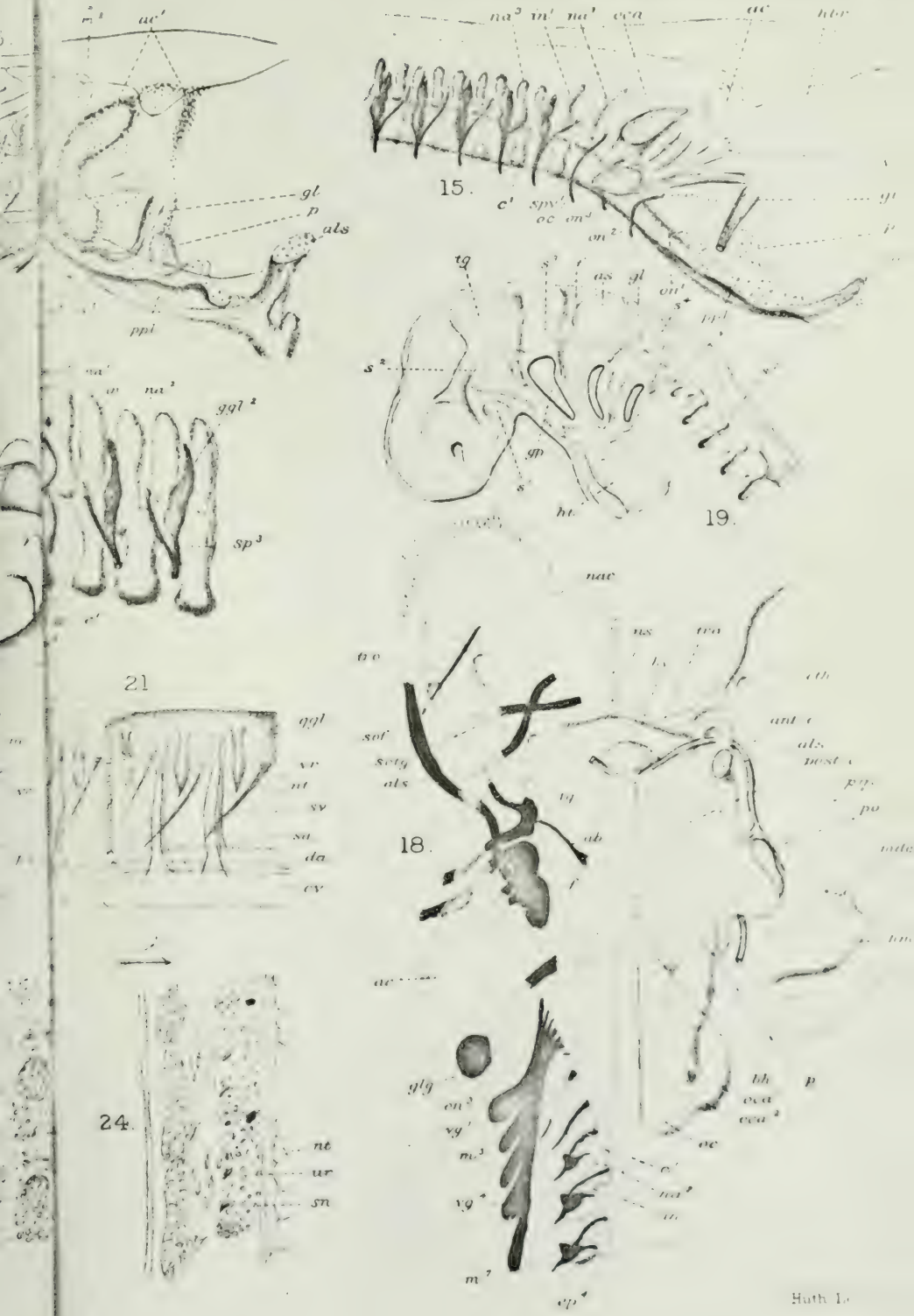
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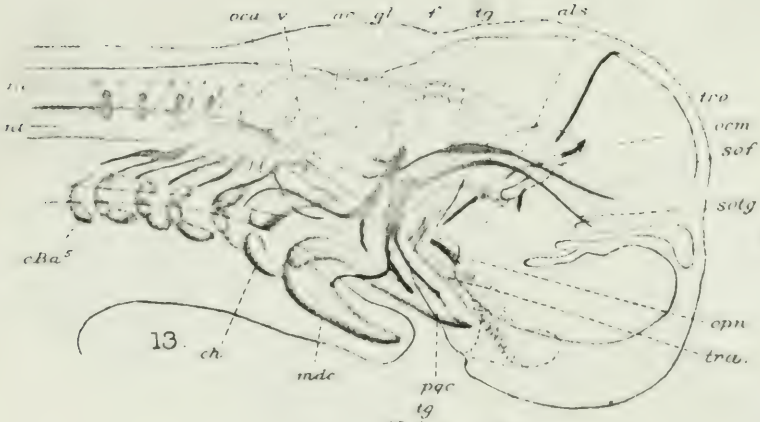

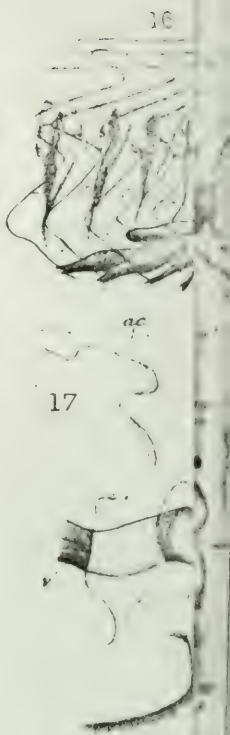

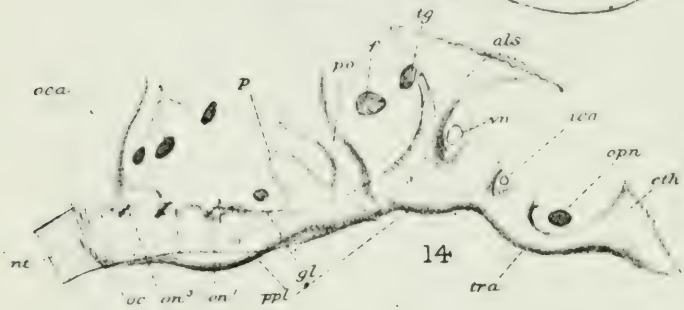

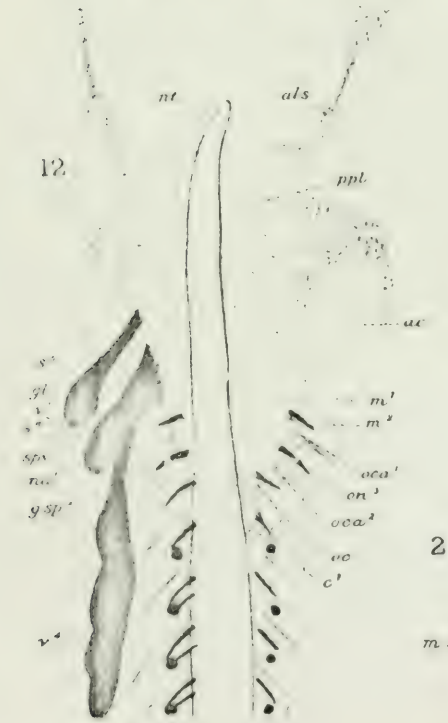

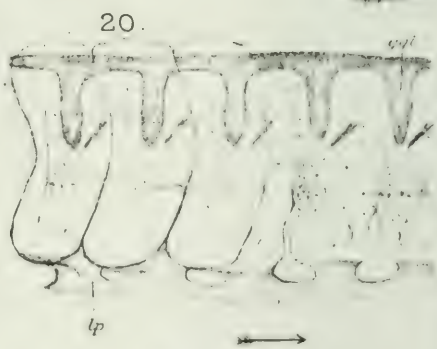

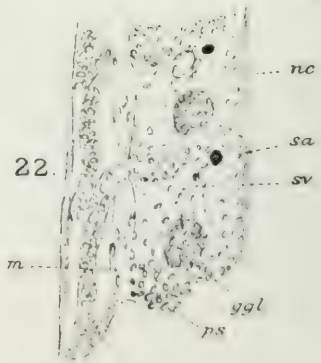

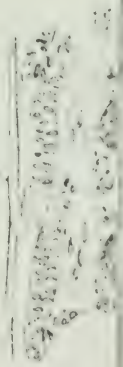

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mesenteries, but also figures muscle banners on the secondaries, a feature which, if it occurs, is unique among the species anatomically investigated. New species were added to the genus, but no additional anatomical investigations were made on it till, in 1897, Kwietniewsky (22) described and gave a short anatomical account of Phellia ternatana, and in the same year Haddon (16) and Maguire (24) did the same for P. sollasi from the island of Funafuti In the following year Kwietniewsky (23) described the anatomy of P. ambonensis and Haddon (17) that of P. vermiformis and gansapata. The result of these several investigations may be summarised by saying that they corrected Gosse's and Verrill's error in attributing cinclides to the genus; they confirmed Verrill's observation that gonads are borne only on the twelve primary and perfect mesenteries; they drew attention to the small size and imperfect development of the remaining mesenteries; showed that they have only parietal and no longitudinal retractor muscles, and that they can be arranged in secondary, tertiary and quaternary orders, but the last order is always incomplete. Kwietniewsky suggested, as an addition to the definition of the family, that the fourth cycle of mesenteries is only represented by half the full number, since the quaternary septa are absent in all the loculi adjacent to the primary septa. The authors, furthermore, agreed in describing a mesoglœal sphincter muscle, extending over the upper third of the column, thicker below in the scapus, but thinner above in the capitular region. The outcome of this work was a new definition of the Phelliinæ by both Kwietniewsky and Haddon, both now including the subfamily in the Sagartiidæ. I quote Haddon only, as the two authors agree in all but unessential particulars. "Phelliinæ" (sic, Kwietniewsky gives Phellinæ, Verrill); "Sagartiidæ, with usually an elongated column, the capitular part of which is delicate and extensile; body-wall provided with a cuticle, but without any solid or hollow processes, such as tubercles, vesicles or suckers; no cinclides. Tentacles simple, neither very numerous nor very long. Only six pairs of perfect

mesenteries which alone are fertile. The remaining mesenteries are usually freely developed. The retractor muscles are very strongly developed on the primary mesenteries. Acontia usually feebly developed, and emitted only through the mouth. Strong mesogloeal sphincter muscle." Similar as their definitions are, the two authorities differed widely in respect of the forms included in the sub-family. Kwietniewsky, and with him Simon and Carlgren, included Chondractinia, Hormathia, Chitonactis and other forms, which Haddon placed apart in another sub-family Chondractiniinæ on the good ground that, whereas in Phellia the primary mesenteries alone are fertile, in the Chondractiniinæ they are always sterile, and only the well-developed lesser mesenteries are fertile. There can be no doubt that the judgment of the British author is correct.

Recently, in 1911, Wilsmore (**31**) has given a careful anatomical description of Phellia brownii and capitata from New South Wales, in which she has confirmed and extended the observations of her predecessors.

As may be inferred from this short historical preface, the genus Phellia has received a larger share of attention to its anatomical character than most other genera of Actinians. None the less, I am again dealing with the subject at some length. Among the Actinians collected by Dr. Willey in New Guinea were five species referable to the sub-family, four of which I have placed in the genus Phellia, and for the fifth I have erected a new genus. It was not possible to determine the systematic position of the forms except by a study of the anatomy; dissection gave few results, so I had recourse to sections, and in the study of these my attention was directed to a number of details, from which, as I think, inferences as to the systematic position and affinities of the Phelliinæ may legitimately be drawn. Therefore, although I must necessarily traverse a considerable extent of old ground, I have not scrupled to set out my observations in full, and must ask the reader's pardon if some parts of them are of no great novelty and interest.

Accepting Haddon's exclusion from the Phelliinæ of the forms which he has placed in the Chondractiniinæ, and accepting provisionally his definition of the former group, I will proceed to the discription of the species contained in Dr. Willey's collection, reserving further discussion for the latter part of this paper.

Genus PHELLIA, Gosse.

Phellia castanea, n. sp.

Single, fixed (?). Scapus minutely transversely wrinkled and furrowed; covered by a chestnut-red cuticle in which are imbedded numerous grains of reddish, black, and colourless and transparent quartz sand. The upper sixth of the scapus introverted in contraction. Column cylindrical, tapering slightly towards the base. Capitulum in contracted state very short, longitudinally ridged. Base or physa thin, concave; in the single specimen examined filled with quartz sand impacted with mucus into a solid mass. Tentacles 24, in two cycles of 12 and 12; short, conical, deeply transversely wrinkled in contraction. Peristome deeply concave, very thin. Mouth gaping.

Length of contracted specimen, 9 mm.; greatest diameter, 5 mm.; diameter at base, 3·3 mm.

Locality: Rakaiya, New Britain.

The above description, limited to external characters, requires the following explanation. In many species of Phellia a considerable part of the upper or distal part of the column is introverted in contraction, as in Edwardsia, and Wilsmore (**31**) describes the whole of the involuted portion as the capitulum. This description, I take leave to think, is an error. The terms "capitulum," "scapus," and "physa" were first used by Gosse (**14**) in his definition of the genus Edwardsia: "Column long, slender, cylindrical, divided into three distinct regions, of which the two terminal are retracted within the central one. Anterior region forming a short thick pillar (capitulum) of less diameter than the central, and more delicate. Central region (scapus) covered by a

skin (epidermis) more or less thick and opaque. Posterior region (physa) thin, pellucid, inflatable like a bladder; imperforate (?)."

Haddon (**15** and **17**) and others have extended the use of these terms, without much discretion, as I think, to the description of Actinians, in which there is no very obvious distinction of the column into three, or even into two regions. Gosse does not use the terms "capitulum" and "scapus" in his definition of the genus Phellia, but his figures (loc. cit., Pl. 7, figs. 1 and 2; Pl. 12, fig. 8) show as clear a division into an anterior more delicate region and a central region covered by an epidermis as in any of the Edwardsiæ illustrated in the same plates. It seems legitimate, therefore, to apply the terms "capitulum" and "scapus" to the Phelliidæ, but with their original signification. The term "scapus" should be limited to that part of the column clothed by an epidermis, and the term "capitulum" to the distal part of the column not so clothed and of a more transparent and delicate structure. That Gosse recognised this distinction is shown by his account of the habits of Edwardsia callimorpha (beautempsii) loc. cit., p. 257: "If rudely touched the disc was suddenly withdrawn; the capitulum, and then the upper two-thirds of the scapus, disappearing in rapid succession by a process of introversion." The capitulum, therefore, is not that part of the column introverted in contraction, but the more delicate distal region of the column, not covered by an epidermis. The two regions are distinct enough in Phellia castanea, but the capitulum is very short and so deeply infolded in contraction that its limits are not easy to determine in transverse sections. It is recognised by the thinness of the mesoglœa; the small size of the sphincter muscle, here reduced to a few circular fibres imbedded in the mesoglœa; and the low columnar layer of ectoderm, with a distinct external limiting membrane but no trace of an epidermis. The introverted portion of the scapus is readily distinguished by the abundant epidermis, reddish-brown in stained sections; by the sparse and modified ecto-

derm underlying the epidermis; and by the thickness of the mesoglœa, which, in this region, is specially thickened to form six longitudinal ridges corresponding to the exocœles of the six pairs of primary mesenteries. From these ridges secondary branched projections radiate towards the centre of the cavity of introversion, and each whole ridge with its projections seems to correspond with the "soft nose-like projections of the capitulum," described by Wilsmore (**31**) in Phellia browni.

In P. castanea the sphincter muscle is mesoglœal; not very strongly developed, thickest at the rim of the introverted region of the scapus and thinnest in the capitulum. It is somewhat thickened at the bases—i.e. the morphological inner sides—of the six mesoglœal ridge-like thickenings described above, but it does not extend deeply into these thickenings nor into the secondary projections from them, as described by Wilsmore for P. browni.

Mesenteries.—These are best described as macromesenteries and micromesenteries.

The Macromesenteries are twelve in number, forming six pairs, two of which are directives. All the macromesenteries are well developed, attached throughout its length to the actinopharynx; are provided with well-developed parietal muscles; and bear conspicuous longitudinal retractor muscles. The latter are reniform in section; have the usual Actinian arrangement, that is to say, they are dos à dos in the directive, vis à vis in the remaining mesenterial pairs; and the mesoglœal pleats to which the muscle fibres are attached give a characteristic dendritic figure in transverse section. In the distal three-quarters of the column the mesenterial arrangement does not require any special description, but, in the proximal third the macromesenteries in regular sequence diminish in size and lose first the large reniform muscle banner lower down the plicated free edge with its mesenterial filament, and finally on the inner wall of the column are reduced to relatively low ridges in which only the parietal muscles can be distinguished.

Faurot (13) has remarked that the "Actinies pivotantes," a group in which he includes the genera Edwardsia, Halcampa, Peachia, Ilyanthus, and Eloactis, are characterised by the gradual diminution in width of the macromesenteries towards the basal end, and by the diminution in size and final disappearance of the longitudinal retractor muscles in the narrow ends of the mesenteries. He has also (loc. cit., p. 91) laid stress on the fact that in Halcampa (as was first observed by R. Hertwig (19)), Peachia (= Siphonactis), Cereus pedunculatus (= Sagartia bellis), and Chitonactis coronata, two couples[1] of mesenteries, namely, those which appear fifth and sixth in order of development, are recognisably narrower and shorter than the other eight protocnemes: "Il en résulte une disposition qui, sur les coupes transversales, permet de reconnaître facilement les huit premières cloisons formées chez l'embryon. Cette disposition existe aussi, quoique moins évidente, sur les Hexactinies adultes."

Similar observations have been made by G. N. and A. F. Dixon (10) on Bunodes verrucosa, Actinia mesembryanthemum, and Cereus bellis.

A similar embryonic condition of the protocnemes is very clearly exhibited in the basal third of Phellia castanea, and in other undoubted members of the genus Phellia described in this paper. As is shown in Pl. 4, fig. 15, at a distance of about one-third of the length of the contracted specimen from the basal end the ventral members of the dorso-lateral and ventro-lateral macromesenterial pairs diminish in size; the large, reniform muscle banner is reduced and finally disappears; the plicated free edge of the mesentery becomes narrower, loses the filament, and eventually dies out altogether, so that a transverse section taken somewhat below this level shows only eight macromesenteries with the muscle banners oriented as in Edwardsia. At a somewhat lowel level the two directive pairs of macromesenteries follow

[1] Throughout this paper I use the terms "pair" and "couple" in Faurot's sense.

suit (see Pl. 4, fig. 19, for P. phassonesiotes), leaving only four macromesenteries with muscle banners, and these correspond with the two couples of protocnemes formed first in Actinian development. Close to the base all the macromesenteries are reduced, and are recognisable only by the somewhat larger size of their persistent parietal muscles.

This reduction and final disappearance in regular succession of the muscle banners and mesenterial filaments in certain macromesenterial couples is of importance, for it suggests that in Phellia and in the other genera enumerated by Faurot and the Dixons the stages with four and eight protocnemes are of some duration in ontogeny, and that the two mesenterial couples which make up the hexameral arrangement are added comparatively late in life. It also has the practical advantage that it enables one to determine the orientation of the animal, and to define the "dorsal" or sulcular and "ventral" or sulcar aspects with much greater precision than is possible in most hexameral Actinians.

Previous authors have noted the fact, and it has become part of the definition of the sub-family Phelliinæ (Haddon (**17**), Kwietniewsky (**22**)), that gonads are borne only on the twelve macromesenteries. This is true of Phellia castanea, with the difference that in the single specimen contained in Dr. Willey's collection there are only ten pairs of fertile mesenteries, the ventral members of the ventrolateral pairs being sterile. The specimen is a male, and in every fertile mesentery some of the sperm-follicles contain spermatozoa, others showing only the earlier phases of spermatogenesis. It is possible that the absence of gonads in a single couple of macromesenteries may be an individual peculiarity, or that in this case the development of the germ-cells in this particular mesenterial couple may have been retarded. But I could find no trace of germ-cells in this sterile couple, and am inclined to the opinion that these, the latest of the macromesenteries to be developed, are definitely and permanently sterile in P. castanea, but must admit that the evidence in favour of this opinion is not very definite.

It is noticeable that in P. castanea the ventral members of the dorso-lateral and ventro-lateral pairs are the only macromesenteries that give off acontia, therefore the last-named organs are confined to the macromesenteries latest in order of development. In two other species in Dr. Willey's collection undoubtedly belonging to the genus Phellia none of the macromesenteries bear acontia, but according to Maguire (**24**) all the macromesenteries bear acontia in P. sollasi. Neither Kwietniewski (**22** and **23**) nor Wilsmore (**31**) makes any definite statement on this subject with reference to the species that they have respectively studied. It would seem that the distribution and development of acontia varies within wide limits in the genus.

There are both labial and parietal mesenterial stomata, the former minute, the latter of considerable size. Both lie at about the same level, close below the oral disc. The acontia pass freely through the parietal stomata from one inter-mesenterial space to another.

Micromesenteries.—These are sixteen in number, all of them small, and for the most part consisting of low ridges projecting from the body-wall into the cœlenteron, each ridge consisting of the central mesoglœal lamina with lateral folds, to which the muscle fibres of the parietal muscles are attached, the whole covered by undifferentiated endoderm. The micromesenteries, however, are of different sizes, and some of them are so far advanced beyond the others in development that they bear mesenterial filaments, acontia, and even traces of longitudinal retractor muscles. Their structure and arrangement require detailed consideration.

There are three micromesenteries in each dorso-lateral sextant[1]: of these the median, marked *dl.* 1 in Pl. 4, figs. 14, 15, 16, is the largest, bears a short mesenterial filament, trefoil-shaped in section, has a trace of a longitudinal

[1] The six pairs of macromesenteries divide the cœlenteron into six equal radial exocœlic chambers, which may be conveniently described as sextants, and I shall use this name for descriptive purposes throughout this paper.

retractor muscle, and gives off an acontium; the most dorsal of the three, marked *dl.* 2 in Pl. 4, figs. 14, 15, 16, has a well-developed parietal muscle, but bears neither filament nor acontium; the most ventral of the three, marked *dl.* 3 in Pl. 4, figs. 14, 15, 16, is very small, and even the parietal muscle is rudimentary.

There are similarly three micromesenteries in each lateral sextant: of these the median, marked *l.* 1 in Pl. 4, figs. 14, 15, 16, is the largest, and bears filament, acontium, and trace of the longitudinal retractor muscle; the most dorsal of the three, marked *l.* 2 in Pl. 4, figs. 14, 15, 16, bears an acontium, but a mere trace of the filament and longitudinal retractor muscle; the most ventral, marked *l.* 3 in the same figures, is very rudimentary.

There are two micromesenteries in each ventro-lateral sextant: of these the more ventral, marked *vl.* 1 in Pl. 4, figs. 14, 15, 16, is the larger, and bears acontium, filament, and trace of longitudinal retractor muscle; the more dorsal, marked *vl.* 2 in Pl. 4, figs. 14, 15, 16, has a well-developed parietal muscle but no trace of filament or acontium.

Pl. 3, fig. 12 A, represents a transverse section of the median micromesentery of the right-hand lateral sextant magnified 385 diameters. The section passes some little distance above the level of the enterostome, and shows the mesenterial filament, trefoil-shaped in section, the acontium, and at *l.r.m.* the slightly plicated edge of the mesoglœal lamina, to which longitudinal muscle fibres, appearing as dots in section, are attached. Pl. 3, fig. 12 B, is a similar section of the micromesentery marked *l.* 2 in Pl. 4, fig. 15, i. e. the most dorsal of the three micromesenteries in the right lateral sextant. This section is taken at a considerably lower level in the column than that depicted in Pl. 3, fig. 12 A, and is some distance below the enterostome. It shows the rudiment of an obscurely tri-lobed filament, which, three sections lower down, is produced laterally into an acontium and then disappears, and at *l.r.m.* the slightly plicated edge of that part of the mesoglœal lamina lying between the parietal muscle and the

filament, to which longitudinal muscle fibres are attached as in Pl. 3, fig. 12 A.

There can be no doubt that in both sections the muscle fibres *l.r.m.* represent the rudiment of the longitudinal retractor. The two sections, though taken at different levels, are placed in their relative position to one another, and it will be seen that the longitudinal muscles are vis-à-vis, so there can be no doubt that these two micromesenteries, though in different stages of development, constitute a "pair," and have the normal arrangement of the longitudinal retractor muscles. Similarly, in the dorso-lateral sextant, the orientation of the rudimentary retractor muscle fibres on *dl.* 1, Pl. 4, fig. 15, show that they are paired with *dl.* 2, and in the ventro-lateral sextants there is similar evidence that *vl.* 1 pairs with *vl.* 2.

In making the identification of the rudiment of the longitudinal retractor muscle in certain micromesenteries, I dissent from the view put forward by Kwietniewsky (**23**) that the musculature on one side of a micromesentery is longitudinal and on the other side parietal. As is shown in all the figures of transverse sections 14–24, musculature of the macromesenteries consists of the large, reniform expansion nearer to the axial than to the peripheral end of the mesentery, and confined to one side of it. This is the longitudinal retractor, and separated from it by a long tract in which the mesentery is very thin and usually without any trace of mesoglœal plications or muscle fibres, is a muscle symmetrically disposed on both sides of the peripheral end of the mesentery. In a true transverse section the fibres of these latter muscles are cut through obliquely, and equally so on both sides. In this respect there is no difference between the more peripherally situated muscle on one side or the other. These obliquely disposed fibres, distant and very distinct from the longitudinal retractors, are associated with the body-wall or paries and may properly be called parietal. When comparison is made with a micromesentery it is clear that, with the exceptions above described, the only muscles of the latter are the equivalents of the

parietal muscles of the macromesenteries, and that, as a rule, longitudinal musculature is absent. In many Actinians e. g. conspicuously in Siphonactis, the parietal muscle on the side opposite to that which bears the longitudinal muscle is specially well developed and borne on a distinct offshoot of the mesoglœa. When thus differentiated it constitutes the parieto-basilar muscle of Hollard.

I should add here that in all the micromesenteries in which they are present the filaments are very short. The filament depicted in Pl. 3, fig. 12 A, extended over twenty-two sections 10μ in thickness and therefore had a length of only ·22 mm. The rudimentary filament of Pl. 3, fig. 12 B, was only ·1 mm. in length.

I have given a lengthy description of the micromesenteries because the detailed study of them has convinced me that, in the genus Phellia, they are formed in regular sequence, not in couples of pairs as in most dodecameral Actinians, but in couples of singles; one member of a couple right, the other left, of the median plane passing through the directives. The account given above affords sufficient evidence that the micromesenteries *dl.* 1, *l.* 1 and *vl.* 1 are the first to be formed in each sextant. The inner end of each of them has grown centripetally and has developed a trilobed filament, an acontium and a rudimentary retractor muscle. The next oldest micromesenteries in developmental sequence are *dl.* 2, *l.* 2 and *vl.* 2. Of these the couple *l.* 2 is the most advanced in development and has acquired rudimentary filaments, acontia and rudimentary retractor muscles, these structures appearing at a considerably lower level than in the case of *dl.* 1, *l.* 1 and *vl.* 1. I have shown that *l.* 2, *l.* 2 are on the way to form pairs with *l.* 1, *l.* 1, and there can be little doubt that the couples *dl.* 2, *vl.* 2, though they have not acquired filament, acontia or retractor muscles, stand in a similar relation to the couples *dl.* 1, *vl.* 1. The rudimentary couples *dl.* 3, and *l.* 3 are unpaired.

The interest of these observations lies in the fact that, though they eventually become paired, the micromesenteries

of Phellia are, at their initiation, formed in couples and thus repeat the developmental rhythm characteristic of the protocnemes of all the Dodecactiniaria. To this extent they resemble the micromesenteries of the Edwardsidæ, which, as I have recently shown (4) are formed in couples of singles, but differ from those of Phellia in never forming pairs. In P. castanea the mesenterial arrangement is nearly that of Halcampa, but differs in that there are two additional micromesenterial couples, one in the dorso-lateral and one in the lateral sextants, and also in the fact that the fertile macromesenteries do not correspond in the two genera. There is another and considerable difference in that the adult Halcampa has only twelve tentacles, half as many as the number of mesenteries, whereas in P. castanea and in the other species of Phellia described anatomically in this paper and by other authors the number of tentacles corresponds closely to the number of mesenteries.

The tentacles in P. castanea are short, simply infolded over the oral disc and not invaginated in retraction as are the tentacles of Edwardsia. As ascertained by the study of sections of the retracted specimen they are twenty-four in number, arranged in three cycles of 6 + 6 + 12, those of the two innermost cycles being endocœlic and the twelve tentacles of the outermost cycle exocœlic. There are no tentacles in connection with the rudimentary micromesenteries in the dorso-lateral and lateral macromesenterial exocœles, hence the total number of tentacles falls short by four of the total number of mesenteries. As seen in transverse sections of the retracted specimen, the large endocœlic tentacles communicate with the cœlenteron by wide openings, which, in the case of the primary endocœlic tentacles of the innermost cycle, occupy the whole width of a chamber included in a macromesenterial pair, and, in the case of the secondary endocœlic tentacles occupy nearly the whole space between two adjacent macromesenterial pairs. Consequently, the exocœlic tentacles forming the outermost cycle are squeezed in at the edge of the disc between the bases of the primary and secondary

endocœlic tentacles, and their cavities communicate by narrow openings with the cœlenteron. The capitular extremities of the rudimentary micromesenteries of the dorso-lateral and lateral macromesenterial exocœles extend for a short distance into the basal parts of the cavities of the more ventral exocœlic tentacles in those chambers and come into close relation with the macromesenteries contiguous to them. The significance of these details will be pointed out later on in this paper.

The ectodermal musculature of the tentacles is not specially well developed in P. castanea. On the outer faces of the tentacles it is weak, the longitudinally disposed muscle fibres being supported by very short processes of the mesoglœa and extending downwards as far as the upper edge of the capitulum. On the inner or oral aspect of the tentacles the ectodermal musculature is much more highly developed in the endocœlic tentacles; its fibres, supported by conspicuous branched processes of the mesoglœa, diverge outwards at the base of each tentacle and are inserted on the adjacent radial lines marking the attachments of the macromesenteries to the disc. The muscle fibres of the inner faces of the exocœlic tentacles converge to form a distinct bundle inserted close to the attachment of the adjacent macromesentery to the edge of the peristomial disc. The effect of this disposition is that the tentacles are flexed inwards towards the peristomial disc by the contraction of the strong muscle fibres of their adoral surfaces, whereas the weaker muscles of their aboral surfaces come into play during the slower movements of expansion. It is further evident that the insertions of the adoral bands of tentacular muscles on the upper edges of the macromesenteries ensures the co-adaptation of function of the powerful longitudinal retractors of the macromesenteries with the muscles of the tentacles and disc. When the former contract they forcibly pull down the disc and bring about the introversion of the capitulum and upper region of the scapus; the muscles of the inner faces of the tentacles, contracting at the same time, bend the tentacles inwards over the peristomial

disc, and as invagination proceeds cause them to assume the dependent vertical position with tips downwards shown in Pl. 3, fig. 1, *t*.

The ectoderm of the tentacles is abundantly furnished with nematocysts. These are all of the small spiral variety [type I of Matthai (**25**), spiral cnidæ or cnidæ cochleatæ of Gosse], measuring 39·5 μ in length by 5·2 μ in diameter (see Pl. 3, figs. 6 and 7). I have found no other kind of nematocyst in the tentacles of any of the Phelliinæ that I have examined. Between the nematocyst-laden layer of the ectoderm and the muscular layer is a fibrillar nervous layer, very thin on the external faces of the tentacles where the muscular layer is weakest, but much thicker on the inner faces where the muscular layer is strongest. The nematocysts are also much more abundant and more closely crowded together on the inner surfaces of the tentacles. These relations, taken in conjunction with the fact that one never finds the spiral thread extended, suggest that this type of nematocyst is tactile rather than urticant in function. Concerning this question, and the structure and development of the different types of nematocysts found in Actiniaria, I shall have something to say in another place.

It may be noted here that P. castanea has fewer tentacles, and correlated with this fewer mesenteries, than any other known species of the genus. P. phassonesiotes and P. allantoides described in the sequel, have 36 and 44 tentacles respectively; P. sollasi, teste Haddon, has 48–54; P. browni, teste Wilsmore, has over 40; P. capitata has 39, plus several buds; and P. ambonensis, teste Kwietniewsky, has 70 tentacles.

The peristome in P. castanea, as may be seen in Pl. 3, fig. 1, is deeply concave and the mouth gapes widely. In this it agrees with Gosse's description of P. murocincta, the type species of the genus; "disc a deep cup bounded by the thick feet of the inner tentacles" (**14**, p. 135). This description is in every respect applicable to P. castanea, and I am disposed to consider the concave peristome and

gaping mouth as generic characters. Gosse says of P. gausapata, "disc a deep cup or funnel," and I gather from Wilsmore's figures that a similar description would be applicable to P. browni and P. capitata, though she does not make any mention of this feature in her text. The description is certainly applicable to the other species of Phellia described in the continuation of this paper. Gosse, it is true, describes Phellia picta as having "disc nearly flat or slightly concave," but in a note on this species in the appendix to the 'Actinologia Britannica' he says, with reference to additional examples sent to him from Banff: "The epidermis is very thin and deciduous and altogether the species seems intermediate between the true Phelliæ and such Sagartiæ as coccinea." It is therefore probable that Gosse's Phellia picta is not a member of the genus Phellia.

The peristomial disc proper, that is to say, the area between the mouth and the bases of the tentacles is very thin in P. castanea, and I could find in it no trace of muscle fibres or nervous layer, nor are any nematocysts to be found in this region. Kwietniewsky (**22** and **23**) and Wilsmore (**31**) give elaborate descriptions of the musculature of the "disc" which are perfectly consistent with the account given above of the musculature of the bases of the tentacles, but I do not find, either in P. castanea or in the other species that I have examined, that the muscles extend over the peristome as defined above. The difference between my account and theirs probably consists in this, that they include the whole area between the mouth and the upper edge of the capitulum under the term "disc," whereas I only include the area between the mouth and the bases of the tentacles in the term "peristome." My use of the term is more consistent with Gosse's description of P. murocincta, quoted above.

The actinopharynx, as may be seen in Pl. 3, fig. 1, is large; its walls transversely wrinkled, probably as the result of contraction. Longitudinal ridges corresponding to the insertions of the macromesenteries are not very prominent.

Dorsally, there is a distinct gonidial groove or sulculus, in which the epithelium is so far differentiated that it contains no nematocysts, very few gland cells, and the ciliated cells are more closely crowded and bear longer cilia than elsewhere. A similar differentiation is found ventrally in the region bounded by the ventral directive macromesenteries, but here the epithelium bulges slightly into the cavity of the actinopharynx; this is probably the result of contraction, and one may say that a ventral groove or sulcus is present, but that the dorsal groove or sulculus is the more clearly differentiated (Pl. 4, fig. 14).

The general features of the mesenterial filaments and gonads are sufficiently well shown in Pls. 3 and 4, figs. 1, 15, and 16, and as far as macroscopical characters go they are normal and require no special description. The acontia are well developed and are loaded with large nematocysts as is shown in Pl. 3, fig. 12. The single specimen of P. castanea was very well preserved for microscopical purposes, but I must postpone a description of histological details to another place.

Phellia phassonesiotes, n. sp.

Single, elongate; the column divisible into capitulum, scapus and expanded base. Capitulum short; thin-walled. Scapus of leathery consistency; pinkish-brown in colour; thickly encrusted with calcareous sand; its upper sixth introverted in contraction; tapering towards the base. Expanded base firmly adherent below to a piece of dead coral; its free edges folded and puckered. Tentacles 36; the two inner cycles of six and six distinct, the two outer cycles of six and eighteen incomplete and indistinct.

Length of contracted specimen 30 mm.; greatest diameter 6·5 mm.; least diameter near base 3 mm.

A single specimen from Pigeon Island, New Britain. The specific name is derived from the locality; *φάσσα*, a pigeon; *νησιώτης*, an islander.

The expanded base of this species may also be described as a physa, but I have hesitated to apply this name to it because it can hardly be brought under Gosse's original definition as "thin, pellucid, inflatable like a bladder," and it is adherent. As shown in Pl. 3, fig. 2, it is of the same pinkish-brown colour as the scapus, and has a few grains of sand attached to it, so that it can scarcely be distinguished from the scapus on external examination. Histologically, however, it presents very different characters. The mesoglœa of the scapus is pitted and furrowed in every direction, and in section appears to be produced into numerous lobed processes covered by a cubical or very low columnar ectoderm, which is everywhere externally covered by the brown friable and apparently structureless layer called the "epidermis." In the region of the expanded base these characters change somewhat abruptly. The mesoglœa, though thickened in some places, is generally thin; its surface is smooth, and not produced into lobed processes; the ectoderm is thick and composed of elongated columnar cells, amongst which are claviform granular gland cells; there is a well-marked external limiting membrane, staining blue in picro-indigo-carmine; but the yellowish-brown epidermis is absent. This histological differentiation is apparent both on the face and on the adherent surface of the basal expansion.

Macromesenteries.—These, as in P. castanea, are twelve in number, with two pairs of directives, and the reduction and disappearance of the muscle banners of couples V and VI just below the level of the enterostome, and further down the reduction and disappearance of the muscle banners of couples III and IV is very well marked, and is clearly shown in Pl. 4, figs. 18 and 19. All the macromesenteries are fertile, the single specimen being a male. I could not find any evidence of acontia being given off from the macromesenteries. The longitudinal retractor muscles are of very large size, and markedly reniform in transverse section, the reniform outline having a deep hilus, within which the mesentery is attached. The dendritic character of the mesoglœal

folds bearing the muscle fibres is pronounced and characteristic, and I have tried to represent it faithfully in Pl. 4, figs. 17, 18, and 19, but the drawings are on too small a scale to bring out all the details.

There are distinct labial and parietal stomata in all the macromesenteries; the former small, the latter of considerable size, and at about the level of the rim of the introverted scapus. The mesoglœa is thickened at the lips of the stomata.

The Micromesenteries are twenty-eight in number, arranged as follows: (1) A larger pair in each of the sextants; (2) a smaller pair, lying ventrad of the larger pair, in each of the macromesenterial exocœles; (3) a minute and rudimentary unpaired micromesentery lying dorsad of the larger pair in each dorso-lateral and lateral sextant. The last-named bear neither filament nor acontium, scarcely rise above the level of the endoderm, and only are discoverable in the middle of the column, where they have a vertical extent of about 3 mm. All the other micromesenteries bear trefoil-shaped filaments and acontia, and, as in P. castanea, the filaments are very short, and occur at different levels in the different cycles of micromesenteries. Thus, taking the measurements from the rim of the introverted scapus, the filaments of the ventral members of the larger pairs commence at about 1·2 mm. below this point: those of the dorsal members of the larger pairs at about 1·6 mm. in the dorso-lateral and lateral sextants, but at 3·6 mm. in the ventro-lateral sextants. The dorsal members of the smaller pairs bear filaments at 6 mm. below the measuring point in the dorso-lateral and lateral sextants, but at 8 mm. in the ventro-lateral sextants. The ventral members of the smaller pairs bear filaments at a distance of 7·5 mm. in the dorso-lateral and lateral sextants, but at 9·5 mm. below the measuring point in the ventro-lateral sextants. The micromesenterial filaments are very short in every case, the longest not exceeding 1·5 mm., and some can only be traced in half a dozen sections 10 μ thick.

Every micromesentery with a filament bears an acontium at the lower end of the filament. In the smaller mesenterial

pairs the acontia are short and some of them rudimentary, but the acontia of the larger micromesenterial pairs are long and hang down in the cœlenteron, some of them extending to the base, where they end in a tangle of convolutions.

Applying the same reasoning as in the case of P. castanea for the determination of the order of development of the micromesenteries, the facts enumerated above suggest that the first micromesentery to be formed in each sextant is the ventral member of the larger pair; then follow the dorsal members of the larger pairs in the dorso-lateral and lateral sextants, and somewhat later the corresponding micromesenterial couple in the ventro-lateral sextants. Next in succession are formed the dorsal members of the smaller pairs, those of the ventro-lateral sextants lagging behind those of the dorso-lateral sextants. Then follow the ventral members of the smaller pairs, those of the ventro-lateral sextants still lagging behind the others. Lastly, we get the rudimentary micromesenterial couples on the dorsal sides of the larger micromesenterial pairs in the dorso-lateral and lateral sextants, but in P. phassonesiotes these are not yet developed in the ventro-lateral sextants. If I am right in judging the relative ages of the micromesenteries from the heights at which the filaments appear, the order of succession is the same as in P. castanea, but carried to a further stage, and the evidence points to the micromesenteries being formed in couples, the members of adjacent couples subsequently becoming paired.

These inferences as to the order of succession of the micromesenteries are borne out by a study of the tentacles. There are 36 tentacles in P. phassonesiotes, arranged in cycles of 6 primary entocœlic, 6 secondary entocœlic, an incomplete cycle of 6 tertiary entocœlic, and an outermost cycle of 18 exocœlic. There are no tentacles corresponding to the rudimentary micromesenteries. It is advisable to postpone the discussion of the probable order of formation of the tentacles in this and the other species of Phellia to the latter part of this paper.

As in P. castanea the ectodermic musculature of the inner or oral faces of the tentacles is better developed than that of the outer or aboral faces. In general, the musculature of the tentacles is much better developed in P. phassonesiotes than in P. castanea, and the swollen bases of the tentacles of the inner cycles extend further inwards towards the centre of the disc, giving the appearance of a considerable peristomial musculature. But in this, as in the previously described species, the peristomial wall between the bases of the tentacles and the actinostome is very thin, and no trace of muscular or nervous layers can be detected in it.

The surfaces of the contracted tentacles are deeply wrinkled transversely, and the ectoderm is crowded with somewhat elongate fusiform nematocysts.

The histological condition of the single example of this species was not good, and the tentacles, peristomial disc, and lips of the actinostome were so much crumpled and pressed together that I could not make out details as clearly as in the species previously described. It was evident, however, that the peristome is deeply concave, and the actinostome an elongated oval gaping orifice.

The actinopharynx is relatively short, laterally compressed, its walls thrown into sixteen or seventeen moderately deep longitudinal ridges and furrows, which do not bear any definite relation to the insertions of the macromesenteries. As is shown in Pl. 4, fig. 17, there is a well-marked dorsal actinopharyngeal groove or sulculus, and a less pronounced ventral groove or sulcus. The epithelium was not sufficiently well preserved to allow me to say with certainty that these two grooves are histologically differentiated, but there is some evidence that they are.

The remaining features of the internal anatomy do not call for special description. The acontia are rather thick, and crowded with large nematocysts, nearly all of which were everted. A large scale drawing of these nematocysts is given in Pl. 3, fig. 9.

The sphincter muscle of P. phassonesiotes is mesoglœal

and very thin. It is traceable in the greater part, and is best developed in the distal part of the introverted portion of the scapus, and is reduced to a single layer of circularly disposed muscle fibres lying in the thin mesoglœa of the short capitulum, but thickens again just below the bases of the tentacles. The endodermic circular muscles are well developed throughout the scapus, and in its distal part coexist with the mesoglœal sphincter, but they are so reduced as to be hardly recognisable in the capitulum.

Phellia allantoides, n. sp.

Single, fixed (?); the column divisible into capitulum, scapus, and physa. Capitulum short; thin-walled. Scapus of a dirty brown colour; thinly encrusted with fine sand; its distal portion deeply introverted in contraction; its surface deeply wrinkled; not tapering towards the base. Physa thin-walled; colourless; inflated and deeply pitted laterally and below. Tentacles 44, in four cycles of 6, 6, 10, 22; the two last cycles incomplete.

Length of contracted specimen, 55 mm.; average diameter, 6 mm.

A single specimen from Uvea, Loyalty Islands.

The specific name refers to the sausage-like shape of the contracted animal.

The thin-walled, colourless, inflated basal portion of this species may legitimately be called the physa. Its edges and lower surface are pitted by a number of round or oval depressions, the shape of which clearly indicates that they were occupied by pebbles or shingle. Some small, rounded fragments of shingle were still sticking in the smaller cavities in the basal end of the physa when the specimen came into my hands, but the pebbles occupying the larger depressions at its sides had fallen out. From the nature of the sand encrusting the scapus one may surmise that the animal was embedded in a layer of soft, muddy sand overlying a bed of shingle, and that it obtained a firmer anchorage than the sand

afforded by adhering by means of its physa to the shingly bed.

In all essential respects this species displays the same anatomical features as the two just described, but there are differences in detail.

The surface of the upper or introverted part of the scapus is thickened to form prominent longitudinal ridges, which, as in P. castanea, roughly correspond to the macromesenterial exocœles, and the surfaces of these ridges are again furrowed, so that in section one gets the appearance of a number of branched processes projecting into the central cavity and nearly closing the passage to the mouth. The mesoglœa is greatly thickened in these ridges. The sphincter muscle in this species is largely developed and mesoglœal. It forms a thickish band at the level of the rim of the introverted scapus; is fairly thick from this level as far as the capitulum, sending prolongations into the thickened mesoglœa of the ridges described above. It becomes thinner, but is still a relatively stout muscular band, in the capitulum, and thickens again at the bases of the tentacles.

In this species the muscle fibres of the sphincter are broken up into a number of bands, each of which is surrounded by mesoglœa, thus differing from P. castanea and phassonesiotes, but resembling browni, capitata, ternatana, and ambonensis. I am not, however, inclined to attach much importance for classificatory purposes to the sphincter.

The macromesenteries are arranged in the usual six pairs and all of them are fertile, the single specimen in the collection being a female. The lower ends of the macromesenteries are greatly enlarged, distended with nearly ripe ova, and folded in a most complicated manner, filling up the cœlenteron and distending the proximal half of the scapus. The ovaries, however, do not extend into the physa. The muscle banners are very large in the region of the actinopharynx, and their mesoglœal laminæ are beautifully and regularly branched, forming characteristic dendritic figures

in transverse section (Pl. 4, figs. 20, 21). As described for P. castanea and phassonesiotes, the muscle banners of the fifth and sixth couples of macromesenteries thin out and disappear shortly below the level of the enterostome, and at a somewhat lower level the fourth and third couples follow suit, but the order of their disappearance is not quite as regular as in the two species named. In the physa all the muscle banners are lost, but the macromesenteries still predominate in size. There are small labial and large parietal stomata in the macromesenteries.

The micromesenteries are thirty-four in number, there being six in each dorso-lateral, six in each lateral, and five in each ventro-lateral sextant. In the dorsal and dorso-lateral sextants the middle pair of micromesenteries is the largest, the pair on the ventral side of them next in size, and the dorsal pair the smallest. The dorsal member of the dorsal pair is usually minute. The same rule holds good in the ventro-lateral sextants, but in these only one member of the dorsal pair, and that very minute, is present.

As in D. phassonesiotes, some of the micromesenteries bear very short filaments; these are found at different levels, and the detail is almost exactly the same in the two species. Thus, if the highest level, i. e. the most distal from the base, is denoted by A, and successively lower levels by B, C, etc., the ventral members of the larger central pair in each exocœle bear filaments at level A; the dorsal members of the same pair at level B; the dorsal members of the smaller pairs lying ventrad of the larger pairs bear filaments at level C; the ventral members of these smaller pairs at level D, except those in the ventro-lateral sextants, which bear no filaments. The smallest micromesenterial pairs lying dorsad of the larger pairs in the dorso-lateral and lateral sextants and the single micromesenteries occupying a similar position in the ventro-lateral sextants have no filaments. None of the filaments are more than ·8 mm. in length.

The acontia are very small, and so rudimentary that they are easily overlooked. They are borne on most of the micro-

mesenteries that also bear filaments, but not on all, and their distribution is irregular. The macromesenteries do not bear acontia. Such as they are, the acontia have the usual structure, and are furnished with large nematocysts. Kwietniewsky (**22** and **23**) has recorded a similar reduction almost to the point of disappearance of the acontia in P. ternata and ambonensis, but in sollasi (Maguire (**24**)), browni, and capitata (Wilsmore (**31**)) the acontia are long and conspicuous. There is evidently a wide range of variation in respect of these organs in the genus Phellia.

The tentacles of P. allantoides are relatively large, especially those of the two innermost cycles. They are very muscular, deeply transversely wrinkled in contraction, have distinct muscular nervous and epithelial layers, and the last is crowded with rather elongate spiral nematocysts staining green in picro-indigo-carmine. The relation of the several cycles of tentacles to the macromesenterial and micromesenterial pairs is the same as in P. phassonesiotes, but, as the micromesenterial pairs are more numerous in P. allantoides, the number of tentacles is also greater. There are no tentacles corresponding to the unpaired rudimentary micromesenteries in the ventro-lateral exocœles.

The ectodermic musculature of the tentacles is specially well developed; it is thicker on their adoral than on their aboral faces, but this difference is not as clearly marked as in P. castanea. The muscle fibres are supported by long and thin mesoglœal laminæ, which are secondarily folded so as to give a branched appearance in section (Pl. 3, fig. 13), but there is no anastomosis among the branches as described by Kwietniewsky for P. ternata and ambonensis. The two inner cycles of tentacles are inserted well towards the centre of the disc and the peristome is correspondingly reduced in extent, but, as in the two species already described, the latter is thin, has no muscular or nervous layers, and is deeply concave. The endoderm lining the inner sides of the tentacles and running out in radial lines from their bases towards the actinostome is almost wholly composed of elon-

gated vasiform cells of large size, and filled with deep brown granules of various sizes (Pl. 3, fig. 11). The nature and distribution of these cells, the histological features of which are unexpectedly well preserved, indicate that in the living animal the disc is ornamented with radial stripes of colour, continued up the inner face of each tentacle.

The actinopharynx is short, not more than 4 mm. long in the contracted condition, and is longitudinally furrowed. I could not detect any definite sulcus and sulculus, the grooves at the two ends of the actinopharynx having the same histological features as those on the lateral walls, so far as the state of preservation of the epithelium allowed me to determine.

The only other points that I need call attention to are that the endodermic musculature of the body-wall is strongly developed in all parts of the body-wall, and, as has been noted for other Actinians by Haddon and others, it forms a continuous layer intervening between the peripheral ends of both macromesenteries and micromesenteries and the mesoglœa of the body-wall. The mesoglœal laminæ of the mesenteries, however, are from place to place continued into the mesoglœa of the body-wall. The endoderm muscle fibres are transversely disposed in the capitulum and scapus, but take an oblique direction in the physa. They coexist with the mesoglœal sphincter in the capitulum and introversible portion of the scapus, and here the layer is thin; it is thickest in the scapus, and again thinner in the physa.

The ectoderm of the physa is modified, and consists of tall columnar epithelial cells, among which are numerous club-shaped gland cells with broad external ends, and tapering into fine fibrils internally.

Phellia cylicodes, n. sp.

Single, fixed, wine-glass shaped, tapering from the disc downwards to the physa, which is flattened and expanded. Column divisible into capitulum, scapus, and physa. Capi-

tulum half the length of the entire animal; very thin-walled; transparent. Scapes not encrusted with sand; tapering below; transversely corrugated; with firm but not thick walls. Physa expanded; thin-walled; pitted below where attached to shingle. Colour in spirit, white.

Length, 17 mm.; greatest diameter, 8·5 mm.

A single specimen from Uvea, Loyalty Islands. ♂.

Specific name from κυλικώδης; wine-cup shaped.

The specimen was not well preserved, the tentacles, disc, and capitulum being damaged and in part so macerated that I could not count the tentacles or make anything of the details in the oral region.

Apparently the capitular wall is very thin and devoid of mesoglœal circular muscle fibres. It was for the most part torn away, but the fragments remaining showed only a very thin lamina of mesoglœa, with very low cubical ectoderm and endoderm cells on its outer and inner surfaces. There is a very thin layer of endodermic transverse muscle fibres. The mesoglœa is striated along its inner border, but I could not detect muscle fibres between the striations. I could not find any trace of a mesoglœal sphincter muscle, even at the bases of the tentacles where the mesoglœa is somewhat thickened.

The Macromesenteries are all fertile and furnished with large longitudinal retractor muscles, reniform in section, and exhibiting the usual dendritic pattern of the mesoglœal processes for the attachment of the muscle fibres. Owing to the damaged state of the oral end I could not determine whether macromesenterial stomata are present. The dorsal directives and the ventral members of the dorso-lateral and ventro-lateral pairs of macromesenteries do not extend nearly so far down as the remainder, and lose their muscle banners at about the level of the middle of the scapus, leaving only six mesenteries with muscle banners in this region. Still lower down the ventral directives lose their muscle banners, leaving only the macromesenterial couples I and II. Thus, owing to the early reduction of the dorsal directives there is no region

in which the eight "Edwardsian" mesenteries are prominent as in the three species described above. Short but rather thick acontia, furnished with the large nematocysts characteristic of these organs, are given off from the ventral members of the dorso-lateral and ventro-lateral pairs of macromesenteries just below the point where the large reniform muscle banner ends. I could not find acontia in any other macromesenteries nor on any of the micromesenteries.

The Micromesenteries are twenty-eight in number, and in respect of their arrangement and relative sizes are exactly the same as in P. phassonesiotes. But I could only find filaments on the pairs adjacent to the ventral directives and on the micromesentery next the ventro-lateral macromesenterial pair on the right side. These filaments are low down, in the region of the enterostome. It is probable enough that other micromesenteries bear filaments at a higher level in the capitular region, but, as the walls of the capitulum were largely destroyed, I was unable to find them. There were no acontia in connection with the three micromesenteries on which I found filaments, and from the absence of portions or convolutions of acontia in the intermesenterial chambers, I judge that none of the micromesenteries bear acontia, or if they do, they are rudimentary.

Owing to their damaged condition I was unable to count the tentacles. So far as their condition permitted of observation they have the same characters as regards musculature, nematocysts, etc., as in the other Phellia I have described.

The actinopharynx is long and longitudinally plicated, but I could not distinguish a differentiated sulcus or sulculus. The epithelium is everywhere crowded with long claviform gland cells filled with granules stained green in picro-indigo carmine, their narrower ends external and opening to the surface between the supporting cells.

This species differs from other Phelliæ in the relatively large size of the thin-walled capitulum, in the early reduction of the dorsal directive mesenteries, and, as far as could be

ascertained, in the absence of a mesoglœal sphincter muscle. In all other characters it is a Phellia, and I have not created a new genus for its reception on account of the absence of a mesoglœal sphincter because, owing to its damaged condition, I cannot say anything positive on this point.

Decaphellia, n. gen.

With the characters of Phellia, but the capitulum has no musculature except for a mesoglœal sphincter at its distal extremity, and there are only ten complete macromesenteries bearing longitudinal retractor muscles.

Decaphellia psammomitra, n. sp.

Single, fixed (?); the column divided into capitulum and scapus; the base invaginated to form a cup. Capitulum nearly half the length of the entire animal; very thin-walled; inflated; transparent; colourless in spirit; its surface showing ten longitudinal ribs corresponding to the insertions of the macromesenteries. Scapus divided into two regions; the upper region thinner-walled and thickly encrusted with calcareous sand; the inferior region thicker-walled, deeply and completely corrugated, covered by an epidermis but without encrustation. Base deeply concave; covered with a high columnar epithelium. Tentacles 24, in three cycles of 6, 6, 12.

Length, 7·5 mm.; greatest diameter, 2·75 mm.

Two specimens from Lifu, Loyalty Islands.

The specific name refers to the prominent girdle of sand encircling the upper part of the scapus; *ψάμμος*, sand; *μίτρα*, a girdle.

The absence of muscles of any kind on the wall of the greater part of the capitulum and the reduction of the ventral members of the ventro-lateral pairs of macromesenteries are characters of sufficient importance to justify the creation of a new genus for the reception of this species.

Pl. 5, fig. 24, is a drawing of a transverse section passing through the concave peristome and including the bases of some of the tentacles; as the section falls obliquely the thickened lip of the actinostome is included on the right of the section. The figure shows ten complete macromesenteries, having small but well-defined and prominent muscle banners closely attached to the wall of the disc. Dorsal and ventral directives are present with the muscle banners dos à dos, and the dorso-lateral pairs are complete. The ventro-lateral pairs are incomplete, as their ventral members, though recognisably longer than the very minute micromesenteries, do not reach the disc, and have no trace of longitudinal retractor muscles. Pl. 5, fig. 23, is a transverse section taken through the scapus, a short distance below the enterostome. As the specimen was laterally curved, the section is not truly transverse to the axis of the animal and appears elongated laterally. Nine mesenteries bearing longitudinal retractors are seen, the ventral member of the dorso-lateral pair being reduced on the right side, but not on the left. There is no further reduction, the nine mesenteries in question being continued down to the base without much further alteration except that their mesoglœal laminæ are greatly thickened in the lower region of the scapus and the retractor muscle gradually shifts from a more central to a more peripheral position. Thinking that this unusual asymmetry of the macromesenteries must be an individual peculiarity, I made sections of the second example at my disposal and found exactly the same arrangement, and must therefore conclude that the presence of ten macromesenteries bearing retractors in the upper part of the column and the reduction of the number to nine in the lower part is a characteristic of the species. The macromesenteries are perforated by relatively very large parietal stomata at the level of the upper edge of the scapus, but there are no labial stomata, and in this respect also Decaphellia differs from Phellia, for these perforations are always present in the latter genus.

Neither of the two examples in the collection showed any trace of gonads, and until sexually mature specimens are found there must be some doubt as to the inclusion of Decaphellia in the Phelliinæ, for it is a characteristic of the subfamily that only the macromesenteries are fertile. But from the very small size of the micromesenteries it seems improbable that they should bear gonads in Decaphellia.

Pl. 5, fig. 24, shows the extreme tenuity of the capitular wall. It is composed of a very thin lamina of mesoglœa covered externally and internally by a layer of very flat ectoderm and endoderm cells. There is no trace of transverse endodermic muscular fibres in the greater part of the capitular wall, nor is there the slightest trace of mesoglœal muscle. It is also noteworthy that neither macromesenteries nor micromesenteries exhibit any trace of parietal muscles in any part of the capitulum, but, as Pl. 5, fig. 23, shows, the parietal muscles of all the mesenteries, though not large, are perfectly distinct in the region of the scapus, and correlated with their appearance is the presence of transverse endodermic musculature in this region.

At its extreme distal end, just below the outer cycle of tentacles, the capitular walls thicken, ectoderm, mesoglœa, and endoderm, but especially the mesoglœa, taking their share in the thickening. In this region the endodermic transverse musculature reappears, and there is a distinct mesoglœal sphincter muscle, about ·25 mm. in vertical extent. The muscle fibres of the sphincter are few, relatively coarse, and form a single strand.

The micromesenteries are twelve in number; one pair in each sextant. They are tiny and scarcely recognisable projections from the very thin body-wall in the capitulum; in the scapus they acquire the usual feather-shaped parietal muscles, but never attain to any size, and in most places are mere ridges projecting but little beyond the general level of the endoderm. Their free edges are covered throughout the region of the scapus, but never in the capitulum, by a band of modified endodermic epithelium, in which the cells are

more distinctly columnar, and have more deeply-staining nuclei than the adjacent irregularly shaped vacuolated endoderm cells. At about the level of or slightly below the enterostome the micromesenteries are enlarged in depth, the modified epithelial cells invest their sides and tips and become thinner and columnar, their deeply-stained and closely crowded nuclei forming conspicuous objects in section. At a slightly lower level the modified epithelium covering the now swollen end of the mesentery is deeply puckered and thrown into a series of ridges and furrows constituting a "frill" (Pl. 3, fig. 10), which may be traced for a distance of about ·1 or ·2 mm. and then disappears, the mesentery again becoming a low and inconspicuous ridge. In the region of the frill the swollen extremity of the mesentery gives off a lateral process which is at first slender and somewhat trilobed in section, but after a shorter or longer course is somewhat enlarged in diameter, and displays the histological characters of an acontium, with the usual large nematocysts and gland cells always found in these organs. An acontium of greater or less length is given off from every micromesentery, but none from the macromesenteries.

There can be no doubt that all the elements of the "frill" and the acontium are derived from the endoderm. The acontia are of considerable length relatively to the size of the animal, and pass through the macromesenterial stomata from one intermesenterial space into another, so that their course and origin is difficult to trace in sections: they usually end in a tangled convolution. They can readily be distinguished from the median lobes of the mesenterial filaments by their shape and by the fact that they contain a number of large nematocysts of the type depicted in Pl. 3, fig. 8. These are not present in the mesenterial filaments, but occur in the actino-pharyngeal epithelium.

The tentacles are muscular, the arrangement of the muscle fibres being the same as in the Phelliæ previously described. The muscle-fibres are relatively large, and the mesoglœal laminæ supporting them are unbranched and not very long.

In both specimens at my disposal the tentacles were contracted to mere papillæ and their surfaces deeply transversely wrinkled. The ectoderm is crowded with spiral nematocysts (Pl. 3, fig. 6) so closely packed together as the result of contraction that the other elements of the ectoderm are hardly distinguishable.

By simple inspection one can count twenty-four tentacles arranged in an inner circle of twelve larger alternating with an outer circle of twelve smaller. Sections show that there are three cycles; a macromesenterial endocœlic cycle of six; a micromesenterial endocœlic cycle of six and an outer exocœlic cycle of twelve. The relation of the tentacles to the mesenteries may, therefore, be described as typical. It should be noted that, although the ventral members of the ventrolateral pairs of macromesenteries are incompletely developed, the tentacles corresponding to these pairs are fully developed. In all these characters Decaphellia resembles Phellia, as also in the distinct deeply concave and thin-walled peristome, in which neither muscular nor nervous layers can be distinguished, and in the widely gaping actinostome.

The actinopharynx is long, extending through the capitulum and well into the upper region of the scapus. Both sulcus and sulculus are well developed, and are lined by an epithelium consisting wholly of attenuated flagellate cells, whose flagella, though not very long, are conspicuously longer than the cilia borne by the rest of the actinopharyngeal epithelium. The mesoglœa of the actinopharyngeal wall is thickened at the insertion of each of the macromesenteries, and the epithelium covering these ridges is also thicker than elsewhere, forming ten longitudinal ridges in the upper moiety, but only nine in the lower moiety of the actinopharynx, for the ventral member of the dorso-lateral pair of the right side is already reduced in the latter region. At the enterostome these nine ridges, covered by a highly glandular epithelium in which there are numerous large nematocysts, are continued into the median cnidoglandular lobes of the trefoil-shaped mesenterial filaments.

The base is deeply invaginated and its cupped surface corrugated with irregular ridges and furrows. The ridges are covered with a high columnar epithelium, the cells of which radiate fan-wise from the summit of each ridge and their swollen external extremities are either filled with minute granules staining greyish-blue in picro-indigo carmine or are empty, with more or less collapsed walls. Internally, these cells are prolonged into fine fibres terminating in definite enlargements which I cannot interpret otherwise than as muscles-fibres. The presence of ectodermic muscle-fibres elsewhere than on the tentacles and oral disc is a primitive feature, but in this case there is no ectodermal musculature on the wall of the column as in the Cerianthidæ and in Carlgren's group Protantheæ. The endodermic musculature of the base is highly developed and apparently forms an ill-defined sphincter, some of the fibres of which are here, as elsewhere in the column wall, caught up in and surrounded by irregular processes of the mesoglœa; but one can hardly speak of a mesoglœal sphincter in the base. The ectodermal and endodermal muscle-fibres are connected by very fine but distinct branching fibrillæ, apparently of a nervous nature, which traverse the mesoglœa and some enter into connection with stellate cells imbedded in the mesoglœa.

As is shown in Pl. 3, fig. 5, a tuft of short root-like processes projects from the cavity of the invaginated base. In sections these appear as a tangle of thread-like structures continuous with the cuticular secretion which is everywhere adherent to the surface of the glandular basal ectoderm, but no definite structure could be detected in them. They are doubtless used for attachment. The invagination of the base, a feature common in Actinauge richardi and other Chondractiniidæ, is undoubtedly effected by the powerful longitudinal retractor muscles of the macromesenteries which run right down to and are inserted upon the thick mesoglœal swellings of the lower ends of the macromesenteries just above the spot where the latter are attached to the base. Similar conditions exist certainly in Phellia castanea and probably

in the other Phelliæ which I have described, but the state of preservation of the latter did not admit of so careful a study of detail as in Decaphellia psammomitra. In his definition of the genus Actinange Verrill said of the basal disc that it "may be broad and flat, adherent, or it may be bulbous, clasping mud, or it may ensheathe the branches of Gorgoniæ, etc." From what precedes it is evident that there is a similar capacity for change of form and adaptability to varying conditions in the Phelliinæ. When the longitudinal retractor muscles are relaxed the base may assume a bulbous condition as in Phellia allantoides, and it is then intermediate between the vesicular physa of the Ilyanthidæ and Edwardsidæ, and the more or less flat adherent base of the more common Actinians.

A transverse section through the scapus of Decaphellia is singularly like Faurot's figures of sections of Halcampa chrysanthellum (loc. cit., Pl. 8, figs. 2, 3, and 4) the shape and size of the muscle banners of the macromesenteries, the arrangement and convolutions of the mesenterial filaments, and the number and characters of the micromesenteries being strikingly similar. The main differences in addition to the peculiar reduction in number of the macromesenteries in Decaphellia are the corrugated external surface and the presence of acontia in the latter genus. The acontia are the dividing factor; were it not for their presence one could scarcely hesitate to include Decaphellia among the Halcampinæ, and the conclusion that these forms are closely related is irresistible.

This conclusion is strengthened by a consideration of the external and anatomical features of Halcampactis (Farquhar, 12). This extremely interesting little New Zealand Actinian has a distinct capitulum; a scapus covered in life by a thin, rough, greyish cuticle; a rounded aboral extremity, not clearly marked off from the scapus, but which one must agree with Farquhar in calling a physa. I gather from Farquhar's account that the tip of the physa is invaginated in certain conditions of retraction. The capitulum is capable of intro-

version. There are six pairs of macromesenteries bearing strongly developed circumscribed retractor muscles resembling those of Halcampa and six pairs of micromesenteries alternating with them. Though Farquhar does not say anything on the subject, I gather that the macromesenteries alone are fertile. The tentacles are twenty-four in number; six primary endocœlic, six secondary endocœlic, and twenty-four exocœlic. Acontia are present and are emitted through the mouth only; there are no cinclides. I was in some doubt as to whether I should not place Decaphellia psammomitra in the genus Halcampactis, but H. mirabilis has minute suckers on the body-wall, and, according to Farquhar, no sharply-defined circular muscle. These two characters exclude it from the Phellinæ as defined by Haddon, but, as it has well-developed acontia, it cannot be placed among the Halcampinæ. If, as I venture to suggest will be found to be the case, a study of sections should show that there is a distinct though not necessarily "well-defined" circular muscle, Halcampactis would certainly find a place among the Phelliinæ, always supposing that its macromesenteries alone are fertile. Should the opposite be the case, and its micromesenteries alone be fertile, it would find its place among the Chondractiniinæ, and would connect this sub-family with the Halcampinæ. Further details of its anatomy are greatly wanted.

On the Probable Order of Appearance of the Tentacles in the Phelliinæ.

Faurot (**13**), in his admirable "Études sur les Actinies," gives a detailed account of the order of appearance of the tentacles in Ilyanthus parthenopæus and Tealia (Urticina) felina. Although the final results are different, owing to the assumption of a secondary decameral symmetry by Tealia, the developmental sequence is fundamentally similar in these two forms, and the rule probably holds good for all the Actiniiæ. In the earliest stage of Ilyanthus parthenopæus there are six pairs of mesenteries and

twelve tentacles; six endocœlic and six exocœlic. On the formation of the six pairs of secondary mesenteries a new cycle of six tentacles is formed, prolonging the endocœles of the newly-formed mesenteries. The original exocœlic tentacles are therefore pushed to one side, namely, to the dorsal side, in the dorso-lateral sextants, and to the ventral side in the lateral and ventro-lateral sextants. No new exocœlic tentacles are formed at this developmental phase, but on the formation of the next cycle of twelve tertiary pairs of mesenteries as many new tentacles are formed, prolonging their endocœles. Thus a stage is established in which there are twenty-four pairs of mesenteries and twenty-four endocœlic tentacles corresponding to them, but only six exocœlic tentacles. During this stage, which is of considerable duration, the secondary and tertiary endocœlic tentacles grow more rapidly than the six exocœlic and soon overpass the latter in size. The full number of forty-eight tentacles characteristic of the adult Ilyanthus parthenopæus is attained by the formation of eighteen exocœlic tentacles, one for each exocœle hitherto unprovided with these appendages, and the end result is a regular alternation of endocœlic and exocœlic tentacles. This somewhat devious method of arriving at the simple tentacular symmetry of the adult is explained by Faurot on mechanical principles, but these do not suffice, for, if it were simply a question of growth where there is most room for expansion, one would expect each member of a new pair of secondary mesenteries to be formed, one on the one side, the other on the other side of the base of the primary exocœlic tentacle in each sextant of the first developmental phase. If this were so, the primary exocœlic tentacle would become secondarily endocœlic, and a comparable mode of growth actually does occur in the formation of the calcareous septa in Eupsammid corals (see Pourtalès, **26**; Duerden, **11**; Bourne, **3**). But in the growth of the tentacles in Actiniiæ the primary exocœlic tentacles never are included between the members of a pair of mesenteries and therefore never become endocœlic. So far from the line of least resistance

being followed there is, as Faurot clearly shows, a good deal of crowding in some radii, but room for free expansion in others. The peculiar course of development, therefore, must receive an explanation on historical and phylogenetic rather than on mechanistic principles, and some clue is afforded by the study of the relations of the tentacles to the several orders of mesenteries in the Phelliinæ.

The reader will have observed that, in respect of the number and arrangement of the micromesenteries, Phellia castanea, phassonesiotes, and allantoides form a regular series. In the first-named there are sixteen, in the second twenty-eight, in the third thirty-four micromesenteries. In cylicodes the number and arrangement is the same as in phassonesiotes, and in Decaphellia psammomitra, the examples of which were probably adolescent, as they did not contain gonads, there are twelve micromesenteries. In P. sollasi Maguire describes fifty-five micromesenteries, and the number is apparently greater in P. panamensis Verrill. In all these species there is a distinct dorsi-ventrality in the distribution of the micromesenteries: they are more numerous in the dorso-lateral and lateral than in the ventro-lateral macromesenterial exocœles, and in each sextant the smaller and presumably more recently formed mesenteries appear first on the ventral and later on the dorsal side of pre-existing micromesenterial pairs —a fact which arrested the attention of Kwietniewsky, but he did not attempt to explain its significance.

In the following argument objection may be taken to the fact that I am drawing inferences as to developmental sequences from the comparison of stages observed in different species, and not from stages observed in the development of a single species. I must admit the validity of the objection, but may be allowed to reply that, though it has been desirable, in accordance with the rules of nomenclature, to describe the forms here dealt with as separate species, the possibility of several of them being growth stages of one and the same species is by no means excluded. And even if this

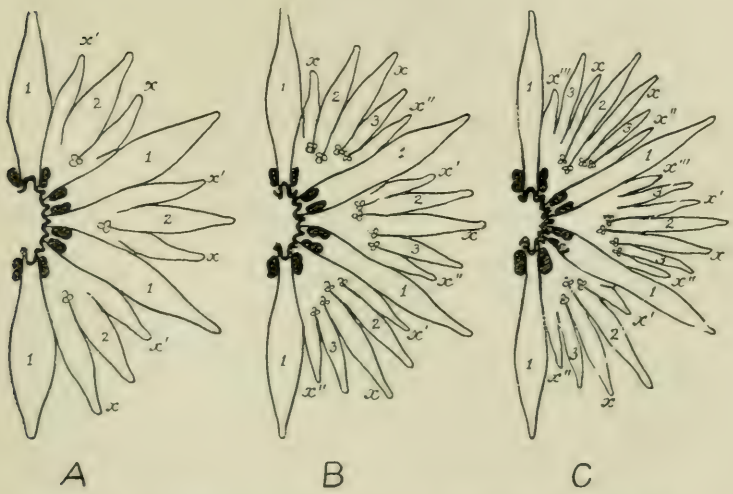
cannot be admitted—I am not inclined to press it—there is much evidence that the Phellia, like many other Actinians, are so far pædogenetic that they increase in size and add to the number of their mesenteries and tentacles long after the attainment of sexual maturity. The differences in the number of micromesenteries, therefore, may fairly be taken, not as specific characters, but as indications of earlier or later growth stages in the individuals examined. To this extent, then, they may be dealt with as if they were a developmental series.

The accompanying text-figures are diagrammatic representations of the relations of the tentacles to the mesenteries in (A) Phellia castanea, (B) phassonesiotes, (C) allantoides. In all the diagrams the primary endocœlic tentacles are marked 1, the secondary endocœlic tentacles 2, the tertiaries 3, and the exocœlic tentacles, in the order of their succession, x, x', x'', and x'''.

Let us first consider the ventro-lateral sextant in A. There are two mesenteries, of which the larger bears a filament and was the first to be formed. Its fellow is shorter, bears no filament, was formed in close association with the primary macromesentery on the dorsal side of it, and is still closely approximated to it. There are three tentacles, of which the central prolongs the endocœle formed by the two micromesenteries and is the secondary endocœlic tentacle, 2. The most ventral, x, is the original exocœlic tentacle, now displaced ventrally by the formation of the new tentacles on its dorsal side. The most dorsal is the secondary exocœlic tentacle growing out in the space between the smaller micromesentery and the adjacent macromesentery. The lateral and dorso-lateral sextants exhibit the same features, but in both there is a very small micromesentery, which, at the edge of the disc, seems to grow out of the angle between x, the original exocœlic tentacle, and 1, the primary endocœlic tentacle ventrad of it. This single mesentery does not form a boundary to any definite intermesenterial chamber, whether an endocœle or an exocœle, and no tentacle has been formed

in connection with it, for there is no intermesenterial chamber to prolong. The next stage in advance is to be found in the ventro-lateral sextant of B. Here the smaller or tertiary pair of mesenteries has been completed by the formation of a second micromesentery on the ventral side, and the new endocœle is prolonged into the tertiary tentacle, 3, to the ventral side of which appears the tertiary exocœlic tentacle,

TEXT-FIG. 1.



Diagrams showing the relations of the tentacles to the mesenteries in A. Phellia castanea; 3, Phellia phassonesiotes; C. Phellia allantoides. 1,2,3. Endocœlic tentacles in the order of their development. x, x', x''. Exocœlic tentacles in the order of their development. For further description see text.

x''. In the lateral and dorso-lateral sextants the arrangement is similar, but here a very small, single micromesentery appears at the edge of the disc in the angle between x' and the adjacent primary tentacle dorsad of it. As before, no tentacle has grown out in connection with the unpaired micromesentery. This condition reappears in the ventro-lateral sextant of C, but in the lateral and dorso-lateral sextants the dorsal pair of tertiary mesenteries is completed

by the growth of another micromesentery on the dorsal side of that already existing in the ventro-lateral sextant. Thus there is a new endocœle prolonged into its appropriate tentacle, and a quaternary exocœlic tentacle is growing out between it and the adjacent primary tentacle.

It is sufficiently evident from Maguire's figures that when the number of micromesenterial pairs is increased their development follows the same rhythm.

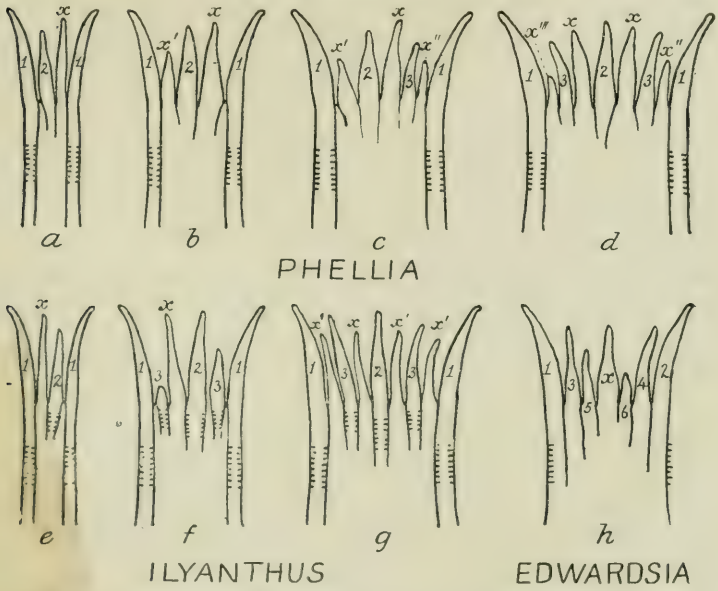
The facts to which I especially wish to draw attention are that the micromesenteries make their appearance in couples and not as complete pairs; that a pair is always established by the growth of an additional micromesentery between the one first formed and the adjacent macromesentery; and that a new endocœlic tentacle does not grow out until the micromesenterial pair of its appropriate endocœle is completed. As a consequence of this successive formation of the parts peripheral growth is gradual, and there is no crowding of the elements in course of formation.

The accompanying series of diagrams, Text-fig. 2, *a–h*, will enable the reader to institute a comparison between the growth processes and succession of the mesenterial pairs and tentacles in Phellia and Ilyanthus. A tangential section through a single sextant is represented conventionally in each figure; the primary, secondary, and tertiary endocœlic tentacles are lettered 1, 2, and 3; x is the primary exocœlic tentacle, and the remaining exocœlic tentacles are lettered x', x'', etc., according to the order of their appearance. The different lengths of the mesenteries indicate their respective ages, the longest being the earliest formed.

In *a* is depicted a sextant bounded by two primary macromesenterial pairs in Phellia. In the preceding stage (not figured) there was a single endocœlic tentacle. In the stage figured a micromesenterial pair has been formed by the development, first of the longer, then of the shorter of the two micromesenteries shown in the diagram. The secondary tentacle, 2, has grown out from the newly formed micromesenterial endocœle, and has displaced the primary endo-

cœlic tentacle, x, towards the right, which is conventionally taken to be the ventral side. In *b* a secondary exocœlic tentacle, x', has sprouted between the secondary tentacle, 2, and the adjacent primary endocœle, and at the same time a new micromesentery has been formed to the right of x, apparently from the angle between it and the adjacent

TEXT-FIG. 2.



Diagrams of tangential sections showing the succession of the mesenteries and tentacles in Phellia, Ilyanthus partheno-pæus and Edwardsia cornea. 1, 2, 3. Primary, secondary, and tertiary endocœlic tentacles. x. The primary exocœlic tentacle. x'–x'''. The remaining exocœlic tentacles according to the order of their appearance. For further description see text.

primary endocœlic tentacle. In *c* a fellow has been added to the new micromesentery on the right or ventral side, and the two constitute a mesenterial pair of the third order, of which the endocœle is prolonged into the tentacle 3, and an exocœlic tentacle, x'', has sprouted from the interval between it and the adjacent primary endocœle. At the same time a single tertiary micromesentery has been formed in the angle between

x' and the left or dorsal primary endocœlic tentacle. This is the condition found in the lateral and dorso-lateral sextants of Phellia phassonesiotes. In *d* a fellow has been added to the tertiary micromesentery of the left side; the exocœle of the pair so formed has been prolonged into the tentacle, 3, and a new endocœlic tentacle, x''', is sprouting from the interval between it and the adjacent dorsal primary endocœle. This condition is found in the dorso-lateral and lateral sextants of Phellia allantoides. It should be noted that new elements are added alternately on the left (dorsal) and right (ventral) side of the primary endocœlic tentacle, x, which eventually becomes submedian in position, though the actual median tentacle in the sextant is 2, which was formed after x. It should further be borne in mind that, for the sake of economising space, more than one growth stage is included in each diagram: it would take double the number to represent each successive addition of micromesenteries and tentacles.

Diagrams, *e–g*, copied from Faurot (**13**), illustrate parallel stages in the development of Ilyanthus parthenopuus. In *e* the two members of a pair of secondary mesenteries with their corresponding tentacle have appeared simultaneously on the ventral side of the primary endocœlic tentacle x. In *f* two pairs of tertiary mesenteries have appeared; one pair, either the larger and earlier in order of appearance, on the ventral side between the secondary tentacle and the ventral primary tentacle; the corresponding rather smaller and later mesenterial pair between the primary endocœlic tentacle x, and the dorsal primary endocœle. Both tertiary endocœles are prolonged into tentacles, but with the exception of the primary exocœlic tentacle x, there are no exocœlic tentacles. There has been no room for these owing to the comparatively rapid formation of the secondary and tertiary mesenteries in pairs, and their development is postponed till the stage represented in *g*, when the three exocœlic tentacles lettered x^1 are formed simultaneously, and make up the full complement of tentacles for the sextant. In this case

the primary exocœlic tentacle *x* is again submedian in position (but on the dorsal instead of, as in Phellia, on the ventral side of the secondary endocœlic tentacle), and the new elements are added alternately on the ventral and dorsal side of it, though not so obviously so as in Phellia. But I think it must be conceded that the growth process in the latter genus has every appearance of being the more primitive, and that the peculiar features of the sequence of tentacular growth in Ilyanthus receive an intelligible explanation if we regard the second method as derived from the first.

Now Phellia has this much in common with Edwardsia that in both the micromesenteries are formed in couples of singles. There is no pairing of the micromesenteries in Edwardsia, but in Phellia the members of adjacent couples combine to form pairs, and it is difficult, though, as I will show, not impossible, to suggest a scheme by which this fundamental difference between the two genera can be bridged over. To make use of Mendelian terminology, it would appear that in Actinian phylogeny a factor for "pairing" was introduced at a certain stage in ontogenetic development. This factor primarily affected the metacnemes; that is to say, all mesenteries formed subsequent to the eight protocnemes, but it carried with it secondary consequences in the relations and mode of succession of the tentacles. For—leaving for the moment out of account the dorsal and ventral directives—as there are no mesenterial pairs in Edwardsia there is no division into endocœlic radii—in which no further growth by addition of parts takes place, and exocœlic radii in which such growth does take place, and in the absence of such a division the metacnemial growth processes of the Edwardsidæ are not comparable with those of the Actiniiæ. But a reference to Text-fig. 2, *h*, founded on my recent demonstration of the sequence of micromesenterial and tentacular formation in Edwardsia carnea (**4**), shows a certain parallelism between the growth principle in the two cases, for in Edwardsia new mesenteries and new tentacles are formed alternately dorsad and ventrad of the primary megacœlic tentacle *x*, which

thus assumes a median position in each growth-sextant, and is actually median when the number of tentacles is an uneven number. And, if one keeps in view this principle of the addition of parts alternately dorsad and ventrad of the median tentacle in each sextant, it is possible to construct a scheme showing the derivation of the Actinian from the Edwardsian mode of growth. For if the first-formed micromesentery in a growth-sextant of Edwardsia—that on the left in the diagram *h*—were formed as a pair instead of a single and if a tentacle grew out of the endocœle thus established, one would get the same relations of micromesenteries to tentacles as in diagram *a*. And, again, if the micromesentery on the right in diagram *h* were, in succession, to be formed as a pair instead of a single, one would get a stage actually represented in Phellia, but not represented in any of the diagrams in the Text-fig. because, as noted above, two or more successive growth stages are represented in each diagram for economy's sake. Such a scheme would be perfectly legitimate for the dorso-lateral megacœles of an Edwardsia, and I have accordingly, in diagram *h*, represented the muscle banners of the macromesenteries in the position they would occupy in such a megacœle. But in the lateral and ventro-lateral megacœles the scheme is somewhat vitiated by the necessity of taking into account the macromesenteries v and vi in order of Actinian development, which, pairing with ii and i, make up six primary mesenterial pairs of the Actiniiæ. The difficulty is not insuperable, for, as I have shown elsewhere, the order of appearance of the micromesenteries is reversed in the ventro-lateral megacœles of Edwardsia carnea, and may be reversed in the lateral megacœles in other species, and one has only to suppose that the mesenteries first formed in these sextants become macromesenteries in order to arrive at the paired hexameral condition. This form of argument, however, is extremely hypothetical, and I do not propose to push it any further. It is sufficient for present purposes if I have succeeded in interpreting the facts of the developmental succession of the

mesenteries and tentacles in Actiniiæ as represented by Ilyanthus by reference to the simpler and more primitive succession observed in the Phelliinæ, and if I have further established certain analogies between the growth processes in the Phelliinæ and those in the Edwardsiæ.

It remains to discuss the relationships of the Phelliinæ with other Actiniiæ, a task which, in the present state of our zoological knowledge, presents considerable difficulties. Proceeding on the accepted methods of systematic zoology, we may accept, as the definition of the family Sagartiidæ, "Actiniiæ provided with acontia," and it follows logically that the Phelliinæ are included in the Sagartiidæ. As to the limits of the Phelliinæ, I think there can be no doubt that the genus Phellia, with Decaphellia and possibly Halcampactis, form a group distinct from the Chondractiniinæ of Haddon, who, in my opinion, has already sufficiently established the validity of the latter family, and a study of sections of Paraphellia expansa, Haddon, has satisfied me that there is little in common between this genus, long since included in the Chondractiniinæ, and the Phelliinæ. Paraphellia is anatomically very similar to Sagartia. As to the nearest affinities of the Phelliinæ, it has been obvious, in the course of this paper, that I regard them as most closely related to the "Actinies pivotantes" of French authors, the Ilyanthidæ of Gosse. But then arises the question as to what forms should be included in this somewhat heterogenosus group. Certainly not the Edwardsiæ, for reasons which I have already given (**4**). Observations that I have made, but not yet completed, on the anatomy of Ilyanthus mitchellii, Gosse, indicate that this species is quite distinct from the others commonly grouped with it. I am doubtful of the near relationship of Siphonactis to Halcampa, and am unable to express an opinion on Eloactis as I have not had an opportunity of studying this genus. There remain the genera Halcampa, Halcampella, and Halianthella, which I prefer to group together in a family Halcampidæ, notwithstanding the

presence of a mesoglœal sphincter muscle in Halianthella, and I agree with Andres in separating these forms from the other "Actinies pivotantes." It is to the Halcampidæ that the Phelliinæ appear most closely related, rather than to the Sagartiidæ, for reasons which I will set out at full length.

The structural and external features which, taken in combination, are the recognisable marks of the members of the Phelliinæ are:

(1) The division of the column into capitulum, scapus, and a more or less inflatable but adherent base or physa. (2) The corrugation of the external surface of the scapus, correlated with the presence of a coriaceous investment known as the cuticle or epidermis. (3) The predominant size and importance of the six pairs of primary mesenteries which alone are attached to the actinopharynx. (4) The dwarfed condition of the secondary, tertiary, and, when present, of the other cycles of mesenteries, which are reduced to little more than a lamina supporting the parietal muscles. (5) The relatively considerable development of the parietal musculature, and its symmetrical arrangement on either side of the peripheral edges of the macromesenteries and micromesenteries. (6) The great development and circumscribed character of the longitudinal retractor muscles of the macromesenteries, and the reniform shape of these muscles in section. (7) The capacity for introverting the distal third of the scapus as well as the capitulum, correlated with the great development of the retractor muscles. (8) The reduction to the point of suppression of the longitudinal retractor muscles of the micromesenteries. (9) The fertility of the macromesenteries only. (10) The sterility of the micromesenteries. (11) The presence of a mesoglœal sphincter muscle. (12) The presence of acontia. (13) The reduction of the peristomial musculature, causing the actinostome to gape.

As far as can be judged from the evidence afforded by simple observation, all members of the Phelliinæ breed true to these characters; but, as I have shown, every character is subject to considerable variation within the group. A critical

study of these marks or characters shows us that, whilst all of them taken together constitute what we call a Phellia, there is hardly one of them that is peculiar to the group, unless it be No. 13. Nos. 9 and 10 are in a large measure peculiar and differential characters, but not wholly so, for, as I have shown, there is probably an infertile couple of macromesenteries in Phellia castanea—a feature which approximates this species to Halcampa, in which the macromesenterial couples I–III are alone fertile. Halianthella, Kwietn., a member of the same family as Halcampa, has all the six pairs of macromesenteries fertile and no others, and thus is in exactly the same condition as Phellia.

On a further analysis of the characters enumerated above, we find that those which are not differential are distributed sporadically in several groups of Actiniinæ, and occur in different combinations in those groups. Thus, character 1 is found in the Edwardsidæ, Halcampidæ, in Ilyanthus, in Siphonactis, and in other forms in various degrees of distinctness. Character 2 is shared by the Edwardsidæ and Chondractiniinæ, and by Aureliania, Gosse; Capnea, Gosse; and Ammonactis, Verrill. Characters 3 and 4 reappear in the Halcampidæ, Metridiinæ, Chondractiniinæ, and in Siphonactis (I do not include the last-named among the Halcampidæ). Character 5 is found in the Edwardsidæ and Halcampidæ, in Eloactis, Andr., and generally in all forms that have very highly developed and circumscribed retractor muscles, but also in some Chondractiniinæ, e.g. Hormathia, in which the retractors are diffuse. Characters 6 and 7 are found in Edwardsidæ, Halcampidæ, and Eloactis, and among the Chondractiniinæ in Paraphellia expansa, which has large circumscribed retractor muscles on the macromesenteries, but, as far as I have been able to observe, no capacity for introverting the upper part of the scapus. Character 8 goes, as a rule, with character 6, but in Paraphellia expansa, and generally in the Chondractiniinæ, longitudinal retractor muscles are present on at least the higher orders of micromesenteries, whether the retractors of the macromesenteries

be specially developed or not. Characters 9 and 10 are elsewhere found only in the Halcampidæ, including Halianthella in this family. Character 11 is found in all members of the family Sagartiidæ (auctt.) except Aiptasia, but also occurs in the Paractidæ and in Halianthella. Character 12 has hitherto been regarded as diagnostic of Sagartiidæ, but it should not be forgotten that analogous, though not exactly similar, structures are characteristic of the Cerianthidæ.

From this analysis it appears that of the thirteen characters enumerated as marks of the group Phelliinæ, no less than ten, and in part an eleventh, recur in the Halcampidæ (including the Halianthinæ); six recur in the Edwardsidæ; four, and in part a fifth, recur in the Chondractiniinæ, four in the Metridiinæ, and two only in the Sagartiinæ. If we may judge of the relationships of animals, and therefore of their places in a natural classification, by the sum of their characters rather than by one or two somewhat arbitrarily selected, the Phelliinæ certainly incline on the balance towards the Halcampidæ rather than towards any other of the sub-families commonly included in the Sagartiidæ.

The main reasons for including the Phelliinæ in the Sagartiidæ are the presence of acontia and of a mesoglœal sphincter muscle. But Halcampactis, in all other respects a Halcampid, has acontia, and Halianthella, in all other respects a Halcampid, has a mesoglœal sphincter muscle.

Are we to regard Halianthella, Halcampactis, and the Phelliinæ as the representatives of a stage in the evolution of Sagartiæ with acontia and a mesoglœal sphincter muscle from a Halcampa-like ancestor? Assuredly not as representatives of the direct line of descent, for in the first place Halianthella has only one and Halcampactis the other of the required characters; and in the second place the differences between the mesenterial arrangements of the Phelliinæ and the Metridiinæ, Sagartiinæ, Chondractiniinæ, and other groups included in the Sagartiidæ are so great as to be fundamental Then the two lines, or possibly the several lines represented by the different families, must have diverged at an early

period from a common ancestral stock which had acquired acontia, and is now possibly represented by Halcampactis, though Halcampactis has not acquired a mesoglœal sphincter, whilst Halianthella, which possesses no acontia, has.

Such an argument assumes that the possession of acontia is a mark of close genetic relationship and involves the inclusion of a heterogeneous group of Actinians, among others Aiptasia, in the family Sagartiidæ, the limits of which have been the subject of much discussion (see Andres (**1**), Hertwig (**19**), Kwietniewsky (**22**), and Haddon (**15**)). But is it not possible that acontia, which cannot be regarded as anything else than a special modification of the ubiquitous mesenterial filament, may have been independently acquired by several groups of Actinians?

We may test this suggestion by inquiring into the distribution of another character, e.g. the mesoglœal sphincter muscle. This is present in all the Actinians that have acontia except Aiptasia and Halcampactis. It is also present in Halianthella, in Ophiodiscus, and in the Paractidæ. It is a character which has been held to be of considerable classificatory importance. If so, it should afford some guide to genetic relationships. But when we take the mesoglœal sphincter into consideration along with acontia to what conclusions are we led? If a mesoglœal sphincter is a sign of descent from a common ancestor, then Halianthella, Ophiodiscus, the Paractidæ, and the Sagartian sub-families of Metridiinæ, Sagartiinæ, Phelliinæ, and Chondractiniinæ are genetically related; the Aiptasiinæ and Halcampactis are not, because they have not got a mesoglœal sphincter. But we have just seen that the Aiptasiinæ and Halcampactis are related to the other sub-families of Sagartiidæ because they have acontia, and that Halianthella, Ophisdiscus, and the Paractidæ are not related because they have no acontia. If acontia are taken as the criterion of descent from a common ancestor, then Aiptasia and Halcampactis must either have independently lost the mesoglœal sphincter or have never acquired it. If the mesoglœal sphincter is taken as a cri-

terion, then the Sagartiidæ and Halcampactis must have acquired acontia independently of the Paractidæ, of Ophisdiscus, and of Halianthella; or these latter forms must at some time have possessed acontia and subsequently lost them. Whichever way one looks at it, there is the question of the acquisition of a new character or the dropping out of a character previously existing.

It will be more logical, however, to regard the phenomena from the following standpoint.

There are four possible combinations, viz.:

(1) + Mesoglœal sphincter, + acontia : Metridiinæ Sagartiinæ; Phelliinæ; Chondractiinæ.

(2) + Mesoglœal sphincter, — acontia : Halianthella; Paractidæ.

(3) — Mesoglœal sphincter, + acontia : Halcampactis; Aiptasia.

(4) — Mesoglœal sphincter, — acontia : Actinia, Anemonia, Bunodes, etc.

(The plus and minus signs stand for presence or absence of the character in question.)

It will be observed that every possible combination is represented and that some of the combinations bring together forms between which no relationship has even been suggested, e. g. Halianthella and the Paractidæ.

We may go a step further and bring in another element, choosing the predominant size and "perfection" of the primary mesenteries only. Then, if we denote the presence of a mesoglœal sphincter by M., the presence of acontia by A., and the perfection of only the first cycle of mesenteries by I., and use the plus and minus signs as before, we get eight possible combinations, viz.:

(1) + M. + A. + I. : Metridiinæ; Chondractiniinæ, Phelliinæ.

(2) + M. + A. — I. : Sagartiinæ.

(3) + M. — A. + I. : Halianthella.

(4) + M. — A. — I. : Paractidæ.

(5) — M. + A. + I. : Halcampactis.

(6) — M. + A. — I. : Aiptasia.
(7) — M. — A. + I. : Halcampa.
(8) — M. — A. = I. : Actinia, Anemonia, Bunodes, etc.

Again, all the possible combinations are represented by described forms of living Actinians. This method of presenting the facts might be carried further by successively introducing new elements, but it would quickly become so complicated as to require mathematical treatment, and I do not propose to pursue the subject in this place.[1]

What I want to emphasise is that we are dealing with unit characters, each of which may be present or absent, and when present may enter into all possible combinations with the other unit characters. In brief, these unit characters have all the properties of, and may legitimately be identified with Mendelian units.

This being the case is it not probable, and more than probable, that among the many "factors" that go to make up the full complement of variable Actinian characters there are some which, when brought together in the germ-cell, lead to the production of that particular outgrowth called an acontium? And if this be the case, does it not follow from the evidence accumulated by the experiments of the last fifteen years, that in any given Actinian germ-cell there may be some of the factors necessary for the production of an acontium, but that they will not lead to the exhibition of that feature in the adult organism unless one or more additional factors are added to them? I submit that the Phelliinæ give considerable support to this view, for in them the acontia show every possible grade between full development and reduction to the point of disappearance, and the most reasonable explanation of this phenomenon is that in some species

[1] The number of possible combinations is 2^a, where a stands for the number of unit characters entering into combination. Thus the addition of a fourth element would give sixteen possible combinations, and if all the twelve elements enumerated above as characters of Phelliinæ but also occurring in other groups of Actinians were taken into account the possible number of combinations would be 2^{12} or 4096.

(or varieties) there is missing a factor required for the full development of the structures in question.

If this be a true explanation, and it is the most consistent with recent researches, we can account for the appearance or disappearance of acontia in groups having very different combinations of other characters, and we get rid, once and for all, of the idea that the presence of this single character is such a positive mark of inter-relationship that all the forms possessing it must be united into a single family. The same reasoning applies to the mesoglœal sphincter and to every other structural feature which can be shown by the methods indicated above to be independently variable. I may claim also that this method of dealing with observational data throws a new light on the phenomena of homoplasy or parallel development, which I have dealt with on previous occasions in connection with other animals. Applying these principles to the subject in hand, it is obvious that the group Sagartiidæ, including Halcampactis, the Aiptasiinæ, the Metridiinæ, the Sagartiinæ, the Phelliinæ, and the Chondractiniinæ must be broken up. Halcampactis, judged by the sum of its characters, takes its place with the Halcampidæ. On the same principle, Aiptasia goes alongside of its obvious ally, Anemonia. The Phelliinæ, as I have shown, must stand apart from the other sub-families, and be approximated to the Halcampidæ. Of the remaining groups the Metridiinæ will be found to share one set of characters with the Chondractiniinæ, another set with the Sagartiinæ, and further analysis may lead to further subdivisions. It is also evident that the same principles will have to be applied to the whole of the Actiniinæ; but I am not in a position, and do not propose, to make such an ambitious attempt now.

If the method of dealing with systematic questions which I have recommended and briefly indicated in the foregoing paragraphs were accepted and generally adopted, it would have a result, unpalatable to many zoologists, of undermining many accepted beliefs on phylogenetic questions. But this, I beg leave to submit, would be wholly advantageous to the

progress of zoology. It is not possible to read Tower's investigations on the evolution of the genus Leptinotarsia and Morgan's critique of the 'Theory of Evolution,' together with much other current Mendelian literature, without experiencing grave doubts as to the validity of a large part of current, systematic, and phylogenetic speculations. All this class of reasoning is open to the fundamental objection taken against it by Morgan, that we collect a large number of "characters," external or anatomical, and arrange them in a series which we call evolutionary, without having any evidence as to the actual relationship by way of descent and inheritance among the different forms constituting our series. On the other hand, breeding experiments show direct genetic relationship between forms that one would never have supposed to have descended one from the other, and contrariwise, more remote relationship between forms which, on accepted methods of systematic criticism, one would unhesitatingly have placed in direct lines of descent. "Çela donne," or, at any rate, doit donner "furieusement à penser."

Having for some years past recognised the force of such criticisms as those mentioned in the preceding paragraph, and being convinced of the importance of bringing morphological and systematic studies into harmony with the principles established by genetic researches, I have endeavoured in the foregoing pages to arrange certain limited morphological and systematic data in conformity with the conclusions reached by the Mendelian school of zoologists, and have indicated a method that seems to me appropriate to the purpose. In so doing I am aware that I am proposing a rèvolution in our methods of envisaging and dealing with morphological and systematic problems, and have only given the barest outline of the plan which I propose to pursue. It is possible, as I perceive from some attempts that I have made in the course of writing these few pages, to carry the ideas I have propounded much further, and to give much greater precision to the methods roughly sketched out above. But the subject is one of great size and complexity, and therefore inappropriate

to the concluding passages of a memoir undertaken with only a limited object in view. My suggestions are, therefore, given for what they are worth in their present state of incompleteness, and a further discussion of the possibility of co-ordinating morphological, systematic, and genetic data must be postponed to a future occasion.

List of the Principal Papers referred to in the Text.

1. Andres, A.—"Actinien," 'Fauna und Flora des Golfes von Neapel,' ix, 1884.
2. Beneden, E. van.—"Die Anthozoen der Plankton Expedition," 'Ergeb. der Plankton Expedition d. Humboldt Stiftung,' Bd. ii, K. e.
3. Bourne, G. C.—"Report on the Solitary Corals collected by Prof. Herdman at Ceylon," 'Ceylon Pearl Oyster Fisheries,' Supplementary Report, xxix, Published by the Royal Society, 1905.
4. ——— "A Description of Five New Species of Edwardsia, Quartr., from New Guinea," 'Linn. Soc. Journ.,' xxxii, 1916.
5. Carlgren, O.—"Studien über nordische Actinien," 'Kongl. Sven. Vet-Akad. Handl.,' xxv, 1893.
6. Carlgren, O.—"Zoantharien," 'Hamburger Magalhaenische Sammelreise,' Hamburg, 1898.
7. ——— "Actiniarien d. Olga Exped. Wissensch. Meeresuntersuchungen," v.
8. Dixon, F.—"On the Arrangement of the Mesenteries in the Genus Sagartia, Gosse," 'Proc. Roy. Dublin Soc.,' New Ser., vi, 1888.
9. Dixon, G. Y.—"Remarks on Sagartia venusta and Sagartia nivea," ibid., New Ser., vi, 1888.
10. ——— and Dixon, F.—"Notes on Bunodes thallia, Bunodes verrucosa, and Sealia crassicornis," ibid., New Ser., vi, 1889.
11. Duerden, J. E.—'The Coral Siderastræa radians and its Postlarval Development,' Washington, 1904.
12. Farquhar, H.—"Preliminary Account of Some New Zealand Actiniaria,' 'Linn. Soc. Journ.,' xxvi, 1898.
13. Faurot, L.—"Études sur les Actinies," 'Arch de Zool. expér. et gén.' (3), ii, 1895.

14. Gosse, P.—'British Sea-anemones and Corals,' London, 1860.

15. Haddon, A. C.—"A Revision of the British Actinia," 'Trans. Roy. Dublin Soc.,' ser. 2, iv, 1889.

16. ——— "Phellia sollasi, a New Species of Actiniarian from Oceania," 'Proc. Roy. Dublin Soc.,' N.S., viii, 1897.

17. ——— "Actiniaria of Torres Straits," 'Trans. Roy. Dublin Soc.,' ser. 2, vi, 1898.

18. Hertwig, O. ü R.—"Die Actinien," 'Jenaische Zeitsch. f. Naturwiss.,' xiii, 1879.

19. Hertwig, R.—"Actiniaria," 'Challenger Reports: Zoology,' vol. vi, 1882.

20. ——— Ibid., 'Challenger Reports Suppl.,' vol. xxvi, 1888.

21. Kwietniewsky, C. R.—"Revision d. Actinien, etc.," 'Jenaische Zeitschr.,' xxx, 1896.

22. ——— "Actiniarien von Ternate," 'Abh. d. Senckenb. naturf. Gesell.,' xxiii, 1897.

23. ——— "Actiniaria von Ambon. u. Thursday Island," 'Denkschr d. Med. nat. Gesellsch. zu Jena,' viii, 1898.

24. Maguire, Dr. Katharine.—"Notes on Certain Actiniaria: (1) The Anatomy of Phellia sollasi, Haddon," 'Proc. Roy. Dublin Soc.,' N.S., viii, 1898.

25. Matthai, G.—"A Revision of the Recent Colonial Astræidæ possessing distinct Corallites," 'Trans. Linn. Soc. Lond.,' xvii, 1914.

26. Pourtalès, L. F. de.—'Illus. Cat. Mus. Comp. Anat.,' Harvard, Camb., Mass., 1871.

27. Stuckey, F. G. A.—"A Review of the New Zealand Actiniaria," 'Trans. New Zealand Inst.,' Wellington, xli.

28. Verrill, A. E.—"Notes on the Radiata in the Museum of Yale College," 'Proc. Connecticut Acad.,' i, 1867.

29. ——— "Descriptions of Imperfectly Known and New Actinians, with Critical Notes on Other Species," Amer. Journ. Sci.,' 4th ser., vol. vii, Pt. I, p. 41; Pt. II, p. 143; Pt. III, p. 205; Pt. IV, p. 375; 1899.

30. Wassilieff, A.—"Japanische Aktinien," 'Abh. Akad. Wiss. Math. Phys. München,' Suppl., Bd. i, 1908.

31. Wilsmore, Leonora J.—"On Some Hexactiniæ from New South Wales," 'Linn. Soc. Journ. Zool.,' vol. xxxii, July, 1911.

EXPLANATION OF PLATES 3, 4 AND 5,

Illustrating Prof. G. C. Bourne's paper on "Some New Phelliinæ from New Guinea."

LETTERING IN ALL THE FIGURES.

ac. Acontium. *aph.* Actinopharynx. *b.* Basal disc or physa. *d. l.* 1–3. Micromesenteries of the dorso-lateral sextant. *ec.* Ectoderm. *ed.* Endoderm. *ed. m.* Endodermic muscle. *fr.* Frill of mesenterial filament. *l.* 1–3. Micromesenteries of the lateral sextant. *l. r. m.* Longitudinal retractor muscles. *m. f.* Mesenterial filament. *mg.* Mesoglœa. *msl.* Muscles of tentacles. *n.* Nervous layer. *ov.* Ovaries. *p. m.* Parietal muscles. *sc.* Sulcus. *sl.* Sulculus. *sph.* Sphincter muscle. *st.* Mesenterial stomata. *t.* Tentacles. *ts.* Testis. *v. l.* 1–2. Micromesenteries of the ventro-lateral sextant. *I–VI.* Macromesenterial couples numbered in the order of their development.

PLATE 3.

Fig. 1.—Phellia castanea, n. sp. The animal has been divided in half by a sagittal section. × 3.

Fig. 2.—Phellia phassonesiotes, n. sp. × $\frac{3}{7}$.

Fig. 3.—Phellia allantoides, n. sp. × $\frac{2}{3}$.

Fig. 4.—Phellia cylicodes, n. sp. × 2.

Fig. 5.—Decaphellia psammomitra, n. gen. et. sp. × 6.

Fig. 6.—A spiral nematocyst from the tentacle of Decaphellia psammomitra × 1040. *n.* Nucleus.

Fig. 7.—A spiral nematocyst from the tentacle of Phellia allantoides. × 1040.

Fig. 8.—A nematocyst from an acontium of Decaphellia psammomitra. × 1040.

Fig. 9.—Everted nematocysts from an acontium of Phellia phassonesiotes, showing the short, everted, barbed thread. × 520. These as well as the nematocyst shown in Fig. 8, apparently belong to type II c of Matthai.

Fig. 10.—Decaphellia psammomitra. A micromesentery showing the "frill" of the mesenterial filament, its ridges crowded with deeply-stained nuclei, and at *ac.* the origin of an acontium. × 230.

Fig. 11.—Pigmented endoderm cells from a tentacle of Phellia allantoides. × 520.

Fig. 12.—Two micromesenteries forming a pair from Phellia castanea. × 385. For full description see p. 41.

PLATE 4.

Fig. 13.—Part of a section through a tentacle of Phellia allantoides showing the highly developed ectodermal musculature supported on branched processes of the mesoglœa. × 385.

Fig. 14.—A transverse section through the actinopharynx of Phellia castanea, showing the arrangement of the macromesenteries and micromesenteries; the mesenterial stomata; the acontia; and the sulcus and sulculus. In this and the succeeding figures the macromesenteries are indicated by roman numerals according to their order of development.

Fig. 15.—The half of a transverse section of P. castanea taken below the level of the enterostome.

Fig. 16.—The half of a similar section to that in Fig. 15, taken near the base.

Fig. 17.—Phellia phassonesiotes; a transverse section through the actinopharynx, showing six pairs of macromesenteries with largely developed longitudinal retractor muscles.

Fig. 18.—P. phassonesiotes; a transverse section taken just below the enterostome; only eight of the macromesenteries retain the conspicuous muscle banners, and these are oriented as in Edwardsia.

Fig. 19.—P. phassonesiotes; a transverse section through the lower third of the column; only the macromesenteries I, I and II, II bear conspicuous muscle banners.

Fig. 20.—Phellia allantoides; a transverse section through the actinopharynx, showing six pairs of macromesenteries with conspicuous muscle banners, and the small micromesenteries in the intervening sextants.

Fig. 21.—P. allantoides; a section through the middle of the column showing the reduction of macromesenteries V and VI.

PLATE 5.

Fig. 22.—Phellia cylicodes; a transverse section just below the level of the enterostome, showing the six pairs of macromesenteries with conspicuous muscle banners and the micromesenteries in the intervening sextants.

Fig. 23.—Decaphellia psammomitra; a transverse section below the level of the enterostome showing only nine macromesenteries with muscle banners, no V being reduced on the right-hand side.

Fig. 24.—D. psammomitra; a transverse section passing nearly through the actinostome. The macromesenteries I–V are complete and bear muscle banners, but the couple VI is incomplete and bears no muscle banners. The body-wall is extremely thin; the micromesenteries, one pair in each sextant, are minute, and in this region have no parietal muscles.

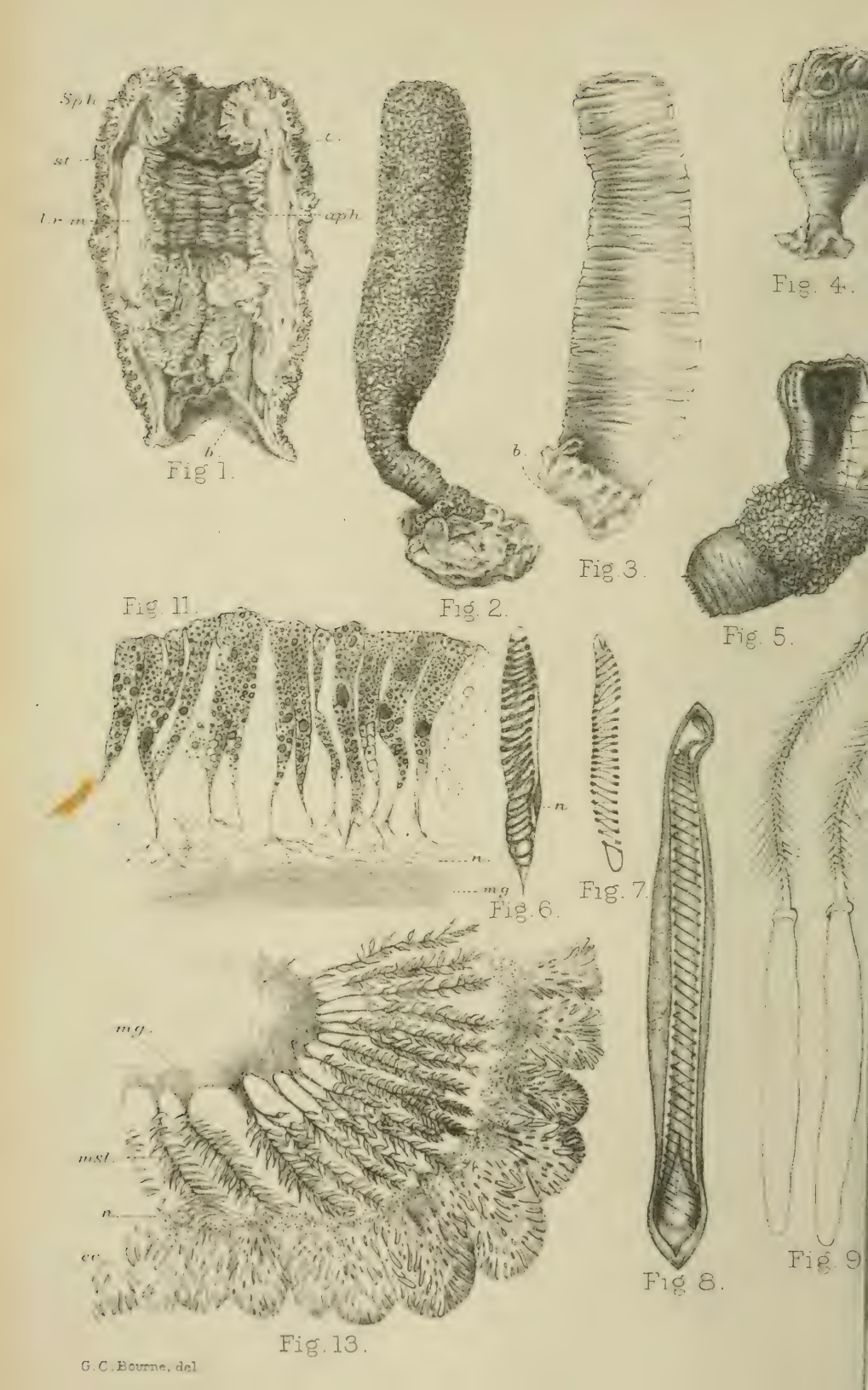

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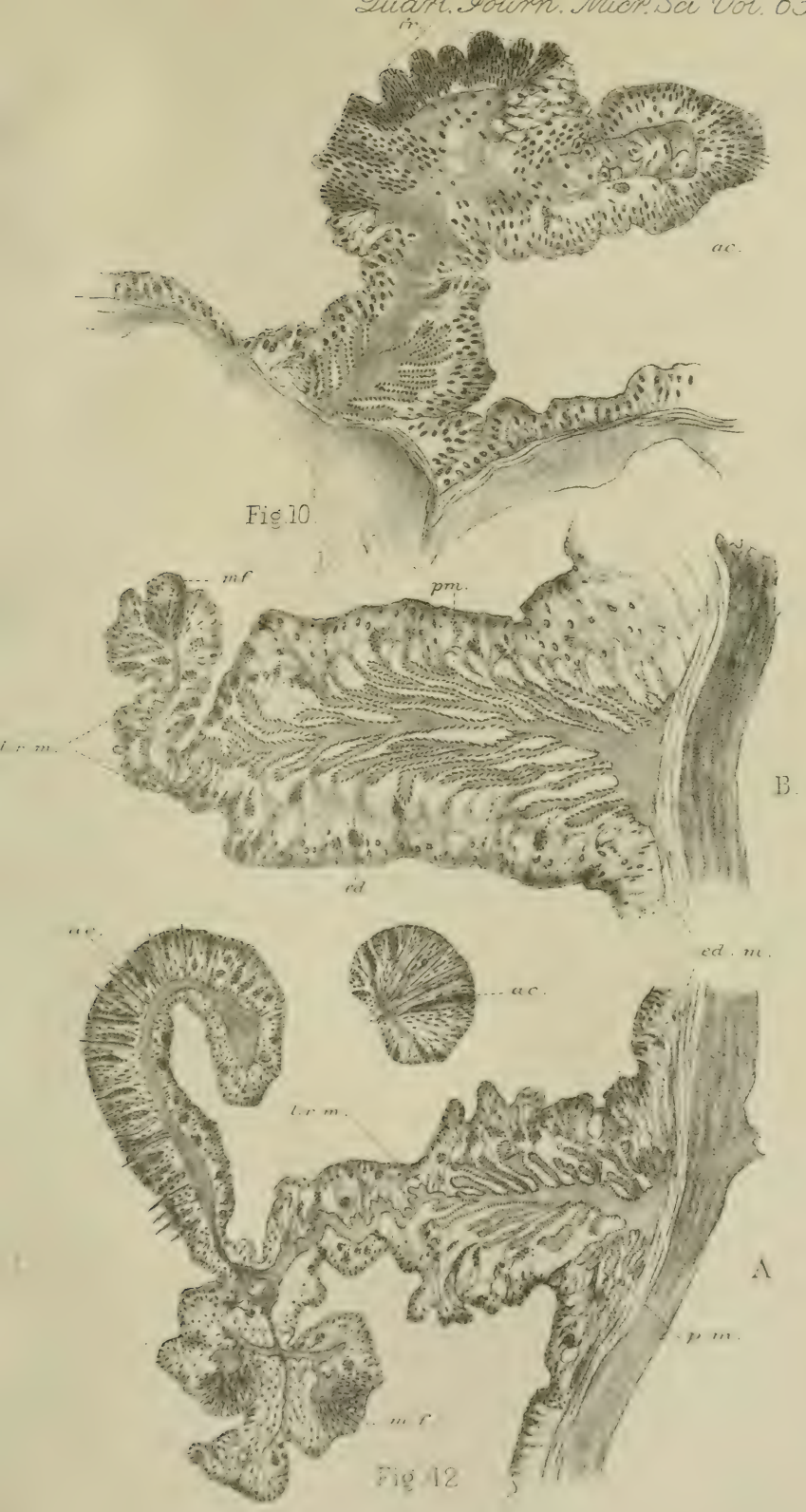
Fig. 13.
G.C. Bourne, del.



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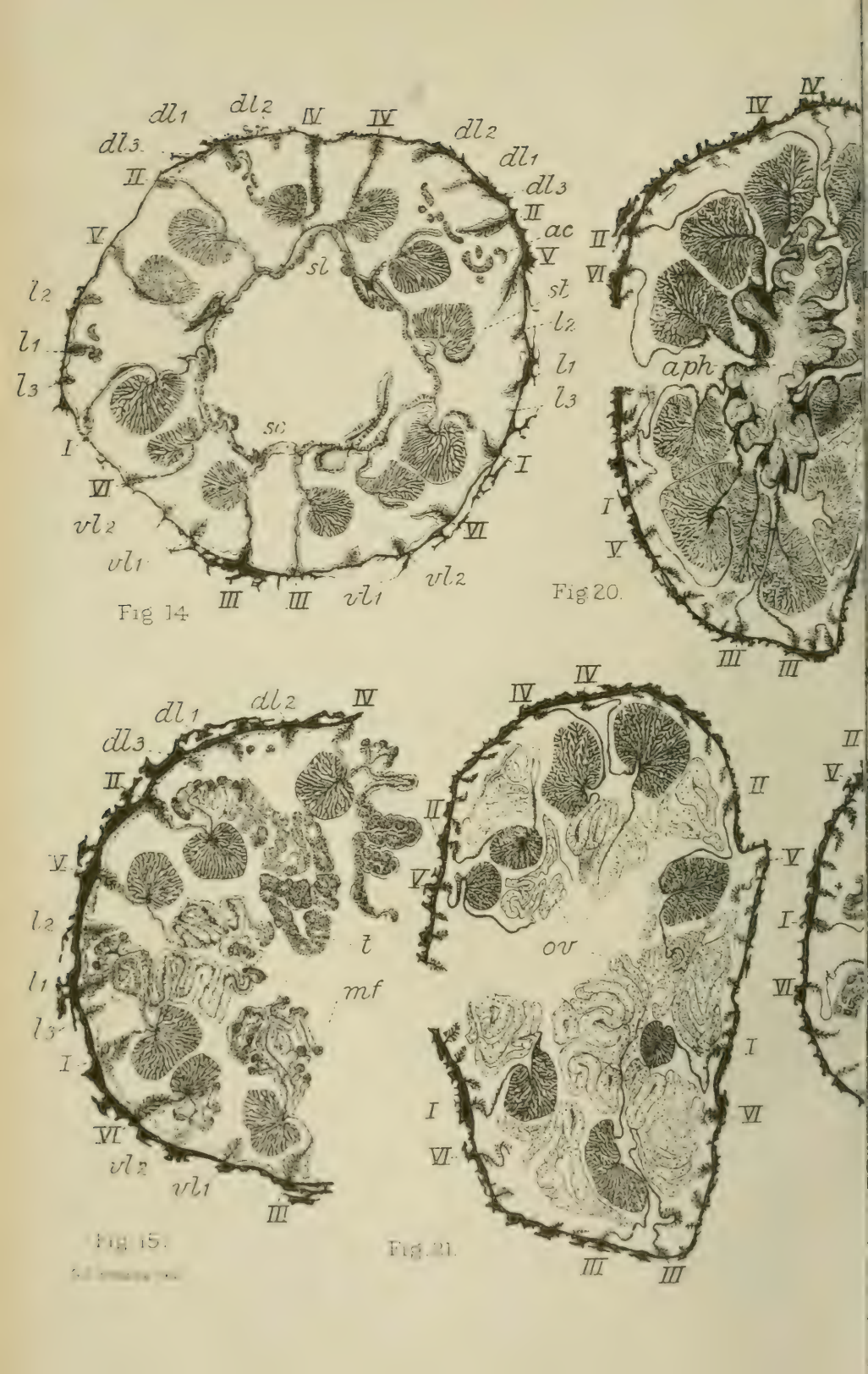

Fig 14.

Fig 20.

Fig 15.

Fig 21.

Fig. 16.

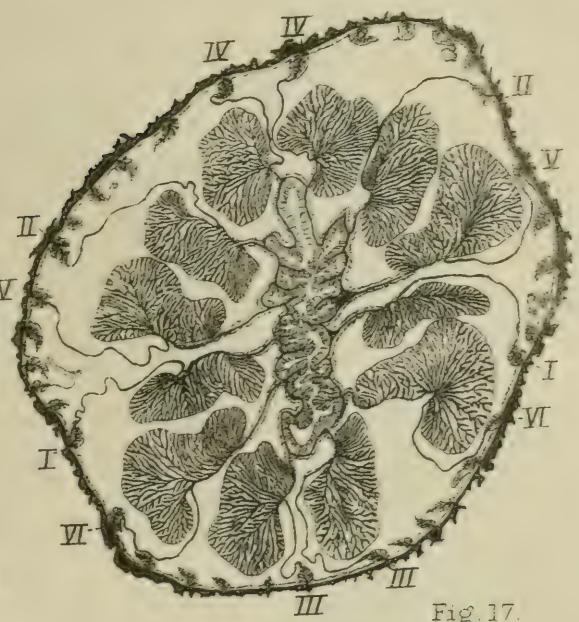

Fig. 17.

Fig. 18.

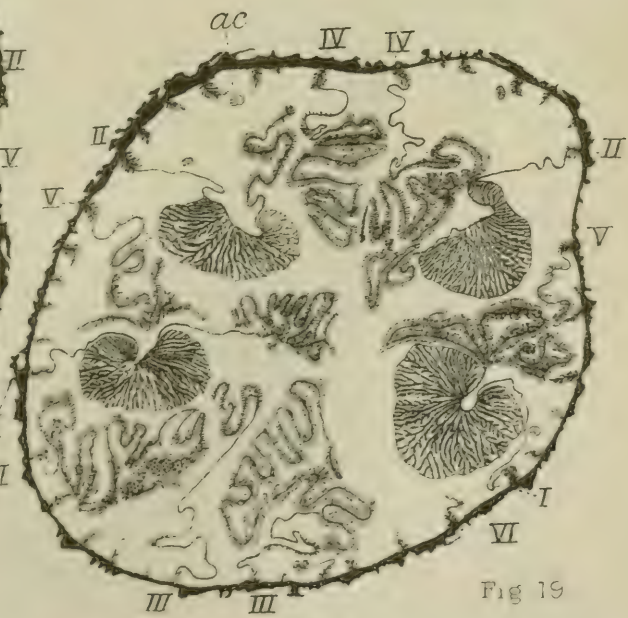

Fig 19

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Fig 22.

Fig. 23

Fig 24.

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Some Observations on the Early Development of Didelphys aurita. (Contributions to the Embryology of the Marsupialia.—V.)

By

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With Plates 6–9.

Introduction.

The observations recorded in this paper are the outcome of the examination of a small amount of early material of Didelphys aurita collected by the Percy Sladen Expedition in Brazil in 1913. The chief object of that expedition was the collection of embryological material of South American mammals, and more especially of the Didelphyidæ. Up to the beginning of 1916, our knowledge of the early development of the latter rested on the well-known account given by the late Emil Selenka in 1886 of that of the Virginian opossum (Didelphys virginiana). Selenka's conclusions, as I have pointed out in my paper on the early development of Dasyurus ('10), were derived from the study of a relatively small amount of material, much of it of very doubtful value, and differ in essential respects from my own, based on the study of an abundant material of Dasyurus, and as we should not expect à priori to find fundamental differences in the early development of two animals so closely related as Didelphys and Dasyurus undoubtedly are, the need for a re-investigation of the early development of the former was sufficiently obvious.

In the interval that need has been met by the publication in March of last year of an extremely valuable account of the early development of Didelphys virginiana, by C. G. Hartman, of the University of Texas ('16).

For another reason, we were anxious to secure developmental material of the Didelphyidæ. It has been suggested that the Australian marsupials have been derived from an ancestral Didelphyd stock, and if that be so, it is of first importance that we should possess a knowledge of the development of those genera of the Didelphyidæ which on anatomical grounds have been regarded by various authorities (Winge ('93), Bensley ('03), Bresslau ('12) and others) as lying nearest the base of the Didelphyd series. The genera in question, Marmosa and Peramys, are small, rat-like forms, remarkable for the entire absence of the pouch so characteristic of most other members of the order. In view of the researches of Bresslau ('12), which demonstrate that the pouch of the marsupial has nothing whatever to do with the pouch or incubatorium of the monotrematous Echidna, it may well be that the non-existence of a pouch in these genera is a further evidence of their primitive character. We were accordingly most anxious to obtain embryological material of these pouchless forms.

Developmental material of non-domesticated mammals just in the precise stages desired by the investigator is at no time very easy to obtain, and that inherent difficulty was enhanced in our own case by the absence of exact data as to the breeding seasons of the South American forms, and of records of their frequency and local distribution.

As it turned out, however, we were fortunate in that we found shortly after our arrival in Rio de Janeiro that Didelphys aurita (the Gambá of the Brazilians), a species widely distributed throughout Brazil and relatively common in certain regions, especially near habitations, was breeding. We collected altogether some forty-six specimens of this form, and of these, eight proved to be pregnant females, nineteen were females with pouch-young, three were non-

gravid females, and sixteen were males. As concerns the pouchless forms, although we succeeded in trapping a number of specimens of Marmosa cinerea, and in securing, thanks to the kindness of Mons. A. Touchon, A.M.I.C.E., a few specimens of Peramys americana and of P. scalops,[1] from the Theresopolis region (Serra dos Orgãos), we unfortunately obtained no developmental material. These forms, though not perhaps rare, are shy, retiring creatures, somewhat difficult to trap, and to secure them in numbers, one would require to live in the regions in which they occur for a considerable time.[2]

The material dealt with in this paper and listed below was derived from six females of D. aurita, and comprises eggs from the unsegmented condition up to the 16-celled stage, and blastocysts of about 1 mm. in diameter. It is thus of a somewhat fragmentary nature, but is, I think, worthy of description, inasmuch as it is derived from a different, though closely allied, species to that from which Hartman obtained his material. Moreover, although my observations, so far as they go, are in the main confirmatory of the latter's results, I believe I am able to supplement his work in regard to certain not unimportant details, e. g. the mode of origin of the characteristic cross-shaped, 4-celled stage.

I have to express my grateful thanks to the Council and Government Grant Committee of the Royal Society and to the Percy Sladen Trustees for generous grants in aid of the Expedition. My friend and colleague, Major G. S. Samson, M.C., R.F.C., B.Sc., rendered me loyal co-operation throughout, and I much regret that existing circumstances have prevented him from collaborating with me in the preparation of the present paper, which forms the first of a series dealing

[1] I am indebted to Mr. Oldfield Thomas for the specific determinations.

[2] For an account of the habits, etc., of the three-striped opossum (P. americana [tristriata]), vide Goeldi, E. A., "Critical Gleanings on the Didelphyidæ of the Serra dos Orgãos, Brazil." 'P. Z. S.' 1894.

with the material collected by the Expedition. I desire also to acknowledge the generous help afforded us by scientific men in Brazil. With the permission of the Minister of Agriculture, we were enabled to utilise the well-equipped laboratory of the Jardim Botanico as our headquarters, and to Dr. J. C. Willis, late Director of the Gardens at Rio, we are under a deep debt of gratitude for unstinted kindness and help during our stay. We are also indebted for invaluable assistance to Dr. A. de M. Ribiero, of the Inspectoria da Pesca, and his staff; to the staffs of the Jardim Botanico and the Museu Nacional; and to Dr. Oswaldo Cruz, Director of the Instituto Oswaldo Cruz, and to Dr. A. Lutz, of the same Institute.[1] Lastly, we desire to thank G. Chalmers, Esq., Superintendent of the St. John del Rey Mining Co., and his staff, for many facilities and much kindness during our stay at Morro Velhyo.

Literature.

At the time the material described in this paper was collected, Selenka's account of the early development of D. virginiana was, as already mentioned, the only one available. That account has already been critically dealt with in my paper on Dasyurus ('10) and also by Hartman in his recent paper, and to these critiques the reader is referred. Shortly after the return of the Expedition to England, I learned from Prof. J. T. Patterson, of the University of Texas, that one of his research workers, Mr. C. G. Hartman, had been collecting developmental material of the American opossum and had obtained a very complete series of stages. I therefore decided to await the publication of Mr. Hartman's results before describing my own fragmentary material. Prior to the issue of Mr. Hartman's paper, however, I had made out that the Didelphys ovum is considerably smaller than that of Dasyurus, that the deutoplasm is eliminated in a different fashion, that there is a cross-shaped 4-celled

[1] Since the above was written, we have seen, with deep regret, the announcement of the death of Dr. Cruz.

stage, and that in the 16-celled stage there is not that clear evidence of the differentiation of the blastomeres into two groups, respectively formative and non-formative, which is so striking in Dasyurus.

Mr. Hartman's paper ('16) appeared in March of last year, and proves to be a very complete and excellent piece of work. In the main, his results are confirmatory of my own for Dasyurus and entirely justify the criticisms of Selenka's work expressed in my paper. Furthermore, his results, taken in conjunction with my own, now justify us in concluding that the early development of the Marsupialia is effected throughout according to a common plan, such differences as exist being differences in detail and not in principle.

Mr. Hartman shows that the ova of D. virginiana, as was apparent from Selenka's description, are considerably smaller than those of Dasyurus; "its egg measures on the average about one-half the diameter, that is, one-eighth the volume of the egg of Dasyurus." They exhibit no obvious polarity inasmuch as there is no polar concentration of deutoplasmic material and in correlation therewith elimination of surplus yolk occurs, not by the separation of a yolk-body at one pole as in Dasyurus, but by the elimination of numerous small masses ("yolk-spheres") from the entire periphery of the blastomeres in the 1- and 2-celled stages, in a manner comparable with that described by Van der Stricht ('09) for the ovum of Vesperugo.

The first cleavage plane divides the ovum into two approximately equal cells, but, whilst there are no recognisable quantitative differences between the blastomeres, Hartman indicates that there is possibly a qualitative difference, inasmuch as he observed in one case that one of the two blastomeres eliminated its yolk-material prior to the other.

The second cleavage plane, he describes as again meridional and at right angles to the first, but the original radial arrangement of the four blastomeres is not retained. The blastomeres assume a spherical form and shift their position until the pairs come to lie at right angles to each other. In

this way, according to Hartman, there is established a cross-shaped 4-celled stage, quite comparable with the corresponding stage of the Monodelphia. It may here be noted that I am unable to confirm the occurrence of such a shifting of the blastomeres as Hartman describes. On the contrary, I am able to demonstrate that in D. aurita the cross-shaped arrangement results directly from the division of the blastomeres of the 2-celled stage in planes at right angles to each other.[1] This cross-shaped grouping of the blastomeres of the 4-celled stage Hartman emphasises as the chief point of divergence in the cleavage of Didelphys and Dasyurus. The succeeding cleavages he regards as "indeterminate, as in the Eutheria." The resulting cells migrate to the periphery and become applied to the zona. At the 8- and 16-celled stages the blastomeres are all apparently similar, and by the end of the fourth cleavage or even earlier they have become arranged in the form of a sphere enclosing the cleavage cavity in which are present deutoplasmic spherules and coagulum. In correlation with the smaller size of the ovum and this arrangement of the cells of the 16-celled stage in the form of a sphere, the blastocyst wall is completed much earlier in Didelphys than in Dasyurus, in about the 40-celled stage in the former as compared with the 125-celled stage, or thereabouts, in the latter. Polar differentiation is now manifested for the first time as a thinning-out of the cells over the hemisphere destined to form the non-formative or trophoblastic ectoderm, the remaining and smaller portion of the blastocyst-wall constituting the formative or embryonal area. Accordingly, just as in Dasyurus, this latter area is from the first freely exposed at the surface, and simply forms part of the wall of the blastocyst. The formation of the entoderm sets in in blastocysts of about ·6 mm. in diameter, as compared with

[1] More recently, Mr. Hartman informs me, in response to a letter of mine, that he has observed in one 3-celled egg, a cross at right angles, and in another, a cross at 45°, so that he is now prepared to regard the cross-shaped 4-celled stage as being produced directly and not by shifting.

blastocysts of about 4 mm. in Dasyurus (there being in Didelphys no such marked period of growth as occurs in the Australian form), and is effected in substantially the same way as in the latter. To quote from Hartman's summary ('16, p. 61): "The entoderm is formed by the proliferation of specialised 'entoderm mother cells' which appear only in the embryonic area. The entoderm mother cells may or may not migrate from their position in the wall of the blastocyst, but in either case they give off by division primitive entodermal cells which spread out at first immediately beneath the embryonic ectoderm and later beyond this area, until they completely line the entire vesicle." He thus confirms in the most striking fashion the account which I gave of the mode of origin of the entoderm in Dasyurus.

We may conclude then that the early development of Didelphys proceeds along essentially the same lines as in Dasyurus, and that such differences as exist are of the nature of secondary modifications only and not fundamental. In both types, cleavage results in the formation of a unilaminar blastocyst, the wall of which consists of formative and non-formative regions, the former destined to furnish the embryonal ectoderm and the entire entoderm, the latter, the trophoblastic or extra-embryonal ectoderm (tropho-ectoderm).

Apart from the very striking difference in the arrangement of the blastomeres of the 4-celled stage in these two forms, the meaning of which I hope to discuss later, the most obvious difference in their cleavage-process is the apparent absence in Didelphys of the unequal qualitative division which in Dasyurus results in the separation of the formative and non-formative cells. As we shall see, however, this difference is more apparent than real, for there are clear indications in earlier cleavage-stages of the existence of two groups of blastomeres which differ in size; indeed, Hartman regards it as highly probable that of the two blastomeres of the 2-celled stage, one is destined to give origin to the formative, the other to the non-formative region of the blastocyst-wall, and that consequently the unsegmented ovum possesses

a potential but concealed polarity. My own observations convince me that no other interpretation is possible.

Almost contemporaneously with Hartman's memoir appeared another paper dealing with "the implantation and early segmentation" of D. virginiana by Spurgeon and Brooks ('16). The authors are insufficiently acquainted with the literature, and do not add greatly to our knowledge. They state that "the ovum of the opossum has marked polarity which is noticeable at the beginning of segmentation," but they bring forward no evidence in support of their statement. In regard to yolk-elimination, they state that "before or during the process of segmentation, the ova have given off fragments or extrusions of cytoplasm or yolk," and they figure a section (fig. 1, p. 389) of an unsegmented ovum with two pro-nuclei, surrounded by a considerable amount of eliminated deutoplasm. No detailed account is given of cleavage. It is stated that the first cleavage-plane is meridional and divides the egg into two equal blastomeres; the second is equatorial, and "divides each of the first two blastomeres into two unequal cells, the smaller ones being at the animal pole. The second divisions are not simultaneous, as is well shown in figs. 6, 8, and 9." The cross-shaped 4-celled stage is not described.

The authors figure (fig. 10) an interesting reconstruction of an 8-celled stage, in which "the four blastomeres at the animal pole are smaller than those at the vegetative pole," and which is strikingly similar to my fig. 1, Pl. 6, representing a model of the corresponding stage of D. aurita.

The only other paper which has appeared in more recent years dealing with the development of the opossum is a note on the didermic blastocyst by the late C. S. Minot ('11), to which reference will be made later (p. 127).

List of Material.

The material described in this paper and listed below was obtained from six of the eight pregnant specimens collected. One specimen yielded a series of blastocysts at the flat

embryo stage, which are not herein dealt with ; another yielded twenty-four eggs from the two uteri, which proved to be entirely abnormal.

Specimen 1.—D. aurita, 24 : x :'13. Jardim de Botanico, Rio. Small ♀, with large mammary glands just after suckling. Left uterus with seven eggs, fixed in picro-nitro-osmic acid (P.N.O.). The eggs (measured in fixing solution) varied in diameter from ·39 to ·47 mm., average = ·42 mm. Right uterus with eight eggs, fixed in Flemming's fluid. The eggs varied in diameter from ·44 to ·47 mm., average of seven = ·45 mm. The ova are unsegmented and proved to be unfertilised.

Specimen 2.—D. aurita, 20 : x :'13. Theresopolis. Pouch and mammary glands very large, milk from nipples. Left uterus with twelve eggs (fixed in P.N.O.), right uterus with nineteen eggs (fixed Flem.). The eggs (measured in fixing fluids) vary in diameter from ·43 to ·51 mm., average of seven = ·458 mm.

Of these eggs, thirty were imbedded and cut into sections. They comprise the following : Unsegmented ova, two (A and D, Flem.) ; 2-celled ova, ten (H, L, P, R, T, Flem.), (D, E, H, K, M, P.N.O.) ; 3-celled ova, two (M, N, Flem.), 4-celled ova, eleven (G, J, K, O, S, Flem.), (A, B, F, G, J, L, P.N.O.).

Out of the thirty, four are undoubtedly abnormal, and one apparent egg proved to be a compact mass of sperms surrounded by albumen and shell-membrane. It was noted, when examining the 2-celled eggs in the fixing fluid, that one blastomere tended to be larger than the other, and this was found also to be the case in the serial sections of the same eggs.

Specimen 3.—D. aurita, 26 : x :'13. Jardim Botanico, Rio. Young ♀ with large mammary glands, just after suckling. Left uterus with ten eggs, fixed P.N.O. Eggs (measured in fixing fluid) vary in diameter from ·5 to ·61 mm., average of six = ·56 mm. Right uterus with ten eggs, fixed in Flem.

The P.N.O. eggs comprised one of 11 cells, three of 14 cells, and six of 16 cells.

The Flem. eggs comprised one 4-celled, one 7-celled, one 8-celled, one 12-celled, one 13-celled, one 14-celled, two 15-celled, and two 16-celled.

Specimen 4.—D. aurita, 1. 12 : vii : '13. Jardim, Rio. Pouch small, not cleaned, hardly tumid. Ten eggs from one uterus, fixed in P.N.O., and twelve from the other, fixed in chrom-aceto-osmic acid. The latter eggs proved to be poorly preserved, and are omitted from consideration. The P.N.O. eggs comprise the following: One 10-celled, one 13-celled, one 14-celled, one 15-celled, five 16-celled, and one 17-celled.

Specimen 5.—D. aurita, 3. 14 : vii : '13. Jardim, Rio. Pouch small, not cleaned, nipples small. Left uterus with twelve blastocysts (fixed in P.N.O.), and right, with sixteen (one small) fixed in chrom-aceto-osmic acid. The blastocysts vary in diameter from 1 to 1·3 mm.; and possess circular embryonal areas, ·5 to ·6 mm. in diameter.

Specimen 6.—D. aurita, 19 : x : '13. Theresopolis. Pouch and mammary glands very large, no sign of milk, nipples retracted. Left uterus with nineteen blastocysts, ten normal, nine abnormal, fixed in Flemming. Diameter of blastocysts, 1·1 mm.; of circular embryonal area, about ·5 mm. Right uterus with sixteen blastocysts, thirteen normal, three abnormal, fixed in P.N.O. Diameter of blastocysts, ·82 to 1·13 mm; of embryonal area, ·4 to ·5 mm.

Methods.

The methods which I utilised in working out the early development of Dasyurus were generally employed. As fixing fluids, picro-nitro-aceto-osmic acid (P.N.O.) and Flemming's strong fluid were employed, with, on the whole, satisfactory results. The ova were attached by ·5 per cent. photoxylin solution to thin slices of brain cortex prior to clearing and imbedding, and sections were stained on the slide by the iron-hæmatoxylin method followed preferably by safranin.

Models of one 8-celled and of three 16-celled eggs were made by the wax-plate method. To Mr. F. J. Pittock I am indebted for valued technical assistance.

Breeding Habits.

The Expedition arrived in Rio in the beginning of July, and we commenced collecting at the Jardim Botanico on July 10th. On the 12th, we obtained two pregnant specimens of D. aurita, one with segmenting eggs (specimen 4 of preceding list) and the other with blastocysts 8–8·5 mm. in diameter. On the 19th, we obtained a female with seven pouch-young measuring in G.L. 18–19·5 mm. and in H.L. 9 mm. In the beginning of September, the pouch-young were hair-clad, the lips had become free and the eyes open, and by the middle of October, at Theresopolis, the young were either just ready to leave the pouch or had already done so. From what we knew of the breeding habits of Dasyurus, we concluded that the Gambá would be of no further interest to the embryologist until the succeeding June. Great, therefore, was our surprise to find in a female, trapped on October 19th (specimen 6 of list), and with a very large pouch and prominent mammary glands that the uteri exhibited the usual signs of pregnancy. On opening them up, thirty-five blastocysts (twenty-three normal, twelve abnormal) were obtained. Subsequently three other early pregnant females were trapped.

These records show that D. aurita has at least two breeding seasons in the year, viz. one in June–July and a second in the latter half of October, some three months or so later, immediately after the July-young have left the pouch. The Gambá would thus appear to differ from the Virginian opossum, which, according to Hartman, "has only one sexual season a year, and normally (i. e. in the wild state) only a single œstrus period," and it would also appear to differ from Dasyurus, which also has been stated to have but one breeding season in the year (Hill, '00 and '10; Hill and O'Donoghue, '13). This discovery of the occurrence of two breeding seasons in the Gambá raised doubts in my mind as to the accuracy of my statement for Dasyurus, and I accordingly applied to my friends in the Zoological Department of the

University of Sydney to determine whether Dasyurus was to be found with pouch-young during the summer months (November–January). Mr. Charles Badham, B.Sc., has been good enough to report that he examined five females in January, "all of which had pouches which showed no signs of young having been recently suckled." There the matter must rest for the present so far as Dasyurus is concerned, but as regards Trichosurus vulpecula and Macropus ruficollis, which we were inclined to believe also bred but once a year, we now possess some further evidence indicating that these two species probably breed twice in the year, the former breeding in New South Wales round about Easter, and again in September, the latter breeding during August–September, and again during December–February, as soon as the young one has vacated the pouch or even before.

As regards other Didelphyds, it may be noted here that we collected over two dozen specimens of Metachirus (M. opossum and M. nudicaudatus). One specimen of the latter species proved to be pregnant, but the vesicles were abnormal. Our records indicate that Metachirus breeds a little later than D. aurita. We obtained a female of M. opossum on August 8th in which the uteri were enlarged and vascular, but ovulation had not taken place, and a female of the same species with pouch-young on August 19th. The above-mentioned pregnant female of M. nudicaudatus was obtained on September 1st, and another non-pregnant female, with enlarged uteri, on September 6th. On August 27th we secured a female of Caluromys philander with young in the pouch.

Description of Material.

(1) Unsegmented Ova.

The material available is not sufficient for a complete study of either the ovarian or the unsegmented uterine ovum. No

females with fully-ripe ovaries were obtained. The unsegmented uterine ova in the collection comprise fifteen unfertilised ova from specimen 1 and two from specimen 2.

As concerns the size of the ovarian ova, measurements of eleven apparently full-grown ova with peripheral nuclei, from the ovaries of a female killed on October 16th, yield average diameters of ·144 × ·13 mm., whilst nine ova from other ovaries average ·14 × ·127 mm. in diameter. According to Hartman, the ripe ovarian eggs in D. virginiana average in diameter in section ·165 × ·135 mm. In Dasyurus, the corresponding measurements range from "·28 × ·126 mm. to ·27 × ·26 mm. (average, ·24 mm.)," from which it is clear that the ovarian ovum of Didelphys is, as Hartman points out, much smaller than that of Dasyurus. It also differs in that it shows no corresponding concentration of fluid deutoplasmic material.

The unsegmented and unfertilised uterine eggs are spherical, and vary in diameter in the fixing solution from ·39 to ·47 mm., the average being about ·43 mm., as compared with an average of about ·45 mm. in D. virginiana (Hartman), and of about ·32 mm. in Dasyurus. The larger size of the Didelphyd egg is due, as Hartman points out, to the much greater thickness of the albumen layer, which here attains a thickness of as much as ·14 mm., as compared with ·015–·022 mm. in Dasyurus. As in the latter, the albumen appears in the fresh state as a uniform, semi-transparent layer, composed of fine, concentrically arranged laminæ embedded in a fluid matrix. In normally fertilised eggs, numbers of sperms are to be seen embedded in it, and quite frequently, also, there may be observed in it a clump of cells apparently derived from the discus proligerus.

The shell-membrane is somewhat thinner than in Dasyurus, measuring about ·0012 mm. in thickness, and in unsegmented and cleavage stages, it always appears markedly wrinkled after preservation (Pl. 8, fig. 17). As Hartman shows (cf. his Table 2, p. 59), it does not exhibit such a marked progressive growth in thickness as does that of Dasyurus, the

maximum thickness attained in Didelphys being only about half that in Dasyurus (·008 mm.), from which it may be concluded that the shell in the former is still more vestigial than in the latter.

The ovum itself is enclosed by a thin, homogeneous zona (about ·001 mm. in thickness), which is always perfectly distinct in eggs preserved in Flemming's fluid, but which tends to undergo dissolution in those preserved in P.N.O.

According to Hartman's description and figures, the cytoplasmic body of the tubal ovum of D. virginiana exhibits a differentiation into three more or less distinct regions, viz. central and peripheral zones of granular cytoplasm, between which is a broad intermediate zone in which numerous yolk-vacuoles and fat-spherules are situated. I regret I am not able to provide an adequate account of the unsegmented ovum of D. aurita. The only unsegmented ovum in my material which may be regarded as approaching the normal is egg A, from specimen 2 of the list. It is unfertilised. It measures in section ·13 × ·117 × ·17 mm, and is thus ellipsoidal in form. Within the zona, and approximately of the same thickness as that, is a very thin zone (occupying what would otherwise be the perivitelline space), composed of a light staining matrix, crossed by fine, radially-arranged lines, and perhaps of the nature of a coagulation product (Pl. 7, fig. 1). Within this zone is a very delicate but perfectly distinct egg-membrane. Hartman has observed the same parts in tubal eggs of D. virginiana. The cytoplasmic basis of the ovum is uniformly very finely granular, and has embedded in it, peripherally, sparse granules, measuring up to just over ·003 mm. in diameter, and each surrounded by a clear, vacuole-like area. These granules stain brownish with osmic acid, and are evidently of a fatty or lipoid nature. They are most abundant in the peripheral region of the ovum and are absent from its central region. The first polar body is present in the form of a minute, flattened cell, spindle-shaped in section, and situated between the egg-membrane and the zona. It contains an irregular, beaded chromatin

mass, and measures ·015 × ·003 mm. in diameter. Adjacent to the first polar body, but to one side of it, is the second polar spindle with an equatorial group of chromosomes (? twelve in number in egg D).

The unfertilised eggs from specimen 1 need not be described in any detail, since they have undergone obvious alteration during their sojourn in the uteri. The ovum has altered in form, and exhibits a marked tendency to flattening on one side; it is separated from the zona, especially on the flattened side, by a space partly occupied by coagulum, and over this flattened region, the zona appears to be thickened and the albumen is more condensed. The chromosomes of the second polar mitotic figure are readily recognisable, but they tend to be scattered. The maximum number counted is twelve; Jordan ('11), from his study of the spermatogenesis of D. virginiana, concludes that the haploid number in that species is nine (eight plus an accessory chromosome). The position of the second polar chromosome group is variable; usually it is situated adjacent to the flattened side, near the periphery of the ovum, but it may also occur in the equatorial region, or, exceptionally, nearer the pole of the convex side. A corresponding variability in the position of the second polar body was observed in Dasyurus, and according to Hartman, in D. virginiana "the position of the polar body is, in the majority of cases, though by no means invariably, at one pole of the elliptical egg."

Hartman states definitely that "aside from the position of the polar body, there is no evidence of polarity in the tubal egg of the opossum such as has been described for ovarian and unsegmented uterine eggs of Dasyurus." Nevertheless, consideration of the cleavage-process leads him to the conclusion that an inherent, if non-visible, polarity probably does exist in the unsegmented ovum—a conclusion with which I am in complete agreement. In this connection, it is, at the least, suggestive that the majority of the ova from specimen 1 should have suffered a similar flattening on one side, the appearance presented recalling at first sight an ovum of

Dasyurus from which the yolk-body (represented by the above-mentioned coagulum) had been separated off.

(2) Two-celled Eggs.

The 2-celled eggs at my disposal are ten in number, all derived from specimen 2 of list. They comprise eggs H, L, P, R, and T, fixed in Flemming's fluid, and eggs D, E, H, K, M, fixed in P.N.O. I propose to give a brief account of each of these eggs.

Egg P, Flem.—The two blastomeres (Pl. 7, fig. 2), enclosed by the distinct but thin zona, are of equal size, each measuring in section ·111 × ·066 × ·096 mm. They are definitely contoured and ellipsoidal in form, flattened against each other where they meet along the plane of cleavage and with convex surfaces where they adjoin the zona. It is to be noted that they largely occupy the space enclosed by the latter. Each possesses a membranate nucleus, situated slightly excentrically and surrounded by a lighter area of cytoplasm. The chromatin is mainly aggregated into an irregular central mass. The cytoplasm of the blastomeres is finely and uniformly granular, and peripherally there are present in it scattered fat spherules, larger and more abundant than those of the unsegmented egg (Pl. 7, fig. 1). There is no sign of active yolk-elimination nor are eliminated yolk-spheres definitely recognisable, the intra-zonal space being occupied by a small amount of reticular light-staining material.

Egg R, Flem.—This egg is on the whole very similar to P. The blastomeres show no appreciable difference in size, each measuring ·114 × ·069 × ·096 mm. The nuclear membrane has disappeared and a group of chromosomes is differentiating in each nuclear area. Yolk-spheres are present, but are not abundant.

Egg T, Flem.—The two blastomeres are here of unequal size, the larger one measuring ·112 × ·08 × ·096 mm., and the smaller ·112 × ·064 × ·096 mm. Each possesses an

excentrically situated group of chromosomes. Yolk-spheres are present in small numbers.

Egg L, Flem.—In this egg also, the blastomeres are of unequal size and the second cleavage has made further progress, each blastomere containing two small nuclei. The larger blastomere (Pl. 7, figs. 3, 4, 5, B) measures ·099 × ·057 × ·104 mm. It contains two small recently reconstituted nuclei, separated from each other by the thickness of one section (= ·008 mm.), the line joining them being slightly oblique to the sectional plane. The smaller blastomere (A) measures ·117 × ·048 × ·088 mm. and shows a definite constriction round its mid-region, best marked on its surface next the zona. The two nuclei lie in a plane almost exactly coincident with the sectional plane (Pl. 7, fig. 4, A). They resemble those of B except that one of the two (the upper in the figure) stains rather less deeply than the others and has apparently shed some of its chromatin into the surrounding cytoplasm (Pl. 7, fig. 5). In correspondence with the disposition of the nuclei, it is to be noted that blastomere B is elongated at right angles to the sectional plane, whilst A is elongated in that plane; in other words, the long axes of the blastomeres along which the nuclei are situated, are disposed at right angles to each other. Consequently, when the division of the blastomeres is completed, we should expect to find the blastomeres of the 4-celled stage arranged not radially as in Dasyurus, but in two pairs, forming a cross-shaped group, and that as a matter of fact is just what we do find. It is a point of some interest that division of the cytoplasm should be initiated earlier in one (the smaller) of the two blastomeres than in the other (the larger). Apart from these differences (size and time of initiation of cytoplasmic division), the two blastomeres appear to be similar, but I agree with Hartman in the belief that one of the two (I believe the smaller) is destined to give origin to the formative region of the unilaminar blastocyst and the other to the non-formative region of the same.

The blastomeres contain sparse fat spherules, whilst elimi-

nated yolk-spheres are present but are not abundant. The polar bodies lie in relation to one pole of the smaller blastomere (Pl. 7, fig. 4, *p.b.*).

Egg H, Flem.—This egg is very slightly later than the preceding. One of the blastomeres (B) is smaller than the other (A). It is divided by a deep constriction into two lobes connected by a narrow bridge. Each lobe contains a small nucleus; in one, the nuclear reticulum is established, in the other it is forming. Blastomere A is less deeply constricted; each half contains a nucleus not yet fully reconstituted, the two being still connected by the remains of the spindle, in relation to which are extra-nuclear chromatin granules.

Owing to the presence of the constrictions and to the sectional plane, it is not possible to obtain accurate measurements. The following are only approximate:

Blastomere A = ·09 × ·063 × ·104 mm.

Blastomere B = ·072 × ·063 × ·096 mm.

The sectional plane cuts the line joining the nuclei of blastomere A almost transversely, whilst it is almost parallel to that joining the nuclei of B. Here, again, we have evidence of the division of the first two blastomeres in planes approximately at right angles to each other.

Yolk-spheres are more abundant than in any of the preceding eggs.

Egg D, P.N.O.—Is not very perfectly fixed. The two blastomeres are of approximately equal size, measuring respectively ·09 × ·063 mm. and ·097 × ·057 mm. Each shows a group of scattered chromosomes in a clear area. It is impossible to determine the planes of cleavage.

Egg H, P.N.O.—The blastomeres are of unequal size. The larger one measures ·096 × ·072 × ·08 mm., its nucleus being in the anaphase of division; the smaller one measures ·096 × ·052 × ·072 mm., and possesses a scattered group of chromosomes (? equatorial plate) in the nuclear area. The sectional plane cuts the spindle axis of the larger blastomere at right angles, whilst it coincides with the chromosome

group of the smaller. If the latter group represents an equatorial plate, then the cleavage-planes in this egg must be held to be parallel to each other. It appears also to be exceptional in that the larger blastomere is in advance of the smaller as regards division.

Yolk-spheres are present in small numbers and are apparently in process of elimination from the surface of the larger blastomere.

Egg K, P.N.O.—The two blastomeres are again of unequal size; the larger one measures ·092 × ·056 × ·088 mm., the smaller ·084 × ·052 × ·08 mm. Both are in the anaphase of division. In the larger blastomere the spindle axis is oblique to the sectional plane; in the smaller, almost at right angles to the same, so that here again the appearances point to the second cleavage-planes being approximately at right angles to each other. Yolk-spheres are present in small numbers.

Egg M, P.N.O.—The blastomeres again differ in size; the larger one measures ·079 × ·069 × ·064 mm., the smaller ·081 × ·054 × ·08 mm. The smaller blastomere shows an equatorial constriction and possesses two quite small nuclei, the line joining them being cut practically at right angles by the sectional plane. The larger blastomere also possesses two quite similar nuclei situated in adjacent sections, and therefore very nearly in the sectional plane. Small yolk-spheres are present.

Egg E, P.N.O.—The blastomeres (Pl. 7, fig. 6-8), differ only very slightly in size, so little indeed that they may be described as approximately equal, the measurements being ·090 × ·057 × ·08 mm. and ·093 × ·06 × ·08 mm. They are in the anaphase of division; in the one, the mitotic figure lies in the plane of section (Pl. 7, fig. 7); in the other, it is approximately at right angles thereto (Pl. 7, figs. 6 and 8). It is thus clear that the second cleavages are effected in planes at right angles to each other.

In the peripheral zone of the blastomeres, there are present more or less spherical, clear vacuolar-like areas of variable size, and either isolated or in contiguity with each other.

These areas are not empty, but contain a light staining material, in which a fat spherule may be situated. This apparent vacuolisation of the peripheral cytoplasm appears to correspond with that described and figured by Hartman for D. virginiana. In eggs preserved in Flemming's fluid such an appearance is not met with, though it may be noted that the fat-spherules usually appear as if situated in a clear vacuole in the cytoplasm. I am inclined to think that the apparent vacuolisation here is an artefact, due to the action of the P.N.O. Eliminated yolk-spheres are present in some abundance, and there are indications that elimination is in progress.

The most important conclusions to be drawn from the foregoing description are (1) that there is a tendency for the first two blastomeres to differ slightly in size as evidenced by measurements of the blastomeres in section, and also by observations made on the eggs immediately after their transference to the fixing fluid, and before the albumen had lost its transparency; and (2) that the second cleavages are effected in two planes at right angles to each other. My observations in these respects supplement those of Hartman, who states that he had "only three normal 2-celled stages taken from two different females" available for examination. In the two eggs, of which figures are given (his figs 9 and 10), the nuclei of the blastomeres are in the "resting" condition, so that he was not in a position to observe the phases of the second cleavage divisions. As regards the first cleavage, he states that "the first cleavage plane divides the egg into approximately equal halves. While no quantitative difference is to be recognised, there seems to be a qualitative difference between the two blastomeres: for in one case the separation of one blastomere from its yolk had not yet been consummated (fig. 9)." In D. aurita, on the other hand, as we have just seen, there is, in six out of ten 2-celled eggs examined, a recognisable quantitative difference between the first two blastomeres, in addition to a presumed qualitative difference.

(3) Three-celled Eggs.

These comprise two eggs, M and N, from specimen 2, fixed in Flemming's fluid, and probably not normal.

Egg M.—Of the three blastomeres, one is large and grooved round its mid-region, each half possessing a small nucleus; the other two blastomeres are smaller and form an unequal pair, the larger of the two measuring ·084 × ·069 × 0·64 mm. and the smaller ·072 × ·045 × ·072 mm. The line joining the nuclei of the paired blastomeres is approximately at right angles to that joining the nuclei of the undivided blastomere.

Numerous yolk-spheres are present, and elimination appears to be in progress.

Egg N.—One blastomere is large, binucleate, and unconstricted. The other two form again an unequal pair, the larger one measuring ·09 × ·06 × ·08 mm., and the smaller ·076 × ·063 × ·064 mm. The nuclei of the undivided blastomere are very minute and probably abnormal. They lie in the sectional plane, as do those of the paired blastomeres, so that completion of the second cleavage would result in a radial arrangement of the four blastomeres.

Numbers of yolk-spheres are present.

(4) Four-celled Eggs.

Eleven 4-celled eggs areavail able for study viz. G, J, K, O, S, fixed in Flemming's fluid, and A, B, F, G, J, L fixed in P.N.O., all derived from specimen 2.

Egg J, Flem.—Diameter as preserved, ·64 × ·35 mm. A very fine stage, in which the four blastomeres are grouped in two pairs, arranged so as to form a cross-shaped figure (Pl. 7, figs. 9 and 10 and Pl. 6, fig. 1).

The measurements of the blastomeres are as follows:

First pair (Pl. 7, fig. 9)	A	·084 × ·06 × ·072 mm.
	B	·084 × ·06 × ·072 mm.
Second pair (Pl. 7, fig. 10)	C	·084 × ·069 × ·08 mm.
	D	·087 × ·051 × ·064 mm.

The measurements show that the two pairs are approximately equal as regards size, whilst as regards their nuclear and cytoplasmic characters, I am unable to detect any differences between them.

The blastomeres appear ovoidal in outline in section, and the members of each pair tend to be flattened against each other where they touch (Pl. 7, fig. 10). Peripherally, they are separated from the enclosing zona by a narrow space which widens out between their poles, these enlargements being largely occupied by yolk-spheres, now very numerous. The blastomeres are definitely contoured, and there is no evidence of active yolk-elimination. Their cytoplasm is finely granular, and is distinguishable into a broad peripheral zone, denser, and more deeply staining and a central lighter staining zone around the nucleus. In the former zone, there occur sparse fat-spherules.

The shell-membrane has a thickness of about ·0015 mm., and the zona, which is distinct, is still thinner.

Egg O, Flem.—Another very fine stage in which the blastomeres show precisely the same cross-shaped arrangement as in the preceding egg (Pl. 8, figs. 11 and 12).

The approximate measurements of the blastomeres are as follows:

First pair (Pl. 8, fig. 11)	A ·078 × ·054 × ·08 mm.
	B ·078 × ·048 × 0·72 mm.
Second pair (Pl. 8 fig. 12)	C ·066 × ·048 × ·064 mm.
	D ·075 × ·057 × ·081 mm.

They show that the two blastomeres of the first pair are together somewhat larger than those of the second pair.

The blastomeres, especially those of the second pair (Pl. 8, fig. 12), are somewhat irregular in contour owing to the presence of lobe-like outgrowths which appear to be connected with the elimination of deutoplasmic material in the form of yolk-spheres. These latter in this egg are specially abundant, some of them reaching a diameter of ·02 mm. In the peripheral cytoplasm of the blastomeres, fat-spherules are relatively abundant.

Eggs G, S, and K, all show the same cross-shaped arrangement of the two pairs of blastomeres, though not quite so typically as in J and O. In G, and in less degree in K, one pair of blastomeres is larger than the other; in S, the two pairs are approximately equal. Yolk-spheres are present in small numbers in K and S, but are practically absent in G.

Of the 4-celled eggs fixed in P.N.O., only A and F need be referred to in any detail.

Egg A, P.N.O.—The four blastomeres are clearly grouped in two pairs, of which one pair (A and B) lies at right angles to the sectional plane, the other pair (C and D) in that plane, the two pairs forming a cross-shaped group.

The measurements of the blastomeres are as follows:

First pair	A	·066 × ·069 × ·048 mm.
	B	·069 × ·063 × ·056 mm.
Second pair	C	·066 × ·051 × ·056 mm.
	D	·06 × ·045 × ·064 mm.

A and B together are slightly larger than C and D. Yolk-spheres are present in considerable abundance, and elimination appears to be in active progress, more especially from the larger blastomeres A and B (Pl. 8, figs. 13 and 14).

Egg F, P.N.O.—The two pairs of blastomeres form a perfectly typical cross-shaped group, and are approximately equal as regards size. Their measurements as as follows:

First pair	A	·06 × ·06 × ·048 mm.
	B	·075 × ·051 × ·056 mm.
Second pair	C	·078 × ·057 × ·048 mm.
	D	·078 × ·054 × ·048 mm.

Yolk-spheres are not very abundant and elimination is not active.

Egg L, P.N.O.—The blastomeres form a fairly typical cross-shaped group, A and B being distinctly larger than C and D, as shown by the following measurements:

First pair	A	·08 × ·064 × ·048 mm.
	B	·088 × ·064 × ·064 mm.
Second pair	C	·064 × ·056 × ·064 mm.
	D	·064 × ·052 × ·064 mm.

Very few yolk-spheres are present.

Eggs G and J, P.N.O., are poorly preserved, and it is impossible to determine with certainty the arrangement of the blastomeres.

Egg B, P.N.O., is exceptional and abnormal in showing an apparent radial arrangement of the four blastomeres. Hartman states that he has "several times seen such eggs consisting of four radially arranged 'blastomeres' or egg fragments." He regards such eggs as abnormal, and points out that the single 4-celled stage described and figured by Selenka, inasmuch as it also shows a radial arrangement of the blastomeres, must likewise be regarded as abnormal, "notwithstanding that it is comparable with the 4-celled egg of Dasyurus." It may be suggested that such eggs in Didelphys are of the nature of "reversions" to the ancestral (Dasyurine) type (*v.* conclusion, p. 135).

The foregoing observations on 2-celled and 4-celled ova of D. aurita conclusively demonstrate that the characteristic cross-shaped arrangement of the blastomeres of the 4-celled eggs is the result not of any shifting of the blastomeres, as Hartman originally believed (*v.* his paper, p. 25), but of the division of the blastomeres of the 2-celled egg in two different planes at right angles to each other. They also show that there is a tendency for one pair of the blastomeres to be slightly larger than the other, this being the case in five out of eight 4-celled eggs examined.

In Hartman's view, "the orientation of the blastomeres in the 4-celled stage presents the chief point of divergence in the cleavage of Didelphys and Dasyurus, the former following the manner of Eutheria." Whilst I am not prepared to agree that it is the chief or most important point of difference, I freely admit that it is the most obvious point of difference in the early cleavage-process in these two forms. Whereas in Dasyurus, the blastomeres of the 4-celled stage are paired (though not very obviously) and radially arranged; in Didelphys, they are definitely grouped in two pairs so arranged as to form a cross-shaped figure, exactly as are the blastomeres

of the 4-celled egg in the Monodelphia. In my paper on Dasyurus, I laid considerable emphasis on the radial and cross-shaped modes of grouping, and accepting the 4-celled stage described and figured by Selenka, as normal for Didelphys, I regarded them as characteristic of the Didelphia and Monodelphia respectively; but it is now clear that this generalisation no longer holds good, and it is also evident that my suggestion as to the influence of the loss of the shell-membrane on the early ontogenetic phenomena in the Monodelphia requires modification so far as concerns its supposed effect on the orientation of the blastomeres in the 4-celled stage.

(5) Yolk-elimination.

It is a very interesting fact that the ovum of Didelphys, although much smaller than that of Dasyurus, nevertheless exhibits a comparable process of elimination of surplus deutoplasm. In D. virginiana, Hartman shows that the process is effected in a somewhat different manner to that in Dasyurus, and I find the same holds true for the egg of D. aurita. In Dasyurus, I have shown that the surplus deutoplasm becomes concentrated at what is morphologically the upper pole of the ovum, and that prior to, or simultanously with, the formation of the first cleavage-furrow, it is separated off in the form of a single spherical mass or yolk-body, composed of an extremely delicate cytoplasmic reticulum holding the fluid deutoplasm in its meshes. In Didelphys, in correlation with the absence of any polar concentration of the surplus deutoplasm, elimination of the latter takes place from the general surface of the unsegmented ovum, and of the blastomeres in the form of numerous small, rounded masses or yolk-spheres, which are of much firmer consistency than the single yolk-body of Dasyurus, and appear equally well preserved after fixation with either Flemming's fluid or P.N.O. They are limited by a thin membrane-like layer or pellicle, within which

is a clear, delicate, reticular matrix embedded in which are numerous fine granules staining deeply with safranin. Occassionally they contain one or more fat-spherules. In D. virginiana, according to Hartman, the separation of the yolk-spheres takes place prior to, and after the completion of the first cleavage, i. e. in the unsegmented and 2-celled eggs, and a comparable statement is made by Spurgeon and Brooks ('16, cf. ante, p. 98). In D. aurita, eliminated yolk-spheres are generally scanty in 2-celled eggs and more abundant in 4-celled and later stages, which seems to indicate that the process is somewhat retarded in that species as compared with the North American form.

As regards the actual process of elimination, Hartman says "the egg gets rid of surplus yolk by forming the new cell-membranes of the two blastomeres at such a distance from the original surface of the egg as to leave a portion of the peripheral cytoplasm rich in yolk outside the blastomeres." This description seems to imply that "the peripheral cytoplasm rich in yolk" is separated off as a continuous mass, which later breaks up into isolated yolk-spheres. His figure (Pl. 5, fig. 9), illustrating the process in a 2-celled stage, rather suggests that the egg in question was not quite normal. My own observations indicate that the yolk-spheres are budded off from a narrow, clear zone which has made its appearance at the exposed surfaces of the blastomeres (Pl. 8, figs. 13 and 14). The figures show undoubted yolk-spheres in direct continuity with the lighter peripheral zone, and within that the apparently unaltered cytoplasm of the blastomeres, containing lighter vacuolar areas, with fat-spherules. Whereas then, in Dasyurus, segregation and elimination of the surplus deutoplasm take place at the upper pole of the ovum, in Didelphys, the corresponding processes are effected at the general surface of the ovum and the early blastomeres. Didelphys, in respect of its yolk-elimination, thus agrees more closely with Vesperugo, as described by Van der Stricht ('09), than with Dasyurus.

(6) Seven to 17-celled Eggs.

The material available, derived from specimens 3 and 4 of list, comprises one egg each of 7, 8, 10, 11, 12, and 17 cells, two eggs of 13 cells, five of 14, three of 15, and thirteen of 16 cells.

Egg B, 26 : x : '13, Flem.—Eight-celled. (Model, Pl. 6, fig. 2, A and B, and Pl. 8, fig. 15).

This is an extremely interesting stage, in which the eight blastomeres are definitely arranged in two superimposed tiers, each composed of four cells, viz. an incomplete ring of four smaller cells (1 and 2, 3 and 4), (2 and 4 being separated by a small gap), constituting what I regard as the upper or formative ring and a ring of four larger cells (5 and 6, 7 and 8), which I regard as the lower or non-formative ring. The four cells of the upper ring are situated opposite the junctions between the four cells of the lower ring, so that the cells of the two rings alternate with each other much as in the spiral type of cleavage, but this alternation is not due, as it is in the latter, to obliquity in the direction of the cleavage spindles but to the derivation of the 8-celled stage from a cross-shaped 4-celled group: each pair of blastomeres of the latter has become subdivided into two pairs by meridional cleavages and the blastomeres subsequently moving apart to enclose the cleavage cavity, the four blastomeres of the one set have come to alternate with the four of the other.

The measurements of the blastomeres are as follows:

Blastomere	1. ·03 × ·051 × ·043 mm.	Upper ring.
	2. ·03 × ·054 × ·039 mm.	
	3. ·036 × ·054 × ·039 mm.	
	4. ·03 × ·054 × ·036 mm.	
	5. ·048 × ·033 × ·048 mm.	Lower ring.
	6. ·042 × ·051 × ·043 mm.	
	7. ·042 × ·069 × ·036 mm.	
	8. ·042 × ·051 × ·051 mm.	

Blastomeres 1 and 2, 3 and 4, 5 and 6, 7 and 8 form pairs. From the above measurements and from the figures (Pl. 6,

fig. 2, A and B), it is clear that this 8-celled stage has been derived from a cross-shaped 4-celled stage in which one pair of blastomeres was larger than the other.

The blastomeres have now moved apart, their outer surfaces lying for the most part in contact with the zona (Pl 8, fig. 15), and they surround a distinct cleavage-cavity, open above and below. The cavity is most extensive between the small cells of the upper ring and its upper opening correspondingly large. Its lower opening (Pl. 6, fig. 2, B), is relatively minute. The blastomeres are in contact with each other only by limited areas of their adjoining surfaces, so that more or less extensive gaps exist between them, more especially between the cells of the two rings. They show evidence of more marked metabolic activity than those of the 4-celled eggs. Their cytoplasm (Pl. 8, fig. 15) is now more coarsely granular in appearance, and contains in addition to very fine granules and sparse fat-spherules, larger granules (up to about ·003 mm. in diameter), which stain homogeneously with iron-hæmatoxylin, whilst, peripherally ,there are present curious vesicular bodies, definitely limited, and measuring up to ·006 mm. in diameter. Their nuclei are large, deeply staining, and evidently in a highly active condition; they are situated excentrically, always towards the outer surfaces of the blastomeres.

Yolk-spheres are abundant and largely fill up the cleavage-cavity. They are most numerous in relation to the smaller cells, but whether this is simply due to the larger size of the cleavage-cavity in their region or is to be regarded as indicative of more active elimination on the part of these cells, it is impossible to decide. They present, on the whole, a more homogeneous appearance and stain more deeply than those of the 4-celled eggs; in addition to occasional fat-spherules they contain one or more homogeneously staining granules of variable size, similar to those in the cytoplasm of the blastomeres.

The shell-membrane measures ·003 mm. in thickness and the zona and albumen are distinct.

Spurgeon and Brooks have figured ('16, fig. 10) an

8-celled stage of D. virginiana, which appears to be very similar to the egg above described. They state that "the four blastomeres at the animal pole are smaller than those at the vegetative pole," and they figure the nuclei occupying the same excentric position in the blastomeres, as was described above. Hartman figures a model of an 8-celled stage of the same species (his fig. 2 B, p. 27), in which, judging from the figure, the cells are of approximately equal size. The figure shows four cells forming an incomplete ring, situated on four more compactly arranged cells. It is stated that seven of the eight cells are in mitosis, whilst one is in the resting condition.

Egg C, 26 : x : '13, Flem.—Seven-celled.

The blastomeres comprise one distinctly larger cell (2) and six smaller, which apparently constitute three pairs, so that $(2 \times 2) + [1 \times 2 + 1 \text{ undivided}] = 7$.

The measurements of the blastomeres are as follows:

Blastomere	1.	·048 × ·036 × ·036 mm.
	2.	·066 × ·06 × ·05 mm.
	3.	·045 × ·048 × ·036 mm.
	4.	0·4 × ·042 × ·036 mm.
	5.	·045 × ·042 × ·036 mm.
	6.	·045 × ·036 × ·03 mm.
	7.	·045 × ·045 × ·03 mm.

Analysis of the serial sections indicates that pairs 3 and 4 and 6 and 7 form one group (A), pair 1 and 5 + 2, another group (B), the plane of division of 1 and 5 lying in the sectional plane, those of 3 and 4 and 6 and 7 at right angles thereto. The relations are probably to be expressed thus:

$$(3 + 4) + (6 + 7) + [(1 + 5) + 2] = 7.$$

Group A perhaps corresponds to the upper ring of the 8-celled egg and Group B to the lower ring, but it is impossible to be certain since the measurements point to an approximate equality of the two pairs of blastomeres of the antecedent 4-celled stage.

There is no definite cleavage-cavity. Yolk-spheres are fairly abundant.

Egg I, 1. 12 : vii : '13, P.N.O.—Ten-celled.

The blastomere relations are probably to be expressed as follows:

$$[2 + 2] + [2 \times 2 + 2 \text{ (undivided)}] =$$
$$[(2, 6) + (5, 7)] + [(4, 8) + (9, 10) + 1 + 3] = 10.$$

Group A. Group B.

Blastomeres 1 and 3 are slightly larger than any of the others, and, like the pairs of Group A, have not undergone the fourth cleavages. They are associated in a group (B) with the paired blastomeres 4 and 8 and 9 and 10, which pairs are distinctly smaller than pairs 2 and 6 and 5 and 7 forming Group A. I conclude that the pairs of Group B have resulted from the fourth cleavage-divisions, and that one group (B) undergoes these divisions before the other (A). The cleavage-cavity is well marked.

Egg B. 26 : x : '13, P.N.O.—Eleven-celled.

The blastomere relations appear to be as follows:

$$[2 + 2] + [2 \times 2 + 1 \times 2 + 1 \text{ [undivided]} =$$
$$[(2, 3) + (7, 8)] + [(4, 5) + (6, 9) + (10, 11) + 1] = 11.$$

Apparent pairs (2 and 3) and (7 and 8) are distinctly larger than the remaining pairs and form one group. The remaining blastomeres form a second group, consisting of three pairs, produced by the fourth cleavages and one blastomere (1), which is undergoing that cleavage. Here, as in the preceding egg, it is evident that one group divides before the other. The blastomeres are applied to the zona, and the cleavage-cavity, which is distinct, contains numbers of yolk-spheres.

Egg F, 26 : x : '13, Flem.—Twelve-celled.

A quite good stage of twelve cells, probably represented by $(4 \times 2) + 4$, but I have failed to make out the grouping satisfactorily after repeated attempts.

Egg H, 26 : x : '13, Flem.—Thirteen-celled.

Three of the blastomeres (11, 12, 13) are larger than the others. They form a group along with a pair produced by recent division, whilst the remainder form a group of four pairs. The relations appear, therefore, to be as follows:

$$[(1, 3) + (2, 5) + (6, 10) + (7, 9)] + [(4, 8) + 11 + 12 + 13] = 13.$$

Blastomeres 4 and 8 both have small recently formed nuclei and are obviously sister-cells; 12 is in the anaphase of division. If the above grouping is correct, then we may conclude, as in the preceding eggs, that one of the groups of the 8-celled stage tends to complete the fourth cleavages prior to the other.

The blastomeres lie in contact with the zona which is distinct and the cleavage-cavity is largely occupied by yolk-spheres.

Egg J, 1. 12 : vii : '13, P.N.O. also consists of 13 cells, but I did not succeed in determining the grouping.

Five eggs of the 14-celled stage have been examined, viz. A, 26 : x : '13, Flem., C, H, and G, 26 : x : '13, P.N.O., and F, 1. 12 : vii : '13, P.N.O. Of these, only A and C need be referred to here.

Egg A, 26 : x : '13, Flem.—Fourteen-celled.

Diameter in spirit, ·52 × ·48 mm. Shell-membrane ·003 mm. in thickness. Albumen and zona distinct. The blastomeres (Pl. 8, fig. 16) lie with their outer surfaces in contact with the zona. They fall into two groups: Group A (formative ?) comprises blastomeres 1, 2, 3, 4, 6, 7, 8, 9, and Group B (non-formative ?), (11, 14), (12, 13) + 5 + 10. Blastomeres of Group A tend to be smaller on the whole than those of B.

Blastomeres 12 and 13 form a pair as do 11 and 14, the nuclei of the latter being small. Blastomere 5 is a very large cell (·075 × ·036 × ·036 mm.) with its nucleus in the early prophase; blastomere 10 is of about the same size, its nucleus being in the resting condition. The lineage-formula is represented by 4 × 2 + (2 × 2 + 2 undivided) and bears out the conclusion that one group (? the formative) completes the fourth cleavages before the other.

The cytoplasm of the blastomeres is very granular and contains very few fat-spherules. Yolk-spheres (up to

·02 mm. in diameter) are abundantly present in the cleavage-cavity.

Egg C, 26 : x : '13, P.N.O.—Fourteen-celled.

Generally resembles A, but the grouping is much less clear. A possible grouping is the following: Group A (2, 6), (7, 10), (11, 12), (14, 15), Group B (3, 5), (9, 13), + 1 (in the telophase) + 4–8 (binucleate but undivided) = 4 × 2 + (2 × 2 + 1 in division + 1 binucleate) = 14.

Three 15-celled eggs, viz. D and J, 26 : x : '13, Flem., and A, 1. 12 : vii : '13, P.N.O., have been examined, but they call for no special description.

Of 16-celled eggs, thirteen are available for study, viz. five from Specimen 4, and eight from Specimen 3. They resemble each other in their general characters, and it will suffice for our purpose if we confine our attention more particularly to the four of which reconstructional models in wax have been prepared. Of these, the most instructive is perhaps E, 26 : x : '13.

Egg E, 26 : x : '13, P.N.O.—Sixteen-celled. (Pl. 8, fig. 18, and Pl. 6, fig. 3, A and B).

The model (Pl. 6, fig. 3) shows that the blastomeres are arranged so as to form the as yet incomplete wall of the hollow spherical blastocyst. They are variable as to size and for the most part are only in partial contact with each other, there being gaps of varying size between them. In particular, there is present on one side (fig. 3, A) a large opening leading directly into the extensive cleavage, or, as we may now term it, blastocyst-cavity, and bounded, except on one side (right of figure) where there is a gap, by five cells (1, 2, 4, 5, 3), and in the region of the just mentioned gap, by three more cells (6, 10, 7), in addition to 1 and 3. On the opposite side (fig. 3, B), the wall is less incomplete. Comparison with egg B, 26 : x : '13 (fig. 2, A) suggests that the large opening here probably corresponds to the opening bounded by the four smaller (presumably formative) cells of that egg and so occupies what is morphologically the upper polar region. If we

accept that interpretation, then it is possible to group the blastomeres in pairs as follows: Formative (?) = 1 and 6, 2 and 4, 3 and 5, 7 and 10; non-formative (?) = 8 and 9, 1 and 12, 14 and 15, 13 and 16.

The measurements of the blastomeres are as follows:

Formative (?)		Non-formative (?)	
1	·045 × ·037 × ·032 mm.	8	·045 × ·021 × ·032 mm.
6	·05 × ·024 × ·032 mm.	9	·043 × ·021 × ·032 mm.
2	·040 × ·035 × ·024 mm.	11	·043 × ·027 × ·04 mm.
4	·051 × ·027 × ·032 mm.	12	·045 × ·029 × ·032 mm.
3	·040 × ·032 × ·032 mm.	14	·043 × ·035 × ·032 mm.
5	·051 × ·027 × ·04 mm.	15	·043 × ·032 × ·032 mm.
7	·040 × ·027 × ·032 mm.	13	·045 × ·027 × ·032 mm.
10	·040 × ·024 × ·032 mm.	16	·045 × ·037 × ·032 mm

They show that at this stage the two groups of blastomeres presumably present are not distinguishable by any marked difference in size, whilst microscopical examination also fails to reveal any obvious cytological differences between them.

The blastomeres (Pl. 8, fig. 18) appear ovoidal in section, their outer surfaces being flattened against the albumen (in this egg, the zona is not preserved). Each possesses a large spherical nucleus situated towards the outer surface of the cell and containing besides spherical granules of chromatin, a large centrally situated darkly-staining mass. The cytoplasm contains numerous spherical granules, most of them staining light red with safranin, the remainder, black with iron-hæmatoxylin and also numbers of fat-spherules. The cleavage-cavity is occupied by numerous yolk-spheres, similar to the cytoplasm in their characters and by coagulum.

The shell-membrane measures about ·0028 mm. in thickness.

Egg I, 26 : x : '13, P.N.O.—Sixteen-celled. (Pl. 6, fig. 4.)

This egg only differs from the preceding in lacking the conspicuous opening on one side. Here, again, the cells are only in partial contact with each other with the result that

the blastocyst-wall is markedly fenestrated. Though it is possible to pair the cells and to group them into two sets, I find it is quite impossible to determine which set is formative and which non-formative. The same holds true for egg E, 1. 12 : vii : '13, P.N.O., of which a model was also made, but it is not figured here since it is essentially similar to that of I.

Pl. 9, fig. 19, taken from egg K, 26 : x : '13, Flem., shows the sectional appearance of the blastomeres, whilst Pl. 8, fig. 17, affords a general view of a section of the entire egg (egg F, 1. 12 : vii : '13) at this stage of development. The thin shell-membrane is characteristically folded, whilst the albumen, it is worthy of note, shows no appreciable diminution in amount as compared with earlier stages, a detail in which Didelphys, as Hartman has pointed out, differs from Dasyurus, in which, by the 16-celled stage, the albumen is largely absorbed.

Egg C, 1. 12 : vii : '13, P.N.O. — Sixteen-celled (Pl. 6, fig. 5).

This egg is slightly later than the eggs described above. The model (fig. 5) shows that the wall of the blastocyst is now practically completely established. The cells are now more flattened and have come into more marked contact with one another by their edges, with the result that the gaps between them are not nearly so conspicuous as in eggs E and I. In the model the apparent poles are occupied each by a single cell (1 and 16), and it is significant that the seven cells grouped round cell 16 are, taking them as a whole, larger than the remaining eight which form the wall of the opposite hemisphere and which vary more in size than those around cell 16. Here again, then, we have an indication of the grouping of the cells of the 16-celled stage into two sets, differing slightly in size.

Pl. 9, fig. 20, illustrates the sectional appearance of the blastocyst at this stage. It is taken from egg D, 1. 2 : vii : '13, P.N.O., in which the blastomeres are still more flattened than in egg C. The blastocyst-cavity is partially occupied by yolk-spheres and coagulum.

Egg G, 1. 12 : vii : '13, P.N.O.

In this egg there are 17 blastomeres (15 + 1 × 2), i.e. one of the blastomeres of the 16-celled stage has already undergone the fifth cleavage. In addition, two blastomeres, situated near each other in one hemisphere, are in process of division, but I am unable to distinguish with certainty the two recently formed blastomeres and to make out the grouping.

Hartman states, with reference to the cleavage-stages of D. virginiana corresponding to those described in the preceding section, that "a thorough search was made for structural evidences of polarity, differences in size, staining qualities, and yolk-content of cells. None were discovered" (p. 39). He records, however, that in three eggs of 6, 10, and 12 blastomeres respectively, there is evidence of more rapid division of the cells at one pole than at the other, and concludes that these eggs exhibit a polarity, "slight though this be," and that "if a comparison with the eggs of Dasyurus in this regard be valid, the more rapidly dividing cells are formative, the others non-formative." He summarises his observations "by the statement that cleavage proceeds in the opossum from the 1-celled to the 16-celled stage superficially somewhat as in the Eutheria and without evident polar differentiation, but that potentially the cells at the poles differ, those of one hemisphere being destined to become the formative, those of the other the non-formative region of the early blastocyst."

My own observations supplement those of Hartman, more especially in regard to the question of polar differentiation, since I have been able to bring forward stronger evidence of its existence in D. aurita than he was able to do in the case of D. virginiana. In many of the segmenting eggs of D. aurita, described in the preceding pages, we have demonstrated the existence of two groups of blastomeres, occupying opposite polar regions of the egg and distinguishable by a difference in their size-relations and in their time of

division, one group, that composed of the smaller blastomeres, completing the fourth cleavage before the other. Apart from the absence of recognisable qualitative differences between them, these two groups are comparable with the upper and lower cell-rings of the 16-celled stage of Dasyurus which exhibit an obvious polarity and which furnish the formative and non-formative regions of the unilaminar blastocyst. Hartman has shown that homologous regions are present in the blastocyst of Didelphys, and I agree with him that all the evidence supports the conclusion that one of the two groups of blastomeres of the segmenting egg, presumably that composed of the smaller cells, is formative in destiny, the other non-formative, and that accordingly these two groups are homologous respectively to the upper and lower cell-rings of the Dasyurus egg.

It may be noted here that in the later 16-celled stages, in which the cells have begun to flatten out, it is always difficult and frequently impossible to determine with certainty the grouping of the blastomeres and to distinguish the future formative and non-formative regions. That two sets of blastomeres, respectively formative and non-formative in destiny, are actually present all the evidence goes to show, but they become temporarily indistinguishable much in the same way as the original, and much more obvious polar differentiation in the segmenting Dasyurus egg becomes obliterated in the blastocyst during its period of active growth. According to Hartman, the definitive formative and non-formative regions first become recognisable in Didelphys in blastocysts of about 40–50 cells, "soon after the fifth cleavage when the blastocyst is just completed," as the result of the thinning out of the cells over the non-formative region.

(7) Later Blastocysts.

The later blastocysts available for examination were derived from Specimens 5 and 6 of list. They vary in diameter from ·82 to 1·3 mm., but are all at essentially the

same stage of development. Blastocysts of D. virginiana of a precisely corresponding stage have previously been described by Selenka ('86) and more recently by the late Prof. C. S. Minot ('11).

As described by these observers, the blastocyst at this stage is bilaminar throughout, and the definitive embryonal area is established over the upper polar region. The area is circular in form (Pl. 8, fig. 21), with a diameter of ·4 to ·6 mm., and is sharply marked off by its denser, more opaque character, from the thinner, more transparent, and more extensive remainder of the vesicle-wall, the extra-embryonal region.

The outer layer of the bilaminar vesicle, the ectoderm, consists of two portions sharply distinguishable by their cytological characters, viz. (1) the embryonal ectoderm, co-extensive with the embryonal area and by its thickness conditioning the appearance of that, and (2) the tropho-blastic ectoderm (tropho-ectoderm) over the remainder of the vesicle-wall. The inner layer, the entoderm, exhibits no such distinction into two parts and appears as a uniform, thin layer, forming a complete lining to the blastocyst cavity.

The blastocyst is invested externally by the persistent shell-membrane, homogeneous in character and measuring from ·0027 to ·003 mm. in thickness. Selenka refers to it as the "granulosa-membran" and Minot as the "zona pellucida," both of which designations are incorrect. Between the shell-membrane and the ectoderm is the space formerly occupied by the albumen and now usually quite narrow and filled by a homogeneous material which stains lighter than the shell-membrane and is obviously quite distinct from it (Pl. 9, fig. 24). This material may be absent over the embryonal area (Pl. 9, fig. 23), and in any case tends to be thickest over the tropho-ectoderm, where, indeed, it may be locally thickened (up to a maximum of about ·07 mm.) in the equatorial or lower polar regions. Selenka refers to it as the "Eiweisschicht," but it lacks the lamination charac-

teristic of the albumen of the earlier stages, and as to whether it represents the remains of that or a coagulum derived from the uterine secretion, I have no means of determining.

Examined in section, the embryonal ectoderm (Pl. 9, figs. 23 and 24) is seen to be composed of a layer of well-defined cubical cells, with finely granular, deeply-staining cytoplasm and large oval or spherical nuclei, possessing a delicate nuclear reticulum and one or more nucleolar-like chromatin masses. It attains a maximum thickness of from ·021–·024 mm. over the central region of the area and thins out peripherally to a thickness of about ·0135 mm. before its junction with the tropho-ectoderm. The outer ends of the cells are either flat as in blastocysts Spec. 6, where they abut against the coagulum (Pl. 9, fig. 24), or they are rounded and in the form of blunt projections, which come into direct contact with the shell-membrane as in blastocysts Spec. 5 (Pl. 9, fig. 23). Minot figures corresponding projections in D. virginiana, but erroneously interprets them as caps of the "more or less granular coagulum" situated in the space between the shell-membrane and what he regards as the true outer end of the ectodermal cells.

In blastocysts Spec. 5, the adjoining surfaces of the cells are in close apposition, but in blastocysts Spec. 6, they are separated here and there by narrow clefts, obvious in sections (Pl. 9, fig. 24), and especially so in whole mounts (Pl. 9, fig. 22).

Examined in stained preparations of the blastocyst-wall, mounted on the flat, the ectoderm of the embryonal area is seen to be separated from the tropho-ectoderm by a definite clear-cut junctional line (Pl. 9, fig. 22), quite comparable to and homologous with that in the corresponding stage in Dasyurus. Minot notes that "the edge of the embryonic shield is very sharp," but Selenka's figures (figs. 1 and 5, Taf. xix) convey an altogether erroneous impression of the actual condition.

As noted by Minot, the embryonal ectodermal cells stain much

more deeply than those of the tropho-ectoderm (Pl. 9, fig. 22). They are, like the latter, polygonal in outline, the cell outlines being clearly visible in preparations of blastocysts Spec. 5 (Chrom-osmic fixation), although not in those of blastocysts Spec. 6, but are of much smaller surface extent than the tropho-ectoderm cells; their nuclei in proportion to the surface extent of the cells are large, and so they appear closely packed. Many of the cells are in process of mitotic division.

The tropho-ectoderm is also composed of cubical cells, polygonal in outline when seen in surface view (Pl. 9, fig. 22), but of greater surface extent and more flattened than those of the embryonal ectoderm. It varies in thickness from ·008 to ·0135 mm. and tends to be thickest over the lower polar region where the coagulum also tends to be most developed. The cytoplasm of the cells is vacuolated and stains lightly. Their nuclei also stain, on the whole, lighter than those of the embryonal ectoderm. When measured in section they are of smaller average diameter than those of the embryonal ectoderm, but when measured in whole mounts, they are found to be of slightly greater average diameter than the latter in correspondence with the greater surface extent of the cells to which they belong. Many of the cells are in process of division (Pl. 9, fig. 22).

The entoderm (Pl. 9, figs. 23 and 24) is similar in character throughout its extent, and appears as a very thin layer of large flattened cells with correspondingly flattened nuclei which stain more deeply, are wider spaced, and have in whole mounts a greater average diameter than those of the tropho-ectoderm. It is very closely applied to the inner surface of the ectoderm, such separation as occurs in sections being artificial.

Through the kindness of Prof. C. F. W. McClure, of Princeton University, I have had the opportunity of examining two blastocysts of D. virginiana, belonging to the same batch as two of the vesicles studied by Prof. Minot. They measure 1·03 mm. in diameter and possess a circular embryonal area ·69 mm. in diameter. They differ in no

essential respect from the vesicles of D. aurita described above.

Prof. Minot was greatly struck by the occurrence in the blastocysts he examined of little gaps in the tropho-ectoderm closed by the underlying entoderm. "He says: "One is forced to the conclusion that the ectoderm is not continuous, but that there are small gaps in it, each of which is filled up by the entoderm." I have observed the same gaps in the opossum material before me, the gaps varying in size from ·02 to ·04 mm. in diameter and having an oval, angular, or irregular form. I have also seen similar gaps in the tropho-ectoderm in Dasyurus and in one blastocyst of D. aurita. In my opinion these gaps are due to some defect in the growth of the cellular wall and are of no morphological significance.

I must confess I fail to follow Minot when he makes the statement: "Still more remarkable are the cytological differences between the two forms [Dasyurus and Didelphys] in the extra-embryonic area" ('11, p. 299), and proceeds to discuss the same. So far as I have observed, the extra-embryonal regions in blastocysts of corresponding developmental stages in these two Marsupials exhibit no cytological differences of moment or such as would invalidate their strict homology.

Conclusion.

In my view, the most important general result which follows from Hartman's work on Didelphys and my own on that form and Dasyurus, is the demonstration of the fact that the early ontogeny in these two Marsupials, the only two so far adequately investigated, is effected according to a common plan. Such differences as exist are, in my opinion, of the nature of secondary modifications only, and not fundamental, and the result, as I hope to show below, of abbreviation of the developmental cycle. I have shown, further, that the early development in other Marsupials (Macropus, Perameles) agrees in essentials, so far as it is known, with that of Dasyurus.

These concordant results would therefore seem to justify the general conclusion that there is one common mode of early development characteristic of the Didelphia as a group, just as there appears to be a common but divergent and derivative mode characteristic of the Monodelphia.

The early ontogeny of the Didelphia is characterised before all by the fact that cleavage, in the absence of a morula-stage, results directly in the formation of a unilaminar blastocyst, the wall of which consists over opposite polar areas of formative and non-formative regions, the former destined to furnish the embryonal ectoderm and the entire entoderm of the blastocyst, the latter, the trophoblastic or extra-embryonal ectoderm (tropho-ectoderm). The formative cells, the homologue of the inner cell-mass of the Monodelphia, are always freely exposed at the surface of the blastocyst, and are never, even temporarily, enclosed by the tropho-ectoderm, as appears always to be the case in the Monodelphia.

The significance of the first-mentioned difference in the relations of the formative and non-formative cells in the Didelphia and the Monodelphia I have discussed at some length in the section of my paper on Dasyurus, entitled "The Entypic Condition of the Eutherian Blastocyst," p. 111. The term "entypy" ("Entypie des Keimfeldes") we owe to Selenka ('00, p. 203), and from his definition (*v.* footnote on p. 112 of my paper) I understood him to mean that the term was to be applied generally to designate the characteristic Monodelphian developmental condition in which the formative cells are completely enclosed by the tropho-ectoderm. It would seem, however, that that was not what Selenka had in view. At an informal discussion during the meeting of the Institut International d'Embryologie at Freiburg i. Br. in 1912, the late Prof. A. A. W. Hubrecht and Prof. F. Keibel pointed out to me that, in their opinion, Selenka employed the term "entypie" in a limited sense only, viz. to designate the condition of so-called "inversion of the germ-layers," as seen in the blastocyst-stage of certain rodents, e. g. Mus, Cavia, and did not intend it to be applied in the way I had

done in the paper referred to. Hubrecht, indeed, in his memoir of 1908, refers to the term in the above sense. With reference to the condition of the blastocyst in Tupaia, where the embryonal shield is bent V-wise on itself so as to enclose the cleft-like primitive amniotic cavity, closed by the covering portion of the trophoblast, he says: "This arrangement possesses suggestive points of comparison with what has been called by Selenka ('00 *a*, p. 201) the "entypie" of the embryonic shield, such as it exists in many rodents" ('08, p. 10).[1] Bryce has also interpreted the term in the same sense; he says ('08, p. 31): "In another and considerable series of mammals, the inversion persists rather longer, and the cavity never opens out on the surface of the blastocyst but remains roofed in by the trophoblast layer. This condition was named by Selenka 'entypy of the germinal area.'"

It would, therefore, appear that my former use of the term "entypy" was incorrect, and that the developmental condition in the Monodelphia to which I had applied that term still lacks the designation to which, as an important diagnostic character, it would seem to be entitled, and the same holds true for the contrasted condition characteristic of the blastocyst of the Didelphia and the Ornithodelphia. The term "entypie," so far as I can gather, appears to be derived from the Greek τύπος, signifying form, image, etc., and in attempting to frame terms to express the conditions in question, I have thought it best to proceed from the root of entypy as a basis, that term having become incorporated in the literature. Accordingly, I venture to suggest the term Phanerotypy to designate the condition of the blastocyst in the two subclasses of the Ornithodelphia and Didelphia, where "the formative cells are freely exposed and constitute from the first part of the blastocyst wall, just as those of the Sauropsida form part of the general blastoderm" (Hill, '10, p. 112), and the term Cryptotypy to designate the developmental condition characteristic of the Monodelphia in which

[1] Italics mine.

the formative cells are completely hidden or enclosed by the tropho-ectodermal mantle. Selenka's term Entypy now falls into line in its original sense as designatory of the exaggerated cryptotypy of rodents with so-called "inversion of the germ-layers." If the reader will substitute cryptotypic for entypic, wherever that occurs in my former paper ('10, p. 111, et seq.), my unwitting misinterpretation of Selenka's meaning will stand fully corrected.

Finally, a word or two by way of comparison of the cleavage-process in Dasyurus and Didelphys. As the result of his study of the cleavage-process in the latter form, Hartman arrives at the interesting and highly significant conclusion that it is "highly probable that the cells of each hemisphere [destined to form the embryonic and the non-embryonic regions, respectively, of the blastocyst] are lineal descendants of one or other of the blastomeres of the 2-celled stage." That conclusion is supported in the strongest fashion by the evidence recorded in preceding sections of this paper. My observations show that in six out of ten 2-celled eggs examined, the first cleavage division is an unequal one, one of the blastomeres exceeding the other in size; that in five out of eight 4-celled eggs described, one pair of blastomeres is slightly larger than the other, the two pairs of blastomeres being so arranged as to form a cross-shaped figure; that the 8-celled egg described, consists of two pairs of larger and two pairs of smaller blastomeres, forming two distinct groups just as in the 8-celled stage of D. virginiana described by Spurgeon and Brooks ('16), and that in later cleavage-stages, up to, and including the 16-celled stage, two corresponding groups are recognisable, one of which tends to complete the fourth cleavage before the other. That evidence demonstrates, conclusively to my mind, that the two cell-groups in question are derived, as Hartman has suggested, one from each of the blastomeres of the 2-celled stage. Now, if the evidence for the conclusion, that one of these groups furnishes the formative region of the completed blastocyst, the other, the non-formative, be

accepted as adequate, then there is no escape from the further conclusion that the first cleavage division which in D. aurita tends to be an unequal one, is a differential cleavage, resulting in the production of the parent cells of the future formative and non-formative regions. I therefore agree with Hartman in holding that the unsegmented ovum of Didelphys is potentially polar in its constitution.

Certain very interesting deductions follow from these conclusions. In the first place, it becomes evident that the fourth cleavage in Dasyurus is the homologue of the first cleavage in Didelphys. In Dasyurus, I have shown that the fourth cleavage is the differential cleavage; it is unequal, qualitative and horizontal or equatorial in direction, and results in the formation of two superimposed cell-rings, each of eight cells, viz. an upper ring of smaller, less yolk-rich cells, destined to furnish the formative region, and a lower ring of larger, more yolk-rich cells, destined to give origin to the non-formative region of the unilaminar blastocyst. In Didelphys, the evidence as set forth by Hartman and myself shows that it is actually the first cleavage which is the differential cleavage; it tends to be unequal in D. aurita and results in the production of two blastomeres, one formative in destiny, the other, non-formative.

Then, in the second place, it follows, whether we postulate an inherent polarity in the unsegmented ovum of Didelphys or not, that the first cleavage in that form must be horizontal or equatorial like the homologous fourth cleavage in Dasyurus, since the one blastomere is destined to give origin to what is morphologically the upper polar region of the blastocyst and the other to the remaining larger and lower portion of the same (Hartman).

In the third place, it follows that the cleavage-process in Didelphys has undergone abbreviation as compared with that of Dasyurus, since the first three cleavages in the latter are omitted, the fourth cleavage becoming the first.

It will be generally agreed, I think, that the Dasyurus type

of cleavage represents the ancestral mode from which that of Didelphys has been derived. That view is supported by the consideration that the ovum of Dasyurus, in respect of its size and markedly polar constitution, exhibits more primitive features than that of Didelphys, and by the further consideration that such a shifting forward of an ontogenetic process, as is presumably seen here in Didelphys, to an earlier period of development than that to which it originally belonged is a phenomenon which is familiar to the embryologist, and of which a reasonable interpretation can be given, viz. that it is of the nature of an adaptive response, since it results in an abbreviation of the developmental cycle, whereas, on the contrary view that the Dasyurus type is the derivative one, it is difficult to imagine what possible advantage is gained by a shifting back or retardation of the ontogenetic process in question.

On the assumption, then, that Dasyurus exhibits the more primitive condition, we would appear to have displayed in the cleavage of Didelphys yet another instance of what Sir Ray Lankester in his classical essay, "Notes on Embryology and Classification" ('77, p. 411), originally termed "precocious segregation"; "'precocious,' since it is the acquirement of a condition in the developing organism, in virtue of heredity, at an earlier period of development than that at which such acquirement was attained by its forefathers through adaptation. The tendency to precocity in this sense, in regard to important structural arrangements, has been insisted on by Haeckel in discussing what he terms 'heterochrony in the palingenetic phenomena of ontogeny.'"

That the resulting acceleration in development is directly related as an adaptive response to the reduction in size which the ovum of Didelphys has undergone, and the consequent earlier completion of the blastocyst, as compared with Dasyurus, there can be little doubt, and just as I was led to regard the occurrence of the cross-shaped 4-celled stage and the morula stage in the development of the Monodelphia as "cleavage-adaptations of prospective significance in regard

to the entypic [cryptotypic] condition" ('10, p. 115), so I would interpret the cross-shaped 4-celled stage in Didelphys as a corresponding but independently acquired adaptation, induced by the shifting forward of the differential cleavage, the object of which is perhaps to ensure the separate grouping of the precociously segregated formative and non-formative cells.

The facts and considerations herein set forth, taken in conjunction with what we know of the early ontogeny in the Monodelphia, forcibly suggest the view that the first cleavage in the latter may in all cases have the same differential value as in Didelphys; indeed, the observations of Hubrecht ('08) on the cleavage of Tupaia, where the morula stage is described as consisting of a single central cell, regarded as the parent cell of the inner cell-mass and an investing layer of trophoectoderm, seems to show that that is actually the case in this form.

List of References.

Bensley, B. A. (1903).—"On the Evolution of the Australian Marsupialia; with Remarks on the Relationships of the Marsupials in General." 'Trans. Linn. Soc.,' 2nd Ser.; 'Zool.,' vol. ix, pt. 3.

Bresslau, E. (1912).—"Die Entwickelung des Mammarapparates der Monotremen, Marsupialier und einiger Placentalier. III. Entwickelung des Mammarapparates der Marsupialier, Insectivoren, Nagethiere, Carnivoren und Wiederkäuer," 'Semon, Zoolog. Forschungreisen,' Bd. iv, lfg. 7.

Bryce, T. H. (1908).—"Embryology," 'Quain's Elements of Anatomy,' vol i.

Hartman, C. G. (1916).—"Studies in the Development of the Opossum Didelphys virginiana, L. I. History of the Early Cleavage. II. Formation of the Blastocyst," 'Journ. Morph.,' vol. xxvii, No. 1.

Hill, J. P. (1900).—"On the Fœtal Membranes, Placentation and Parturition of the Native Cat (Dasyurus viverrinus)," 'Anat. Anz.,' Bd. xviii.

——— (1910).—"The Early Development of the Marsupialia, with Special Reference to the Native Cat (Dasyurus viverrinus)." 'Quart. Journ. Micr. Sci.,' vol. 56.

Hill, J. P., and O'Donoghue, C. H. (1913).—"The Reproductive Cycle in the Marsupial Dasyurus viverrinus," ibid., vol. 59.

Hubrecht, A. A. W. (1908).—"Early Ontogenetic Phenomena in Mammals and their Bearing on our Interpretation of the Phylogeny of the Vertebrates," ibid., vol. 53.

Jordan, H. E. (1911).—"The Spermatogenesis of the Opossum (Didelphys virginiana), etc.," 'Archiv f. Zellforschung.' Bd. 7.

Lankester, E. Ray (1877).—"Notes on the Embryology and Classification of the Animal Kingdom, etc.," 'Quart. Journ. Micr. Sci.,' vol. 17.

Minot, C. S. (1911).—"Note on the Blastodermic Vesicle of the Opossum," 'Anat. Rec.,' vol. 5.

Selenka, E. (1886).—'Studien über Entwickelungsgeschichte der Thiere' IV (1 and 2),. "Das Opossum (Didelphys virginiana)," Wiesbaden.

——— (1900).—'Studien über Entw. der Thiere, H. 8 Menschenaffen.' "III. Entwick. des Gibbon (Hylobates u. Siamanga)," Wiesbaden: C. W. Kreidel.

Spurgeon, C. H., and Brooks, R. J. (1916).—"The Implantation and Early Segmentation of the Ovum of Didelphis virginiana," 'Anat. Record, vol. 10.

Van der Stricht, O. (1909).—"La Structure de l'Œuf des Mammifères (Chauve-souris, Vesperugo noctula)," Troisième Partie, 'Mem. de l'Acad. roy. Belgique,' 2me Serie, t. ii.

Winge, H. (1893).—"Marsupiaux fossiles et vivants de Lagoa Santa. Minas Geræs, Brésil," 'E Museo Lundii,' Andet Bind. Første Halsbind. Kjobenhavn.

EXPLANATION OF PLATES 6–9,

Illustrating Prof. J. P. Hill's paper on "Some Observations on the Early Development of Didelphys aurita."

[All the figures are from material of D. aurita. Figs. 1–20 and 22–24 I owe to the skill of Miss M. Rhodes, the cost of the same being defrayed from a grant from the Dixon fund; for Fig. 21 and Pl. 6, figs. 2–5 (the former drawn from photographs of the blastocyst, and the latter from models of segmenting eggs), I am indebted to Miss E. A. Steele.]

List of Common Reference Letters.

alb. Albumen. *cl. c.* Cleavage (Blastocyst) cavity. *emb. ect.* Embryonal ectoderm. *ent.* Entoderm. *p. b.* Polar body. *sh. m.* Shell-membrane. *tr.-ect.* Tropho-ectoderm. *y. sph.* Yolk-spherules. *z. p.* Zona.

PLATE 6.

Fig. 1.—Reconstruction of Egg J, Flem., 20 : x : '13 (see Pl. 7, figs. 9 and 10), to show the two pairs of blastomeres arranged so as to form a cross-shaped figure. × 400.

Fig. 2, A and B.—Views of model of 8-celled egg B, Flem., 26 : x : '13. × 400.

Fig. 3, A and B.—Views of model of 16-celled stage, Egg E, P.N.O., 26 : x : '13. × 480.

Fig. 4.—View of model of 16-celled stage, Egg I, P.N.O., 26 : x : '13. × 400.

Fig. 5.—View of model of 16-celled stage, Egg C, 1. 12 : viii : '13. P.N.O. × 400.

PLATES 7–9.

Fig. 1.—Section of unsegmented ovum, showing first polar body and second polar spindle. Drawn from two sections ($\frac{7}{1}$ and $\frac{1}{2}$). Egg A, Flem., 20 : x : '13. × 500.

Fig. 2.—Section of 2-celled egg. Egg P, Flem., 20 : x : '13. Section $\frac{6}{1}$. × 500.

Figs. 3, 4, and 5.—Sections $\frac{7}{1}$, $\frac{1}{2}$, and $\frac{3}{2}$. Egg L, Flem., 20 : x : '13. 2-celled in division. × 500.

Figs. 6, 7, and 8.—Sections $\frac{5}{1}$, $\frac{7}{1}$, and $\frac{2}{2}$. Egg E, P.N.O., 20 : x : '13. 2-celled in division. × 500.

Figs. 9 and 10.—Sections $\frac{3}{2}$ and $\frac{8}{2}$. Egg J, Flem., 20 : x : '13. 4-celled. × 500.

Figs. 11 and 12.—Sections $\frac{6}{1}$ and $\frac{1}{2}$. Egg O, Flem., 20 : x : '13. 4-celled. × 500.

Figs. 13 and 14.—Sections $\frac{3}{1}$ and $\frac{13}{1}$. Egg A, P.N.O., 20 : x : '13. 4-celled, showing yolk-elimination. × 500.

Fig. 15.—Sections $\frac{9 \text{ and } 10}{2}$. Egg B, Flem., 26 : x : '13. 8-celled, showing blastomeres 5, 6, 7, and 8. Cf. Model, Pl. 6, fig. 2, A. and B. × 500.

Fig. 16.—Section $\frac{6}{1}$. Egg A, Flem., 26 : x : '13. 14-celled.

Fig. 17. Section $\frac{3}{2}$. Egg F, P.N.O., 1. 12 : vii : '13, to show the structure of the entire egg. × 200.

Fig. 18.—Sections $\frac{6 \text{ and } 7}{1}$. Egg E, P.N.O., 26 : x : 13. 16-celled. Cf. Model, Pl. 6, fig. 3, A. and B. × 500.

Fig. 19.—Sections $\frac{8 \text{ and } 10}{2}$. Egg K, Flem., 26 : x : '13. 16-celled. × 500.

Fig. 20.—Section $\frac{3}{2}$. Egg D, P.N.O. 1. 12 : vii : '13. 16-celled. × 500.

Fig. 21.—Upper polar view of entire blastocyst, 19 : x : '13. Showing embryonal area. × About 30.

Fig. 22.—Drawing of whole mount, blastocyst *y*, 19 : x : '13, P.N.O. Showing junctional line between the embryonal ectoderm (*emb. ect.*), and the tropho-ectoderm (*tr.-ect.*). × 400.

Fig. 23.—Section of embryonal area of blastocyst *a*, P.N.O., 3. 14 : vii : '13. Section $\frac{8}{3-2}$. × 300.

Fig. 24.—Section showing part of embryonal area of blastocyst C. P.N.O., 19 : x : '13. Section $\frac{6}{9-1C}$. × 800.

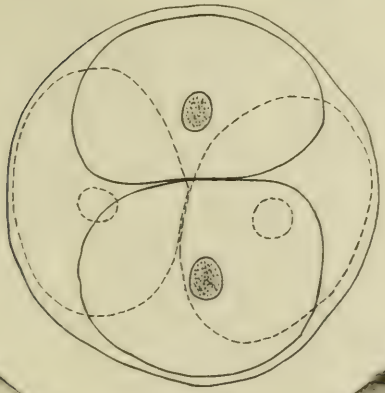

Fig. 1.

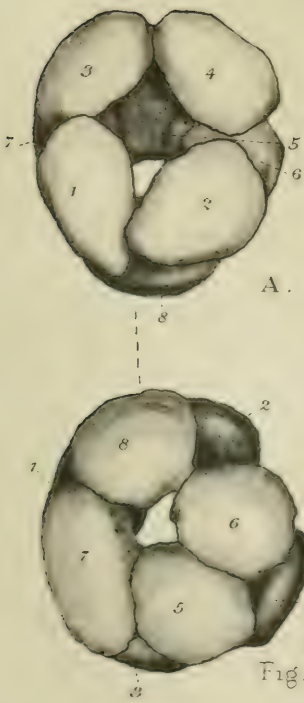

Fig. 2.

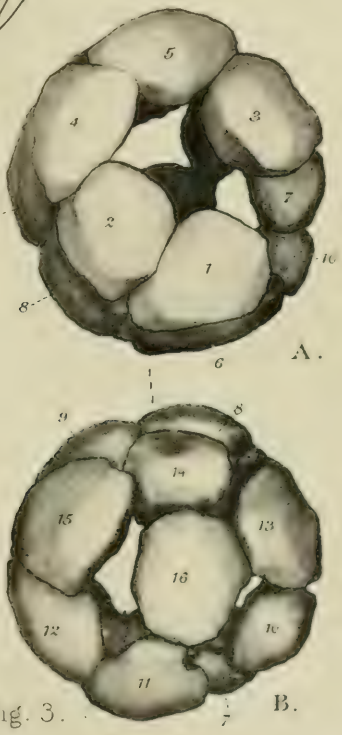

Fig. 3.

Fig. 4.

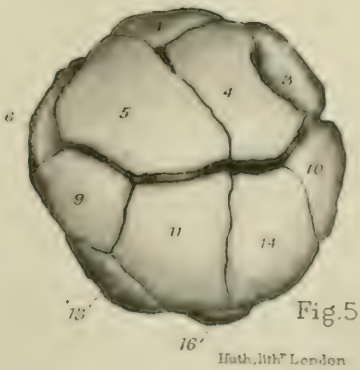

Fig. 5

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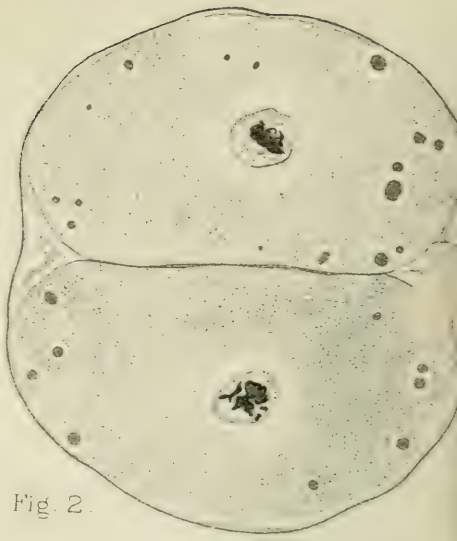
Fig. 1.



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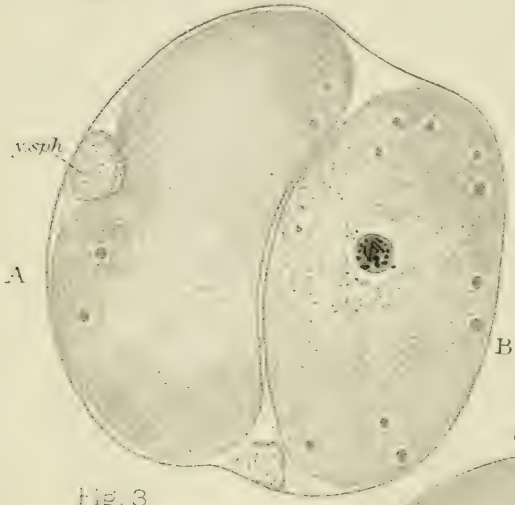

Fig. 3

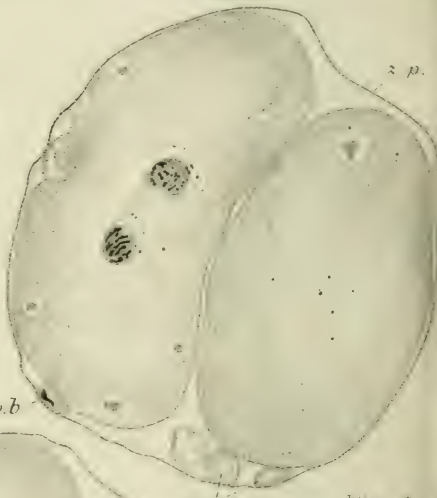

Fig 4.

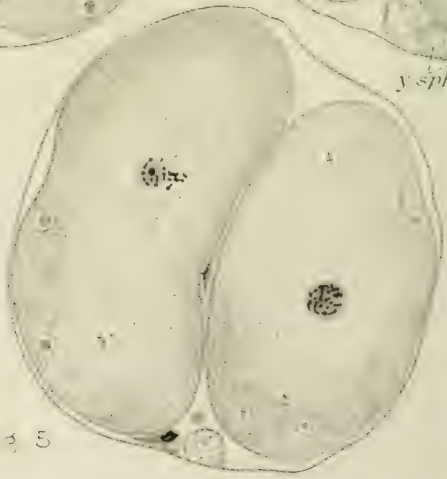

Fig 5

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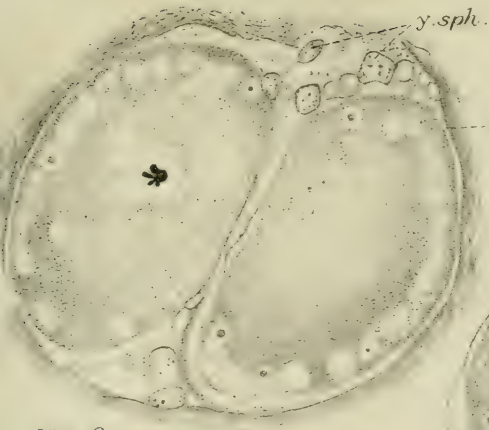

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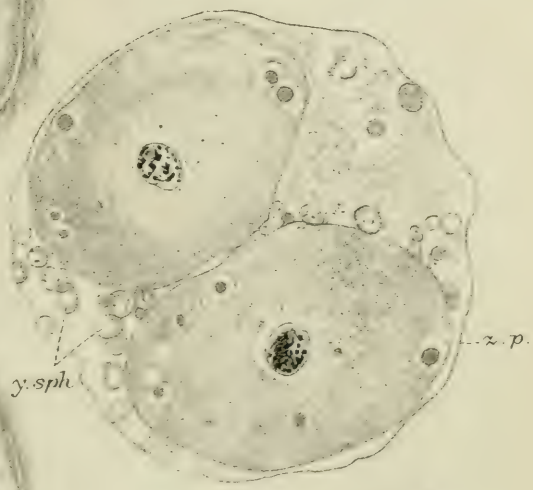

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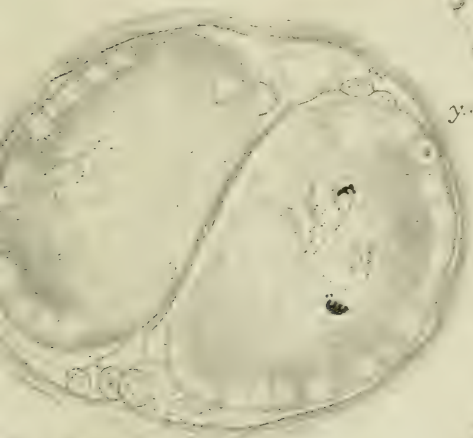

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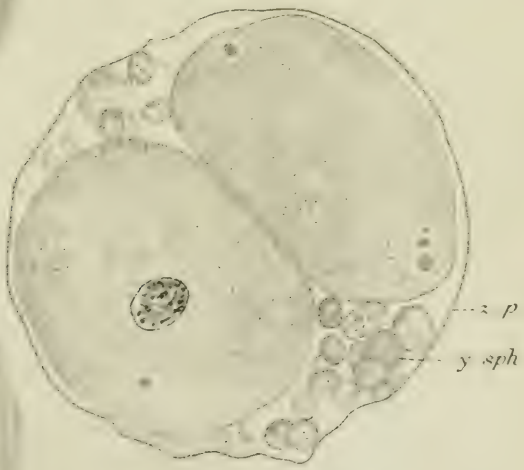

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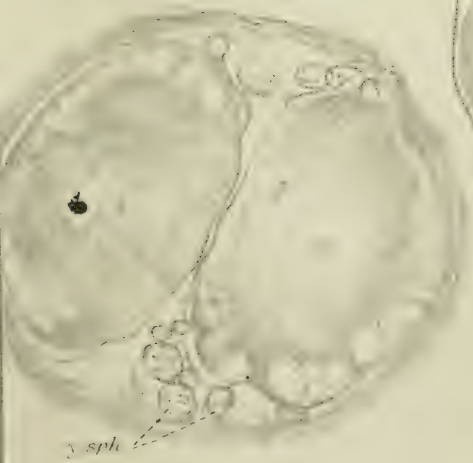

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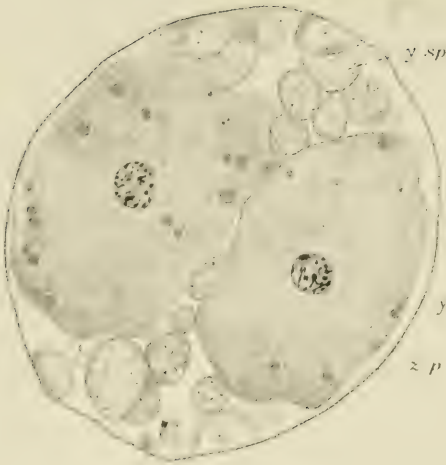

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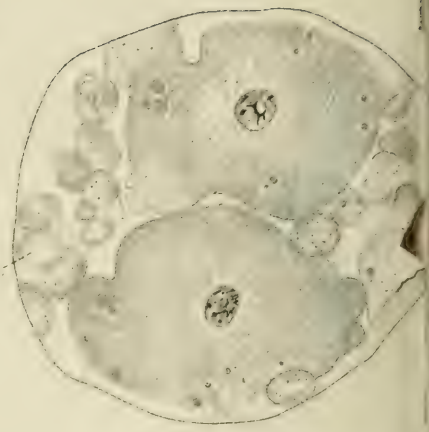

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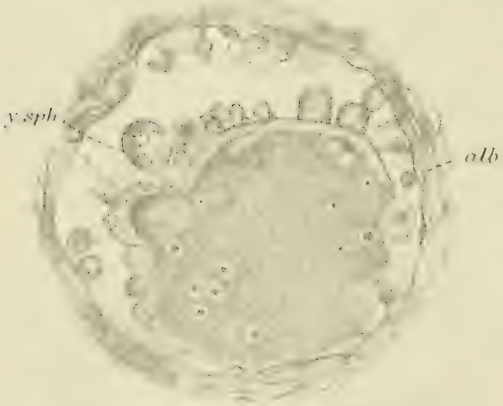

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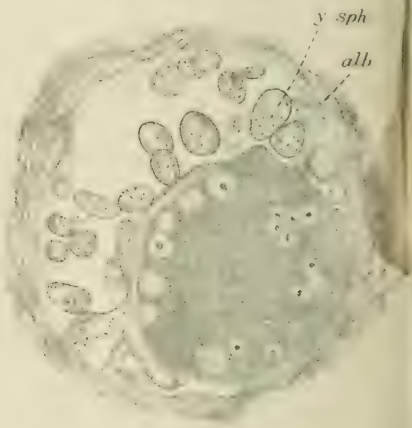

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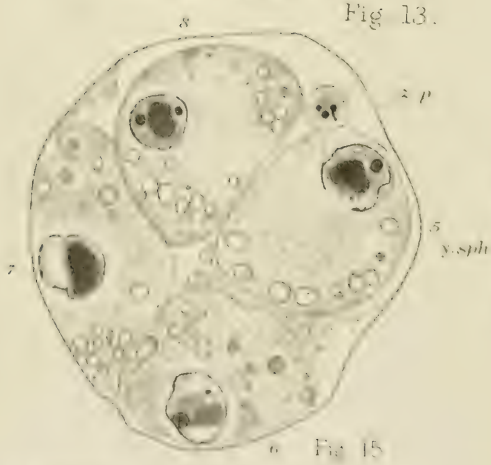

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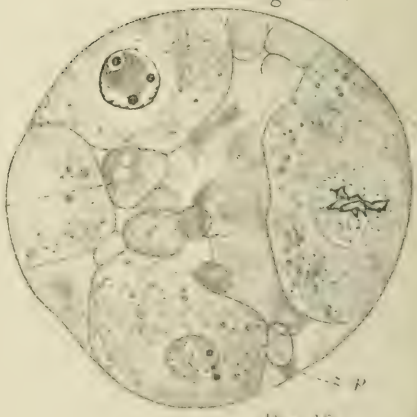

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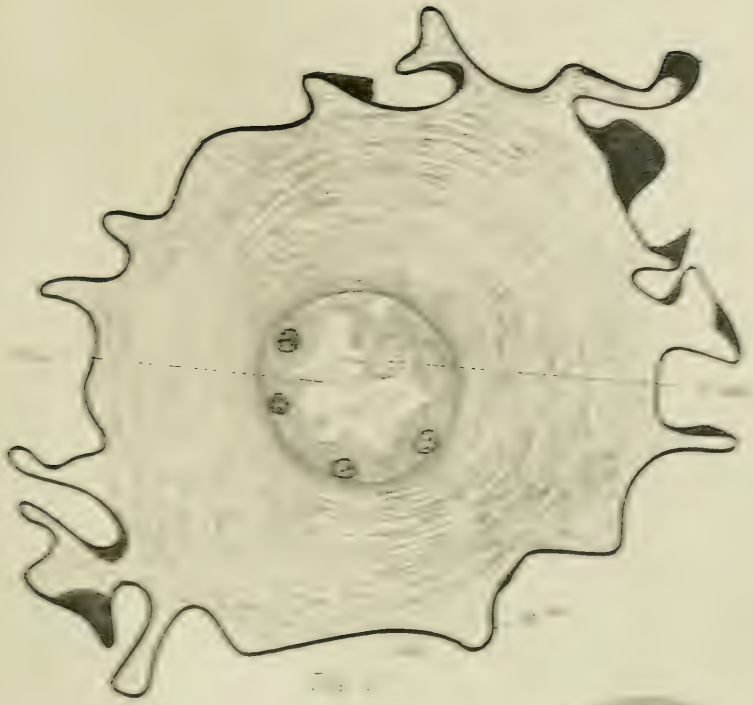

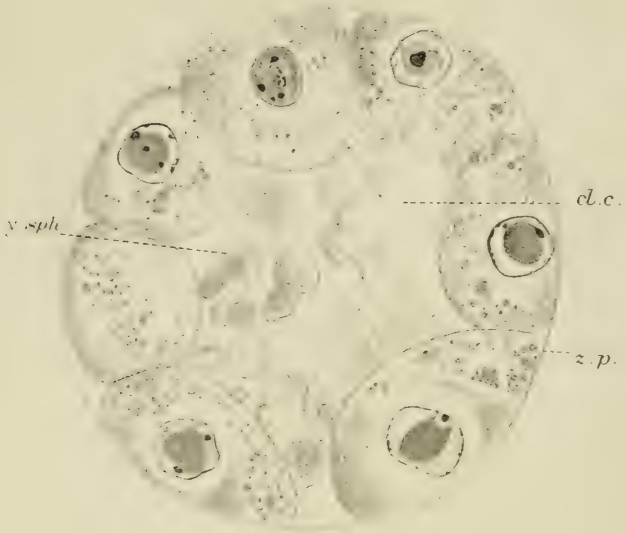

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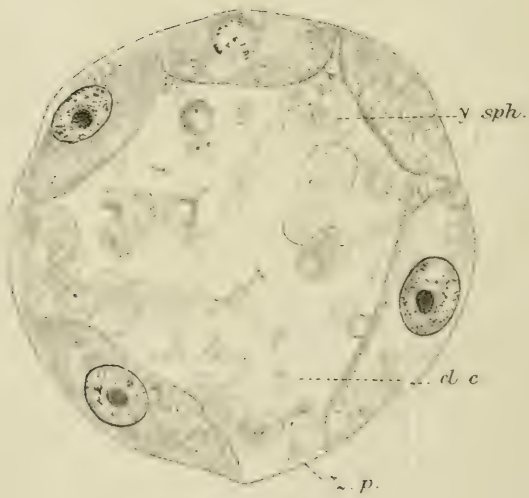

Fig. 20

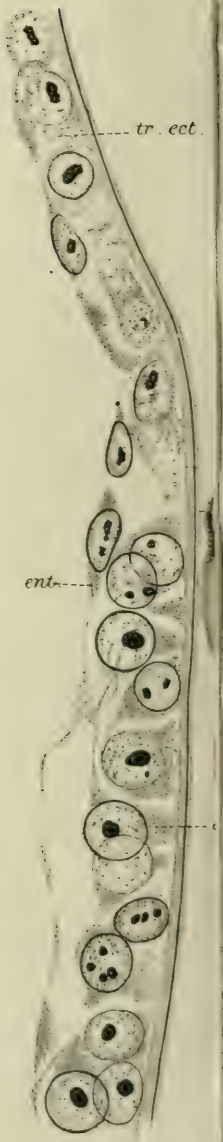

Fig. 24.

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Fig 22.

Fig. 23.

Bath. London.

Note on Eggs and Embryos of the South African Myxinoid, Bdellostoma (Heptatretus) hexatrema, Müll.

By

J. D. F. Gilchrist, M.A., D.Sc., Ph.D.

With Plates 10–12.

THE problem of the exact relationship of the Cyclostomes to Amphioxus on the one hand and the Gnathostomes on the other has been rendered more difficult of solution on account of the lack of knowledge of the early stages of the Myxinoids. While the Lampreys have been very thoroughly investigated in this respect, there remains some considerable obscurity with regard to the details of the development of the Hag-fishes. Of the two chief genera, Myxine and Bdellostoma, embryological material has only been procured in the case of the latter, and that so inadequately that a detailed comparison with other groups of animals is not yet possible. Sufficient, however, is known to show that the development differs essentially from that of the Lampreys, thus rendering it all the more necessary that a fuller knowledge of it should be obtained.

Various species of Bdellostoma are found at widely separate localities—California, Alaska, Chile, South Africa, New Zealand, and Japan—and, though they occur in abundance at such places, their eggs and embryos are little known. In the case of the Californian B. stouti our knowledge of early stages has been considerably advanced by Dean (2), Price (6), and others. Eggs and some embryological material

are recorded from Japan by Dean (3). The eggs of a species of Bdellostoma are also recorded from Chile (5), (7). Otherwise, so far as I am aware, no other material of this kind has been procured. The discovery, therefore, of some naturally deposited eggs with embryos of the South African species is of interest. Repeated attempts to find these have hitherto been in vain. The probable appearance of such eggs has been described to fishermen and others likely to have come across them, but none knew of such objects having been found in the sea. Recently, however, Mr. Cripps, the Cape Province Fishery Officer, found and recognised the eggs from the description given, and placed them in methylated spirits, the only preservative available at the time.

The eggs, five in number, were found on August 23rd, 1916, in a small bay ("Fiddle Bay") on the west coast of South Africa, in which were rocks interspersed with mud and sand. They were found near low water mark, partly embedded in mud, two of them only being visible on the surface. It proved on examination that these two alone had any contents; those embedded in the mud were empty, their contents having been removed in all probability by some predaceous animal, for in each of the empty shells there was a small, irregular aperture, with edges torn in such a way as might have been done by the radula of a mollusc. These openings are seen in two eggs in Pl. 10, fig. 1, from a photograph of the eggs as received. The eggs were attached to each other by their anchoring filaments, the attachment having been rendered more secure by the presence of the cast-off byssi of some bivalve. The threads of these were in some cases attached to the surface of the egg, and in others to small pebbles. These byssi, two or three of which were found at the end of each egg, were probably those of Mytilus crenatus, Lam., a small specimen of which, 18 mm. in length, was attached at the point of junction of three egg-cases. All these circumstances seem to point to the fact that the group of eggs had been detached from the place in which they were naturally deposited.

Two of the eggs (Pl. 10, fig. 1, *a* and *e*), which were not embedded in the mud, contained embryos in an advanced stage. These were at each end of the chain. One (*a*) has been cut open, and the contained embryo placed alongside of it, as shown in the photograph.

Dimensions of Egg.

The five eggs are very uniform in size, being about 30 mm. in length and 12 mm. in greatest breadth. This is somewhat longer than those of Bdellostoma stouti, which are stated by Dean to range from 14·3 to 29 mm., and decidedly larger than the eggs of the Bdellostoma from Chile, which were 25 mm. at most. Müller records an ovarian egg of a Bdellostoma from the Cape of Good Hope 31 mm. in length, and I have confirmed this by examination of well-advanced ovaries, in which some eggs were even 33 mm. in length, but much narrower than the naturally deposited egg. The extra three millimetres in the ovarian egg can hardly be looked on as casting doubts on the identity of the present eggs, and this is conclusively proved by the characteristics of the embryo.

The breadth of the egg (12 mm.) is decidedly greater than that of B. stouti, which varies from 6·8 to 10·5 mm.

Shape of Egg.

The egg-shell is slightly bent on its long axis. This is most clearly shown by the fact that if the planes of the two polar rings, which are approximately at right angles to the long axis of the egg, were produced, they would meet at a distance from the egg of about two or three times its length. This asymmetry of the egg appears also very distinctly in well-developed ovarian eggs, the concave or straight side being sometimes next the wall of the mesovarium, but more frequently on the opposite side. Dean, on the other hand, has not found in B. stouti a notably asymmetrical ovarian egg, nor has he seen this asymmetry in newly deposited eggs, and he is inclined to believe that the asymmetry may be due to

the difference in physical characters of the two sides of the outer egg-membranes, and acquired after deposition.

General Structure of the Shell.

The shell shows a number of small, superficial markings, usually circular or polygonal, and of varying sizes. They may, however, vary considerably in shape, some being elongate, and occasionally elongate and bent on themselves. That these markings may assume an elongate form is of special interest, as will be seen later on. The markings are brought about by the hard and dark brown outer surface dipping down into the substance of the shell so as to appear superficially as dark lines, and in sections as a number of short columns. These are continued inwards as colourless columns, and constitute the zona striata described by Cunningham (1) and Mark (4) in Myxine and by Dean (2) in Bdellostoma. Dean uses the name "villi" and "filaments," but it will be more explicit to use here the term "columns" for these structures, and, for their terminal pigmented ends, the term "heads of columns."

Below this columnar layer there is, as in B. stouti, a layer of stratified material, consisting of from seven to nine broad bands or strata with finer lamellations, and below this stratified layer is a thin but tough homogeneous layer, in which, at least in the greater part of the egg-shell, no differentiation can be seen.

The following regions of the shell may therefore be recognised: (1) The heads of the columns; (2) the columnar layer; (3) the stratified layer; (4) the homogeneous layer. A useful distinction also is that the first and the last, to a lesser extent, are of a yellowish or dark brown colour. The third, or stratified layer, is further distinguished from the others in chemical composition, as it can readily be dissolved when boiled in caustic potash. These distinctions, it will be seen, are necessary, as the different layers occur in very different proportions in the egg-shell of the Cape Bdellostoma.

Papillæ or Projections on the Shell.

Scattered over the surface of the shell are a number of minute, projecting points or papillæ, which can be seen in the dry condition with the naked eye. About fifty were counted, with the aid of a lens, across the shell in one case. They occur all over the egg, close to and on either side of the opercular ring. That these small projections are made up of a number of columns, and not of one enlarged column, is readily seen from the fact that the polygonal markings can be traced from their basis to their tips (Pl. 11, fig. 3). The tips by transmitted light appear as clear points under the microscope, and the dark lines forming the polygonal markings end abruptly round the periphery of the clear spot or are continued on it as faint lines or disconnected dots. A section through the projection (Pl. 11, fig. 4) shows that its formation is due to the increase in thickness of the stratified layer. The columns towards the centre of the projection are somewhat crowded together, and, at their distal end, are mostly devoid of the dark pigment characteristic of the heads of the columns elsewhere, thus giving rise to the clear spot seen by transmitted light in a surface view.

The Opercular Rings.

The eggs differ from those of B. stouti and those recorded from Chile by Putnam (7) in having an opercular ring at each end of the egg; in this respect, however, agreeing with the eggs recorded from Chile by Plate (5) and from Japan by Dean (3). The opercular rings are not equally developed. One is well marked in all the eggs, and is situated about 6 mm. from the end of the egg. The other is well marked in three of the five specimens, but in one (Pl. 10, fig. 1, *e*) only a slight trace of it can be found. In specimen *a* there are two well-marked rings, one 4·5 mm. from the terminal filaments, the other only 1 mm. from the filaments; in specimen *b* the rings are also well marked, the one being 4 mm. from the terminal filaments, the other 1 mm.; in *c* the distance is

again 4·5 mm., but there is only a very indistinct mark of a second ring ·5 mm. from the filaments; in *d* there are two rings, one well marked, and 4·5 mm. from the filaments, the other imperfect, being evident on one side only, where it is 1 mm. from the filaments; in *e* there is again a well-marked ring, about the same distance from the filaments, but there is no definite second ring, though there is a faint indefinite mark, about 1 mm. from the base of the filaments.

The rings appear in a superficial view as dark brown lines or bands forming a slight ridge round the egg-shell. The polygonal markings occur on either side, but, as they approach the ridge, they become somewhat broken up into lines and dots, and on the ridge itself they cannot be seen, just as in the case of the tips of the shell-projections.

The diameter of the large opercular ring is very constant, being about 10 mm., while that of the smaller varies from 5·5 mm. to 7 mm. These measurements are, of course, no indication of the shape of the egg, for the smaller ring is nearer the end.

The structure of the opercular rings is of special interest. In B. stouti they do not differ essentially in structure from the rest of the egg-shell, for, as Dean (**2**) states, in the process of rupture at the ring a slight fissure first appears between the bulbous tips of the outer or columnar layer; this extends downwards between the columns, and, about the time of hatching, passes into the middle stratified layer, and the process of warping, thus brought about, doubtless causes rupture of the inner non-striate membrane. In the present material, however, the rings are of a much more definite nature.

Their structure may be best made out by first examining sections of varying thickness without special treatment, and then observing the changes brought about by treatment with caustic potash. Results may be obtained more quickly by using hot or boiling solution, but it was found more instructive to observe the changes brought about by prolonged reaction. In thick unaltered sections in the long axis of the egg the ring appeared as a broad band or pillar through the

substance of the shell, and of the yellowish colour characteristic of the outer part of the shell. This part, the heads of the columns (Pl. 11, fig. 5, *h. col.*) is somewhat different here, as was also observed in a surface-view, in which dark lines were broken up or fragmentary. The columnar layer has changed its character, being much reduced or absent, as no distinct columnar structure was observed here. The stratified layer has, however, greatly increased in thickness, and extends upwards almost to the heads of the columns. At the point where the strata meet the pillar, they are bent outwards, and in thin sections they appeared to end here; in thicker sections, however, they were seen to be continued across the pillar (Pl. 11, fig. 5). If now the section be treated with caustic potash, the stratified layer is entirely dissolved away, and the structure of the pillar becomes much more evident. It consists of a clear structureless groundwork similar to the substance of the homogeneous layer, with which it is continuous. It becomes narrower towards the outer surface, where it appears to merge into the layer of the heads of the columns, though it cannot be distinctly followed here. Running up the centre of this pillar is a very distinct dark brown line, which meets the layer of heads of the columns at its distal extremity, and, at its proximal extremity, passes into the homogeneous layer. On each side of this line there are others running parallel to it, and of the same colour, but much thinner. They do not reach the outer surface, but, towards the inner surface, they become more numerous, and finally spread out at the proximal end, and merge into the homogeneous layer.

It appears from these observations that the ring-structure is brought about by the great development of the homogeneous layer, as a consequence of which the stratified layer, though carried somewhat outwards, has not diminished, but rather increased in breadth, leading to a reduction or disappearance of the columns of the columnar layer, and a modification of the heads of the columns. The brown line, which runs across the shell from inner to outer surface, and

the thinner lines on each side of it, suggest the structure of the columnar layer, but they are more intimately connected with the homogeneous inner layer. Functionally it is doubtless connected with the rupture of the shell and the throwing-off of the terminal capsule at the time of hatching.

Structure of Polar End of Shell.

The polar ends of the shell consist of the same layers as the body of the shell, but in such different proportions and arrangement that they require special notice.

From the ring towards the apex of the shell the homogeneous layer is at first in the form of a thin almost colourless membrane, but increases somewhat abruptly in thickness (Pl. 11, fig. 6) and assumes a yellow tinge, till near the apex it is the most conspicuous part of the shell-structure (Pl. 11, fig. 7). Stout offshoots are given off from it to the polar filaments, and, at the micropylar region, it constitutes practically the whole thickness of the shell. It still retains its homogeneous structure, which, however, becomes slightly granular in parts.

The stratified layer does not increase much in thickness, but it comes to lie near the outer aspect of the shell, and it is interrupted by the above-mentioned offshoots of the homogeneous layer, so that in section it appears more or less in patches. It may be seen to run up into the bases of the filaments (Pl. 11, fig. 7).

The columnar layer has become very much reduced, and at most places the columns have disappeared, but their hard-coloured heads are well-developed, though somewhat modified at places, as noted below.

The Micropyle and Micropylar Funnel.

The micropyle is not a simple canal, but is composed of definite and well-marked parts (Pl. 11, fig. 7, *mp.*). It lies in the homogeneous layer. The distal end, by which it opens to the exterior at the base of the wide micropylar funnel (*m.f.*), consists

of a well-defined straight tube narrower than the rest of the tube. It then expands into a more or less spherical dilatation, and is continued to the inner surface as a tube, somewhat wider than the first portion. Where it meets the inner surface of the shell, it expands into a funnel-shaped opening.

The micropylar funnel (Pl. 11, fig. 7, *m.f.*) is very similar to that in B. stouti, being cup-shaped; it cannot be described strictly as a funnel, as the bottom is somewhat flattened, as in this species and also in Myxine (**1**). It is wider than in B. stouti, being about ·4 mm. in greatest diameter as against ·25 mm. in this species. Its depth cannot be accurately determined, as its sides pass gradually into the bases of the surrounding filaments. The base and part of the sides are made up of the homogeneous layer, but, on its sides, may be seen in surface-view the polygonal markings, which occur in a more or less modified condition over the whole surface of the egg. They probably also occur on the base, but a surface-view of this region was not obtained. The markings are very superficial, and do not apparently extend inwards to the underlying layer. The funnel was filled with a structureless extraneous substance, the nature of which was not determined. This substance was also found forming a hard white encrusting layer on the shell between the bases of the filaments (Pl. 10, fig, 2).

The Anchor-Filaments.

The anchor-filaments of the Cape Bdellostoma are characterised by their shortness, the longest being 3·5 mm. They are, however, more numerous than in other species, there being about a hundred at each end. They are arranged more or less concentrically at the animal pole of the egg (Pl. 11, fig. 8). In the specimen drawn there were 117 of these filaments.

The outer filaments are shorter, and some are mere projections, apparently homologous with the projections on the

body of the shell, and have no anchors. In most cases, however, those without anchors have had them broken off.

There appears to be some considerable difference of opinion as to the nature of the anchor-filaments. A comparison of the egg-case and its filaments with those of Elasmobranchs, suggested by Thompson and considered unfavourably by Putnam and Cunningham, has little to justify it. Dean puts forward a very definite suggestion, which seems to be supported by the material at his disposal. It is that "the anchor filaments are homologous with the villi of the outermost (definitive) shell layer, zona striata. The anchor represents the highly-specialised bulb of the villus, its stalk the filament of the latter." In other words, the anchor-filament is a greatly enlarged and specialised column of the columnar layer, and the anchor is its head. The facts in favour of this view seem to be that in B. stouti no trace of the villi are found in or on the shell immediately surrounding the filaments, and that the filament with its anchor is constituted of the same elements as the villus or column of the columnar layer. Thus the filament was found to be homogeneous in its structure, with no trace of the stratified layer. There were, indeed, some fine striations found on the surface, but as these were merely superficial, and visible only under a high power of the microscope, no particular importance was attached to them. The surface of the anchors showed no pits, dots, nor irregularities, being smooth like the surface of the bulbous tip of the villus.

The egg-cases of the Cape Bdellostoma differ from those of the Californian species in these particulars, and this can be seen without minute examination by sectioning. Thus the polygonal markings, indicating the heads of the columns, can be clearly seen extending up over the surface of the egg-case towards the polar end and between the bases of the outer filaments. They extend on these bases, and, if such a filament and its base be cut out and flattened under a cover-glass (preferably after boiling in caustic potash) so as to obtain a clear surface-view, it is seen that the markings

extend up over the base to the stem of the filament (Pl. 11, fig. 9). From the base upwards they, however, gradually change from a polygonal to a more elongate shape, till, on the stalk itself, they appear as elongate striations. These are not specially fine, and can readily be seen with a Zeiss A objective. Towards the anchor they divide into groups of two or three according to the number of anchor-lips or projections; they spread out over the under side of these and end abruptly at their margins (Pl. 11, fig. 10). Between the bases of the more closely set inner filaments the markings were not polygonal, but somewhat elongate, as on the filaments themselves. The striations on the filaments are therefore apparently modified polygonal markings, and are the drawn-out heads of the columns.

If, now, the distal surface of the anchor be examined, no striations are seen, but a number of dark spots are very clearly visible, extending, not only over the central part, but on to the upper surface of the projecting parts or flukes of the anchor, where, however, they become fainter (Pl. 11, fig. 10). They suggest similar markings seen on the projections and the rings of the egg.

All this can be seen without more minute examination, but is made much clearer in sections. Thus a longitudinal section through the filaments shows that not only the stratified layer, but also the homogeneous layer, passes up into the bases of the filaments (Pl. 11, fig. 7). These branches of the homogeneous layer passing into the filaments are very conspicuous in such sections owing to their brownish colour, and were noted by Dean, who, however, describes them as the continuations of the bases of the filaments into the substance of the shell. Transverse sections of the filament itself (Pl. 12, fig. 11) indicate clearly that these elements are continued up into its main stem. The columnar layer is here well developed, though the caps of the columns are not so well marked. Internal to this is the stratified layer, and, occupying the centre, the homogeneous layer. The elements were seen only after prolonged treatment with caustic potash or by

slight heating. In untreated sections the filament appears to be solid and structureless, as Dean describes them in B. stouti, and, if actually boiled in the solution, unless the section is very thick, nothing is left except the outer striated layer representing the heads of the columns, as Dean describes and figures (his fig. 13).

Sections of the anchor (Pl. 12, fig. 12) show that here, also, the stratified layer is present, and constitutes the main body of this part, the outer surface being made up of the harder heads of the columns. The hook-like projections or flukes of the anchor are difficult to section, but they are apparently made up entirely of the heads of columns, which would account for their hard, tough nature. Their upper surface shows a number of dots, similar to those of the upper surface of the anchor generally, and their lower surface shows the striations characteristic of the surface of the filament.

The reasons for believing that the filament represents a modified villus or column of the zona striata, and the anchor its terminal bulbous end or head, seem to be convincing enough in the case of the Californian Bdellostoma, but, as is apparent from the above description, these reasons do not exist in the case of the Cape species, in which both the filament and its anchor consist of the columnar and stratified layer, along with the homogeneous layer (in the filament, but apparently not in the anchor). It may be suggested that the condition in the Californian species is a specialisation and modification of the more primitive condition found in the Cape species, and that the homogeneity of the filament and anchor are secondarily acquired. The fine striations in the filament noted by Dean may be significant in this respect.

Respiratory Apertures of Egg-Case.

Though the embryo of Bdellostoma is abundantly supplied with yolk, and presumably has, like the yolk-laden embryos of Elasmobranchs, a comparatively long period of development, there are are no special embryonic respiratory

organs, as in this group. More remarkable is the fact that there is apparently no means of introducing an adequate current of water for respiratory purposes, as in the Elasmobranchs. The absence of special embryonic respiratory organs may be compensated for by the highly vascular nature of the surface of embryo and yolk, or the functional activity of the gill-pouches at an early stage; but only somewhat uncertain indications of canals through the egg-membranes have been detected. It can hardly be doubted that a free supply of water must in some way pass through the tough egg-case, yet Dean could find no clear proof that canals passing through the shell existed. In the case of the Cape Bdellostoma no clearer indications of canals passing through the egg-case were seen, but there were numerous longitudinal fissures, some appearing as mere cracks in the surface, others passing completely through the egg-shell (Pl. 10, fig. 2). They became fewer near the opercular rings, and were not so marked on the polar side of the rings.

The mechanism whereby water can be drawn in through the fissures may well be the movement of the much-flattened body, though in the advanced embryo there is little space between the egg-case and the yolk in which this can take place.

External Characters of Embryo.

Of the two embryos one is slightly more developed than the other; both extend completely round the egg in the direction of its long axis; in one the tail overlaps the head-region by about one-third of the length of the egg (Pl. 12, fig. 13), while, in the less advanced one, this overlapping was only about one-fifth. The egg and embryo form a mass, which, like the egg-capsule, is somewhat curved, the more curved side representing the back of the embryo, which is still attached to the yolk. On the ventral side the embryo is quite free from the yolk, the point of separation, both in the case of the free head-region and the tail, being the extremities of the yolk. These free portions of the embryo are,

however, closely applied to the yolk, and lie in a shallow, wide furrow on itsventral aspect. Both portions are much compressed, and are somewhat twisted on themselves at the points where they become free from the yolk. This twisting is more marked in the tail-region, which comes to lie flat on its left side against the yolk. The compression thus is from side to side, so that the mucous sacs of the right side are visible on a surface-view. The anterior and longer free end of the embryo does not, however, undergo so much torsion, and this region is flattened, not laterally, like the tail-region, but dorso-ventrally, so that the mucous sacs are applied to the yolk, and are not visible from a superficial view. That there is a slight twisting, however, is shown by the fact that the mucous sacs of the right side are much closer to the margin of the body than those of the left.

These sacs are very prominent at this stage, and appear as little hillocks. They are specially well marked on the yolk, where about forty were counted. Here they lie about midway between the dorsal and ventral margin of the egg, being somewhat nearer the dorsal, so that they are visible in a dorsal, but not in a ventral view of the egg. They are thus further removed from the main axis of the embryo than in Bdellostoma stouti.

The length of the anterior free end from snout to the last gill-opening is 26 mm.; the length of the middle portion, which is attached to the yolk, is 21 mm.; the length of the posterior free end, from anus to end of tail, is 15 mm.; the total length of the embryo is, therefore, 62 mm.

Structure of Embryo.

As only two embryos were procured, it did not seem advisable to sacrifice more than one for detailed examination and sectioning. Owing to the advanced stage of these embryos, it was not to be expected that they could throw much further light on the development of the Myxinoids, and, so far as an examination has shown, the embryo, unlike the shell, does

not exhibit any primitive or specially instructive features. For an adequate examination a complete series is desirable, and this will doubtless be procured at some future time. One or two points, however, may be noted in connection with certain features of this stage.

The anterior free end of the embryo, when stained and viewed from the ventral aspect, shows that the various organs are well developed. Three pairs of tentacles and the tentacular skeleton are practically as in the adult; the cartilages of the naso-pituitary canal and of the olfactory capsule are well developed. The notochord is seen in transverse section to be much compressed from above downwards, and is apparently at this stage a very flexible organ, adapting itself readily, not only to the flattening, but also to the twisting of the body at the tail-region.

The condition of the excretory system is, however, of more interest at this stage. Its main features can readily be made out in a stained preparation. The pronephros measured about ·58 mm. in length, and occupied about one and a half segments of the body, judging by the position of the spinal ganglia. The mesonephros, consisting of a long segmental duct and twenty-two tubules with their glomeruli, was clearly made out. It is believed that the Myxinoids differ from all other Craniates, in that they alone have the mesonephric tubules strictly segmental. In this embryo, however, the anterior tubules were obviously much nearer each other than the more posterior, and were apparently not strictly segmental. This was confirmed by a series of sections, which showed that, between the posterior end of the pronephros and the first following spinal ganglion (right side), there were two tubules opening into the segmental duct; between this and the second spinal ganglia two tubules open into the segmental duct, and two between the second and third ganglia, there being thus six mesonephric tubules in three segments of the body behind the pronephros. Posterior to the sixth tubule of the mesonephros, there was one tubule to each segment of the body to the end of the mesophrones.

In front of the first tubule of the mesonephros is a tubule of special interest (Pl. 12, fig. 14, tu_1.). It appears in the stained preparation as a somewhat elongate straight tube, passing from the segmental duct into the pronephros, where it ends in a glomerulus. At the point where this tubule touches the segmental duct, the latter is interrupted (Pl. 12, fig. 14, *s.d.*), though immediately in front of and behind this point it has a complete lumen. There is, however, no distinguishable communication between the tubule and duct as in the tubules of the mesonephros. In front of this tubule and now completely in the pronephros is another (Pl. 12, fig. 15, tu_2.) of a similar nature, connected proximally with another glomerulus, and distally ending near the segmental duct, which is, however, now solid (Pl. 12, fig. 15, *s.d.*). The third tubule of the pronephros is also attached proximally to a glomerulus, and distally seems to end blindly. At the bases of the other tubules is a mass of vascular tissue, which might be the result of fusion of a number of glomeruli, while, at the distal end, they open by large nephrostomes into the body-cavity, the segmental duct having disappeared.

No undoubted trace of the further continuation of the segmental duct into the pronephros, after its disappearance at the distal end of the second last tubule, was discovered. Though the tubules sometimes expanded into wide cavities (Pl. 12, fig. 15 *tu.*), and were connected with each other, there appeared no sufficient reason to regard these as traces of the segmental duct (cf. Price (**6**)).

Summary.

(1) Five naturally deposited eggs of the Cape Bdellostoma have been found, two containing well-advanced embryos.

(2) The eggs are larger than those of other species, the anchor-filaments are shorter, and there are two polar rings.

(3) The general structure of the shell is similar to that described for other species, and there are numerous small projections on its surface, as in some species. These consist

of the columns of the columnar layer modified at the apex of the projection.

(4) There are numerous small fissures in the shell, probably respiratory apertures.

(5) The polar rings have a definite structure, differing from that of the rest of the shell, in that the inner layer becomes greatly enlarged, and the outer layer much reduced.

(6) The anchor-filaments are not homogeneous in their structure, but consist of all the layers of the shell, the chief modification being that the heads of the columns of the columnar layer becomes drawn out so as to appear as striations.

(7) The anchors consist of the modified columnar layer and the stratified layer. On their outer surface the heads of the columns of the columnar layer appear as disconnected dark dots, while their lower surface consists of the same elements as the surface of the filament.

(8) In the embryo the segmental duct occurs at the distal end of the last tubule of the pronephros, but, though having a lumen, does not open into it. It is found also at the distal end of the second last tubule, where, however, it becomes solid, and disappears. It was not found extending further into the pronephros.

(9) The tubules of the mesonephros are not strictly segmentally arranged, in that there are six tubules in three segments of the body behind the pronephros, though there is one tubule for each succeeding segment, as far as the mesonephros extends.

I am greatly indebted to Prof. Bashford Dean for his generous assistance in literature on the early stages of Bdellostoma and allied subjects. As a pioneer in this work, he is deeply interested in the finding of the eggs of the Cape Bdellostoma. Prof. Price has also kindly sent me reprints of his important papers on the development of the excretory organs.

I have also to express my obligations to Mr. P. MacManus, who has redrawn for me figs. 2 and 10.

References.

1. Cunningham, J. T.—"On the Structure and Development of the Reproductive Elements in Myxine glutinosa, L.," 'Quart. Journ. Micr. Sci.,' vol. 27, 1887.

2. Dean, Bashford.—"On the Embryology of Bdellostoma stouti," Kupffer's 'Festschrift,' 1899.

3. ——— "Notes on Japanese Myxinoids," 'Journal of the College of Science, Imperial University of Tokyo,' vol. xix, art. 2, 1904.

4. Mark, E. L.—'Bull. Mus. Comp. Zool.,' vol. xix, 1890.

5. Plate, L.—"Ueber die Eier von Bdellostoma bischoffii, Sch.," 'Sitzber. Ges. Nat. Fr. Berlin,' 1896.

6. Price, G. C.—"Development of the Excretory Organs of a Myxinoid, Bdellostoma stouti," 'Zool. Jahrb. Abth. Anat. Ontog.,' Bd. x, 1897.

7. Putnam, F. W.—"Notes on the Genus Myxine" (p. 127), and "Notes on the Genus Bdellostoma" (p. 156), 'Proc. Bost. Soc. Nat. Hist.,' vol. xvi, 1873.

EXPLANATION OF PLATES 10–12.

Illustrating Dr. J. D. F. Gilchrist's "Note on Eggs and Embryos of the South African Myxinoid, Bdellostoma (Heptatretus) hexatrema, Müll.

PLATE 10.

Fig. 1.—Photograph of eggs of Bdellostoma as received. *a–e*. The eggs. a^1. Embryo removed from *a*.

Fig. 2.—Egg showing papillæ, polar rings, anchor-filaments and fissures.

PLATE 11.

Fig. 3.—Polygonal markings on surface of egg up to clear surface of papilla.

Fig. 4.—Vertical section through papilla. *col. l.* Columnar layer. *hom. l.* Homogeneous layer. *pap.* Papilla. *str. l.* Stratified layer.

Fig. 5.—Section across polar ring. *col. l.* Columnar layer. *h. col.* Heads of columns. *hom. l.* Homogeneous layer. *str. l.* Stratified layer.

Fig. 6.—Longitudinal section through shell of egg near outer filaments, showing change in layers. *f.* 1. Filament cut at side. *f.* 2. Filament cut near middle and broken.

Fig. 7.—Longitudinal section through apex of shell showing micropyle. *f.* Filament. *h. l.* Homogeneous layer. *m. f.* Micropylar funnel *mp.* Micropyle.

Fig. 8.—Arrangement of filaments at animal pole of egg. *a.* Surface-view. *b.* Longitudinal section.

Fig. 9.—Surface-view of part of base and stem of an outer filament, to show change from polygonal markings to longitudinal striations.

Fig. 10.—Anchor showing longitudinal striations on filament passing up to under surface of anchor, and dark dots on upper surface.

PLATE 12.

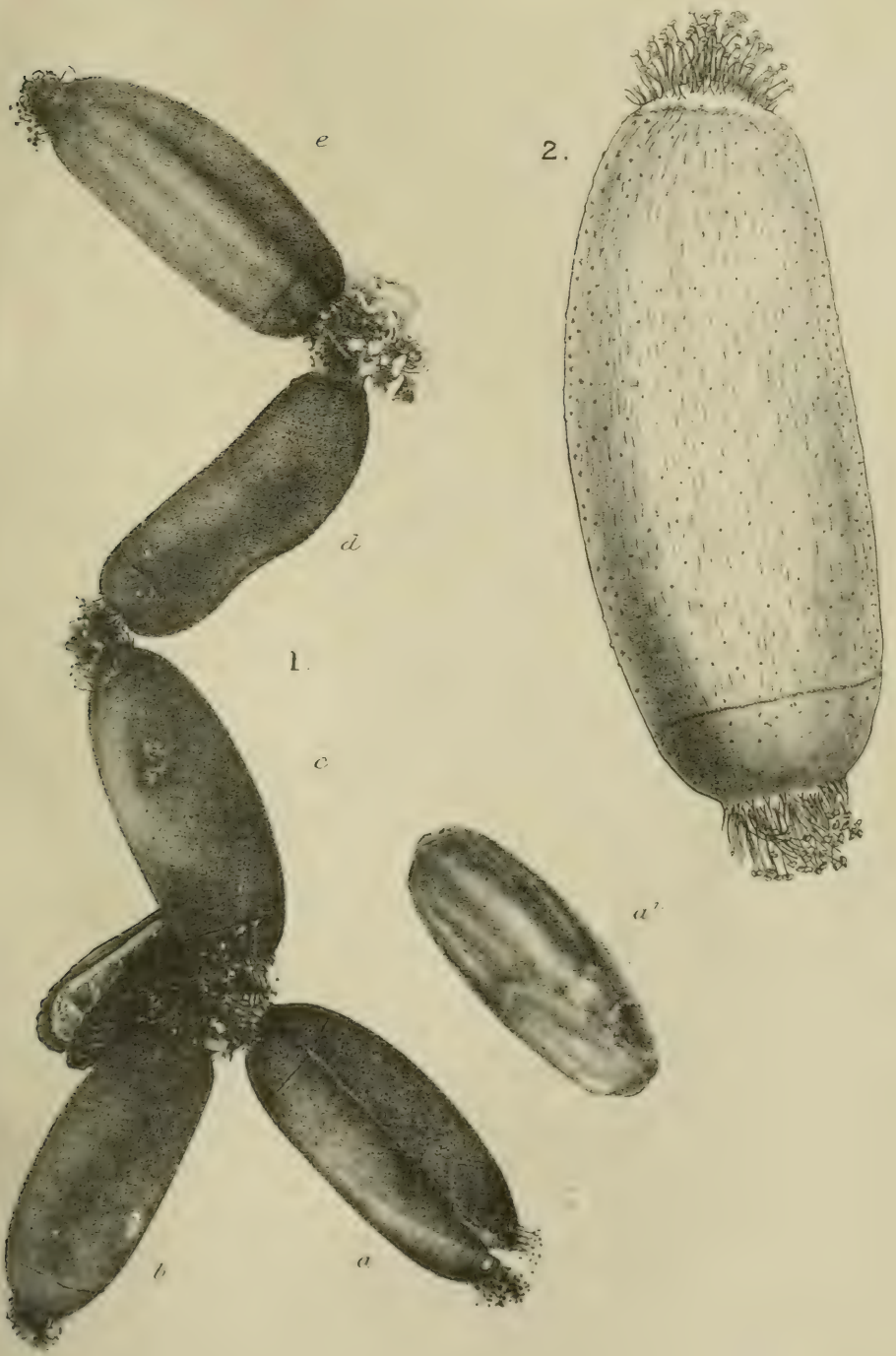
Fig. 11.—Transverse section of filament. *col. l.* Columner layer. *hom. l.* Homogeneous layer. *str. l.* Stratified layer.

Fig. 12.—Vertical section of anchor. *h. col.* Heads of columns. *str. l.* Stratified layer.

Fig. 13.—Ventral and two lateral views of embryo.

Fig. 14.—Transverse section of pronephros at last tubule. *b. c.* Body-cavity. *bl.* Blood-vessel. *bo. c.* Bowman's capsule. *gl.* Glomerulus. *s. d.* Segmental duct. tu_1. Last tubule of pronephros.

Fig. 15.—Transverse section of pronephros at second last tubule. tu_2. Second last tubule of pronephros. *tu.* Tubule showing enlargement. Other letters as in fig. 14.



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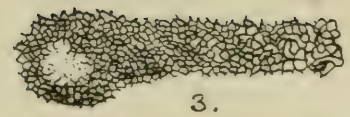
GILCHRIST — BDELLOSTOMA EGGS & EMBRYOS.



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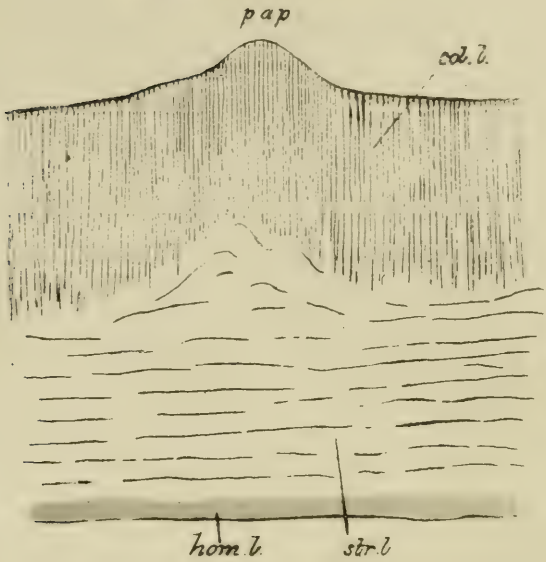

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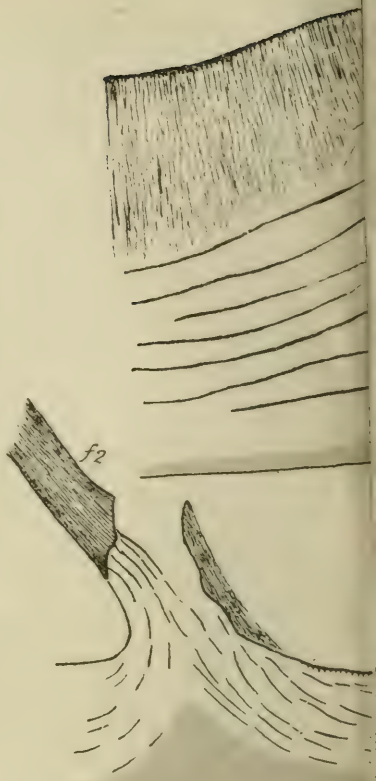

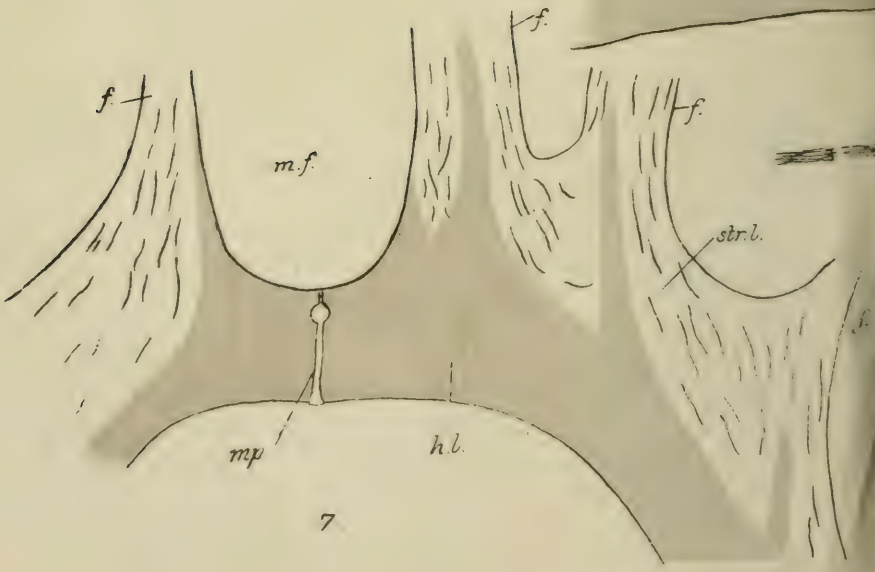

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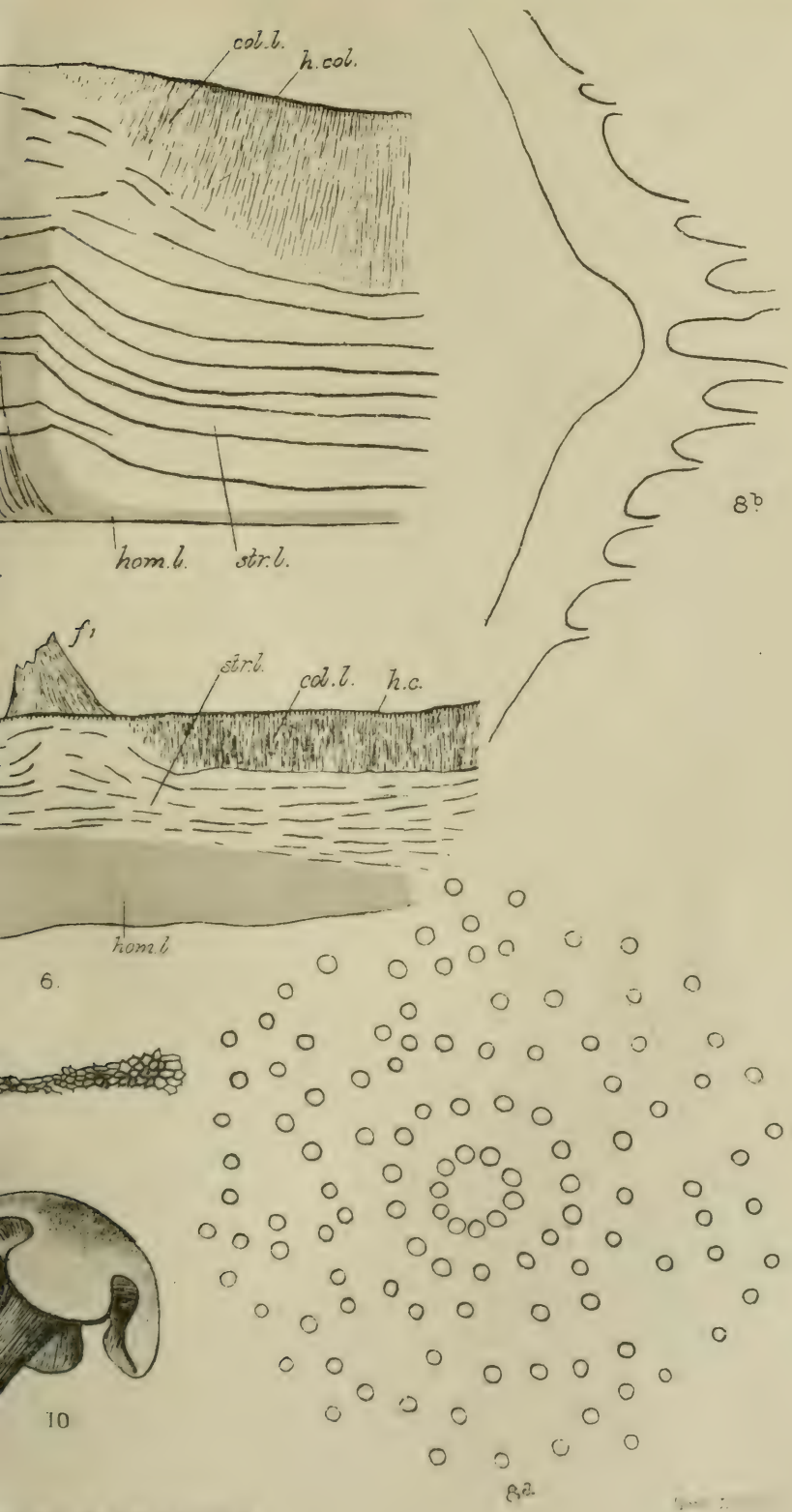
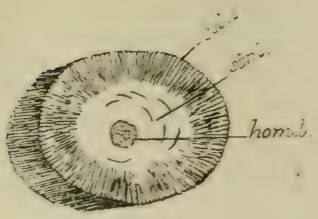


EGGS & EMBRYOS



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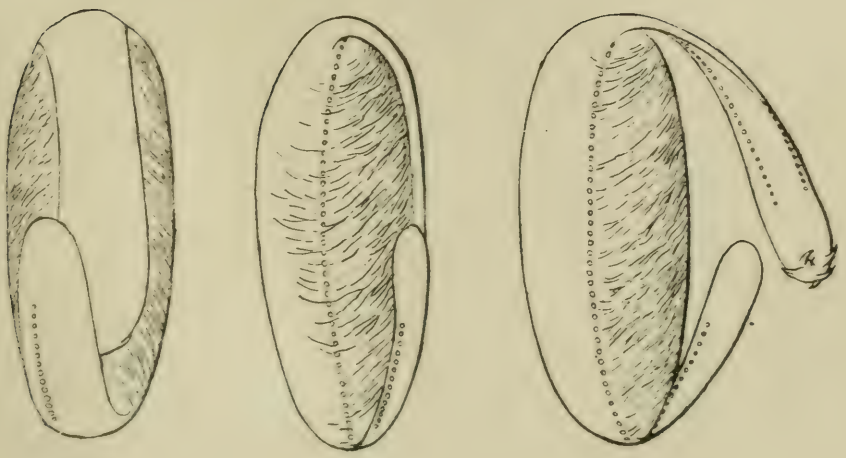

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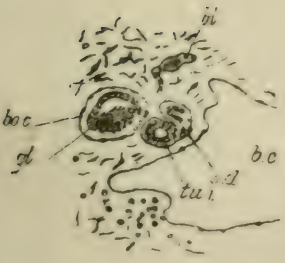

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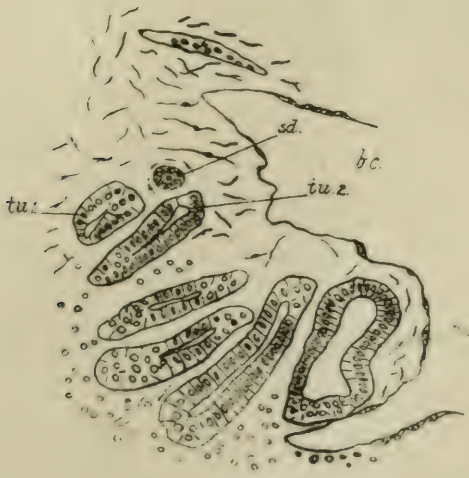

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GILCHRIST — BUELLOSTOMA EGGS & EMBRYOS

The Segregation of the Germ-cells in Trichogramma evanescens.

By

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With Plate 13 and 1 Text-figure.

CONTENTS.

INTRODUCTORY.

IN a previous paper (4) I described a number of stages in the development of Trichogramma evanescens, but at that time I was unable to follow out the segregation of the germ-cells. Since then I have collected more material, and in this paper I am able to describe the missing stages.

As far as I am aware, this is the only work that has been

done from the minute cytological aspect on the segregation of the germ-cells, and the segmentation of the egg in a monembryonic egg parasite. The presence of a germ-cell determinant at the posterior pole of the egg adds to the interest of a study of this form.

Previous Work.

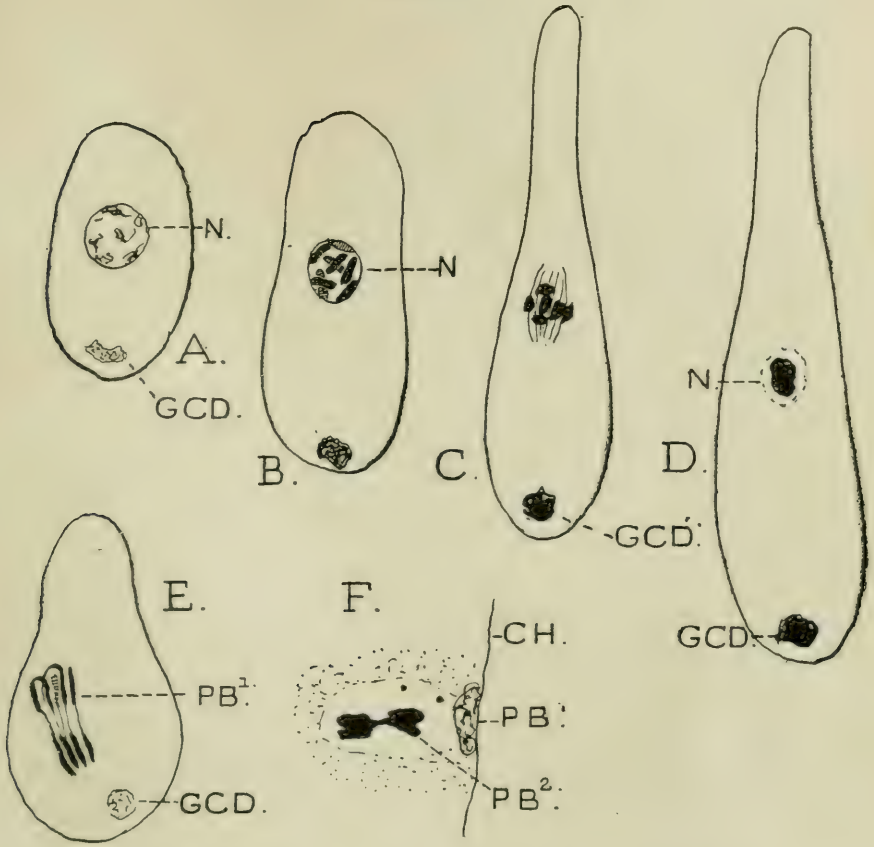
Silvestri (1) has examined a form, Oophthora semblidis, which is another species of the genus Trichogramma, Oophthora being a synonym for the latter.

Oophthora parasitises the eggs of Mamestra brassicæ.

The young ovarian egg contains a normally reticulate nucleus and this condenses to form a solid mass of chromatin surrounded by zona. (For this process see Text-fig. 1.) This solid nucleus may, according to Silvestri, regain its reticulate open structure ((1) p. 74, Pl. 1, fig. 6), but the polar body is drawn dividing without a clear aster or centrosomes almost as I have drawn it in my previous paper. After the extrusion of the polar body or bodies, the egg nucleus looses its solid form and becomes normally reticulate, as does the sperm nucleus if the egg has been fertilised. The two fuse, and Silvestri distinctly draws a normal zygote nucleus.

The zygote nucleus now divides with a fairly normal spindle, apparently with centrosomes ((1), p. 74, Pl. 1, figs. 13 and 14), but without asters around the centrosomes. Subsequently the segmentation nuclei divide many times by mitosis and produce a large number of normally reticulate nuclei. These migrate to the surface of the egg, leaving a few in the centre. After migration the somatic nuclei condense to form a solid mass of chromatin surrounded by a lighter sphere. The segregation of the germ-cells is brought about by several of the segmentation nuclei wandering down to the region of the germ-cell determinant. In another later paper (3) Silvestri has examined several other monembryonic parasites whose development is similar to that of Oophthora. In all there is a distinct germ-cell determinant.

TEXT-FIG. 1.



A., *B.*, *C.*, and *D.* are consecutive stages in the formation of the solid oocyte nucleus from a normal reticulate nucleus (*A.*). The chromosomes appear (*B.*), and a spindle is formed (*F.*), which is not typical; gradually the chromosomes, instead of dividing and passing to the poles of the spindle, conglomerate and entirely fuse to form the solid nucleus in *D.* (Original, from an Aphid parasite, probably a Chalcid.) *E.* Extrusion of first polar body in Ageniaspis (Encrytus) showing the non-typical "spindle." (Martin (5).) *F.* Anaphase of second polar body in Trichogramma showing the non-typical "spindle." (4). Smaller capitals used as reference letters have the following significations: N. Nucleus; GCD. germ-cell determinant; PB¹. and PB². first and second polar bodies; CH. Chorion.

Technique and some Remarks on the Sizes of the Eggs.

On Pl. 13 the eggs in figs. 1, 2, 3, 4, 6, and 18 are all drawn to the same scale. It will be noticed that their sizes differ often quite considerably. In Pl. 13, figs. 9, 10, 11, 12, 13, 14, and 19 are drawn from the widest part of the egg. Here, also, wide differences in size occur. If Pl. 13, figs. 2 and 3, and figs. 9 and 19 be compared it will be seen that the amount of chromatin differs considerably in bulk in the several eggs. Moreover, the germ-cell determinants in Pl. 13, figs. 1, 2, and 7 are of a different kind from those drawn in the other figures.

When I sent a number of Trichogrammas for identification to the Rev. J. Waterston he pointed out that there might possibly be more than one species, but that this could not be ascertained without closer examination. Nevertheless, it should be remembered that all the eggs in the ovary of the insect are graded in size, the lowermost eggs being largest, while those higher up the ovarian tube would be somewhat smaller. In connection with the amount of chromatin in the egg, an explanation might be found in the number of polar bodies extruded (whether one or two) or in whether the egg was fertilised or not.

Technique was the same as that previously used, only some material was fixed in Carnoy, while other was treated for differing times in Gilson-Petrunkewitsch (from three to twenty-four hours).

Certain Stages in the Later Ovarian Oocyte.

In a very large number of forms (1, 3, 4, 5, 6, 7) it has been found that the egg nucleus while in the ovary, just before deposition, undergoes a most remarkable process. This is shown in Text-fig. 1. At first the oocyte nucleus is normally reticulate, but soon chromosomes appear in it (Text-fig. 1, *A*. and *B*.). The nuclear membrane now breaks down, and an imperfect "spindle" becomes established; in some cases the spindle seems to have fibres and a doubtful

aster with centrosomes (Martin (**5**)), in other cases (Hegner (**6**) and the forms I have examined) there are apparently either spindle fibres without a centrosome at each pole, or only a clear space in the egg. In this region or on the imperfect spindle the chromosomes lie, and at this stage, instead of splitting and their halves pulling apart as in normal mitosis, the chromosomes gradually close up together and fuse to form a blackly-staining mass (iron hæmatoxylin). The "spindle" now disappears (Text-fig. 1, *C.* and *D.*) and the nucleus appears to be quite solid. When the first polar body is in process of formation, in most cases no distinct centrosome or aster appears. The chromosomes may appear at this stage (Martin (**5**)), but each individual chromosome divides by itself; in other cases, no chromosomes could be found (**4**) and the polar bodies seem to be extruded by an amitotic figure.

Text-fig. 1, *E.*, is a copy of Martin's figure of Encyrtus showing the remarkable "spindle" of the first polar body.

The Germ-cell Determinant.

(*a*) In the Unsegmented Egg.—In the previous paper I described the germ-cell determinant in the unsegmented egg. It was pointed out that the germ-cell determinant arose as a cloud of granules, which seemed to make their appearance spontaneously in the cytoplasm, and that in the egg before the formation of the polar bodies, the determinant stained densely black with iron hæmatoxylin.

I am able to add a few more facts to my original description; in some of the eggs I have examined, the determinant instead of being solid was found to be formed of a number of separate regularly-shaped granules (Pl. 13, figs. 1, 2, and 7). In these cases it was of the same size and appearance as the solid determinant, the individual granules staining very darkly.

(*b*) During the Formation of the Germ-cells.—After the segmentation nuclei are from fifteen to twenty-five in number, they have begun to pass to the poles of the egg.

Several which go to form the future germ-cell nuclei lie near the determinant. This is seen in progress in Pl. 13, figs. 4, 8, and 15, and so far the nuclei themselves are unaltered. In Pl. 13, fig. 16, the nuclei (*G.N.*) in close proximity to the germ-cell determinant have begun to alter and to differ from the other nuclei (*B.N.*) which form the blastoderm.

It is just at and after this stage that the germ-cell determinant begins to alter.

In Pl. 13, fig. 6, an interesting stage is drawn. The blastoderm nuclei are very chromatic, while at the pole of the egg is seen the germ-cell determinant. The latter had become basin-shaped, and in the hollow of the basin lie about half a dozen paler nuclei; the edges of the hollow cup-like determinant have become frayed and pieces are breaking off (*F.G.C.D.*). In Pl. 13, fig. 17, the same egg is drawn at a higher power after the microscope had been focussed down a little. The determinant is seen in optical section and in its hollow lie the pale germ-cells. The fragments of the determinant (*F.G.C.D.*) are here plainly seen, while the difference between blastoderm nuclei (*B.N.*) and germ-cell nuclei is marked.

In Pl. 13, fig. 5, another egg is drawn at a slightly earlier stage. The determinant has become a little irreglar in shape, its spherical contour becoming altered to give place to the basin shape. Very often the posterior end of the egg becomes constricted as in Pl. 13, figs. 5, 16, and 18, and marks the place where the germ cytoplasm joins the blastoderm cytoplasm. It is very difficult to say how this constriction is brought about, but it is probably caused by a contraction of the cytoplasm in the form of a band around the posterior end of the egg. In other forms (Encarsia, Silvestri (**3**)) the constriction and consequent protrusion of the germ-cells is marked. After the stage drawn in Pl. 13, fig. 17, the determinant fragments more, and gradually becomes absorbed; but before this happens each germ nucleus has formed around it a cell wall containing part of the cytoplasm from the end of the egg.

The loss of chromaticity of the germ-cell nuclei is synchronous with the dissolution of the germ-cell determinant. In some eggs it is not a little difficult to say how many nuclei are forming germ-cells at the posterior pole of the egg; and, moreover, at such a stage as that in Pl. 13, fig. 16, it becomes somewhat difficult to distinguish between blastoderm and future germ-cell nuclei.

The germ-cell determinant appears to be of a plastic nature, for were it not so the occurrences which take place as depicted in Pl. 13, fig. 17, would never happen. Moreover, in many cases the germ-cell determinant in the unsegmented egg, or during the time the egg is segmenting, often shows curious lumps on its surface, as in Pl. 13, fig. 3 or 16 at *XX*. In a few cases one can find large stray pieces of germ-cell determinant at a stage before the latter begins to fragment (Pl. 13, fig. 4, *G.X.*), and in a very large number of examples one finds small granules scattered in proximity to the germ-cell determinant (Pl. 13, figs. 1, 3, and 15, *G.*). These granules occur here and there throughout the egg (Pl. 13, figs. 9, 12, and 13). The exact nature of such bodies is unknown to me. In my previous paper I found them near the second polar body "spindle" ((**4**), Pl. 13, fig. 11 *A*). I feel sure that these grains are not centrosomic in nature, but those found in the segmenting egg may be broken off pieces of chromatin.

In Pl. 13, fig. 19, is drawn a transverse section through the middle of an egg; the latter seemed about to give off a polar body, but the granules (*G.*) were not, I consider, the degenerated polar bodies. In Pl. 13, fig. 9, such granules were present in large numbers.

The Segmentation of the Egg.

In my previous paper (**4**) I described the egg just after deposition and the extrusion of the first polar body. In Pl. 13, figs. 9 and 19, are transverse sections of two eggs; in both the nucleus is not of the usual reticulate kind, but is formed of an irregularly-shaped, darkly-staining chromatin lump. In Pl. 13, fig. 19, the nucleus has gone to the surface

of the egg and seemed to be elongating preparatory to the formation of a polar body. In Pl. 13, fig. 9, the nucleus was distinctly constricted in the middle as if it were dividing by amitosis; in my figure of the second polar body in my previous paper (**4**) the "spindle" is a dumb-bell-shaped chromatin mass, quite unlike any stage in mitosis.

In Pl. 13, fig. 1, the egg contains two densely staining, apparently solid nuclei; at *P.* is an elongate body, which is the remains of either one or two polar bodies; in Pl. 13, fig. 13, another egg is drawn in transverse section; the nuclei are chromatin lumps, and at *X.* the nucleus is constricting as if in amitosis. In Pl. 13, fig. 2, all the nuclei are apparently in division, and all are solid dumb-bell-shaped figures. Another egg is drawn in Pl. 13, fig. 3; at *X.* one nucleus is distinctly constricting; this is more plainly shown at a higher power in Pl. 13, fig. 8.

I have examined a large number of eggs; stages in division are rather difficult to find, but in no one case did I find any stage resembling karyokinesis; without exception every nucleus in early segmentation stages is solid, blackly staining, and no amonnt of extraction reveals a reticulate structure. When the nuclei are from twenty to fifty in number they gradually disperse as division goes on, till they wander towards the poles of the egg (Pl. 13, figs. 2, 3, and 8). The germ nuclei are then segregated as previously explained; those which happen to lie near the germ cell determinant eventually forming germ-cells. As soon as segmentation has gone on to such a stage that nuclei occupy the egg in the manner drawn in Pl. 13, figs. 5 and 10, segmentation stops; no further nuclear divisions take place till at a later stage. When the egg has reached the stage when the nuclear division temporarily ceases and the end of segmentation arrives, it contains some 120 to 150 nuclei. The number seems very variable. Just at or before this final period differences become apparent in the nuclei. In Pl. 13, fig. 15, nearly all the nuclei are solid, but at *B.N.* one nucleus has become frayed at its edges, and a nuclear membrane becomes

demonstrable; the same and later stages of this process are seen in Pl. 13, figs. 5, 16, and 17. By this process all the nuclei eventually become spherical or ovoid structures containing coarse chromatin lumps.

Now it is constantly found that the chromatin lumps in the nuclei are specially oriented after a while. The main mass of chromatin is seen to lie outwards, while inwards is left quite often a single grain, or two grains, of chromatin, as shown in Pl. 13, fig. 14.

The segmentation nuclei lie throughout the whole region of the egg, as shown in Pl. 13, fig. 10, or in an earlier stage in Pl. 13, fig. 3. When the changes in the solid nuclei take place, which cause the formation of the roughly reticulate nucleus to appear, many, if not all, the nuclei lying in the central middle region of the egg seem to undergo this process, but soon afterwards they become dark again, as shown in Pl. 13, figs. 4, 10, 11, 12, and 13. In Pl. 13, fig. 10, this is seen in progress; there are thirteen nuclei in the field, four are markedly blackly staining; one of these lies at the periphery of the egg. In the centre are two black nuclei, and one healthy one; in Pl. 13, fig. 11, all the central nuclei had shrunken and become black (staining), and one at the upper peripheral region had also changed in this way. In Pl. 13, fig. 4, of the twenty-two central nuclei, three were of the rough reticulate form, while all the others were solid; this figure shows that many centrally disposed nuclei do become roughly reticulate before they degenerate. In Pl. 13, figs. 5, 6, 12, 14, and 18, subsequent stages are drawn.

The fate of these discarded chromatin lumps has already been followed out (**4**).

The Peculiar Form of the Blastoderm Nuclei and its Significance.

As far as I am aware, no other case is known in which such a peculiarly oriented somatic nucleus is found. In Pl. 13, figs. 10, 11, 14, 17, and 18, the usual form of somatic nucleus

is seen; there is nearly always an inner grain and an outer larger lump to the nucleus (see (4) previous paper). In several cases the outer lump was present (Pl. 13, figs. 4 and 12), but no inner lump could be found. I am quite at a loss to account for the remarkable form of the nuclei in this Hymenopteron. The only suggestion I can make is that the inner granules represent that chromatin, which is unnecessary and will be extruded, and the reason for the special orientation of this little granule inwards is that the inner region of the egg is at this period the dumping-ground of waste matter. As I pointed out before (4) this inner matter is later extruded. It should also be remembered that the egg at this period is growing without vitellophags or yolk which are found in other insect eggs.

The Central Nuclei which Degenerate (Pl. 13, figs. 4, 5, 6, 10, 11, 12, 14, and 18).

In an ordinary insect's egg, after the zygote nucleus has divided many times in the interior of the egg, a large number of these nuclei migrate to the surface to form the blastoderm. Certain others are left inside the egg, and these form the yolk cells or vitellophags, whose function is to make ready for absorption the stores of yolk and fat which fill the egg. These nuclei, with their island of surrounding cytoplasm, live long after the formation of the mesenteron, and in the majority of cases become very large in the process of attacking the yolk stores.

There seems to be little doubt that the centrally placed nuclei which degenerate in the case of Trichogramma are the representatives of the vitellophags of other holometabolous insects. The reason they degenerate seems to be that they have no yolk to utilise for the food of the embryo, since the latter gains its nutriment in another manner. I consider the subsequent extrusion of a part of the centre of the egg containing these nuclei has no other significance than that of a method of removing from the egg waste matter (previous paper (4), p. 162).

The Cytoplasm.

The eggs of all the figures, with the exception of Pl. 13, figs. 9, 18, were fixed for four hours in Gilson-Petrunkewitsch: those of Pl. 13, figs. 9 and 18, were fixed overnight in the same fixative; in the latter drawings the vacuoles were more markedly reticulate, while in the other figures the vacuoles were smaller, evener, and the entire cytoplasm less "stringy"; this is the usual effect, which is due to the length of exposure to the solvent properties of the acetic-alcohol of the fixative. When the egg reaches the stage drawn in Pl. 13, figs. 10 and 12, each nucleus is often seen to be surrounded by a halo of cytoplasm. This seems preparatory to the establishment of a cell-wall boundary between each nucleus (see Pl. 13, fig. 18). I could not find the boundary line till just before the stage drawn in this figure, and, moreover, this line did not entirely surround the nucleus, only passing inwards and ending abruptly. This does not apply to the germ nuclei, which are surrounded by a complete cell-wall. It seems that in later stages this cell-wall around the outer peripheral edge of each blastoderm nucleus is obliterated when the germ-layers are being formed. In certain other forms Silvestri (**2**) describes a like occurrence.

Discussion.

Germ-cell Determinant.—It is not proposed to discuss the determinant at any length in this paper, but it can be pointed out that this structure cannot consist altogether, if at all, of mitochondrial matter. This seems to be shown by the fact that Carnoy and Petrunkewitsch and Flemming-acetic apparently do not dissolve away any part of the determinant. The latter appears to react to fixatives and stains in much the same way as Amphibian yolk discs, but at present I take the view that the germ-cell determinant of Trichogramma consists in part or whole of cytoplasmic material.

Though it is difficult to be sure, I think that the function of the determinant is nutrimental. The germ-cells early become segregated from the somatic region of the egg, and while the somatic nuclei are, so to speak, undergoing phenomenal changes, losing chromatin, becoming altered from time to time or occasionally degenerating, the germ nuclei remain resting. It is tempting to consider that the dense germ-cell determinant is, during this time and later on, acting as a reserve store, while many changes take place in the somatic nuclei. The latter must needs procure their nutriment from the Donacia (host) egg, and during this they are also becoming disposed to form the germ-layers. The function of the determinant seems to be that of preventing the germ-cell nuclei from being exposed to the uncertain conditions existing elsewhere in the segmenting egg and embryo.

It is also noteworthy that the germ nuclei at first differ not at all from the other segmentation nuclei, and certain nuclei become germ-cell nuclei only because of their accidental position in the egg, towards close of segmentation, in the region of the posterior pole of the egg.

With regard to the important question of the segmentation of the egg, it seems clear that typical mitotic spindles do not exist at first. There seems to be a great many interesting facts with regard to the nuclear behaviour during early stages in many of the parasitic Hymenoptera, and I feel sure that further research on this subject would repay the expenditure of time and patience needed to study these interesting forms. It may be possible that the stages I have found which look so much like amitosis are really anaphases during which the chromosomes have fused, but it is now an established fact that in certain forms no centrosome or normal spindle exists at special periods of segmentation (**1**, **4**, **5**), and I consider that neither centrosomes nor mitotic spindles of any sort will be found in Trichogramma in early stages.

Bibliography.

1. Silvestri, F.—"Contribuzioni alla conoscenza biologica degli Imenotteri parasitici i–4," 'Boll. Scuol. sup. Agric. Portici,' T. 1 and 3.

2. ——— "Prime fasi di svillupo del Copidosoma buyssoni (Mayr), Imenottero Calcidide," 'Anat. Anz.,' Bd. 47.

3. ——— "Struttura dell'ovo e prime fasi di sviluppo di alcuni Imenotteri parassiti," 'Boll. Scuol. sup. Agric. Portici,' vol. x, 15.

4. Gatenby, J. Bronté.—"The Embryonic Development of Trichogramma evanescens," 'Quart. Journ. Micr. Sci.,' vol. 62, pt. 2.

5. Martin, F.—"Zur Entwicklungsgeschichte des polyembryonalen Chalcidiers Ageniaspis (Encyrtus) fuscicollis, Dalm.," 'Zeit. wiss. Zool.,' Bd. 110.

6. Hegner, R.—'The Germ-cell Cycle in Animals,' Macmillan, 1914.

7. ——— "Studies on Germ-cells IV," 'Journ. Morph.,' xxvi, 15.

EXPLANATION OF PLATE 13,

Illustrating Mr. J. Bronté Gatenby's paper on "The Segregation of the Germ-cells in Trichogramma evanescens."

Lettering.

X. dividing nucleus (?). *B.N.* Blastoderm nucleus. *D.N.* Degenerated nucleus. *F.G.C.D.* Fragments of germ-cell determinant. *G.* Granule of unknown nature. *G.C.* Germ-cells. *G.C.D.* Germ-cell determinant. *G.N.* Germ-cell nucleus. *N.* Nucleus.

Figs. 1, 2, 3, 4, 6, and 18, drawn at magnification of 1383. Figs. 5, 7, 8, 9, 10, 11–17, and 19 drawn at magnification of 2100. Fixed either in Carnoy or Gilson-Petrunkewitsch, stained in iron hæmatoxylin.

Fig. 1.—Segmentation stage, two nuclei, at *P.*—either one or two polar bodies came into focus at a different level. Germ-cell determinant granular.

Fig. 2.—Later stage, about ten nuclei, apparently dividing by an amitotic figure. Germ-cell determinant as in Fig. 1.

Fig. 3.—Later stage, nuclei "nesting" except at *X.*, where one seems to be dividing. Germ-cell determinant solid.

Fig. 4.—End of segmentation, inner nuclei necrotic. Staining blackly.

Fig. 5.—End of segmentation, showing segregation of germ-cell nuclei near determinant. At *D.N.* are the inner degenerated nuclei.

Fig. 6.—Blastoderm stage nearing the time when the germ-cells become completely isolated.

Fig. 7.—Posterior pole of egg showing granular germ-cell determinant.

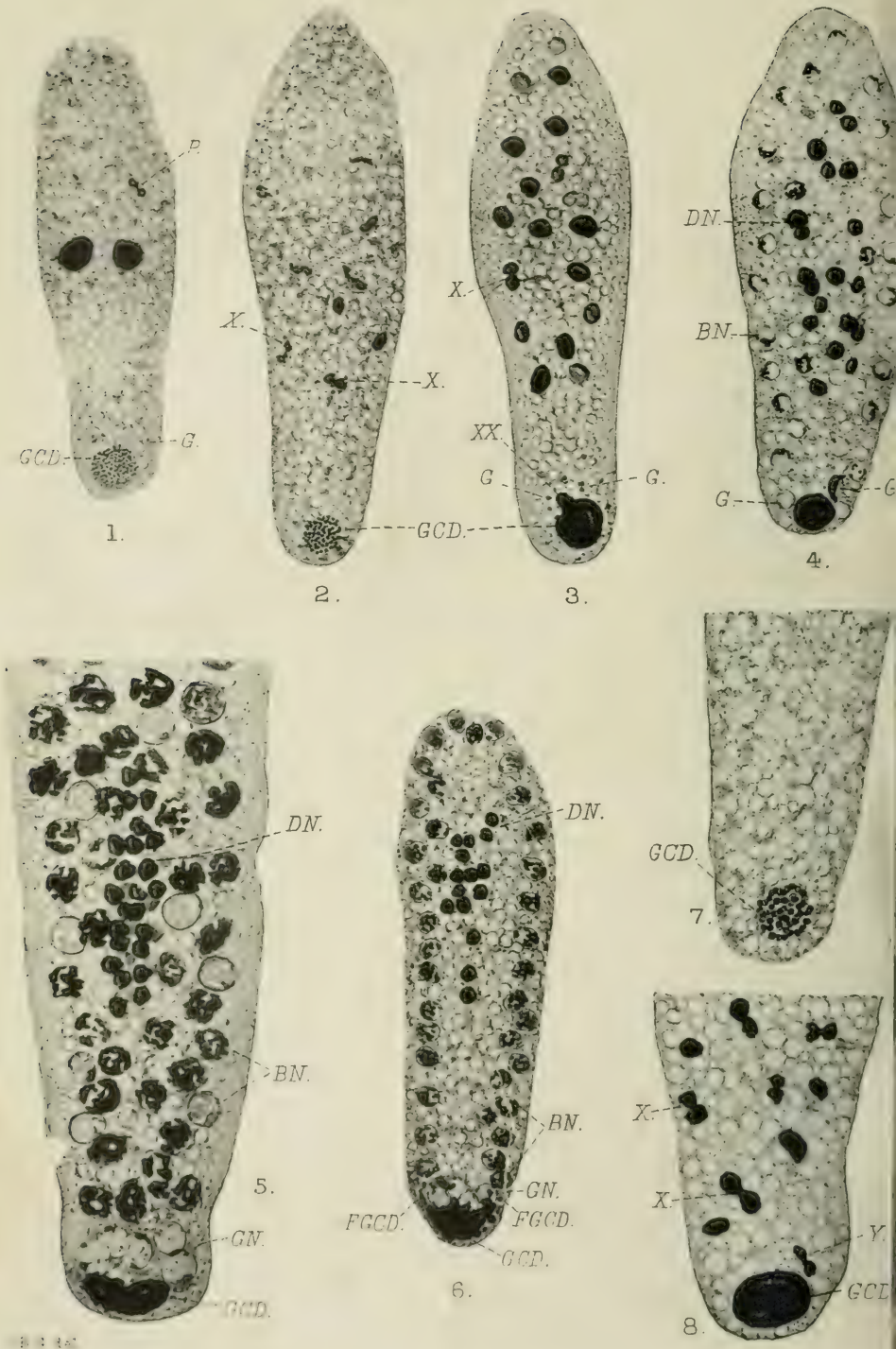
Fig. 8.—Lower part of posterior pole of egg showing dumb-bell shaped "spindles" *X.*, at *Y.* future germ nucleus, cut in half, another part in the next section.

Figs. 9–14.—Transverse sections of mid-region of egg. Fig. 9, egg just laid, nucleus apparently beginning to constrict preparatory to formation of first polar body. Figs. 10, 11, 12, and 14, stages in segmentation showing peculiar nuclei. Fig. 13, early segmentation stage showing irregular nuclei.

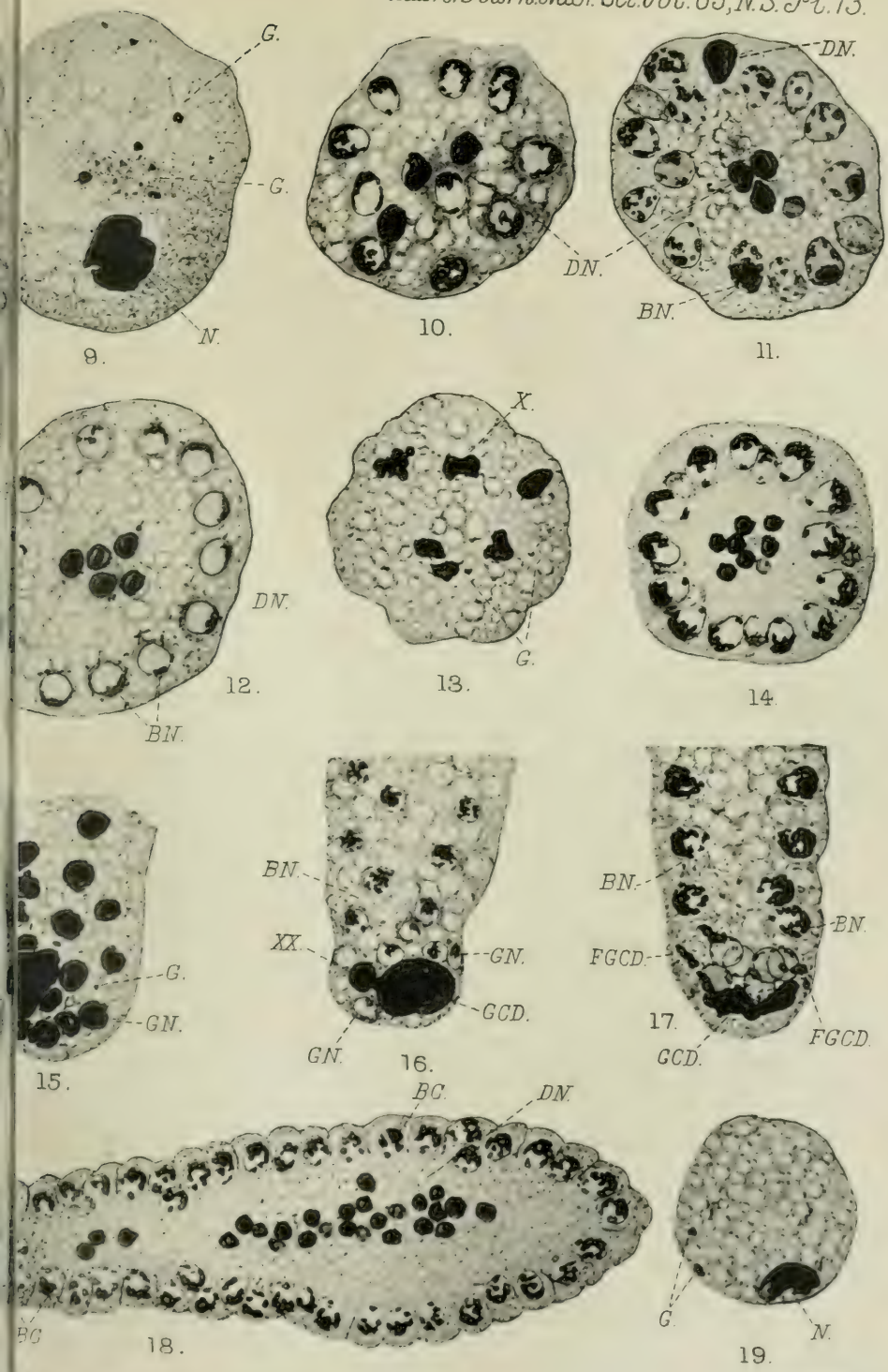
Figs. 15, 16, and 17.—Three stages in the segregation of the germ-cells. Fig. 17 drawn from same egg as that in Fig. 6, only at a lower focal level.

Fig. 18.—Blastoderm after segregation of germ-cells.

Fig. 19.—Unsegmental egg, nucleus near surface preparatory to extruding polar bodies.



GATENBY — SEGREGATIO



LLS IN TRICHOGRAMMA.

Huth, London

Polyembryony in Parasitic Hymenoptera: A Review.

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With Plates 14 and 15.

CONTENTS.

INTRODUCTORY.

AMONG the specially remarkable facts in the study of embryology that of polyembryony or germinogony in Parasitic Hymenoptera is not the least noteworthy. Polyembryony consists in the production from one single egg, by a process of gemmation, of a large number of separate embryos. Polyembryonic species of Hymenoptera are known from the Chalcididæ and the Proctotrypidæ; many of these insects are jet

black or brownish creatures about 1 or 2 mm. in length, with gaudily sheeny wings and bodies; in many cases they are so small as to be almost invisible to the naked eye, in others these insects are much larger, but British species above 5 mm. in length are not common.

In connection with the word Hymenoptera one naturally associates thoughts of the wonderful habits and life-histories of Ants, Bees, and Wasps, but among such parasitic forms as the Chalcids one finds instincts and wonderful life-histories just as remarkable as in other forms. Within the last decade a great many new facts have been ascertained with regard to polyembryony, principally by Marchal (**5**), Silvestri (**9**), Martin (**14**), and Patterson (**13**).

Bionomical.

Holometabolous insecta from several orders are known to be the hosts of polyembryonic Hymenoptera, but the majority of the latter parasitise moth or butterfly larvæ. Pieris brassicæ is a martyr to all kinds of hymenopterous parasites.

Prof. Martelli (**17**) has given a list containing fifteen parasites and fourteen hyperparasites on this common Lepidopteron, and of course all such parasites have a value from an economic viewpoint. In the Oxford district Apantales glomeratus[1] is responsible for the slaughter of thousands of Pierid caperpillars, while a hyperparasite Mesochorus pallidus takes a large toll on the parasitic Apantales. In the small extent of a little cabbage-patch one then realises that wars and counter-wars are raging between the various parasitic fauna. The polyembryonic parasite seeks among the leaves of the food-plant of the host caterpillar for eggs of its host. The Hymenopteron alighting on these eggs pierces some of them with its ovipositor and lays in them one or more of its eggs. This is shown in Pl. 14, figs. 1 and 2. *B*. Each egg eventually gives rise to many larvæ.

The parasite's egg (or eggs) generally lies inside the sub-

[1] Monembryonic

stance of the host egg. The latter segments as usual and eventually the small moth larva emerges from the egg. Outwardly the host larva resembles others of its kind, but there is this difference—inside its body lies a small germinal mass derived from the parasite's egg, which later breaks up into a very large number of embryonic masses or gemmules, which give rise to larvæ, and which are destined later to destroy the unfortunate host larva.

If a parasitised larva nearly full-grown be opened up, it will be found to contain a large number of parasitic larvæ which have been nourished at the expense of the host tissue. These larvæ may be all derived from the one egg, laid by a parasite inside the egg laid by a moth or butterfly. The purpose of this short paper is to describe how this process is brought about.

After the parasitised larva has grown a good deal, in some cases when the observer is well acquainted with the behaviour of the healthy non-parasitised larvæ, certain differences in the reactions of the older parasitised larvæ can be detected. Such larvæ may often be found to be "sleepy," that is, they are not so active as their healthy fellows; moreover, in certain cases where the host larva is delicately tinted in transparent colours, parasitised specimens can easily be found, for the fat body which, in thin-skinned larvæ causes a definite appearance, is in these cases altered or absent.

The parasitised larva in later stages of its life is merely an empty skin full of parasites.

Generally everything except brain, etc., gut, and absolutely necessary organs are eaten up; a noteworthy fact is that the tracheal tubes are left quite intact. In fact, the parasites eat up everything but that absolutely necessary to keep their "vache à lait" living.

When the parasites have used their unfortunate host long enough to enable them to grow sufficiently they either kill their host by eating up its vitals or by boring holes in its sides in order to emerge from its body.

They now pupate near or inside the skin of their host.

Specimen Description of the Life-cycles of Host and Parasite (from Marchal).

Hyponomeutus mahalebellus is a moth whose caterpillar is the host of the parasite, Encyrtus fuscicollis, the polyembryonic Hymenopteron.

(1) The moths emerge from their cocoons in the last days of July.

(2) Encyrtus fuscicollis commence to emerge early in August.

(3) Hyponomeutus lays its eggs early in August, and the Encyrtids by August 15th are at their busiest parasitising these eggs. By August 22nd the parasites are rare.

(4) The parasitised eggs segment and develop, and by the end of September the contained moth embryos are ready to hatch.

The young larvæ do not emerge till next spring, but winter under cover of their egg cases.

(5) By spring the moth larvæ awaken, and the parasite's eggs contained in their bodies begin to develop. The trophoamnion is formed (see p. 180).

(6) By the end of spring the parasitic polygerminal mass is well formed (Text-figs. 11 and 12), and the host cyst (Kyste adventice of Marchal) or covering of host-cells around the parasitic mass is completely differentiated (see p. 183).

(7) By June or July the gemmules have metamorphosed into larvæ; the latter have broken out of their sheaths and the parasitised Lepidopterous larva is killed and its viscera eaten up (see p. 186).

(8) Encrytids pupate and finally emerge in August in time to parasitise other moth eggs.

With regard to the numbers of individuals produced from one host caterpillar, Howard ('Pr. U.S. Nat. Mus.,' xiv), affirms that in the case of Plusia brassicæ 2500 Litomastix truncatellus were hatched out of one caterpillar carcase. Giard ('Ann. Soc. Ent.,' Fr., 1898), writes that he

found nearly 3000 individuals from one larva of Plusia. Of course not all these arose from one Litomastix egg (**9**). Patterson (**13**) got on the average 175 individuals of Copidosoma from one Gnorimochema larva; the number from one caterpillar carcase varied from 25 to 395.

Early Development of the Egg of the Polyembryonic Species Ageniaspis (Encyrtus) fusicollis (Dalm.), parasite of Hyponomeutus malinellus or Hyponomeuta cognatella, etc. Marchal (**5**), Martin (**14**), Silvestri (**9**).

The newly-laid egg resembles that of the majority of parasitic Hymenoptera. The nucleus is of the condensed type. If the egg has been fertilised it contains a solid, bluntly comet-shaped spermatozoon. There is also the germ-cell determinant. The solid nucleus now breaks up into chromosomes and the first polar body forms. The spindle of this does not appear to have the usual centrosomes at its poles. The polar figure generally lies towards the upper central region of the egg. The oocyte nucleus again divides to form the second polar body, and at the same time the first polar body itself enters into division stages. Pl. 14, fig. 5: In this figure both spindles are in telophase, the sperm lies near the second polar body spindle, and the germ-cell determinant takes up its position in the interior of the lower part of the egg.

After the extrusion of the second polar body the matured egg nucleus assumes the resting reticulate appearance, while the three polar bodies (the first one divided) lie in the upper part of the egg in the form of three condensed solid plates, Pl. 14, fig. 6: If the egg was fertilised, the sperm, as is usually the case, breaks into a reticulum and takes its place next the egg nucleus. Pl. 14, fig. 6: The germ-cell determinant keeps closely in this region (*G.C.D.*). When the zygote nucleus is formed (fertilised egg), or when the matured egg nucleus resumes its reticulate shape (unfertilised egg), a most remark-

able process begins; a definite region of the cytoplasm of the egg round the segmentation nucleus becomes separated off from the rest of the egg, Pl. 14, fig. 7. This region contains the segmentation nucleus and the germ-cell determinant (*G.C.D.*).

The egg now contains two distinct regions:

(1) The embryonic region with the nucleus and the germ-cell determinant (Embryonic ooplasm).

(2) The polar ooplasm so-called, with the polar nuclei.

The plate-like chromatic masses derived from the polar bodies (Pl. 14, fig. 6, *P.B.*[1], *P.B.*[2]), now begin to alter. Just as the fused chromosomes of the telophase of mitosis absorb a nucleoplasmic fluid and become reticulate (nesting nucleus), so do these remains of the extrusion of the polar bodies become, so to speak, blown out and frothy in shape (Pl. 14, fig. 7). The individual nucleus-like bodies derived in this way gradually become larger and finally join up (Pl. 14, figs. 7 and 8).

The original egg-shape is now finally lost, and the germinal body becomes more or less spherical. The embryonic region at the same time is not inactive, chromosomes appear in the segmentation nucleus (Pl. 14, fig. 8), and the embryonic cell divides. Only one of the daughter cells gets the germ-cell determinant—the latter going over to one cell quite intact (Pl. 14, fig. 9).

A further discrimination may now be made: The polar ooplasm and the modified remnants of the maturation divisions of the oocyte nucleus now form an investing nutrient capsule or tropho-amnion. The inner region is the embryonic part with the embryonic or germinal blastomeres (Pl. 14, figs. 9 and 10).

Pl. 14, figs. 7 and 8, mark the parting of the ways betwixt polyembryony and monembryony, and I will now describe Silvestri's case in Litomastix truncatellus, which is in certain ways intermediate between the monembryonic and the polyembryonic method of segmentation. The polar bodies are given off in the usual way, and at first are disposed exactly as in Pl. 14, fig. 6, for Ageniaspis (Encrytus).

Presently, however, the three elements approach and fuse to form a solid nucleus (Pl. 14, fig. 3 *P.N.*). The segmentation nucleus in this figure has divided, and the egg is divided into three sub-equal blastomeres. The germ-cell determinant lies in one, another has no determinant, while the third contains the polar nucleus. The two lower blastomeres are the embryonic ooplasm, the upper one the polar ooplasm. The latter subsequently gives rise to the tropho-amnion. The stage in Pl. 14, fig. 7, may now be compared with that in Pl. 14, fig. 3, and it will be noticed that in the former the method of separation of the two ooplasmas is more specialised than in the latter.

The polar nucleus in Pl. 14, fig. 3, now swells out to form a normal reticulate nucleus, which later gives rise to chromosomes and to a perfectly typical spindle. This divides, and in Pl. 14, fig. 4 the two nuclei derived from the first division are dividing again in the typical manner. In about the 8-cell stage the germ-cell determinant fragments and forms a halo around the spindle of the dividing cell which happened to receive the germ-cell determinant. This infusion of the determinant into the cytoplasm of the two daughter cells causes the latter to be somewhat conspicuous for a time (Pl. 14, fig. 4, *G.C.*, *D.C.*). Such a stage as that drawn in the latter figure is interesting, for it will be noted that the two cells containing the germ-cell determinant have entered a resting condition while their fellows around them are in the telophase of mitosis. Subsequently, however, they begin to divide again; their cytoplasm clears up, and as far as is known they form merely part of the germinal mass just as other cells do. These blastomeres containing the germ-cell determinant are later lost to sight among the other cells, and there is no evidence that they form the gonads of the future larvæ.

In Pl. 14, fig. 13, is a later stage. The polar ooplasm or tropho-amnion has many nuclei in a syncytium, while the embryonic mass has also segmented further, cell-walls being formed after each division.

The thread of the story of Ageniaspis may now be picked up again. In Pl. 14, fig. 11, is a further stage; the tropho-amnion has now become divided into an inner granular zone (endoplasm) and an outer clear zone (ectoplasm), while outside the entire germinal mass is a cellular sheath formed by the tissues of the host (*H.*).

The tropho-amniotic nucleus has constructed to form several minor polar nuclei ($P.N.^2$, $P.N.^3$), while the embryonic cells are becoming divided up into parts containing several blastomeres. These blastomeres lie in a cavity in the tropho-amniotic mass, in a fluid which in later stages is coagulable with certain fixatives.

The outer covering (*H.*) in Pl. 14, fig. 11, is of different appearance in various forms. In Martin's case it is early developed from cells of the body-cavity of the caterpillar, and is stout at first; in Marchal's case it is found developing in the same way and later forms a very definite outer sheath to the ramifying germinal mass (Pl. 14, fig. 12, and Pl. 15, fig. 15).

In the form examined specially by Silvestri, the outer or host-layer in later stages becomes very thin, much thinner than in Marchal's case (Pl. 14, fig. 14, *H.*).

In Pl. 14, fig. 13, is a later stage in Litomastix; the polar nuclei in the polar ooplasm (tropho-amnion) are now very numerous and soon will form a fairly even sheath around the embryonic ooplasmic region (E.R.).

In Pl. 14, fig. 12, a figure is given from Patterson's case in Copidosoma.

The tropho-amnion now forms a thick sheath (*T.A.*) to the inner embryonic masses (*E.R.*), while here and there adhere parts of the host fatty tissue (*F.T.*). There is also a non-nucleated inner sheath around each mass (blacked in below in Pl. 14, fig. 12) of doubtful relationship to anything described in other forms.

In all the cases examined a process begins about this time by which the spherical or ovoid germinal mass gets divided up into smaller masses. This is brought about by constrictions, primary, secondary, and tertiary, and so on, till in

some cases a ramifying figure becomes formed. The constricting process has not yet begun in Pl. 14, fig. 12, but subsequently this germinal mass will become divided up like that in Pl. 14, fig. 14. In some cases the most remarkable ramifying "sausage-like" structure such as that in Pl. 14, fig. 15, is produced, and this structure later becomes more and more attenuated, and is found to ramify throughout the whole hæmocœl. of the host caterpillar; when the parasitised caterpillar is opened one finds this peculiar body as drawn in Pl. 15, fig. 16, *P.M.* In other cases this attenuated ramifying appearance is never so marked, and the germinal mass is a shapeless, elongate body. It should be noted (Pl. 14, figs. 11 and 12) the embryonic cells are divided into parts before the tropho-amniotic layer begins to construct the mass into smaller regions.

The organ seemingly most concerned in the production of the construction of the germinal mass into smaller regions is the modified tropho-amniotic layer (Pl. 14, figs. 12 and 14), and as the constricting layer meets through the germinal masses, it carries in the outer layer derived from the host tissues (host-layer *H.*).

A word or two must now be written in connection with the structure of the tropho-amniotic layer. This sustains throughout the character of a nutrient membrane; the appearance of a heavy granulation in it is explicable on this view. Pl. 14, fig. 11, endoplasm (*E.N.P.*), and in Pl. 15, fig. 14, it will be seen that the tropho-amniotic layer around each constricted off mass is very granular and deeply-staining (*T.A.*).

In the two cases where the polar nuclei either divide by mitosis regularly (Pl. 14, fig. 13, etc.) or the polar nuclei form a frothy structure in the polar ooplasm (Pl. 14, fig. 11, etc.) the end result as far as the nuclei is concerned is nearly the same. In both cases the nuclei become very numerous either by mitoses or by amitosis, and the tropho-amniotic layer becomes well supplied with nuclei. In Patterson's case the nuclei are very even and regularly disposed as compared with

the cases described by Marchal and in one of Silvestri's forms.

Formation of Larvæ from the Inner Embryonic Masses (Marchal (8)).

The two periods of greatest interest to the embryologist in the remarkable history of this development are the stages just after the polar bodies and those of the formation of the germ-layers from the embryonic masses or gemmules.

The latter in Marchal's case were often over a hundred in number, all derived from the gemmulation of a part of the original egg.

At this period they lie inside the membranes formed by the host (*H.* in Pl. 14, fig. 11) and by the tropho-amnion (*T.A.*) and form long strings of embryonic masses or gemmules. The several scores of embryos, together with their membranes, form the germinal cord or polygerminal cordon (Pl. 14, figs. 14 and 15).

When the germinal cord is like that in Pl. 15, fig. 15, and about 3 to 4 mm. in length the gemmule or embryonic mass contains about twenty to forty cells. Each embryonic mass is really a morula, a ball of cells of the same size. During this time the cells in each morula divide constantly by mitosis, and gradually the subspherical morula becomes a distinctly ovoid mass containing sixty to eighty cells of the same general size.

From this time the embryo ceases to be represented by a simple morula, and the germ-layers commence to form. The embryo now tends to a discoid form, so that it has completely defined relations, as shown in Pl. 15, fig. 18. Its shape is reniform thanks to a depression which becomes formed in what corresponds to its dorsal region (Pl. 15, fig. 18).

The side opposite the depression corresponds to the ventral face of the embryo.

These relations are exactly the opposite of what have been described so far by other authors who have studied Chalcids or Proctotrypides.

On the convex border representing the ventral face runs a longitudinal furrow, which is not much marked in the middle region of its course, but which hollows out at the level of the stomodæum and of the posterior extremity of the body. This furrow is the representative of the mesodermal groove of other Insecta (Pl. 15, figs. 19 and 20).

Soon other furrows in a transverse direction make their appearance; these represent the incipient segmentation of the body of the embryo.

In each morula, at the largest size it attains, and at the time when the dorsal furrow (or hilus), which gives the reniform shape to the morula, begins to become hollowed out, those cells in the central region of the embryo become modified, lacunæ become formed between them, and they form a loose sort of mesenchymatous tissue.

The nuclei of these cells become very large, and the protoplasm becomes vacuolated to form a sort of network. Such modified cells form the endoderm (Pl. 15, fig. 21).

As this process has been going on the outermost cells of the morula become grouped like an epithelium, in a row. These cells constitute the ectoderm and the mesoderm, which, because of the condensation or telescoping up of the stages of development, appear to be formed synchronously Pl. 15, fig. 21).

The ecto-mesoderm, so-called, is well developed on the ventral side; in the latero-dorsal side it is not so well marked The ventral region, where the ecto-mesoderm is thickest, corresponds to the germinal band in other insects.

In short, this particular kind of development of the germ-layers in the gemmule or embryonic mass of Encyrtus is remarkable for the rapidity with which organogeny takes place, and for the manner in which the various processes are condensed, in that they occur almost synchronously. The primitive characteristics of the developmental processes of Encyrtus are masked by the occurrence of a process of delamination.

The central endoderm cells form at once the wall of the

mesenteron, and a group of yolk-cells destined to remain inside the mid-gut till the end of embryonic development (Pl. 15, fig. 21).

From the above description, which is nearly a literal translation of Marchal's work on this section of the development, it will be seen that the proctodæum and stomodæum are formed normally from invaginated ectoderm cells. The mesoderm and endoderm are formed, so to speak, in situ from the outer central and inner central regions respectively of the morula, and in this way the developmental processes of Encyrtus are remarkable.

In Pl. 15, fig. 22, is an advanced embryo showing the normal arrangement of the organs after the germ-layers are properly differentiated. This is an embryonic Litomastix, and is, in certain ways, different from Marchal's Encyrted larva (Pl. 15, fig. 21). The hilus (*h.*) is not so deep in the former, while the silk gland (*s.g.*) in Marchal's form is much more marked in this early stage.

The Independent Life of the Larvæ in the Hæmocœl. (Bugnion (3)).

In Encyrtus towards the end of June, at a time when the host caterpillars spin their cocoons, the young parasitic larvæ, now fully formed inside the tropho-amnion, break the tubular cyst in which they lie, and float free in the body-cavity. At this time also they undergo their first ecdysis, leaving the cast-off skin inside the remains of their epithelial membranes.

They are now 1 to 2 mm. in length. According to Bugnion, these larvæ for the time being are satisfied with imbibing the hæmocœlvic fluid of the host's body, but later, when they become nearly full-grown, they eat the more important organs of the host, and so kill it. Some species pupate inside, others outside the host's skin. According to Silvestri, in Litomastix there are two sorts of larvæ—sexual and asexual—derived from one polygerminal mass. The asexual larvæ are used to tear up the host tissues for the benefit of the sexual. As some doubt has been cast on this part of Silvestri's work,

nothing more need be said till this matter has been properly confirmed.

Abortive Embryos (Patterson (**13**)).

As has been described, the embryonic areas are derived from fragmentation of an original solid polynuclear mass. Patterson especially describes how certain of these embryonic masses are much smaller than the others, and form abortive or degenerating embryos. In some cases this production of abortive embryos seems to be due to the fact that the subdivision of the egg has been carried too far, and such embryonic masses contain too few nuclei. In other cases degeneration is apparently due to the lack of proper nutrition. Most polygerms lie in a thick layer of fatty tissue, others are nearly bare of the latter, and it is an observed fact that in such cases mortality of the embryos is very high. In one of Patterson's polygerms, with little surrounding fat tissue, there were more than 100 embryos, not more than thirty to thirty-five of which had developed normally. In Pl. 15, fig. 23 are drawn the outlines of six embryos from one caterpillar; according to Patterson, only those of the sizes from *c.* to *f.* would have developed; those of the sizes in *a.* and *b.* would degenerate.

Summary.

Before entering upon any discussion or comment on the above description of Polyembryony, a summary of the main facts known will be given.

(1) Polyembryony in the Hymenoptera parasitica is process whereby the single egg, instead of producing a single embryo, often produces several score or more.

(2) The polyembryonic Hymenoptera are generally small insects about 1 mm. in length.

(3) The polyembryonic parasite lays from one to ten or more eggs in the ovum of the host.

(4) This oviposition does not kill the host eggs. Larvæ

hatch from the latter in the normal way, but contain the eggs of the parasite, generally in the hæmocœl cavity.

(5) The parasite's egg gives off polar bodies, and may or may not be fertilised in the normal way.

(6) The polar bodies rest for a time, but then break into activity, forming an actively growing mass, or collection, of nuclei.

(7) That part of the egg cytoplasm containing the segmentation nucleus separates off from the outer part containing the active polar nuclei, and the germ-cell determinant goes to the former, but later becomes absorbed and lost to sight.

(8) The polar cytoplasm or ooplasm containing the polar nuclei forms an investing sheath around the contained embryonic ooplasm, which later gives rise to the embryos. The polar ooplasm nourishes the inner embryonic mass and acts as an amnion or placenta. Hence the name tropho-amnion.

(9) The nuclei of the tropho-amnion derived from the original polar body nuclei become very numerous by division, and the tropho-amniotic cytoplasm becomes very granular in the region of the nuclei.

(10) Certain cells of the embryo, either hæmolymph or fat cells or both, form an outer covering to the parasitic germinal mass. This host-covering later becomes much stretched and epithelial in character. In some forms it is not well developed.

(11) The primary embryonic cell separated off at the time when the polar nuclei begin to become active, has already divided many times to give rise to many germinal masses. The parasitic body lying inside the host hæmocœl. may now be called a polygerm.

(12) The polygerminal embryonic masses, keeping on dividing till as many as a hundred or more masses may be produced, later become constricted into areas each containing an embryonic mass surrounded by two membranes, the outer host-epithelial and the inner tropho-amniotic layer.

(13) The shape of the entire polygerminal mass differs in

different forms. In some it is a ramifying cylindrical body, in others a shapeless mass, constricted here and there by the outer membranes.

(14) Each separate germinal mass is now a spherical or ovoid morula containing a score or more cells. The latter keep dividing.

(15) The embryonic or germinal mass now begins to differentiate further; it loses its sub-spherical shape and becomes elongate, while dorsal, ventral, and lateral sides of the future embryo can be distinguished.

(16) The stomodæum and proctodæum are formed by invaginations of the two extreme ends of a ventral groove. The ectoderm is formed by a rearrangement of the outer cells of the morula. The endoderm and mesoderm are formed in situ by a modification of the more centrally-placed cells of the embryo.

(17) The larvæ at a later stage break away from their membranes and are free-living for a time. They later eat up nearly everything in the host-caterpillar's body and then pupate inside (in some cases, however, apparently outside) the body of their host.

In this review I have not so far given any data with regard to the sexes of parasites emerging from one caterpillar. Broods may be purely male or female, or mixed. In Patterson's cases 55 per cent. of all broods were female. Moreover, the average number of females emerging from a single carcase is 198 as compared with 175 for males. Some of the mixed broods doubtless arose from two or more eggs, fertilised and unfertilised; but Patterson makes the interesting suggestion that such mixed broods may also arise from a single fertilised egg by a process of disjunction of the sex chromosome during early cleavage stages (13). As far as is known fertilised eggs produce females, unfertilised males.

Discussion.

(For the following I alone am responsible).

The egg of the insect has been long considered one of the

most highly organised of all animals. In the ovary we can recognise its relations, where the future embryonic regions will lie and so on (**18**).

In no insect eggs, however, do organ-forming substances such as those found in Ascidia, appear to be demonstrable; the germ-cell determinant alone marks definitely the position of the germ-cells. Nevertheless, it is probably true that the other regions of the egg, though on a microscopical basis apparently homogeneous, are divided up into future endoderm, ectoderm, and mesoderm regions, or even into organ-forming regions. This seems directly proven by Hegner's (**18**) experiments with beetle eggs: "When the anterior or posterior parts of freshly-laid eggs are killed (with a hot needle) the material remaining alive develops that part of the embryo which it would have produced if the eggs had remained intact. No regeneration of the part which would have been produced by the killed region takes place." This result should be compared with what happens in polyembryony where the entire anterior end of the egg is voluntarily discarded, as far as organogeny is concerned.

It is remarkable to find that in the polyembryonic Hymenoptera a large region of the egg is entirely discarded. In fact, just that region of the egg which would have formed the head, brain, etc., of the embryo is rejected. There is no reason for supposing that the polyembryonic egg is differently organised from the monembryonic—in fact, just the reverse. The middle region, or middle region plus the lower pole of the egg, alone gives rise to the scores of embryos. We cannot recognise any special plan of division in the segmentation process; even the germ-cell determinant after passing into what in monembryonic species (**15**) would be the germ-cells, later becomes lost, and in view of the remarkably haphazard method of formation of the inner embryonic masses there seems little possibility that any special care is taken to ensure that the "germ-cell determinant" cells become the gonads of the future embryos. As far as we can tell, they may just as well form cells of the gut or ectoderm; Silvestri, however,

without any direct evidence, thinks that the "germ-cell determinant" cells might form the future germ-cells of each embryo.

The following conclusions I at present consider to be justified with regard to polyembryony:

(1) The "germ-cell determinant," being possibly a nutrient cytoplasmic-mass, has no other effect than that of temporarily stopping mitosis in the cells which happen to contain it. There is no evidence for supposing that the "germ-cell determinant" cells later form the germ-cells of each embryo.

(2) There is absolutely no evidence in polyembryonic species of a "germ-track"; everything is, in the first place, subservient to the production by haphazard divisions and fragmentations, of numerous morulæ, without any discoverable definite regions. Differentiation of germ-layers follows later.

(4) Mere position in the morula is all that seems to determine whether this or that cell will be an endoderm or ectoderm cell, etc.

(5) Marchal's description clearly shows that differentiation of germ-layers only takes place after the formation of the solid morula, and I can find no evidence for supposing that the inner cells differ in any way except in position from the outer cells of the morula.

From an examination of the literature on polyembryony, and from my personal knowledge of the development of a monembryonic form, I foreshadow that when more species of both monembryonic and polyembryonic forms are examined it will be found that some may be either polyembryonic or monembryonic according to season of the year, or to some condition of host egg or caterpillar. It is already known that the number of larvæ produced from one egg of different species may differ greatly; those forms with fewest larvæ from one egg are the ones in which one might expect to find transition between monembryony and polyembryony.

In certain monembryonic species the polar bodies are long

in degenerating, and, moreover, pieces of egg may live apart for a long time (15). There are still a great many parts of the polyembryonic development which should be carefully studied again; such are especially those questions connected with the segmentation of the egg, the fate of the "germ-cell determinant" cells, and the formation of germ-layers. Moreover, the "asexual" larvæ of Silvestri should be re-examined, and their true nature properly determined.

With regard to the possible place of origin of polyembryony in Hymenoptera, Marchal ('Arch. Zool. Expt. et Gener.,' 4e serie, tome iv) shows that in the monembryonic form Synopeas rhanis, the cortex of the ovum is separated in much the same way as in Pl. 14, figs. 7 and 8. The inner part of the egg, plus the segmentation nucleus, a very small part of the egg, gives rise to one embryo, instead of the many in polyembryonic forms. The cortical zone of the egg is, as far as I can ascertain from Marchal's description, supplied with nuclei from the revived polar bodies. In Synopeas rhanis the blastomeres do not part and become free in a fluid, but instead keep together, and eventually form the organs of one larva.

Bibliography.

1. Ganin, M.—"Beiträge zur Erkenntniss der Entwickelungsgeschichte bei den Insecten," 'Zeit. f. wiss. Zool.,' xix, 1869.
2. Kulagin, N.—"Beiträge zur Kenntniss der Entwicklungsgeschichte von Platygaster," 'Zeit. f. wiss. Zool.,' lxiii, 1898.
3. Bugnion, E.—"Recherches sur le developpement postembryonnaire, l'anatomie et les mœurs de l'Encyrtus fuscicollis," 'Recueil zool. suisse,' v. 1891.
4. Giard, A.—"Sur le développement de Litomastix truncatellus," 'Bull. Soc. Ent. Fr.,' 1898.
5. Marchal, P.—"La dissociation de l'œuf en un grand nombre d'individus distincts chez l'Encyrtus fuscicollis," 'C.R. Soc. Biol.' (10e S.), v, 1898.
6. ——— "Le déterminisme de la polyembryonie spécifique, etc.," 'C.R. Soc. Biol.,' lvi, 1904.

7. Marchal, P.—"Sur la formation de l'intestin moyen chez les Platygasters," 'C.R. Soc. Biol.,' lvi, 1904.

8. ——— "La Polyembryonic specifique ou Germinogonie," 'Arch. Zool. Exp.,' 4e serie 2, 1904.

9. Silvestri, F.—"Contribuzioni alla conoscenza biologica degli Imenotteri parassiti. I. Biologia de Litomastix truncatellus (Dalm.)," 'Ann. R. Sc. Agr. Portici,' vol. vi, 1906; 'Boll. Labor. Zool. R. Sc. Agr. Portici,' vol. i, 1906, pp. 17–64.

10. ——— "Contribuzioni, etc. II. Sviluppo dell' Ageniaspis fuscicollis (Dalm.). III. Sviluppo dell' Oophthora semblidis," 'Boll. Lab. Zool. R. Sc. Agr. Portici,' vol, iii, 1908.

11. ——— "Notizie preliminari sullo sviluppo del Copidosoma Buyssoni (Mayr.)," 'Monitore Zool. ital.,' anno xxi, n. 11–12.

12. ——— "Prime fasi di sviluppo del Copidosoma Buyssoni (Mayr.) Imenottero Calcidide," 'Anat. Anz.,' xlvii, 1914.

13. Patterson, J. T.—"Observations on the Development of Copidosoma gelechiæ," 'Biol. Bull.,' vol. xxix, 1915.

14. Martin, F.—"Zur Entwicklungsgeschichte des polyembryonalen Chalcidiers, Ageniaspis (Encyrtus) fuscicollis," 'Zeit. f. wiss. Zool., Bd. 110, 1914.

15. Gatenby, J. Bronté.—"The Embryonic Development of Trichogramma evanescens, etc.," 'Quart. Journ. Micr. Sci.,' vol. 62, part 2.

16. ——— "The Segregation of the Germ-cells in Trichogramma evanescens," 'Quart. Journ. Micr. Sci.,' vol. 63, part 2.

17. Martelli, G.—"Contribuzioni alla Biologia della Pieris brassicæ (L.), etc.," 'Boll. Labor. Zool. Portici,' v. i.

18. Hegner, R. W.—"The Effects of Removing the Germ-cell Determinants from the Eggs of Some Chrysomelid Beetles," 'Biol. Bull.,' vols. xvi and xx.

EXPLANATION OF PLATES 14 AND 15,

Illustrating Mr. J. Bronté Gatenby's "Review on Polyembryony in Parasitic Hymenoptera."

EXPLANATION OF LETTERING.

A. Amnion of host caterpillar. *B.L.* Separated blastomeres of polyembryonic egg. *br.* Brain. *CH.* Chromosomes. *coag.* Fluid in which embryonic blastomeres lie and which coagulates in fixatives. *D.* Dorsal. *ECTP.* Ectoplasmic or clear region of polar ooplasm.

E.M. Embryonic mass. *END.* Endoderm. *ENP.* Inner or granular region of polar ooplasm. *ER.* Definitive embryonic region of egg. *F.* Space filled with fluid. *FC.* Adipose tissue cell of host. *F.PN.* Female pronucleus. *G.* Gut of host caterpillar. *G.C.D.* Germ-cell determinant. *G.C.D.C.* Cell which contains germ-cell determinant. *G.L.* Granular layer around each embryonic mass. *H.* Layer of cells around the polyembryonic mass formed by cells of the host. *h.* Hilus or dorsal depression in the kidney-shaped larva. *Lit.* Litomastix imago ovipositing in ova of Plusia. *M.PN.* Male pronucleus. *N.ch.* Nerve cord. *PD.* Proctodæum. *P.E.* Egg of host moth, Plusia. *P.M.* Parasitic mass or polygerminal cordon in hæmocœl of Plusia caterpillar. *P.N.* Polar nucleus. *P.OP.* Polar ooplasm. *SP.* Spermatozooni *st.* and *STD.* Stododæum. *TA.* Tropho-amnion, or polar ooplasm. *TU.* Tracheal tube of host larva. *V.* Ventral. *Z.N.* Zygote nucleus.

PLATE 14.

Figs. 1–4. (All after Silvestri.)

Fig. 1.—Litomastix truncatellus (Dalm.) in the act of ovipositng in the egg of a moth, Plusia gamma, L. (Much enlarged.) *Lit.* = Parasite. *P.E.* = Plusia egg.

Fig. 2.—Section through egg of Plusia containing an embryo nearly eady to hatch. The substance of the host (Plusia) caterpillar contains many Litomastix eggs. Three lie in the mouth and stomodæum of the caterpillar and will probably never hatch out. One lies just behind the brain (*Br.*), two more lie beneath the nerve-chord, while another has been oviposited outside the body of the host.

Fig. 3.—Three-cell stage in the segmentation of the egg of Litomastix. The three polar bodies (*P.N.*) (compare fig. 6) have now fused to form a single solid mass of chromatin; no other nucleus lies in this upper blastomere or polar ooplasm (*P.O.P.*). One of the lower blastomeres contains the germ-cell determinant (*G.C.D.*), the other only a segmentation nucleus ($B.L.^2$). (Four comp. eye-piece, $\frac{1}{15}$ semi-apocromat., camera lucida.)

Fig. 4.—Same species as in last figure, but at 8 blastomere stage. The two cells which received the germ-cell determinant (*G.C.D.C.*) are temporarily resting. The polar body had divided once, and in the present figure the two cells derived from the first mitotic division are themselves now dividing by mitosis. (Drawn in same way as before.)

Figs. 5–10. (All after Martin.)

Fig. 5.—Egg of Ageniaspis. The first polar body ($P.B.^1$) has been given off and is dividing again; the second polar body is being formed, the upper part of this spindle being the second polar body, the lower

part the female pronucleus. The egg contains a sperm (*SP.*) and a "germ-cell determinant" (*G.C.D.*).

Fig. 6.—Later stage, polar bodies given off; the pronuclei, male (*M.PN.*) and female (*F.PN.*) are about to unite. The "germ-cell determinant" lies near (*G.C.D.*).

Fig. 7.—Zygote nucleus formed (*Z.N.*), the polar bodies have begun to form nuclei (at *P.N.*), and the polar ooplasm (*P.OP.*) is separated by a space from the embryonic ooplasm or region (*E.R.*). The embryonic ooplasm contains the zygote nucleus and the "germ-cell determinant."

Fig. 8.—Polar nuclei fusing to form the tropho-aminotic nucleus (*P.N.*); the chromosomes are appearing in the segmentation nucleus (*CH.*).

Fig. 9.—The embryonic nucleus has divided by mitosis to form two cells, only one of which contains any "germ-cell determinant"; the complete fusion of the secondary polar nuclei has taken place to form a mass in the polar ooplasm (*P.OP.*) or tropho-amnion. The elongate shape of the egg is being lost.

Fig. 10.—The embryonic cells are now three in number, one of which contains the germ-cell determinant. The tropho-amniotic or polar nucleus is becoming compressed before it begins to divide into regions by amitosis. The three blastomeres are not adherent to one another.

(All figures drawn with camera lucida eye-piece 4 and a $\frac{1}{12}$th o.im. Slightly reduced.)

Fig. 11.—Polygerm or multiple germinal mass at later stage. Polar or tropho-amniotic nuclei divided into three ($P.N.^1$ to $P.N.^3$). The three blastomeres in fig. 10 have divided to form nine cells. The polar ooplasm now has an outer clear zone, ectoplasm (*ECTP.*), and an inner granular zone, endoplasm (*ENP.*). Outside the polar ooplasm or tropho-amnion is a membrane (*H.*) derived from cells of the body-cavity of the host caterpillar. (At about same magnification as previous figures; after Martin.)

Fig. 12.—Polygerm (after Patterson) of Copidosoma, showing end phase of embryonic-region formation. At *F.* are fat cells of embryo, at *coag.* a precipitate, at *G.L.* a granular layer (purposely blacked in below to show it), and at *TA.* the tropho-amniotic layer. (Nucleated membrane, polar ooplasm.) × 480, somewhat reduced from original.

Fig. 13.—Litomastix germinal mass, to follow stages in figs. 3 and 4. Polar nuclei now very numerous. Embryonic nuclei (*E.R.*) very numerous, germ-cell determinant cells lost among other cells of embryonic region. (After Silvestri.)

PLATE 15.

Fig. 14.—Polygerminal mass of Litomastix in optical section, showing granular tropho-amniotic membrane (*T.A.*), embryonic regions (*E.R.*), each of which gives rise to an embryo, and the outer host-membrane (*H.*). (After Silvestri.)

Fig. 15.—Embryonic chain or cordon (polyembryonic mass) of Encyrtus, all derived from one egg. *T.U.* Tracheal tube of host. × 63. (After Marchal.)

Fig. 16.—Larva of Hyponomeutus opened and containing several chains of Encyrtus (*P.M.*). × 3. (After Marchal.)

Fig. 17.—Part of the same preparation as that in fig. 15, more magnified, showing inner embryonic masses (*E.R.*), tropho-amnion (*T.A.*), and outer membrane (*H.*), derived from host.

Fig. 18.—Embryonic mass from side after assumption of reniform shape. The hilus (*h.*) of the "kidney" represents the dorsal surface (*D.*) of the embryo; at *st.*, just above the hilus, is the stomodæl depression. × 159.

Fig. 19.—Advanced embryo viewed in profile from the left side; the hilus (*h.*) is the dorsal side, at *st.* is the stomodæum, at *pd.* the proctodæum, and signs of external segmentation are appearing. *V.* Ventral side. *D.* Dorsal. × 159.

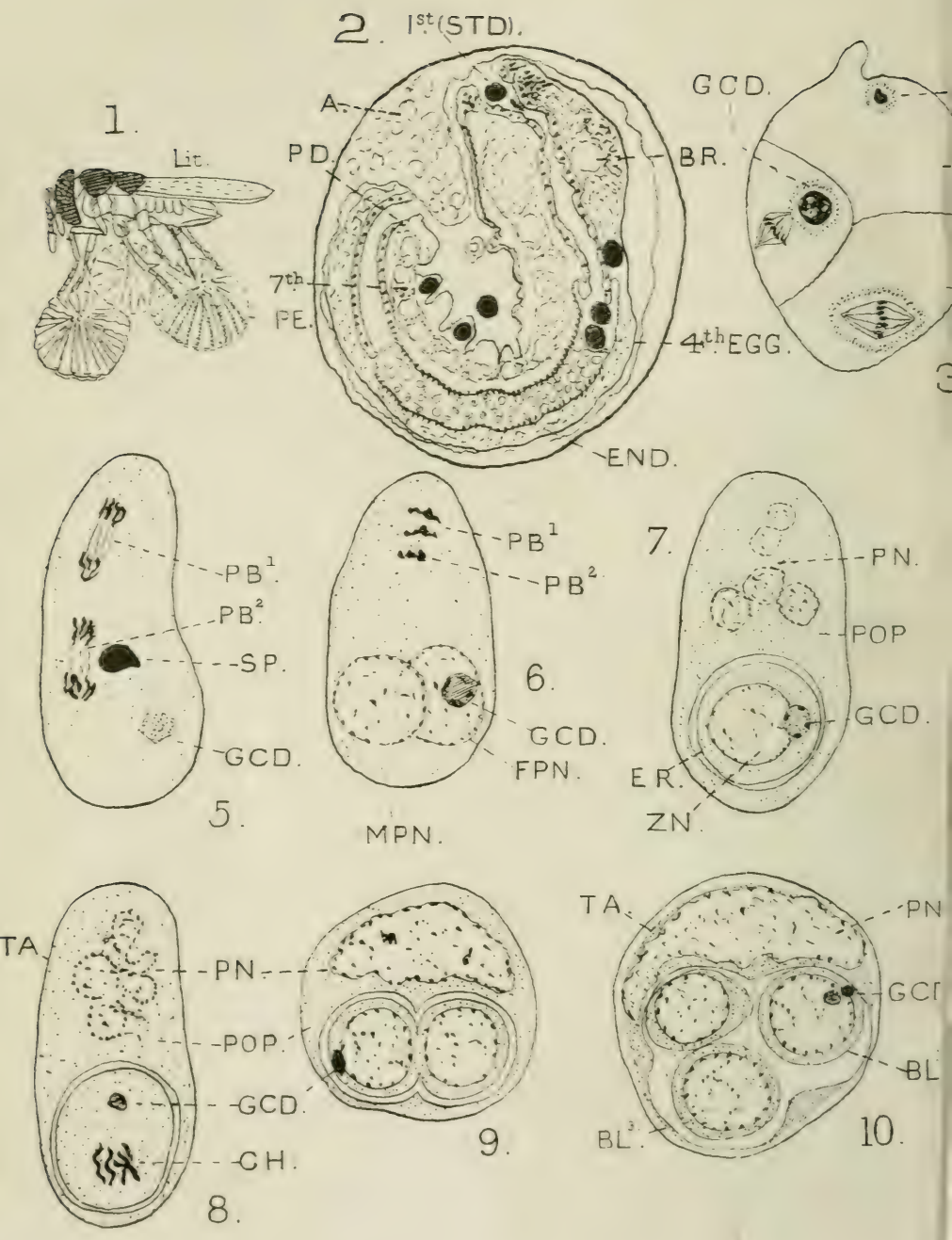
Fig. 20.—Same stage viewed from dorsal face. *st.* and *pd.* as before. ×159.

Fig. 21.—Slightly oblique longitudinal sagittal section of an embryo such as that in figs. 19 and 20. At *sg.* is the silk (salivary) gland; at *st.* the rudimentary stomodæum; at *pd.* the proctodæum; at *h.* the hilus. The endoderm consists of the inner large scattered cells (*end.*). The inner edge of the ectoderm (*ect.*) is not clearly defined as yet, and many of the cells at *m.*, which are forming the mesoderm series of organs, are still intercontinuous with ectoderm cells. At *x.* is a region where cells might be ectoderm, endoderm, or mesoderm, their true nature not yet properly defined. × 340.

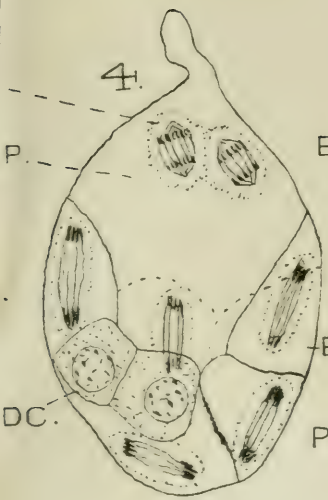
(All these figures after Marchal.)

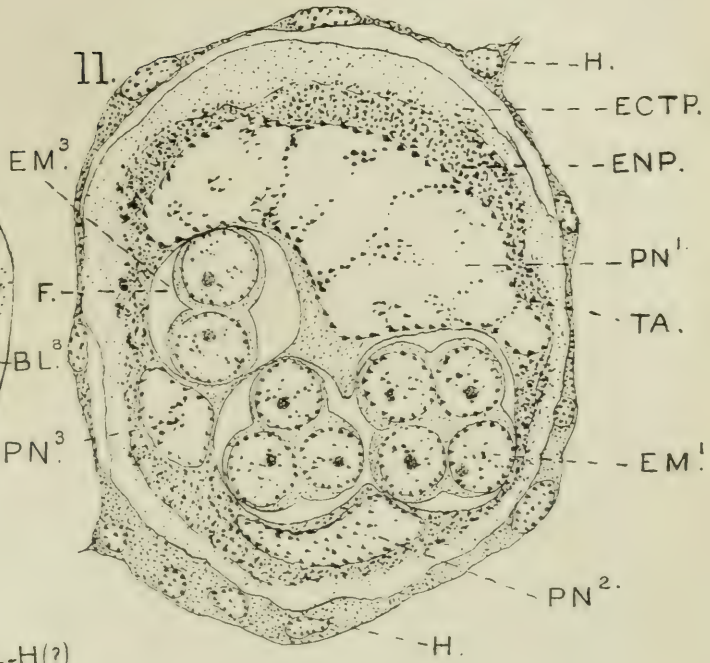
Fig. 22.—Later stage of embryo of Litomastix after Silvestri. Nearly all organs clearly defined. *st.*, *pd.*, *end.*, *m.*, *D.* and *V.* as before. *br.* Brain; *nch.* nerve cord; *mal.* malpighian tubule.

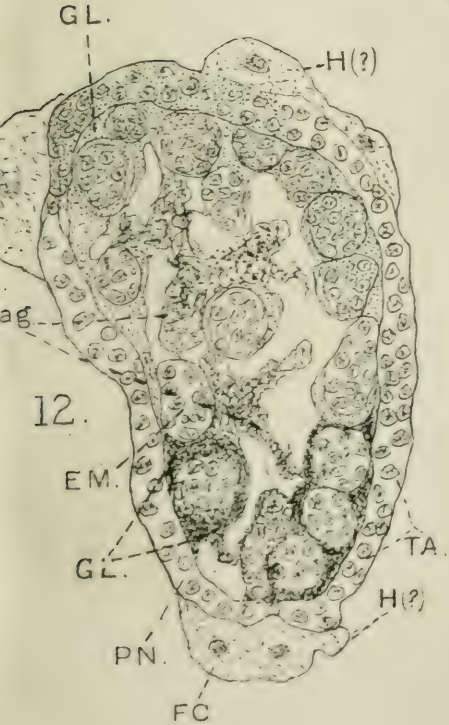
Fig. 23.—*a.* to *f.*, six Copidosoma larvæ free in the body-cavity of same host, to show great variation in size of larvæ from one caterpillar. (After Patterson.)

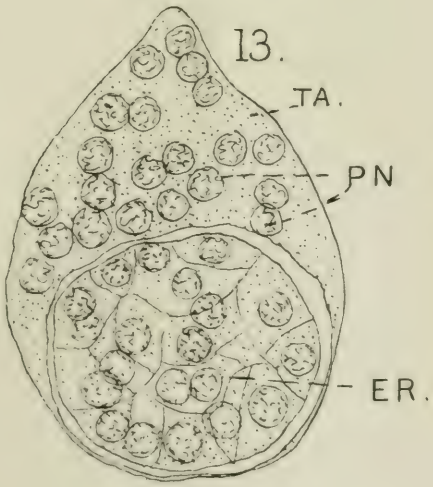


GATENBY—POLYEMBRYONY

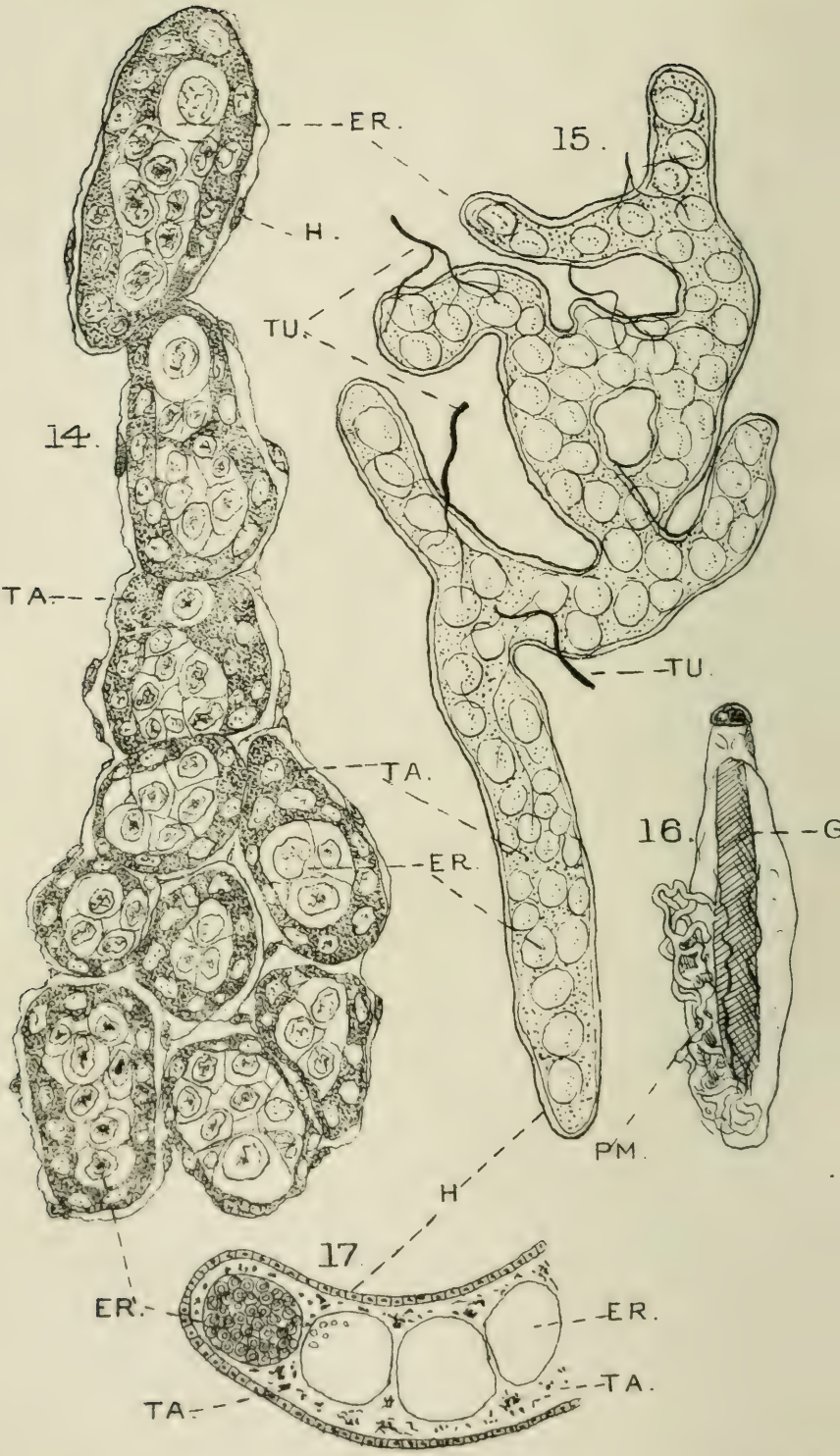








IE PARASITIC HYMENOPTERA.



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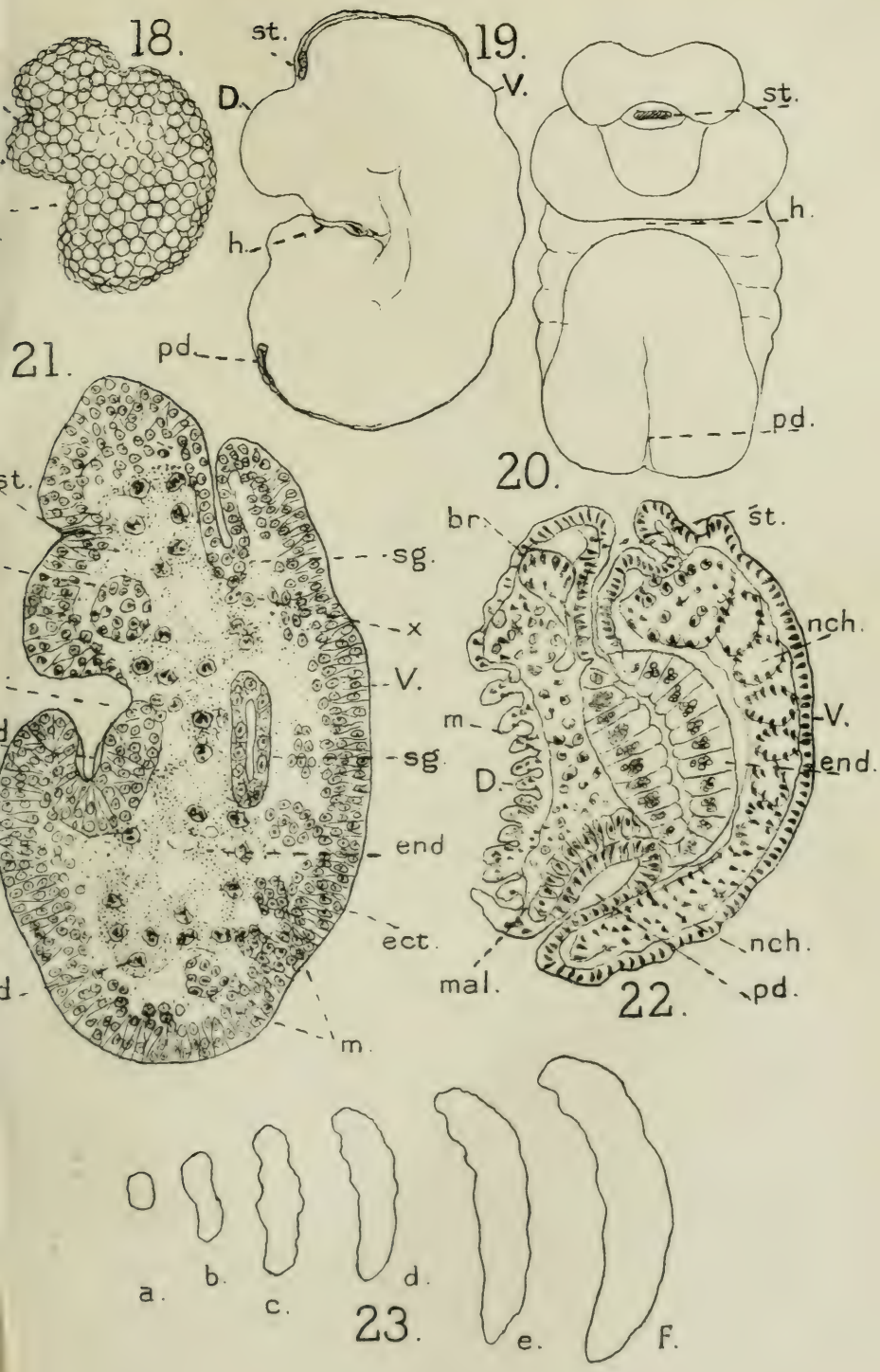
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The Cytoplasmic Inclusions of the Germ-Cells.

PART III. THE SPERMATOGENESIS OF SOME OTHER PULMONATES.[1]

By

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With Plates 16–18 and 3 Text-figures.

TABLE OF CONTENTS.

[1] Part of the cost of the production of this paper was defrayed by a grant kindly made by the Vice-Principal and Fellows of Jesus College, from certain funds which have been put at the disposal of the College by Prof. E. B. Poulton. It is a pleasure to me to thank Prof. Poulton and the College authorities for this grant.

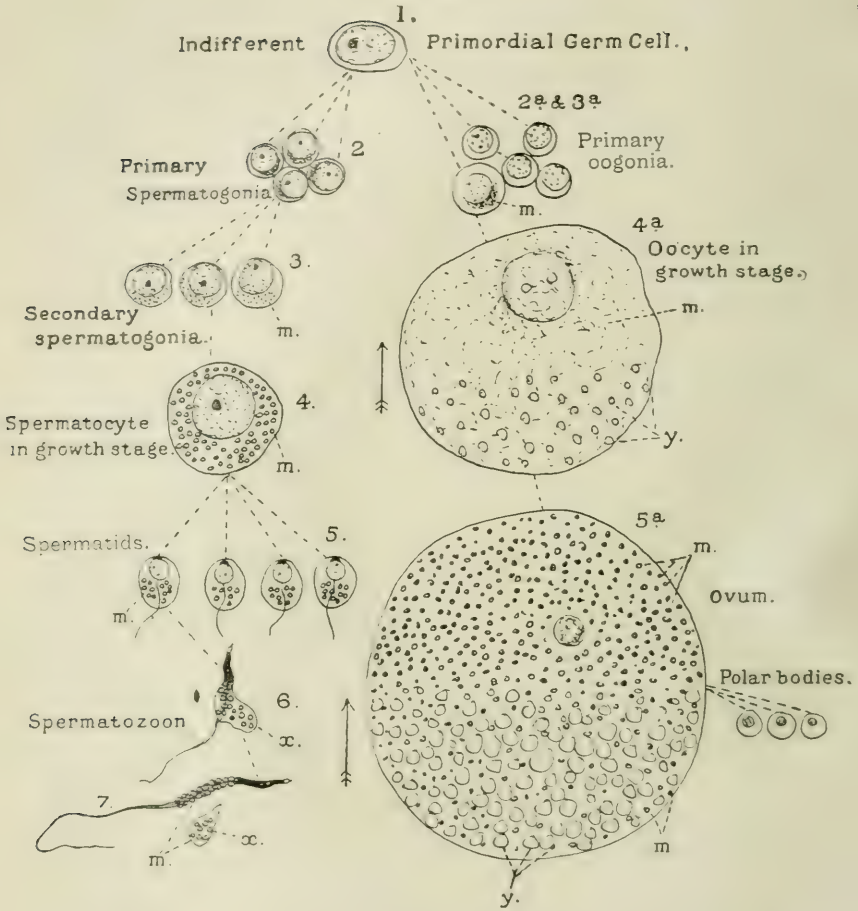
Introductory.

In the two previous papers of this series I have thoroughly examined the mitochondria and other cytoplasmic bodies in the gametogenesis of certain moths and butterflies, and of the garden snail. The aim of these papers, and of those which will follow, is to describe, to analyse, and if possible ascertain the functions of the various bodies in the cytoplasm. In my paper on the germ-cells of Lepidoptera, I described for the first time the remarkable acroblasts, bodies which give rise to the perforatorium, and I also gave the first correct account of the mitochondria during the metamorphosis of the spermatid into the spermatozoon. In the snail (Helix aspersa) I showed how and when the mitochondria first appear in the gametogenesis, and I further registered the discovery of two sorts of mitochondria which I found in the spermatid. This work has now gone far enough to enable us to give a cursory review of the mitochondria and several other bodies in gametogenesis—that is to say, a review of the behaviour of the plasmatic bodies during the time which the indifferent germ-cells are growing and differentiating to form eggs and spermatozoa. In Helix the indifferent germinal cells did not seem to contain any body which could safely be regarded as the rudiment of the future mitochondria; just as in most other cells in a resting condition, the nucleus had to one side of it a denser region of the cytoplasm which I identified as the archoplasm, or the usual dense cytoplasmic sphere which surrounds the centrosome of the cell. Apparently, though

I could not be sure, this region in which lay the archoplasm and centrosome, was where the first sign of the mitochondria appeared.

The mitochondria in these cases were not found to appear from the first as granules; the first sign of their coming was in the formation around one side of the external surface of the nuclear membrane of a dense cloud; this cloud was apparently at first more fibrous than granular, but little by little in it could be seen to appear grains of a definite nature. These were the mitochondria; they gradually became clearer, and soon the dense cloud which generally spread around the total periphery of the nucleus seemed to be absorbed, and the individual granular mitochondria were left (refer to Text-fig. 1). In spermatogonial divisions the mitochondria were seen to be fairly equally, though in a haphazard manner, divided between the daughter-cells. After the primary divisions such as all germ-cells undergo (multiplicatory phase), the mitochondria spread out throughout the cytoplasm and in the spermatogonial cell, remained granular and spherical (Text-fig. 1); in the next or growth stage of the germ-cell the spermatogonium or oogonium passes imperceptibly into what is called the spermatocyte or oocyte of the first order. In this growth stage the mitochondria were thought to increase in number, though because of their small size this could not be ascertained. In the spermatocyte, as the whole cell grew in size, so did the mitochondria become larger and more conspicuous; in some cases there was absolutely definite evidence for believing that each mitochondrial granule in the spermatocyte consisted of two parts—a cortical stainable area and a chromophobe medullary zone. In the oocyte the mitochondria from the first (just about or after contraction figure or synizesis, of the nucleus), became flocculent and finely granular, and of a different appearance from those of the spermatocyte (Text-fig. 1). In the latter the growth stage is followed immediately, as is well known, by two divisions. These two divisions, which give rise to four smaller cells, are called the maturation or first and second spermatocyte

TEXT-FIG. 1.



Diagrammatic scheme of gametogenesis with special reference to the Mitochondria. At 1 is an indifferent primordial germ-cell. After the sex of the embryo is determined, the primordial germ-cells become either primary oogonia or primary spermatogonia. At 2 are several primary spermatogonia derived from the original undifferentiated germ-cell. Each primary spermatogonium divides to give rise to several secondary spermatogonia (3). In some animals the earliest germ-cells contain some mitochondria (chick, frog ?), in others (probably the majority) the earliest germ-cells may not contain differentiated mitochondria (Helix, Insecta ?); in the latter cases the mitochondria appear in the cytoplasm as a cloud, at some

divisions. In one of them (second) the chromosomes are halved, in the other (first) whole chromosomes are bodily separated; this is the reducing division. In these two divisions the mitochondria lie in the cytoplasm around the mitotic figure or amphiaster, and each of the daughter-cells gets a certain number of the individual mitochondrial grains (Text-fig. 1). There is no evidence for supposing that each daughter-cell gets half of a mitochondrium, as is the case in ordinary mitosis with the chromosome. The spermatocyte divisions finished, there are left four cells each containing an approximately equal amount of the mitochondria; these four

stage between 1 and 3 in Text-fig. 1. Between 3 (secondary spermatgonium) and 4 (spermatocyte) is the growth stage, during which the mitochondria become larger and in all probability divide by fission, and so increase in number. The spermatocyte divides twice to give rise to four spermatids (5). Each spermatid has sorted out to it one-fourth of the granules of mitochondrial nature in the spermatocyte (5, 6, and 7). Now come the spermateleosis stages (see page 209), which have no parallel in the development of the ovum. The mitochondria partly form the tail of the sperm (*m.*) and partly slough off (*x.*).[1] At 7 is the ripe sperm. On the right are the oogenesis stages. There are not always the same grades in the multiplicatory phases in the oogonium (2 A and 3 A) as occur generally in the spermatogonial generations. Some of the oogonial cells in some animals appear to become specialised to nourish one of their fellows, which is then the young oocyte (4 A). The mitochondria disperse out, becoming flocculent or in the form of fine rodlets or strings. Yolk begins to appear, and in certain forms already examined (frog, etc.) both yolk disclets and mitochondria tend soon to become segregated in certain regions. The arrow points from the vegetative to the animal pole; the mitochondria in 4 A are as yet flocculent and fine. In some forms they remain so, in others they later become coarser and spherical (5 A), and though these granules are dispersed in both animal and vegetative poles, it is generally found that they tend to lie mostly in the upper or middle regions of the egg. The polar bodies are given off, and apparently never contain any important part of the mitochondrial grains (Rana). At 5 A the ripe egg is drawn, the yolk (*y*) and mitochondria (*m*) being shown roughly as they are found, but these stages have never been studied thoroughly enough for one to give a diagram which would apply generally. The drawing in 5 A, however, serves to show that the mitochondria are not evenly dispersed throughout the egg plasm.

[1] It should, however, be pointed out that in some forms, possibly all Insecta and Prosobranchia for instance, there is no sloughing off of any mitochondrial matter. This may apply also to Mammalia and Scorpions. The case of Mammals (Mus and Cavia) is doubtful.

cells or spermatids now begin the last stage of the history of the formation of the ripe spermatozoon: the nucleus shrinks and undergoes certain staining changes, while from the centrosome grows the axial filament or tail of the spermatozoon. The mitochondria in all animals so far studied (with the sole exception of Peripatus capensis (**15**), now group themselves in some way or other in connection with the head centrosome and the axial filament, so as to form a definite part of the tail of the sperm in the region behind the nucleus. I have been able to show that in the case of Pulmonate Mollusca part of the mitochondria of the spermatid is always sloughed off with the rejected cytoplasm; this is a fact of the very first importance. (See description to Text-fig. 1.)

In the case of the egg, during growth the flocculent mitochondria gradually disperse through the furthermost parts of the cytoplasm, and they may later become more granular and larger. They do not metamorphose into yolk disclets, nor do they disappear; in some forms as Ascidia, the mitochondria during segmentation are segregated in special regions of the egg. In the maturation division of the egg the mitochondria take no very definite part as far as is known. In some unpublished work on the maturation of the egg in Rana temporaria I found that the minute polar body cytoplasm did not contain any mitochondria, but only the chromosomes of the maturation. No mitochondria are rejected in this way, as chromatin is rejected. This also is a fact of some importance. (For Oogenesis, refer to right side of Text-fig. 1.)

In both oogenesis and spermatogenesis there are in all animals certain other cytoplasmic bodies[1] which generally differ from both chromatin and mitochondria in their staining and fixing affinities. Whether such bodies in different orders and families of animals are homologous and identical one cannot at present say. In spermatogenesis such bodies may ultimately form part of the ripe sperm, but their function is at present obscure.

It will now be seen that in the genesis of egg and of sperm

[1] These are dealt with in a forthcoming paper.

the mitochondria are a constant and regular factor; they are undoubtedly of prime importance and there is no doubt that the correct solution of their function would be one of the most valuable of modern contributions for zoological and histological literature.

I regard the study of the mitochondria as being of great importance to the embryologist. Not only is their behaviour in gametogenesis worthy of notice, but in the methods which I have described here and in my previous papers, and which I have tried myself to improve, the embryologist has an instrument which will enable him to ascertain many remarkable facts relating to the division of the cytoplasm in the segmentation of the egg and in early organogeny.

In the case of delicate marine eggs especially should alcohol and acetic acid be rigidly avoided. Observers who study marine animals should carry out experiments with osmic acid, chromic acid, bichromate of potash, and formol mixtures. In some cases, two or three of these substances might be used successively.

Terminology.

It appears that there is still great confusion in the terminology used by various writers, and unwittingly I myself may have added to the disorder. The main factors in producing such confusion seem to be that, with the advent of improved technical methods our conceptions as to the origin and homologies of numerous bodies in different forms have had to undergo drastic alteration, and that older terms have been used several times by various authors in differing shades of meaning. It is merely in order to clear away some of these cobwebs that I give the following explanations and propose the following terms. Apart from the question of the mitochondria, one example will serve to show how great a confusion exists in many branches of cytology: The "centrosome" ((centriole?) or central spot in the asters of a mitotic figure), when in the "resting" cell has around it a cloud of stainable matter. This apparatus (or hypothetical bodies

conceived in connection with it) has been called Archoplasm, Archiplasm, Centroplasm, Attraction Sphere, Centrosphere, Astrosphere, Idiozome, Kinoplasm (Macrocentrosome), Periplast, etc. (see Wilson's "Cell," p. 323, etc.).

This is most complicated, and the various shades of meaning conceived by the inventors of these words are very difficult to understand. Unfortunately, however, this is not all; in the same connection there are the confused questions as to the relationship of "archoplasm" or "idiozome" to the acrosome or head of the sperm. Moreover, some of these words have others so like them, used in a totally different sense, that needless confusion is introduced, e. g., idiosome and idiozome.

In this short glossary no attempt is made to give exhaustive and useless references.

Apart from the fact that numerous terms have been coined for bodies of identical nature in different animals, there are certain terms which have been invented by observers of the Protista. For instance, the word centroplast refers in the Heliozoa to a body which Schaudin showed in Acanthocystis to act in mitosis exactly as the Metazoon centrosome. Schaudin's account has lately been confirmed by Dobell ('Quart. Journ. Micr. Sci.,' vol. 62, part iv), who, curiously enough, instead of seeing in this added support to Schaudin's theory on the phylogeny of the centrosome, is sceptical with regard to the view that the centroplast of Heliozoa is homologous with the centrosome of Metazoa.

Glossary.

Apyrene, Eupyrene, Oligopyrene Spermatozoa, and Typical and Atypical Spermatozoa.—Eupyrene and Typical spermatozoa are ordinary normal sperms.

Atypical spermatozoa may be either "Apyrene" when the nucleus for some reason has altogether degenerated and is absent, or "Oligopyrene" when by some process only part of the chromosomes go to form the sperm nucleus ('Quart. Journ. Micr. Sci.,' vol. 62, part iii, p. 465). Some authors are in favour of discarding Meves' terms Oligopyrene, Apy-

rene, and Eupyrene, in favour of the simpler terms Typical and Atypical spermatozoa. I am in agreement with this, especially in view of the fact that I deny that such spermatozoa have the significance attached to them by Meves and others (Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, p. 481).

Archoplasm, or Archoplasmic Zone.—The dense mass of protoplasmic material gathered around the centrosome in the form of a sphere. Meves' word "idiozome" is applied to the large archoplasmic zone of the spermatid centrosome, and as there is no doubt that the archoplasm of both somatic and germ-cells is identical, it is suggested that Meves' term "idiozome" be discarded in favour of the word "archoplasm," or archoplasmic zone. Other words which have been used in almost if not quite identical sense as Archoplasm are "attraction sphere" and "astrosphere," or simply "sphere"; the former is quite commonly used in connection with germ-cells. Not only is Meves' term superfluous, but it is too much like the word "idiosome," which means the same as pangen or biophore.

Acrosome and Acroblast.—The perforatorium or acrosome is the small body or cap at the extreme tip of the ripe sperm. This body has been traced back by me to the young spermatocyte ('Quart. Journ. Micr. Sci.,' vol. 62, p. 438) and is in all earlier stages separate from the nucleus. It is only later that it becomes fused to the head of the metamorphosing sperm nucleus. It is proposed to call any body found in the cytoplasm of the male germ-cell which finally gives rise to the acrosome or perforatorium—an acroblast ('Quart. Journ. Micr. Sci.,' vol. 62, p. 418, footnote), and it is not till the acroblast or acroblasts have fused to form the cap of the spermatid nucleus that they (or it) form the acrosome. On Pl. 25, fig. 41, of 'Quart. Journ. Micr. Sci.,' vol. 62, I should call the bodies *A.B.* acroblasts, the fused body in Pl. 25, fig. 42, *A.C.*, an acrosome.

Cytoplasmic Bodies, or Plasmatic Bodies.—Terms apply to all cell elements other than the nucleus (Chromatin). This would include centrosome, archoplasm, mitochondria,

and the various other bodies of an enigmatic nature found in the cytoplasm of germ-cells. I do not think that yolk discs or fat globules come under this term; I would call the latter "deutoplasmic bodies," for they are obviously not such definite structures as the mitochondria or chondrioplasts.

Chondrioplast.—The archoplasm in the germ-cells of some animals is often studded over its periphery with a number of banana-shaped rodlets or batonettes. The whole apparatus (archoplasm included) has been called the "nebenkern" (see "Nebenkern" in this glossary). The term "chondrioplast" is singular number and refers to one of the rods or batonettes. The adjective would also be chondrioplast, the entire rods together would form the chondrioplasm. The rods would be spoken of as chondrioplast batonettes or rods, or simply chondrioplasts. The name also draws attention to the fact that these bodies are akin to the mitochondria in fixing and staining affinities. What was originally called the "nebenkern" of pulmonates would under this nomenclature be spoken of as "chondrioplasts and archoplasm."

Chondriosome, Chondriokont, and Mitochondrium.[1]—The bodies signified by these names[2] have been found in the cytoplasm of the germ-, nerve-, gland-, blood-, muscle-, and connective-cells, etc., of all animals. They are in the forms of rods or granules, are dissolved by acetic acid, alcohol, chloroform, and ether, resist nitric acid (circa, 3 per cent.), are sometimes turned brown by osmic acid (rarely black), are preserved by the chromium salts, osmic acid, and formalin, and are stained intra vitam by janus green, neutral red, etc. The mitochondria in fresh unstained material look like slightly refractive rods or granules and can often be noticed in flowing movement.

[1] Regaud and Fauré-Frémiet both consider the mitochondrium chemically a combination of phospholipin and proteid.

[2] Mitochondria (μίτος, a thread; χόνδρος, a grain) are granular; chondriokonts (κοντός, a pole) are rod-like. Chondriosome (σῶμα, a body) is a general term applied to both varieties. The word mitochondrium is now used for a body of any shape and is more frequently used than chondriosome.

It must particularly be noted that there are other cytoplasmic bodies such as chondrioplasts ("nebenkern" batonettes of Pulmonates), acroblasts, and certain siderophile granules, which are not exactly mitochondrial, but which are very nearly of the same chemical nature. All these bodies and the mitochondria act in almost the same way under treatment of acids and fat solvents.

The mitochondria can be distinguished from these bodies only by watching their behaviour, or by the fact that such pseudo-mitochondrial elements are of a different size or shape from the mitochondria. In certain cases mitochondria cannot histochemically be distinguished from the zymogen granules of giant cells.

Nebenkern.—This term has been applied to at least three different cell elements.

(1) The mitochondrial body in the spermatid of Insecta ("Macromitosome," 'Quart. Journ. Micr. Sci.,' vol. 62).

(2) The chondrioplasts and the archoplasm of Pulmonate and other Molluscs. (These are not the mitochondria.)

(3) The secondary nuclei in the egg of an ant, Camponotus (Blochmann): such secondary nuclei are unrelated either to chondrioplasts or mitochondria.

(4) The micronucleus of some Ciliates has been called "Nebenkern" by Butschli (1876) and by Hertwig later.

(5) The enigmatic secondary "nuclear" body in Paramœba eilhardi has been called "Nebenkern" by Schaudinn.

(6) The term has also been applied to cytoplasmic structures in gland and nerve cells.

It is clear that the greatest confusion exists with regard to the word "Nebenkern"; in fact, some authors have applied this term in an extremely slipshod manner to any enigmatic body lying at any time near the nucleus. A convenient rendering of the term "Nebenkern" would be "paranucleus." The body in insect and other spermatids called "Nebenkern" was at first thought to be derived from spindle-fibres; it is really mitochondrial, and I have already proposed the term "macromitosome" for it (see "Macromitosome").

The word "Nebenkern" has been used so loosely that the application of it to any structure occurring in gametogenesis only produces confusion, and it is recommended that cytologists discard it in favour of other well-defined terms.

Nest.—The primary spermatogonium (see "Spermatogonium") gives rise by a number of divisions to a group of cells (secondary spermatogonia), which generally all develop side by side in the form of a "nest" or definitely isolated group of cells. (For a spermatogonial nest see Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, Pl. 25, fig. 46.)

Middle Piece.—This term is used in connection with that region of the sperm just behind the head centrosome. In the spermatozoon of mammals, the nucleus is generally a spatulate shape; behind it comes a short region which lies between the head and second centrosome. This part is apparently the mitochondrial region of the sperm-tail, and is, strictly speaking, the middle piece; behind this region is the rest of the sperm-tail. In Molluscs and certain other animals there is no such arrangement; in these cases the entire tail has formed around it a mitochondrial sheath, and thus the entire tail in the mollusc is the homologue (as far as mitochondria are concerned) of the short region behind the head centrosome of the mammalian sperm. In all sperms there is a region behind the nucleus where the head centrosome lies—the second or tail centrosome in some molluscs and insects seems to become sloughed off or absorbed—but it should be remembered that there would be difficulty in identifying a special middle piece such as in mammals, throughout the animal kingdom. This is particularly the case with Invertebrates.

Macromitosome.—In many Arthropods, and apparently in all the Insecta, the mitochondrial bodies of the spermatid become grouped to form either a remarkable spireme or a definite figure of some kind, or macromitosome. This body has been called a "Nebenkern," but for reasons given above, under the heading of "Nebenkern," it has been thought

better to avoid the further use of this confusing term. (For a macromitosome, see 'Quart. Jour. Micr. Sci.', vol. 62, Pl. 23, fig. 17, *M.D.*)

Spermatogonium, Spermatocyte, Spermatid, Spermatozoon.—For all these terms, see Wilson's Text-book on 'The Cell.' In using the words primary spermatogonium and secondary spermatogonium, one refers to the fact that in many animals the primordial or indifferent germ-cells in the future testis generally can be distinguished as undergoing a definite number of division grades. Thus the embryonic or primordial testis, which may often be distinguished from the primordial ovary by its shape or by the manner in which its cells are grouped, contains a certain number of cells—or primary spermatogonia; generally these are not as yet grouped in cysts or nests (see 'Nest'). Later, they undergo further divisions, and become grouped in cysts; in all probability one primary spermatogonium gives rise by itself to a group of secondary cells or secondary spermatogonia, which become grouped to form a definite cyst of cells. The secondary spermatogonia generally pass on to the growth stage, becoming spermatocytes (see Hegner, 'Germ-cell Cycle in Animals,' Macmillan, 1914).

Spermatogenesis.—This term applies in all modern literature to all the changes which lead to the formation of a ripe sperm from a primordial germ-cell. There is a specially important period in spermatogenesis known as the "metamorphosis of the spermatid into the spermatozoon." This period is especially important, since up to the spermatid stage the sperm-cell is like any other cell in appearance; at this period there begin those remarkable changes which end in the formation of the spermatozoon. As there is no special term for the expression "metamorphosis of spermatid into spermatozoon," I propose the term "spermateleosis"[1] to mean the changes during which the ordinary spermatid cell becomes converted into the peculiar flagellate organism known as the spermatozoon. This term is really needed in cyto-

[1] From σπέρμα, and τελέω, I finish.

logical literature; many of the papers on spermatogenesis deal especially with this stage, during which the nucleus and cytoplasmic bodies become so profoundly altered.

Spireme: Macromitosomal or mitochondrial spireme.—The word spireme means something coiled, or a skein. Generally applied to the nucleus at a time when the chromatin forms a coil. I have used it in connection with the mitochondrial coil (macromitosome) of Lepidoptera.

Spindle-bridge.—The spindle fibres in the telophase of division often condense to form a body joining the two daughter-cells long after the division has taken place. This body is the spindle-bridge. Equivalent to the German "Spindelrestkörper" and the French "reste fusiorial." For a spindle-bridge, see Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, Pl. 25, fig. 46, *S.B.*

For a number of other terms widely used in cytologcial literature, one may consult the excellent "Glossary" in Wilson's text-book on 'The Cell,' where an extensive bibliography is given.

Material, Methods Used, and Species Studied.

The technique used by me hardly differs from that already described in the previous paper (**1**). Where any differences exist, I have indicated in the section of the paper immediately concerned.

The Pulmonates studied were as follows: Helix aspersa, H. nemoralis, H. rufescens, Limax maximus, L. agrestis, Arion ater, and A. hortensis. I also sectioned and studied less thoroughly Limax marginatus. It will be noticed that I have chosen in each genus a large species and a small one. This does not apply to Testacella, where I could only get one species.

The length in millimetres of the species examined by me is as follows, the average being given:

Limax maximus	125
Limax agrestis	35

Arion ater	57
Arion hortensis	20
Helix aspersa	60
Helix nemoralis	40
Helix rufescens	17
Testacella haliotoïdes	65

In different localities the average sizes of the slugs (Limax and Arion) vary considerably: the figures given for L. maximus represent the average size of about six specimens used by me; very often L. maximus attains a size of 6 in. (153 mm.).

Following the classification given by Pelseneer in 'Lankester's Treatise on Zoology,' part v, Mollusca (**2**), we find that the Families, Helicidæ, Limacidæ, and Arionidæ, all belong to the Tribe Holognatha. In the latter the jaw is simple, without a superior appendage. Testacella is grouped in the possibly polyphyletic Tribe, Agnatha; in this there are no jaws, the radular teeth are narrow and pointed.

In the Limacidæ and Arionidæ differences in external morphology are not at first very obvious. Both Limax and Arion are "slug-like." In Arion the pulmonary opening is towards the front end of the mantle, while in Limax it is nearer the hind end; in Arion there is a tail mucous gland, which is absent in Limax. The Limacids have a definite shell covered by the mantle (shield), but in the Arionidæ the shell is amorphous, being formed of granules. Though the differences between the Limacidæ and the Arionidæ are not great, they are distinctive when once grasped, and it is impossible to confuse the two genera. Nevertheless, it must be said that the two genera are undoubtedly very closely allied. Testacella, in outward appearance, is quite distinct; the Helicids are also quite a well-marked type. How far the outer morphological differences are correlated with the differences in the germ-cells and mitochondria will be shown below, and I will endeavour to analyse how far important are the size differences with relation to the mitochondria in the three genera in which I have been able to study giant and small forms.

The Arionidæ (the Post-nuclear Granules).

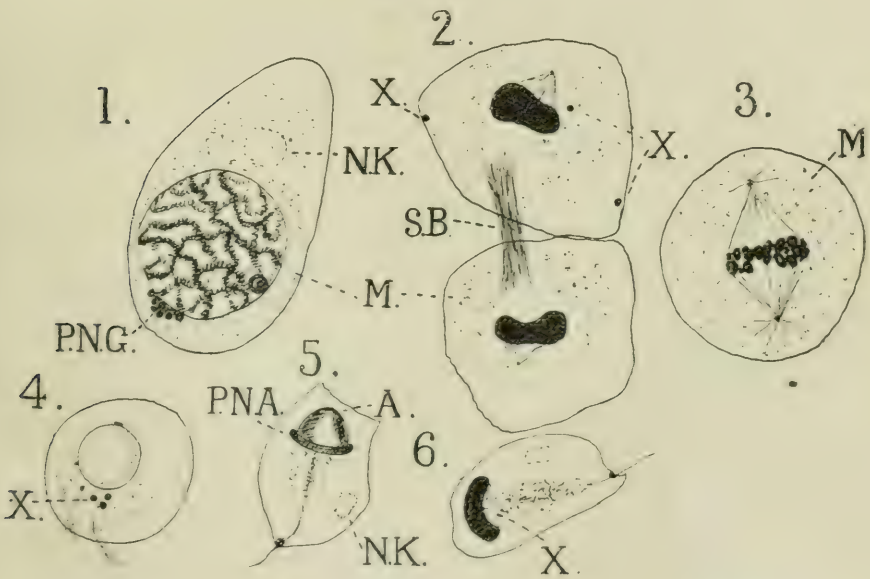
The case of the Arionidæ will be treated first because it is in this Family that certain new bodies found by me are best developed.

If the metamorphosing spermatid of Arion ater or A. hortensis be examined at such a stage as that in Pl. 16, figs. 15 or 16, it will be noticed that at the back of the nucleus lies a darkly staining, coarsely granular body marked *P.N.G.* From their position I have called these structures the post-nuclear granules. When once the appearance of the post-nuclear granules has been learnt, it is an easy matter to find them in the spermatocyte, especially in sections which have been differentiated till the mitochondria are greyish. The granules may then be easily discovered, since they retain the stain more than the mitochondria and, consequently, appear black (Pl. 16, fig. 15, *P.N.G.*). The position in which I have drawn these granules, both in Pl. 16, fig. 11, and in Pl. 16, fig. 12, is quite characteristic. In a bundle of spermatocytes attached to a part of the germinal epithelium it will almost invariably be found that the chondrioplasts lie towards the epithelium, while the post-nuclear granules are towards the lumen of the gland. If the spermatocytes are examined just at or after the prophases of the heterotypic division, the post-nuclear granules (hereafter called P.N. granules) are very clear, as in Pl. 16, fig. 11).

Now, if the Arion's ovotestis be preserved for eighteen to twenty hours in my modification of Flemming, then cut 6 μ in thickness and stained with eosin and toluidin blue, the mitochondria are reddish, the chromatin is blue, and the P.N. granules blue. The slide is first flooded with a ·5 per cent. solution of eosin, and warmed till steaming for about half a minute. The superfluous eosin is drained off and a ·75 per cent. solution of toluidin blue added. This is heated in the same way for about one minute; afterwards the toluidin blue is poured away, the slide drained and blotted. It is then quickly differentiated in 90 per cent. alcohol, dipped in absolute,

then into xylol, and mounted. The P.N. granules stain just as densely as the chromosomes in the metaphase, and can be picked out with a one-sixth objective quite easily. The mitochondria are stained by the eosin a red colour, but are not sharply differentiated. The chondrioplast also is vaguely reddish in colour like the mitochondria. By this means it has been possible to follow out the history of the P.N. granules.

TEXT-FIG. 2.



The ovotestis was fixed in Flemming fluid without acetic acid, and stained in eosin followed by toluidin blue. The mitochondria stain vaguely reddish, the post-nuclear granules (*P.N.G.*) of the spermatocyte are blue (Fig. 1). In the prophases of the maturation division and during division (Fig. 2) the post-nuclear granules cannot be found. In rare cases one can find granules (*X.*), which I do not think are the post-nuclear grains. In Fig. 3 a second maturation division metaphase is drawn showing mitochondria vaguely represented, and absence of post-nuclear grains. In Fig. 4 is a young spermatid in which the *P.N.Gs.* are grouped behind the nucleus (*X.*). In Fig. 5 the *P.N.Gs.* have fused to form a collar behind the nucleus: the post-nuclear matter stains less densely, now a purplish colour, and by the stage in Fig. 6 the zone behind the nucleus marked (*X.*) appears to stain reddish, no blue colour being perceptible. *N. K.* Chondrioplast. *S. B.* Spindle-bridge.

These first make their appearance a little before the prophases of the heterotypic division begin. They reach their maximum size and density between the stages drawn in Pl. 16, figs. 11 and 12, when they form a more or less grape-like mass, disposed as already explained. When the spermatocyte enters the prophases of the first division the P.N. granules are nowhere to be found, and they certainly disappear before the chondrioplast batonettes become disturbed prior to nuclear division.

Afterwards there is to be found in the young spermatid a number of granules which are almost certainly of the same nature as those in the spermatocyte, though it is impossible to follow them through the maturation divisions to make quite sure.

In Arion hortensis I could count eight granules in the spermatocyte at the pachytene stage.

After their reappearance in the young spermatid, the P.N. granules are generally found grouped, as shown in Pl. 16, fig. 13, in A. hortensis. At this stage they form an irregularly spherical mass of granules, the same size as the mitochondria, but staining more heavily. In the next stage the P.N. granules move towards the nucleus and become grouped to form a plate, as shown in Pl. 16, fig. 14. They are then spread out like a layer of marbles, whose individual elements are fairly regularly, but not quite evenly, disposed. The centrosome divides at about this time, but I have not specially studied the events connected with the centrosome at this period. A little later the P.N. granules close up together and form a solid plate, whose centre is pierced by the first centrosome and the axial filament (Pl. 16, fig. 15). The granules have hitherto been separated from the nucleus by a small space. In the next stage the post-nuclear apparatus becomes applied to the posterior surface of the nucleus, as shown in Pl. 16, fig. 16. The P.N. apparatus is now at its most conspicuous stage and cannot be overlooked. In Pl. 16, figs. 15 and 16, it will be seen that apparently the substance forming the P.N. apparatus is much larger in extent than that

in the early spermatid, as for instance, in Pl. 16, figs. 13 and 14. Very often it is difficult to ascertain whether there is a dividing line between the mitochondria behind and the post-nuclear grains. In such a case as that in Pl. 16, fig. 16, often it looked as if the thickened P.N. disc had grown at the expense of some mitochondrial granules. Very soon after the formation of the solid disc behind the nucleus, the mitochondria become grouped to form a mass at the rear of the P.N. apparatus.

Gradually the spermatid nucleus becomes basophil, and the P.N. apparatus and acrosome become for a time obscured. When the nucleus again stains oxyphil the post-nuclear apparatus is found to have shrunken considerably, and it now forms a very small perforated disc just behind the first centrosome (see Pl. 17, fig. 27, *P.N.A.*).

There is no reason for doubting that the P.N. granules give rise to an apparatus which takes part in the formation of the ripe sperm, and their position in the latter is just behind the first centrosome.

In the figures given in Text-fig. 2 it might be thought that what I have described as P.N. granules are really a form of multiple centrosome, and the disappearance of the P.N. granules at the prophases of division might be explained by supposing that these structures form the centrosomes of the asters. Against the view that the P.N. granules are centrosomes there are three irrefutable facts.

(1) The centrosome is always near or inside the archoplasm, and centrosomic activity always arises within the archoplasm (see especially Bolles Lee's work, and my Pl. 16, fig. 5).

(2) The centrosome of the dividing pulmonate germ-cell is never multiple.

(3) The centrosome, in later stages of the spermatid, can be seen to be quite separate from the P.N. granules (for instance, Pl. 16, fig. 16).

It is clear, therefore, that the P.N. granules are not of a centrosomic nature.

A Comparison of the Mitochondria of Eight Species of Pulmonates.

In the first place it should be remarked that the Pulmonate Mollusc is not the best form for instituting a comparison in size of the mitochondria, principally because, in different parts of the ovotestis, the mitochondria tend to differ often quite considerably in size. This is because of the varying conditions of nutrition and the varying number of cell divisions which the generations of cells undergo. But, by examining a large number of cases, it becomes possible to gain a good idea of the average size of the chondriosome elements.

In my previous paper (1) I made a special study of the size differences in Helix, and my figures there are sufficiently explanatory. I have also found differences in Limax and in Helix rufescens. In all my preparations, here and there I found cells in which the mitochondria were undoubtedly in the form of hollow spheres (Pl. 16, fig. 4, and Pl. 17, fig. 24), and there is little doubt that the mitochondrial element of the Pulmonates consists of two parts—an inner, less colourable core, and a cortical layer of chromophil matter.

Comparison of the mitochondrial bodies of all the species treated here shows that in a given order, such as the Pulmonata, there is a general agreement in mitochondrial size, though the sizes of the animal forms may differ markedly.

The manner in which the mitochondria become grouped in the spermatocyte divisions, the grouping of these bodies in spermateleosis (p. 209), and their final disposition is throughout similar in every case. The same applies to their behaviour with regard to fixatives and to stains.

Though there is a tendency throughout for the sperm-tail to be spirally twisted, this tendency is more pronounced in Testacella than in Limax. In the slug-like forms there is a constant tendency for the mitochondria to become divided into two batches, one of which retains its position near the head of the sperm, while the other drifts down to the tail (see Pl. 17, fig. 22). This occurs strikingly in Arion hor-

tensis, apparently less in A. agrestis, and never in the Limacids. Such differences as can be demonstrated are, however, quite small. In Limacids, for instance, the mitochondria very characteristically become grouped behind the post-nuclear disc, as shown in Pl. 16, figs. 7, 8, and 10, while in other forms this grouping, though quite evident, seldom occurs so strongly as to produce perfectly constant and compact figures. In every form so far observed there is a residuum cast away at the end of spermateleosis. This is shown in Pl. 16, fig. 9, at *R.*; in Pl. 16, fig 3, this residual globule is drawn to a much higher scale (see Pl. 17, fig. 26). The mitochondria in such globules generally stain lightly, and rarely quite so sharply, and appear to have become altered. In Helix aspersa I found that they became larger as they were gradually sloughed down the tail. Curious as it may seem, in Limax agrestis especially, I am inclined to believe that the mitochondria sloughed off in the residual globule are as numerous as those to be found in the spermatid. I am convinced that the mitochondria of Pulmonates do not bodily form the tail sheath as seems to be the case in Mammalia.

With regard to the important question of the formation of the tail of the sperm, in relation to the part played by the mitochondria, I carefully studied the case in Testacella haliotoïdes.

With quite low powers it is evident that the sperm-tail of Testacella is spirally twisted. In Pl. 17, fig. 25, the head and upper region of the nearly ripe sperm is depicted. If favourable examples be chosen it will be found that there is an inner rod (*C.R.*) surrounded by a spirally twisted sheath (*S.P.*). The spiral goes in the opposite direction to the hands of a clock; I found no exceptions to this.

I have noticed that it is a most difficult matter to ascertain the exact origin and relationship of these elements in the tail. In Pl. 17, fig. 30, is drawn a sperm at a stage when the spiral sheath (*S.P.*) is partially formed. Between the letters *X.–X.* and *Y.–Y.* is a region where the spiral gradually disappears;

now, inspection of the region *Z* shows that there is already formed around the axial filament a thicker rod; the latter seems almost certainly identical with the central rod drawn in Pl. 17, fig. 25, at *C.R.*

Careful examination of the region *X.* and *Y.* in Pl. 17, fig. 30, shows that the spirally twisted part of the sperm appears to be an outer purely cytoplasmic sheath, while the inner core (*Z.*) is mitochondrial in nature; nevertheless, I am unable to speak with certainty. In Pl. 17, figs. 31 A, B, C, and D, I have drawn various regions of the tail at different stages; Pl. 17, fig. 31 A, is typical of a lower region of Pl. 17, fig. 31, and Pl. 17, fig. 31 B is the kind of loose spiral, apparently formed of two strands, neither of which stained more heavily than its fellow.

Pl. 17, fig. 31 C, is a mid-region in a fully-formed sperm. Pl. 17, fig. 31 D, is another upper region in an early sperm. Examination shows that at first the spiral is loosely twisted, but later on it becomes telescoped up, and then appears as in Pl. 17, fig. 31 C. At such a stage it is impossible to form an idea as to the elements forming the spiral. Pl. 17, figs. 31 A and D appear to show that there is an inner dark rod around which the spiral part is coiled. In sperms at the stage drawn in Pl. 17, fig. 30, the spiral appears to fade into the region just where the cytoplasm is being "skimmed off" the rod; it should be pointed out that there are mitochondrial granules in this region as well.

The later sperm is drawn in Pl. 17, fig. 26, the beads of cytoplasm containing the plasmatic elements are eventually sloughed off. These beads contain granules which were the mitochondria of the spermatid, but they now often appear changed; the changes are sometimes ones of size, at other times ones of staining power. The chondrioplast does not seem to take direct part in the formation of the sperm-tail, though it is impossible to form a quite definite opinion. The behaviour of the mitochondria at the time when the post-nuclear apparatus is formed is of some importance. It has been noted in every case that the chondrioplasts take up a

position intermediate between the head and tail of the lengthening sperm; it is also evident that in such a case as Arion hortensis the mitochondria become divided into two groups. In Helix aspersa it has already been shown that the smaller mitochondria lie nearest the head centrosome in the lengthening sperm. All these facts mean that at the time when the sperm is lengthening some body at the posterior end of the ovoid nucleus is exerting influence on the mitochondria and chondrioplasts. The latter take up the position indicated in Pl. 17, fig. 22, probably because this is the region where the forces causing the lengthening tendency, and the force being exerted from behind the nucleus on the plasmatic bodies, are balanced. In the same way the smaller size of the micromitochondria enables them to be influenced more easily than the larger mitochondria, and hence one finds the grouping indicated in the figures in this and my previous paper. The probable reason why the mitochondria in Arion become split into two groups is that the forces being exerted from the region of the nucleus only succeed in attracting part of the mitochondria while the outgrowing tail end of the cell carries away the rest (Pl. 17, fig. 22).

In some cases, just at the prophases of the spermatocyte mitosis, the mitochondria seem to be definitely gathered around the archoplasm; the attracting body is probably the centrosome, which is breaking into activity.

In all Pulmonates examined by me it has been found that there exists a remarkable period during which the staining of the tail alters very greatly. If such a portion of the ovotestis wall as that drawn in Pl. 16, fig. 9, be examined, it will be found that the spermatids at different periods in spermateleosis do not agree in staining affinities. In Pl. 16, fig. 9, at 1 are spermatids in the stage drawn in Pl. 16, figs. 7 and 8; at 2 are spermatids drawn at a stage earlier than that in Pl. 16, fig. 6; and at 3 are spermatozoa nearly ripe. It is quite evident that the latter stain with greater intensity than any other stages. This increased affinity for stains is due to the tail sheath having been formed; it is

just at the time of formation of the sheath that this increased affinity is noticeable.

A Comparison of the Chondrioplast[1] Elements of the Eight Species of Pulmonates.

I have found that each species possesses the well-known chondrioplasts in the form of siderophil banana-shaped batonettes. The number of rods can be fairly easily counted in the spermatids of some species.

The numbers seem as follows:

Limax agrestis, 2 batonettes in the spermatid.
Limax maximus, 2; rarely 3.
Arion ater, 4 to 8.
Arion hortensis, 4 to 8.
Helix aspersa, 6 to 8.
H. nemoralis, 6 to 8.
H. rufescens, from 5 to 7.
Testacella haliotoïdes, from 6 to 14.

From the case of the Limacidæ especially it is evident that in the spermatocyte divisions the number of batonettes is halved at each mitosis. The Limax spermatid has two rods, the spermatocyte eight (Pl. 16, figs. 1 and 7 or 10). There is a greater difference between the chondrioplast of L. agrestis and L. maximus than between these bodies in the two Arionidæ. In L. maximus the batonette is slim, while in L. agrestis it is much stouter and more curved. In the Helicids and Testacella the rodlets are often approximately of the same type. In Arionids the chondrioplast rods form a fairly characteristic body, being stumpy; their ends appear to fuse and cause the body of individual batonettes to be less well defined than in other forms (see Pl. 16, figs. 15 and 17). In Testacella the rodlets, though very like certain Helicid types, are yet finer and more numerous. In fact, it can safely be said that each family has a distinct type, and even specific differences may be found (e.g. Limacids).

[1] Chondrioplasts and acroblasts will be discussed in the light of "Golgi-Kopsch Apparatus" in a future part of this series.

Though it cannot be quite definitely stated, since there is no accurate method of judging, in all probability the amount of chondrioplast material is about the same in the eight species; that is, of course, proportionately to the size of their cell.

In every case the chondrioplasts take up their position half-way between nucleus and tail centrosome in the stages drawn in Pl. 16, figs. 7 and 8, 16 and 17, and Pl. 17, figs. 19, 21 and 22.

This has already been commented upon; in certain cases (Arion hortensis) it seems as if the chondrioplasts were partly responsible for the sorting out of the mitochondria into two groups. In Pl. 17, fig. 18, the chondrioplasts at *X*. mark the position where the division into two groups takes place. In Pl. 17, fig. 20, the chondrioplasts are not at this place, but in Pl. 17, fig. 22, they are all in the characteristic intermediate position.

With regard to the very remarkable differences in the shape and size of the individual chondrioplasts in Helix aspersa, which were described in the previous paper of this series, and which might be thought due to a faulty technique, I have found in Limax agrestis especially the batonettes are very regular in size and in the amount of curvature. Faulty preparations never seemed to alter the size or curvature of the rods. The rods being stuck on an archoplasmic sphere I at first naturally thought that bad fixation of the sphere might cause the curvature of the rod to alter. Limax agrestis was a good form in which to study this, and my observations reinforce me in the view already taken after my "intravitam" tests (previous paper, p. 597) that the rods in Helicids especially may vary in size, number, and curvature to a remarkable degree.

For variation in chondrioplasts of Arion rufus compare Pl. XX, fig. 32, etc., with fig. 38 in the paper of Fauré-Fremiét (**3**). In one case the batonettes are straight or banana-shaped, in the latter case they are bent circular.

The Fate of the Chondrioplasts in the Maturation Divisions.

In my previous paper I was unable to come to a satisfactory conclusion with regard to this question. Fauré-Frémiet (3), in his valuable paper, figures a maturation division metaphase showing the batonettes at the poles of the spindle. Now it has also been suggested that the batonettes have something to do with the formation of the amphiaster (3), and up to date no satisfactory account of this important point has been given. After carrying out some experiments, I believe I have ascertained correctly why some authors are at variance, and what becomes of the batonettes during division. If the Pulmonate ovotestis is left overnight in chromo-osmic fixatives, and stained as directed in my last paper (twelve hours iron alum, twenty hours in hæmatoxylin), the batonettes are generally not to be found during metaphase, and they disappear during the prophases.

If the material is fixed several days, as for Benda's method, in chromo-osmic fixatives, and left twenty-four hours in mordant and twenty-four hours in hæmatoxylin, the chrondrioplast rods are found attached to the asters, as drawn by Murray (1) and Fauré-Frémiet (3).

It is evident, therefore, that during prophases of the maturation kinesis, the batonettes as well as the mitochondria lose much of their affinity for stains, and cannot be demonstrated without recourse to specially heavy chromatisation of the material, and long mordanting and staining.

These facts show :

(1) That the chondrioplast batonettes, unlike the mitochondria, are attracted specially by the centrosomes.

(2) That the chondrioplast batonettes undergo some chemical change during the prophases of division, and do not resume their normal reactions to fixatives and stains until after the formation of the spermatid nucleus.

(3) That the chondrioplast rods are like the mitochondria, in that they become changed somehow during the maturation

divisions, and that this all supports my contention that the chondrioplast batonettes are somewhat akin to mitochondria.

(4) That the chondrioplast rods, being demonstrable by special methods during maturation divisions, do not take direct part in the formation of the amphiaster, though it is possible that some substance in them might have been withdrawn to give rise to part of the amphiaster.

(5) That since the chondrioplast batonettes disappear altogether (with certain staining methods (1)) during division, while the mitochondria, though also affected, do not become altogether lost with these weaker staining methods, it therefore follows that the chondrioplast elements are not quite similar to the mitochondria in their behaviour during karyokinesis and in their chemical nature.

The Micromitochondria.

One object in carrying out an examination of all the Pulmonate forms that I could procure, was thoroughly to examine the remarkable micromitochondria. The latter were first described in my previous paper. The question as to their possible occurrence in other pulmonates besides the Helicidæ is important.

In the form Helix aspersa further study shows that if the cell after staining in iron hæmatoxylin is differentiated, there is a stage which can be reached at which the micromitochondria stain less heavily than the macromitochondria. In Pl. 17, fig. 19, I have drawn a spermatid of Helix aspersa showing the micromitochondria at M^1.[1] The micromitochondria are paler than the macromitochondria ($M.^2$); at first it may be thought that this paleness is due to the fact that the latter being larger than the former take more time to differentiate, and so appear darker. I do not think this is all; in Pl. 17 fig. 19, at *X.*, is a micromitochondrium, staining palely, and at *Y.* is a macromitochondrium hardly larger than the granule at *X.*, but staining quite blackly. The micromito-

[1] Good microphotographs of these have been obtained.

chondria cannot be said to stain less heavily than the macromitochondria, because they are less exposed to the stain. The micromitochondria appear to be less dense than the macromitochondria, and the attractive force exerted either by the post-nuclear plate or the anterior centrosome causes the plasmatic elements to be arranged in order of density or specific gravity. It might then be suggested that the mitochondria of Helix aspersa vary greatly in size in the individual cell, and that such a force tends to sort them out in order, the smaller ones being near the nucleus. In all the clearest cases I could find there was no even transition between micromitochondria and macromitochondria. The division as drawn in my Fig. 19, on Pl. 17, was very clear and sharp. Moreover, the individual size of the mitochondria in a given cell of a pulmonate is fairly regular, as drawn in all my figures. (For an exception to this, see (1) Pl. 32, fig. 24 A, where the macromitochondria vary somewhat in size.) I have little doubt that the micromitochondria are present in the spermatocyte, but it is not till the sorting-out force caused by the body near the nucleus is applied that these granules become apparent.

In another section of this paper I stated the view that the mitochondria of Pulmonates do not bodily form the sheath of the sperm-tail. This undoubtedly applies to the larger mitochondria, but in later stages of spermateleosis in Helix aspersa it appears that the micromitochondria do become bodily applied to the axial filament, which seems to have a great attraction for them.

In Helix nemoralis a curious condition is found (Pl. 17, figs. 20 and 21). The part which in H. aspersa is occupied by the micromitochondria is here taken by about the same amount of mitochondrial matter, but the grains are the same size and stain in the same way as those lower down (Pl. 17, fig. 21). Speaking correctly, there are no micromitochondria as found in H. aspersa, but there are granules which act similarly to the micromitochondria. Attention should be drawn to the case of Arion hortensis (Pl. 17, fig. 22, *M.X.* and *M.Y.*),

where the mitochondrial granules, though of the same size throughout, are always sorted out into two nearly equal halves, which take up proximal and distal positions in the spermatid. In H. nemoralis the proximal mass of granules near the nucleus is never so large as the distal mass. In the case of Testacella haliotoïdes, the mitochondria rarely divided into separate masses, but become strewn evenly along the axial filament (Pl. 17, fig. 27).

In later stages in H. nemoralis the distal mass (Pl. 17, fig. 21, *M.Y.*), becomes cast down the lower length of the spermatid, while the proximal mass keeps its position. The chondrioplasts remain about half-way between for some time, but gradually pass down the tail.

I examined Helix rufescens (Pl. 17, fig. 23). In some cases, just behind the nucleus, one finds a greyish zone, which may represent the micromitochondria; in Pl. 17, fig. 23, $M.^1$, this collar is very plain. In many other spermatids no such zone can be found, and there is great difficulty in making quite sure whether this area behind the nucleus is really formed of granules.

Besides Helix aspersa, H. nemoralis, and H. rufescens, I examined H. arbustorum and H. cantiana (?). In H. arbustorum there were no micromitochondria, but the spheres were strewn evenly along the length of the cell. The other species, H. cantiana, seemed to be like H. rufescens, and I am uncertain as to whether micromitochondria were present or not, for their germ-cells were too small for successful study. As far as the Helicids are concerned, it will probably be found that completely differentiated micromitochondria are only present in the larger species. Owing to lack of material I have been unable to examine the case in H. pomatia. In other forms I found that in Limax agrestis the axial filament was often surrounded by a granular coat (Pl. 16, fig. 8, $M.^2$). In other cases (Pl. 16, fig. 7) no coat could be found, but such cases as that drawn in the previous figure were so clear that I believe there is good evidence for supposing that micromitochondria, though

extremely minute, may be found in other forms of Pulmonates. Unfortunately, the germ-cells of the slugs are too small for satisfactory study of such a very difficult matter. It must be rememembered that even in the case of Helix, where the germ-cells are large, other observers have overlooked the micromitochondria.

Micromitochondria in Alizarin-Crystal Violet Preparations.

The micromitochondria of Helix aspersa are so remarkable that many Benda alizarin-violet preparations were made in order to examine the case with this well-known stain. In this process the ovotestis of Helix is left for a week in Flemming with much reduced or no acetic acid at all, and then treated for twenty-four hours in a mixture of equal parts of pyroligneous acid and 1 per cent. chromic acid, then for twenty-four hours in a 2 per cent. solution of bichromate of potash. After washing for twenty-four hours sections are made in the usual way in paraffin wax and mordanted twenty-four hours in iron alum, then stained for twenty-four hours in sulfalizarinate of soda. This tints the sections a dark reddish-brown; the nucleus is in various shades of brown at the different periods, and the mitochondria are also brownish. Such stained preparations, after removal from the alizarin, are rinsed and then stained in crystal violet; they are then a dense violet colour, and differentiation is carried out in a 30 per cent. solution of acetic acid. When the violet is washed away so that the reddish shade of the alizarin can be seen appearing in the nuclei, the acetic acid is completely washed away in water. The slide is blotted, passed through absolute alcohol, where it further differentiates to bergamot oil, then to xylol balsam. Though such preparations have neither the definition nor the transparency of the iron hæmatoxylin ones made as directed in my previous paper (1), they show the nucleus and cytoplasm in colours different from the chondrioplasts and mitochondria.

Iron hæmatoxylin, of course, stains everything in black and grey.

The alizarin brown shade in the nuclei is not affected by the crystal violet, which, however, is able to stain the brown mitochondria a dense violet. That this is not a substitution of violet for brown is shown by the fact that preparations overdifferentiated in acetic acid (i. e. crystal violet washed out too much) have the mitochondria faintly brown; the violet simply smothers the brown of the mitochondria and chondrioplasts, but is easily removed from the nuclei on washing in acetic acid. On Pl. 18, fig. 34, I have drawn a spermatid as nearly as possible to the colours it stained. The macromitochondria are deep violet; the archoplasm, around which the violet chondrioplast rods are grouped, stains light reddish-brown; the nucleus is very dark reddish-brown, the posterior centrosome blue to reddish-violet, the cytoplasm is light brown, while, finally, the micromitochondria stain a purplish-brown—if anything, they tend to be more purplish-violet than brown. The reason for this is probably the same that caused the micromitochondria in Pl. 17, fig. 19, to stain grey. They are possibly less dense than the macromitochondria, so the acetic acid caused the crystal violet to be extracted more from them than from the macromitochondria. The drawing of the spermatid on Pl. 17, fig. 34, was made by enlarging the camera lucida outline to twice the original size (now × circa 8000). The other drawings on this plate are × 4000, and are to the same scale as all the other drawings of these stages. The figures 32, 33, 35 of Pl. 18 were drawn from cells in the same region as the spermatid in Fig. 34.

Changes in the Mitochondria during the Spermatocyte Divisions and during Other Stages.

If the plates of this paper be examined it will be seen that in the metaphases of mitotic division (Pl. 16, fig. 4 and Pl. 17, fig. 24) the mitochondria are of the hollow sphere variety.

Moreover, in the critique of corrosive sublimate acetic fixation given in the table on page 233, it will be seen that I have remarked that the mitochondria are most susceptible to destruction during cell division. The briefest study of the staining and fixing reactions of the mitochondria show that during division of the cell some change takes place in these bodies. In some cases the change may be also one of growth in size (e. g. Pl. 16, fig. 4). Generally it is one of stainibility. The mitochondria generally stain less heavily, and are revealed as spheres containing a chromophobe, medullary zone. In corrosive acetic fixed material, stained in iron hæmatoxylin, cells in division and about to divide either have no mitochondria visible or the latter are much distorted; other cells quite near, in growth stages, and whose mitochondria are equally exposed to the fixative, have these bodies fairly well preserved. The corrosive acetic test shows that in Pulmonates there are two periods when the mitochondria change: (1) During the maturation divisions, when they become less resistant to injurious fixatives; (2) when they have grouped around the spermatid axial filament in the stage of Pl. 18, fig. 27, etc., when they become extremely resistant to injurious fixatives.

In the Discussion (p. 247) I have attempted to throw a little light on these interesting and important periods.

The Affinities of the Helicidæ, Limacidæ, Arionidæ, and Testacellidæ judged from a Study of the Cytoplasmic Inclusions.

In a previous paper (**4**) on the Lepidoptera I showed that the mitochondrial elements, though slightly different in different species, did not supply sufficient reliable evidence for classifying groups of moths or butterflies. The same is even more evident with regard to the Pulmonate Mollusca. It will almost certainly be found after more critical studies have been made of numbers of species from other groups, that with probable rare exceptions, the mitochondria will

rarely provide any certain basis for settling a disputed question of specific differences between any two animals, or even for arranging a tree of ancestral relationship in various groups of animals.

The mitochondria in all the pulmonates are much alike, so much so that they provide no method in themselves of distinguishing between species. The smaller or micromitochondria when they can be demonstrated are characteristic, but since I have not had the opportunity of examining H. pomatia and other larger Helicids, I cannot bring forward any evidence with regard to any specificity they may have in different forms. The post-nuclear elements show that the Limacids and Helicids are alike in having these bodies slightly developed, while Testacella is intermediate between Limax and Arion. The chondrioplasts do not provide reliable evidence; as I have already remarked, in some forms they tend greatly to vary. Generally speaking, Testacella has batonettes more like some types found in Helicids. It never has batonettes like those of Arion or Limax. I have already said that the P.N. apparatus tends to establish relationship between Limax and Helix; the chondrioplast, on the other hand, in Helix is not like that of Limax, and this applies especially to the number of rods.

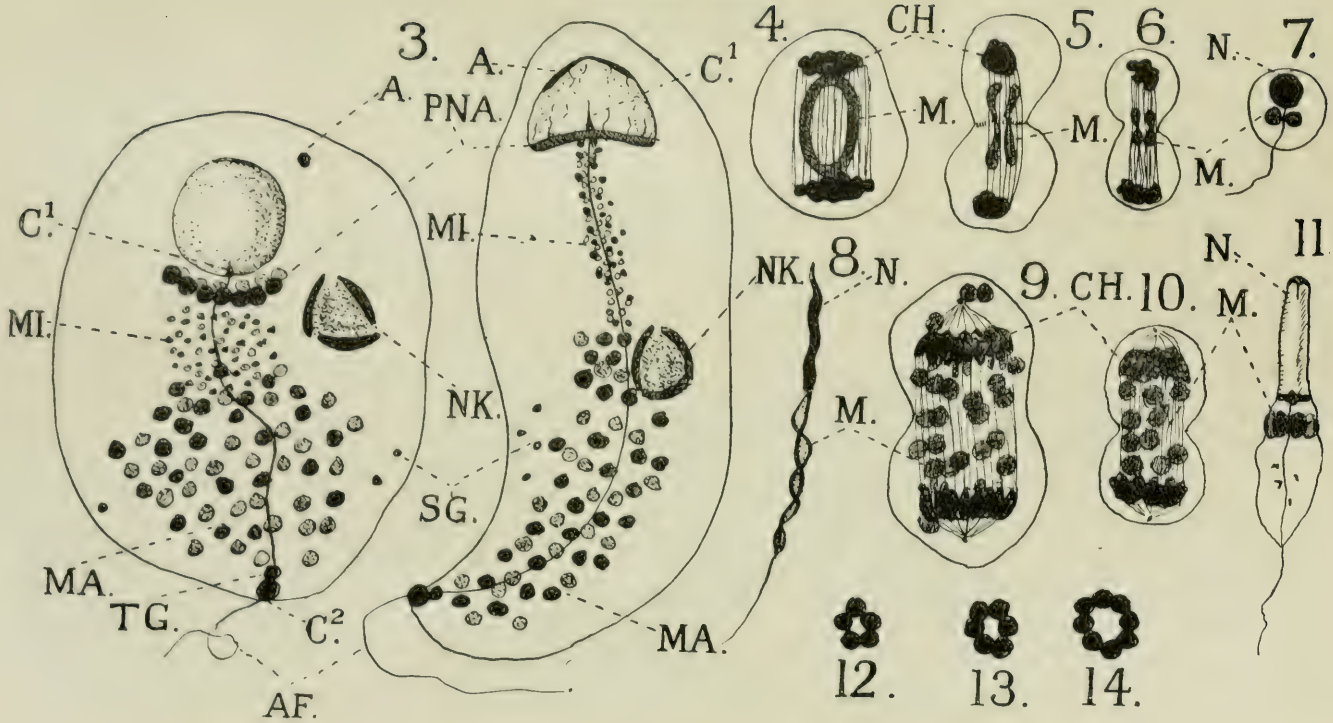
When one views the whole matter, taking into consideration chondrioplasts, mitochondria, and post-nuclear apparatus, it appears that in the Order Pulmonata one finds in every species examined the same individual cell organs, but these cell organs do not all conform to the same type, and, moreover, they appear to have no direct inter-relationship as regards the larger size of one making up for smaller size of another and vice versâ, in any two cells in two separate species. It must be confessed that there is no accurate method of judging this, but that is the general impression I have gathered. For instance, why should the Arion spermatid in Pl. 16, fig. 16, have such a large P.N. apparatus, while the Helicid spermatid in Pl. 17, fig. 20, has such a small one? Did we know the function of this body we might endeavour to frame an

answer, but I myself am at a loss for an explanation. It would be interesting to try to follow such a cell body in all the Orders of Molluscs, if it be present. It might possibly have phylogenetically been derived from certain mitochondria, or it might represent a more important body in other Molluscs. For the present the evidence is too scanty.

Staining Affinities of the Plasmatic Bodies. (See Text-fig. 3).

With regard to the fixing and staining reactions of the various plasmatic bodies, a good deal can be learnt by experiments. The pyronin and methyl green stain introduced by Pappenheim (**5**), is stated by Corti and Ferrara (**6**) to stain chromatin green and cytoplasm red. But in Flemming and Hermann material the reverse takes place, according to the latter observers. I have made many trials with P.M.G. (pyronin and methyl green), and generally, though not always, find that after Fleming-without-acetic acid and Champy, the chromatin does stain green. Corti and Ferrara cannot have

Figs. 3–14.—Diagrammatic plan of the bodies which have been found in Pulmonate germ-cells. The two figures, one on each side of the numeral 3, are spermatids in different stages. *A.* Acrosome. *N.* Nucleus. C^1. Head centrosome. *P.N.G.* Post-nuclear granules. *N.K.* Chondrioplasts and archoplasm. *M.I.* Micromitochondria. *M.A.* Macromitochondria. C^2 Tail centrosome. *A.F.* Axial filament. *T.G.* Terminal granule (a mitochondrium probably). *S.G.* Siderophile granule. Figs. 4–8, five stages from spermatogenesis of a scorpion, Centrurus elixicauda (Wood), after Wilson (**8**). Fig. 4 shows first spermatocyte anaphase, chondriosomes (mitochondrial matter) in form of a ring (*M.*), chromosomes (*C H.*) at poles. Fig. 5 shows ring finally dividing. Fig. 6 shows second spermatocyte division telophase, final division of the chondriosome rods (*M.*). Fig. 7, young spermatid with two chondriosome bodies (macromitosome). Fig. 8 shows early stage in elongation and twisting of the macromitosome (double nebenkern of Wilson) to form the spiral envelope of the flagellum. Figs. 9–14, three stages in spermatogenesis of another scorpion, Opisthacanthus elatus (Gervais), after Wilson (**8**). Figs. 9 and 10 are first and second spermatocyte divisions, showing chondriosomes being divided between daughter-cells in the normal manner without a hoop as in Centrurus. Fig. 11, early spermatid showing macromitosome (nebenkern organ) at *M.* Figs. 12, 13, 14, polar views of macromitosome showing variation in number of chondriosomes (mitochondria) in individual spermatids (see p. 244 of this paper).


3.
A.
PNA.
C¹.
MI.
NK.
SG.
MA.
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C².
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4.
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washed out their material properly. To get a good stain, the Flemming or Champy fixed material must be left overnight in the mixture. With regard to the reactions of this stain my friend, Dr. S. G. Scott, of the Histology Laboratory, writes to me: "Methyl green stains nuclear network, and mucin only, whether in weak or strong, or neutral or faintly acid solution. That is its value as a reagent. If there are any traces of alkali you increase its action, as also by mordanting with tannin or sulphur, or having iodine still in your section. Pyronin, a basic dye, stains all that methyl green stains, and much more besides, on account of its greater dyeing power; but by using a mixture of the two dyes, with excess methyl green, the nuclei are almost pure green, while the other basophil parts of the cell are red with the pyronin. The excess methyl green keeps the pyronin out of the nuclei. Other green basic dyes will not act in the same way, because of their greater dyeing power. I have tried most of these of suitable shade, and failed to get the P.M.G. effect, as the green dye will not limit itself to the nuclei. Thus, while in an Ehrlich or other alum hæmatoxylin, toluidin blue, thionin or safranin preparation, we get all basophil things in one colour, here we find them split up into two sub-groups, the methyl green stainers, and the rest.

"For the practical cell anatomist, the advantage of the method is that it gives a certain distinction independent of shape, between a true nucleolus and a nodal point in the nuclear network, the former always red, the latter always green to black (blackish is a mixture of the two colours)."

That this is of great value I have no doubt, especially in connection with the nucleus. It is a well-known fact that from the spermatid to the formation of the ripe sperm (the spermateleosis stages) the nucleus undergoes profound staining changes. The spermatid nucleus, in pyronin and methyl green preparations is at first green, and gradually becomes bright red, and, finally, again becomes green. Van Geison's picric-acid-fuchsin stain is admirable for showing these changes, used with iron hæmatoxylin. Unfortunately, such

FIXING REACTIONS OF VARIOUS BODIES IN PULMONATE SPERMATID.

Fixative used.	Acrosome.	Nucleus.	Post-nuclear granules.	Mitochondria and chondrioplasts.	Tail granule.	Tail centrosome.
Flemming-without-acetic acid (Gatenby): Chromic acid, Osmic acid	All elements about equally well fixed. The introduction of bichromate of potassium in the Champy tends to make the preparations to stain less well, especially the nuclei and amphiaster.					
Champy's fluid: Chromic acid, Bichromate of potass., Osmic acid						
Corrosive sublimate acetic acid	Well preserved after adherence to nucleus	Well preserved, but not so well preserved as with Champny or F.W.A.	Could not be found in spermatocyte; good after adherence to nucleus; washed-out appearance	Mitochondria ill-defined, but not always destroyed. Cytoplasm vacuolated. During cell division mitochondria generally dispersed. Better during spermateleosis. Chondrioplasts distorted, batonettes tend to coalesce	Generally absent; when perceptible ill-defined	Good.
Carnoy's fluid: Alcohol, Chloroform, Acetic acid	Badly preserved, often quite destroyed	Well preserved, and stain well	Preserved in much the same way as by corr. acet., but often destroyed	Dispersed completely	Absent	Fairly good.

delicate stains as P.M.G. and Ehrlich-Biondi do not work at all well after chromo-osmic, or chromo-bichromo-osmic material, and as many cell inclusions are only properly fixed in the latter media, one at once strikes difficulties.

In Text-fig. 3 I have given two semi-diagrammatic figures of spermatids to show the various cell bodies mentioned in my diagnosis of staining and fixing reactions. When one remembers that several late observers using good mitochondrial stains have overlooked at least two categories of cellular bodies now described by me, it will be understood what difficulty I have had in examining the staining affinities of bodies only with difficulty to be discovered in the best iron alum hæmatoxylin preparations.

In the first place with regard to fixation, Champy and Flemming's fluid without acetic acid are found to preserve perfectly all inclusions. The only objection to these fluids, from the fixation point of view is the fact that they are often too "strong" for the material. For instance, Champy undiluted rarely gives good preparations of the Testacellid ovotestis, but when diluted with distilled water by one-fourth it gives perfect results. In some ways the same applies to the Flemming without acetic acid mixture. My very best preparations of Helicids were got by leaving small pieces of the ovotestis overnight in this mixture diluted by one-sixth or more.

The commonly used corrosive sublimate acetic fixative is unreliable and worthless where delicate cytological results are a sine quâ non. I really only use this as a sort of control to my other chromo-osmic material. Very bright preparations can be made after this fixative, but if only a general chromosome-spindle fixation is wanted Bouin's picro-formol-acetic is superior, especially when diluted by one-third. I consider Petrunkewitsch's fixative, which was really due to Gilson (the former modified the latter's formula), is superior in every way to corrosive acetic, even for whole mounts of embryos, marine animals, etc.

Of course, all the mercury bichloride acetic acid fixatives completely distort and often destroy plasmatic structures of delicate objects, probably only preserving them well in some mammalian tissues (see Waklin Barratt (7)). Even in the latter case I can only say that observers who set out to study mitochondria and other delicate plasmatic structures using ordinary fixatives are doing work which will not be of much use to serious students of the questions of the cell and heredity and general cell problems. To use a simile, such workers are only studying the "bones" of the cell after they have torn away most of the "flesh."

In my table of the effects of corrosive acetic on the cell it will be seen that in the growth stage the mitochondria are generally badly preserved, and in the prophases and during mitosis they are often quite dissolved away. After the spermatid tail is formed and when the granules become grouped around the axial filament, the mitochondria are generally good, but the whole cell has a distinct vacuolated washed-out appearance. The chondrioplast body, especially in the spermatid, is very badly preserved. The acrosome and P.N. granules after adherence to the nucleus are good, but their edge is ill defined and never so well preserved and sharp as with Champy or with F.W.A. Because of the general distortion of the cytoplasm the P.N. granules cannot be made out with any certainty in the spermatocyte.

In Carnoy material there is a general exaggeration of what occurs in corrosive sublimate acetic fixed material. Owing to the accessibility of the cells of the ovotestis to the fixative and the intense fat-dissolving powers of the mixture, the fixation of entire plasmatic bodies is extremely bad. The mitochondria and other plasmatic elements are either quite dissolved away or extremely distorted. The nuclei are well preserved, and the material generally stains well, but is, of course, absolutely useless from the cell inclusion point of view.

Moreover, there is little doubt that alcoholic acetic acid fixatives wash out a great deal of the more soluble contents of

the entire cell. One remarkable fact is that in the most perfectly fixed mitoting cells, in preparations made by chromo-osmic mixtures, the spindle-fibres are often hardly perceptible as such; instead, one finds a clear space in the cell in which one may make out faint regions where the cytoplasmic currents appeared to be flowing when the cell was fixed.

The addition of acetic acid gives a "good" spindle-fibre fixation. That is to say, the fibres of the amphiaster are marked. Do spindle-fibres exist? Or are what we suppose to be spindle-fibres altered and distorted protoplasmic currents? It is a significant fact that substances such as acetic acid and alcohol tend to accentuate the fibrous appearance of the amphiaster. At present I do not wish to add anything to the above remarks, but the matter is worthy of careful attention, and will need special experimentation.[1]

Histologists are wont to use the terms "oxyphil" or "eosinophil," "amphophil," and "basophil," to denote the manner in which different parts of the cell become stained. It might then be asked, How do the mitochondria stain? Are they basophil, amphophil, or oxyphil? If the table on page 237 be consulted it will be seen that the mitochondria generally stain so vaguely with elective stains that it is impossible to say how they become coloured. With such a common stain as Ehrlich and eosin they stain a vague purplish; prolonged immersion in the basic stain gives a bluish colour to the spheres. On the other hand, prolonged staining in eosin followed by Ehrlich or toluidin blue gives a reddish tint to these spheres. It is a fact that use of the common specific stains does not satisfactorily solve the question. In a well washed-out chromo-osmic preparation it is possible to get what would generally be termed a successful Ehrlich and eosin, or Ehrlich and Biebrich scarlet stain; that is, the nuclei and karyosomes are in shades of blue, while the plasmosome and

[1] In their "Étude cinematographique de la division cellulaire." Commandon and Jolly clearly show spindle-fibres in the living dividing Triton leucocyte. 'Journ. Phys. et Path. Gén.,' tome xvii, Pl. 1, fig. 16.

STAINING REACTIONS OF VARIOUS BODIES IN THE SPERMATID OF PULMONATES.

Stain used.	Acrosome.	Nucleus.	Post-nuclear granules	Mitochondria and chondrioplasts	Tail granule.	Tail centrosome.
Pyronin and methyl green	Dark greenish-grey	Green, grey, to red, according to stage of spermateleosis	Black in spermatocyte; dark greenish-grey in spermatid	Ill-defined greenish-grey	Not well-defined	Dark grey to reddish.
Eosin and toluidin blue	Bluish	Reddish to blue, according to stage of spermateleosis	Blue	Reddish, ill-defined	Not defined	Bluish to purple.
Iron hæmatoxylin and Van Geison	Black	Reddish, grey, yellow to black, according to stage of spermateleosis	Black	Mitochondria greyish to black, generally grey; chondrioplasts black	Black	Black.
Ehrlich's hæmatoxylin and eosin	Blue	Blue to reddish, according to stage	Bluish	Purple or bluish	Not defined	Blue.
Alizarin and crystal violet	Violet	Reddish-brown	Violet	Violet; archoplasm reddish-yellow	Violet	Reddish-blue to violet.

the ground of the cytoplasm are in pink, but the mitochondria do not show, or are very faintly pink after prolonged counter-staining. I conclude, therefore, that the colour specificity of the mitochondria probably cannot safely be worked out by using the ordinary cell stains. If crystal violet alone is used on Benda fixed material, the nuclei as well as the mitochondria are violet; but by using alizarin first the violet is generally kept out of the nuclei. It could not, therefore, be stated that the violet itself is specific for any cell element.

The acrosome invariably shows preference for basic dyes, and can undoubtedly be classed under basophil substances. The P.N. granules are the most chromophile of all the plasmatic elements, and in the spermatocyte stain as heavily and in the same way as the chromosomes. From the staining reactions of the P.N. granules I believe one cannot class the latter with the mitochondria. The chondrioplasts almost always behave like the mitochondria, except that it is always a little more chromophile in rest and growth stages, the stain remaining in it for a long time during differentiation, while the mitochondria are grey. This apparent chromophility may be due to the fact that it is denser than the mitochondria, though if the chondrioplast is denser than the latter one would not expect it to behave as it does in spermateleosis (e.g. Pl. 17, fig. 22). Though it should be borne in mind that in regard to the position the chondrioplasts take in spermateleosis, there is the archoplasm in the centre, which would probably alter the behaviour of the whole chondrioplast apparatus. I firmly believe that the chondrioplast batonettes are chemically allied to the mitochondria.

In my previous paper (**1**) and in Pl. 17, fig. 20, *T.G.*, I have drawn a granule often associated with the tail centrosome. Since I described this granule in my first paper I have come to the conclusion that it is a mitochondrium; I have called it the terminal granule, and in certain spermatid bundles it is found in every cell. (See (**1**) Pl. 33, fig. 36.) This granule is possibly carried into the position in which it is found

simply by accident, but it is very difficult to say for certain. It acts and stains like the mitochondria.

These staining and fixing experiments have caused me to take the view that the spermatid of the Pulmonata contains several categories of plasmatic bodies whose chemical constitution, and probably density, is different in each case. A glance at Text-fig. 3 will serve to show how complicated may be the plasmatic elements of the spermatid. At the present time, despite a good deal of work, there is no certain evidence as to the part played by any of the plastid bodies. There are also granules, described by Bolls Lee, which resist acetic acid fixatives and which are siderophil—i.e. stain intensely in iron alum hæmatoxylin. Finally, it may be said under the heading of this section of the present paper, that in the chromo-osmic fixatives, properly diluted and devoid of acetic acid, we have reagents which will fix probably all the visible contents of the cell. It is to be doubted whether the spermatid of the Helicid contains bodies other than those drawn in Text-fig. 3.

Discussion.

The most important facts that can be deduced from a study of the mitochondria in the Pulmonate Mollusca are as follows:

(1) No rigid synchronism exists between the stage of evolution of the nucleus in the growth period of the spermatocyte and the state of development of the mitochondria. (See the previous paper on the Pulmonata (1).)

(2) Though the entire bulk of the mitochondrial matter in a number of cells of the same generation and stage may be nearly the same, the individual granules forming this bulk may not be the same size in all the cells (1).

(3) The cell bulk and mitochondrial bulk have no rigid relationship, for one animal may have mitochondria forming a greater bulk than another whose cell at the same stage is approximately of the same size. (Compare A. ater and T. haliotoïdes.)

(4) The fact that in a much divided cell generation (spermatogonia (1)) the mitochondria tend to be few and large shows that in cell division the individual mitochondria are not themselves divided by any form of fission between the daughter-cells, but are distributed bodily in a haphazard manner. No certain ground for comparison in this way exists between chromosomes and mitochondria.

(5) The size of a given animal form gives no clue as to the probable size of its mitochondrial elements: e.g. among the eight pulmonates examined L. agrestis was nearly the smallest in body size; its mitochondria were nearly the largest of all the species.

(6) The discovery of two kinds of mitochondrial bodies in H. aspersa shows that whatever be the function of these bodies it is such as to call for a division of labour in certain cases.

With regard to other forms, Wilson's (8) discovery in Centrurus (see Text-figs. 4–14) shows that—

(7) In certain rare cases there is a mechanism whereby the mitochondrial matter is equally divided among the four spermatids.[1]

(8) Regaud (9) first pointed out that during the metaphase, especially of the spermatocyte divisions, the colorability of the mitochondria is altered. My own work supports Regaud. Duesberg disputes this (17).

(9) During the spermateleosis stages the mitochondria become changed again, being more resistant to injurious fixatives, and, later, more chromophile.

(10) My work on Lepidoptera (4) showed that the spermatogonial mitochondria may be more resistant to acetic acid and such injurious substances than are those of the growth stages of the spermatocyte.

(11) It seems evident that during growth period of the germ-cells the mitochondria become more numerous. They probably divide by binary fission.

[1] I have also discovered something similar in the typic spermatogenesis of Paludina vivipara.

(12) In some animals at least (insects, scorpions, pulmonates at certain stages, etc.) it is certain that the mitochondria consists of two parts—an inner and either non-colourable or faintly colourable core, and an outer shell of colourable or chromophile matter. The latter part of individual mitochondria often fuses to form threads or rods, which then lie in the apparently liquid chromophobe part formed by the running together of the inner zone. The inner or chromophobe substance in which the threads lie, is probably often overlooked by observers, except when the macromitosome is formed; it is then clear (see Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, p. 426).

In this review of the plasmatic elements in the germ-cells of eight pulmonate Mollusca, I have shown that the mitochondria are, roughly speaking, throughout of the same general type and size; the establishment of the fact that no absolute regularity exists with regard to the numbers and size of the mitochondria at once shows that the part which these bodies play in the germ-cell cycle is not one which necessitates regular dimensions and number as do the chromosomes.

In the case of Opisthacanthus elatus (see Text-figs. 4–14), Wilson (**8**) found that the number of chondriosome elements contained by the spermatid varied as follows: Of two hundred cases, 73 per cent. has six mitochondrial spheres, 16 per cent. had five, and 11 per cent. had seven.

Comparing Centrurus, where division of the mitochondrial matter is caused by a regular mechanism, with Opisthacanthus Wilson says, "it is evident that chondriosome-material having the same origin, fate, and (presumably) physiological significance may be distributed to germ-cells by processes widely different even in nearly related animals."[1] This

[1] In Paludina I have lately found out the remarkable fact that the atypic spermatocytes have mitochondria like those of snails, while the typic have a few huge sausage-shaped mitochondria, "Cytoplasmic Inclusions of the Germ-Cells," Part V.

should be carefully borne in mind by those who would identify with the mitochondria functions needing regular and uniform behaviour in the germ cycle of animals.

The cell elements of Pulmonates which interest us most of all are the mitochondria, the chondrioplasts, and the post-nuclear granules. It appears that the post-nuclear granules may be concerned in the grouping of the mitochondria. This view is somewhat strengthened if one remembers that it is in the forms with the largest post-nuclear apparatus that the mitochondria are longest and closest grouped behind the ovoid spermatid nucleus. Unfortunately, it is not possible to ascertain whether the force which causes the mitochondrial granules to be grouped behind the nucleus is traceable to the centrosome or to the P.N. apparatus. The latter may not supply a kinetic energy of any description, it may have some function in the transmission of some hereditary factor, or it may have some part in fertilisation, at present I cannot produce any definite evidence. It is certain, with regard to the fate of this body during later stages of spermateleosis, that it dwindles and soon becomes a very small hardly noticeable disc near the head centrosome. In Salamandra (10) it is quite certain that the mitochondrial grains are as definitely grouped as in the Pulmonates, and without a post-nuclear apparatus. It may be possible that the P.N. apparatus in other forms has been confused with the centrosome. Bonnevie's (11) case in Enteroxenos östergreni is very curious, and definitely establishes that the centrosomes have an influence over the mitochondria. In later spermatids of Enteroxenos the indefinite grouping of the mitochondria is changed to a disposition in which the latter form a clear spiral. From this and such other evidence it is more likely that the post-nuclear granules are not exclusively for the purpose of shepherding wandering mitochondria into their proper places behind the nucleus; they may have some other and more important function which may be revealed by a study of fertilisation.

Duesberg (12), in a recent paper on the fertilisation in an Ascidian, gives a beautifully illustrated account of the mitochondria in the fertilisation and subsequent cleavage of the egg. The regions, myoplasm, ectoplasm, endoplasm, chymoplasm, and chordaneuroplasm in the egg of the Ascidian, so named by Conklin in his well-known work on these forms (13), are commented on by Duesberg as follows: "Pour moi, ces différentés substances, telles que Conklin les conçoit n'existent pas. Les différentés aspects des diverses régions de l'oeuf et des blastomères dépendent, non pas de l'existence de substances spéciales, mais d'une répartition spéciale des élements figurés de l'ovoplasme. C'est ainsi que le myoplasm de Conklin n'est autre chose qu'une accumulation de mitochondries dans la région correspondante de l'œuf. . . ." In the same paragraph Duesberg also says: "La segmentation de l'œuf des Ascidiens nous montre que la division cytoplasmique est, comme le pense Rabl, un phénomène important, et très compliqué." Duesberg shows clearly that the regions of Conklin are formed by mitochondria, by yolk-grains, or by the absence of one or both of these from certain regions. It will be seen, therefore, that care should be exercised in the use of the words—"organ-forming substances." As far as we now know subsequent work may show that, on the basis of cytological examination[1] at least, the ground protoplasm of the egg is not different in different parts, but only the presence of special granules and droplets causes a pseudo-division into regions.

If this is true, our conceptions with regard to many questions of the "germ-plasm" will need drastic alteration. Further comment at present would be ill-timed; more forms must be exhaustively examined by means of the latest fixatives and stains.

With regard to the mitochondria, there are a number of schools of thought. Some observers rush headlong into the

[1] But from the subsequent developmental occurrences it must be different in different parts.

conception of the mitochondria as "les porteurs des caractères héréditaires du cytoplasme," or "die protoplasmatische Vererbungssubstanz"; others, while agreeing that the mitochondria may be of importance in heredity, are not satisfied with the evidence so far produced; there are even a few observers who either dismiss the mitochondria as "just lipoids" or absolutely deny their presence. It is a curious fact, that whenever a new cell element is discovered, one constantly finds critics describing such as the result of fixation or of staining.

It was not intended, at this juncture of my work, to comment in any way on these various schools of thought, but with regard to the view that the mitochondria represent that part of the idioplasm which is situated in the cytoplasm—a view supported by Benda (**14**), Meves (**15**), and many other prominent workers—it might be pointed out that:

(1) In the conception of units forming idioplasm one would be inclined to expect that such units would behave more regularly in cell division, being correctly distributed, as are the chromosomes. This is rarely the case with mitochondria.

(2) In the conception of units forming idioplasm one would also rightly be inclined to picture such units as more of a uniform size and number, the latter not influenced by, but properly compensated in, a varying number of cell divisions (Helix aspersa (**1**)).

I am quite willing to admit that these two conceptions of the units of the idioplasm might, perhaps, be unnecessary. For instance, the two figures in my previous paper (**1**) Pl. 33, figs. 37 and 40, where the number and the size of the mitochondria differs markedly, might be explained by supposing that the mitochondrium in Pl. 33, fig. 40, contained or represented a mitochondrium in Pl. 33, fig. 37, but to which a part of another mitochondrium had been added in growth. This view is, in my opinion, inadmissible, especially in the light of Wilson's discovery in Opisthacanthus elatus (**8**), where the spermatid sometimes receives five, six, or sometimes seven chondriosome spheres of the same size; compare this with the

case of Centrurus, where the mitochondrial matter is divided evenly.[1]

Broman (**15a**), in a paper which I have been unable to consult myself, suggests that the haphazard division of the mitochondria is a mechanism for producing variation (**12**). Would Broman care to say that Centrurus had no capacity for variation since its chondriosome hoop is equally divided, while Opisthacanthus, also a scorpion, thanks to the haphazard division of its mitochondria, was endowed with special variational powers? Unless the haphazard division of the mitochondria can be explained satisfactorily with reference to functions which we must needs delegate in our minds to cell organs with more perfect distributory mechanism, I cannot conceive the use of raising innumerable ill-digested hypotheses concerning the mitochondria as carriers of hereditary factors of any kind. As organs for producing variation the mitochondria do not appear to me at present to meet the necessary requirements.

The view I at present take with regard to the relationship of mitochondria and nucleus is that the latter produces or is able to produce the former.

In a forthcoming paper I consider that I have shown plainly that this is the case. In the growth of the egg, the mitochondria undergo profound changes, and since the oocyte nucleus consists of moieties from both parents, the male parent as well as the female has had some part in the differentiation of the egg. It is certain that in many forms the tail of the sperm does not enter the egg, and the sperm mitochondria cannot be introduced in that way. Whatever function is performed by the mitochondria, it is a fact that the egg always contains an enormously greater bulk of chondriosome-material than the fully-formed sperm—and even if it be suggested that the mitochondria of the tail are like the nucleus of the sperm—much concentrated, it can be pointed

[1] Lately I have found that in Paludina the mitochondria in the spermatid vary in number from four to seven. See also Retzius' figures of Mollusc spermatozoa, 'Biol. Untersuch.,' xiii.

out that in the spermatocyte and oocyte at the stages when these grains are of approximately the same size and staining power, those in the spermatocyte are immensely less numerous.

In both sexes there is a period of growth of the germ-cells when the number and bulk of the mitochondria become greater, and in many forms it is quite certain that the mitochondria appear only at the beginning of this growth stage, directly under the influence of the nucleus. It is evident, therefore, that there is no complete ground for comparing the nucleus and the mitochondria in the history of development, The nucleus must be passed on from generation to generation. a necessity which would not seem to and does not apply to the mitochondria.

It is evident that this view does not need one to believe that the tail of the sperm is all important in the question of the part (if any) played by the mitochondria in heredity.

I hold the view that even if it cannot be shown in all forms that the mitochondria only appear in the germ-cells at a certain part of the cycle, it is true that even if the mitochondria pass right through the germ-cell cycle, they phylogenetically were derived from the chromatin in the first place.

Minchin (**16**), in his excellent "Introductory Remarks on the Origin of Life," says, after giving a number of reasons for regarding the chromatin as the most important cell constituent: "For these reasons I regard the chromatin as the primitive living substance and hold the view that the earliest forms of life were very minute particles of chromatin, round which in the course of evolution achromatinic substances were formed."

We are aware that not every cell in the animal body contains mitochondrial granules, and the presence of the latter in other cells with a more important function in the body is significant. One might suggest that the mitochondria arose in the evolution of the cell as bodies carrying out, in the cytoplasm, some function of a metabolic nature which hitherto had been carried out within the nucleus, and that this function

might not be in any way directly connected with heredity. We are quite certain that the hereditary function, if I may so call it, is not the only one of the nucleus; there are other functions of a more "domestic nature." Might not the mitochondria be connected with cell-metabolism in some way not necessarily directly concerned with heredity?

The study of the mitochondria has now been carried out well enough to tell us that these bodies are intimately connected in cell-metabolism and secretion in some gland-cells. We are tempted to remark, if the mitochondria are in some way directly connected with the factors of heredity either of nucleus or cytoplasm, what are they doing in gland-cells? A liver-cell or a pancreas-cell is as well supplied with mitochondria as a mammalian spermatocyte, and in gland-cells the mitochondria are often found to undergo great changes at different stages.

In the chromosomes we have a completely satisfactory mechanism for producing certain phenomena which breeding and Mendelian experiments have revealed, and nothing so far described by any of the mitochondria-heredity theorists has seriously brought the chromosome hypothesis into disrepute. I feel convinced that future work will only go to show that the mitochondria are products of the activity of the nucleus and that the so-called factors of heredity, whatever they may be, are borne by the chromosomes. It might be feasible to believe that the mitochondria are deputed by the nucleus to carry out in the cytoplasm important functions, but the fact that the mitochondria are almost certainly produced and influenced by the nucleus, must show us that the problem of the physical basis of heredity merely comes round again to the chromatin of the nucleus.

With regard to the changes undergone by the mitochondria during mitosis, Regaud (**9**), in his valuable paper on "Le Structure des Tubes Seminifères et La Spermatogénèse chez les Mammifères," remarks: "Pendant la metaphase elles (les mitochondries) gardent cette répartition; mais leur grosseur augmente en même temps que leur chromaticité se modifie.

. . . Je pense que, pendant la mitose, les mitochondries se chargent temporairement de certaines substances, probablement voisines de la chromatine, qu'elles abandonnent ulterieurement."

Duesberg (17), reviewing Regaud's work, says: "Je crois devoir faire remarquer: 1. que dans les preparations à la méthode de Benda, les mitochondries des spermatocytes de premier ordre en division ne sont pas plus volumineuses que dans les mêmes spermatocytes au repos."

In many cases, at least, it seems to me that in pulmonates the size of the mitochondria during maturation karyokinesis becomes greater. I feel sure that there is a change in the chemical nature of these bodies at this stage, for their reactions not only to stains (Regaud), but to fixatives become altered. The figure in paper (1) of Helix aspersa first maturation metaphase showing rod-like mitochondria may be an artefact produced, because at this period of change the slightest fault in the mitochondria fixative induces collapse of the spheres, and a consequent rod-like or fibrillar appearance. ((1) Helix aspersa, Pl. 33, fig. 34.)

From my own independent studies on mitochondria, I feel sure that the latter change in some manner at these periods:

(1) Beginning of growth stage of the spermatocyte.

(2) Temporarily during maturation divisions.

(3) During the later stages of spermateleosis.

The question of the change during maturation division is very significant. Why should the mitochondria change during division, and why should the change be most marked during the metaphase? Why also, should the mitochondria at this stage be more easily distorted than before? Something has either come out of or entered into the chondriosome spheres. We are undoubtedly at the beginning of a path, the following up of which may lead us to a better view of the function of these puzzling spheres. This change has been noted in Mammals (Regaud), in Pulmonates (all families I have studied), and in Lepidoptera the mitochondria during division seem to be altered in some way.

I cannot agree with Regaud in the suggestion that during mitosis "les mitochondries se chargent temporairement de certaines substances." I think that they lose something during mitosis, not add anything to themselves. I do not think that the fibrillar appearance of the fixed mitochondria during the metaphase can be altogether explained by supposing that protoplasmic currents induced by dividing cell produces such an effect naturally.

Montgomery (**18**), in the Cape Peripatus, describes how the mitochondrial granules are completely sloughed off and never form an envelope to the tail. This is a most important matter, since Montgomery shows that the globule containing the mitochondria forms at first an investment to the nucleus and not to the hind region of the sperm, as is so often the case, and the entire mitochondrial matter is later sloughed off. Meves (**19**), commenting on this, says: "Nicht nur wegen dieses Endresultates, sondern auch auf Grund der technischen Angaben, welche Montgomery macht, möchte ich bezweifeln, dass es sich bei den von ihm gesehenen Gebilden überhaupt um Mitochondrien handelt. Montgomery gibt an, dass die Mitochondrien sich an seinem Material, welches in Flemmingschem Gemisch und in Sublimat-Eisessig fixiert war. . . ." Meves is incorrect in stating "welches in Flemmingschem Gemisch fixiert war," for Montgomery plainly says in his paper that of his material "some [was] fixed in strong Flemming's fluid diluted with an equal part of distilled water." This is very different from strong Flemming undiluted. As a matter of fact, strong Flemming diluted by half is a fairly good mitochondrial fixative, for the acetic acid is thereby reduced, while the osmic acid and chromic acid are still strong enough properly to preserve the chondriosome matter. Meves' criticism of Montgomery's work does not, therefore, stand.

Were Meves to find fault merely with the shape and size of Montgomery's mitochondria, I might be sufficiently impressed to suspend judgment on this point till another observer had worked on Peripatus, but I am quite aware that strong

Flemming with acetic, even undiluted, often preserves the mitochondria in certain parts of an organ, and I therefore do not consider that the fixation used by Montgomery can be brought forward as a reason for dismissing an important paper which seriously cripples Meves' views on the mitochondria.

I mentioned in the body of this paper that in all the Pulmonata I have studied at least a part of the mitochondrial grains are sloughed off. In Peripatus, Montgomery clearly and finally shows that all the mitochondria slough off. Duesberg (**17**), in the guinea-pig claims that all the grains form a part of the tail sheath, and therefore enter the egg. For my part, giving every respect to Duesberg's very excellent papers, I should like to see another observer work on the mammal without depending on Benda's methods. For instance, Duesberg (**17**), in his paper entitled "Nouvelles recherches sur l'appareil mitochondrial des cellules séminales," has studied Blaps (a beetle). He figures from Benda material the spermatid on Planche 3, figs. 10, 11, 12. The "corps mitochondrial" is a rounded violet body, apparently homogeneous except for a "zone périphérique extrêmement mince." I have no doubt that were preparations of Blaps made by the better Flemming-without-acetic, or Champy-followed-by-iron-hæmatoxylin method, Duesberg would find that the "nebenkern d'apparence homogène" was really a spireme.

In some unpublished researches carried out on the macromitosome ("Nebenkern") of Tenebrio I find that this body is, just as in the moths, a very finely-coiled spireme. Duesberg has committed in Blaps the same error made by Meves (**15**) in Pygaera bucephala. In Coleoptera the spireme is exceedingly fine, and easily mistaken for a homogeneous mass; therefore, careful staining is needed to demonstrate the coil.

Moreover, with regard to the mitochondria of Coleoptera I do not think they form rods as drawn by Duesberg.

From my unfinished observations on Tenebrio I believe

that the mitochondria either exist as spheres containing a chromophobe zone inside or else as elongated tubes containing internally a chromophobe substance. These tubes are formed by the fusion of granules, and when viewed, especially under monocular vision, look like apposed solid rods which are really hollow tubes (I use hollow in the sense that their centre is chromophobe, though it contains a fluid).

In a late paper on a beetle (Passalus) E. L. Shaffer (**20**) has also interpreted the coil of the macromitosome as a homogeneous mass with an outer border and the mitochondria as solid rods. In this he is in agreement with Duesberg, but I believe that more careful attention will confirm the above criticism of Duesberg's work on Blaps.

With regard to the question of the division of the mitochondrial matter in the maturation and other divisions, it is also important to notice that though the mitochondria of the spermatocyte be evenly distributed in quantity to the four spermatids this is not all that is required. Suppose there are six mitochondrial spheres in each spermatid cell, there is no evidence that these six bodies in each are quarters of an original six in the spermatocyte. It rather seems that the six are not individually and qualitatively divided in the spermatocyte. My study on the mitochondria, confirmed by the work of others, shows that the spermatid contains about one-quarter of the spheres found in the spermatocyte, and, moreover, that there is absolutely no special method for distributing the spheres. Nothing even analogous to what is found in the chromosomes can be seen in the vast majority of cases.

A brief return to the Pulmonata will serve also as a warning to the too hasty acceptance of the mitochondrial-idioplasm hypothesis. In the Pulmonate, besides the mitochondria, one finds two other sets of plasmatic bodies, chondrioplast rods and post-nuclear granules which are also approximately evenly divided.

We are certain that the chondrioplast batonettes are cast

off the tail of the sperm, yet these bodies are just as evenly divided as the mitochondria, in some cases undoubtedly more so (Limax agrestis).

Summary.

(1) Eight species of Pulmonates have been carefully studied, and certain stages of four more species examined.

(2) A new set of plasmatic bodies, the post-nuclear granules have been described.

(3) The P.N. granules differ from the mitochondria in their staining affinities, but resemble them in size.

(4) The P.N. granules ultimately form a plate at the rear of the spermatid nucleus. This plate, at first large, gradually shrinks synchronously with the shrinkage stages in the nucleus.

(5) The P.N. granules appear to become non-staining during the maturation division, but it is not known whether more intense chromatization of the material (Benda) might not bring them into evidence.

(6) The mitochondria of the Pulmonates so far studied are of the same general type.

(7) On p. 239 twelve conclusions are given with regard to the mitochondria both of Pulmonates and of other forms.

(8) The chondrioplasts of all the Pulmonates dealt with have been fully examined.

(9) The chondrioplasts of different genera and often of different species tend to differ a little in shape and size.

(10) The chondrioplasts during the prophases of the first maturation karyokinesis lose a great deal of their staining affinity.

(11) In many cases during the metaphase, anaphase, and telophase it cannot be demonstrated without specially heavy chromatization and staining. During these periods it changes in its chemical constitution.

(12) The centrosome is responsible for the sorting out of the chondrioplasts into two equal groups at the prophase of division.

(13) The batonette is not divided by fission, but goes to the daughter-cell complete.

(14) After the final maturation division is over, the chondrioplast rod resumes its lost staining affinity.

(15) The chondrioplast batonettes do not take direct part in the formation of the spindle.

(16) The micromitochondria have been thoroughly re-examined with iron hæmatoxylin and Benda's method.

(17) Their presence is confirmed by all tests that could be made.

(18) They seem to differ in staining capacity from the macromitochondria.

(19) The presence of micromitochondria in other forms is doubtful, because the cells are so small as to make proper examination impossible.

(20) Micromitochondria appear to be present in Limax agrestis and in H. rufescens.

(21) The mitochondria, during the maturation divisions, stain less heavily, and often become larger. The latter fact could not be demonstrated in every case.

(22) On p. 248 I have stated the periods during which the mitochondria appear to alter in staining affinity and resistance to certain fixatives.

(23) The affinities of the several species have been discussed on the basis of their cytoplasmic bodies.

(24) Tables of staining and fixing effects have been given.

(25) From the three papers already published on the "Cytoplasmic Inclusions" it will be seen that strong evidence is being collected against the view that the mitochondria take any part in the transmission of the "factors of heredity."

Bibliography.

1. Gatenby, J. Bronté.—"The Cytoplasmic Inclusions of the Germ-Cells: Part II, Helix aspersa," 'Quart. Journ. Micr. Sci,' vol. 62, part iv.

2. Lankester.—"Treatise on Zoology," Part V, Mollusca, Pelseneer.

3. Faurét-Frémiet.—"Étude sur les Mitochondries des Protozoaires et des Cellules Sexuelles," 'Arch. d'Anat. Micr.,' xi, 1909.

4. Gatenby, J. Bronté.—"The Cytoplasmic Inclusions of the Germ-Cells: Part I, Lepidoptera," 'Quart. Journ. Micr. Sci.,' vol. 62, part iii.

5. Pappenheim.—"Wie verhalten sich die Unnaschen Plasmazellen zu Lymphocyten"? 'Arch. path. Anatomie.,' 166, 1901.

6. Corti and Ferrara.—'Mon. Zool. Ital.,' xvi, 1905.

7. Barratt, Wakelin.—"Changes in Chondriosomes occurring in Pathological Conditions," 'Quart. Journ. Micr. Sci.,' vol. 58, 1913.

8. Wilson, E. B.—"The Distribution of the Chondriosomes to the Spermatozoa in Scorpions," 'Proc. Nat. Acad. Sci. Phil.,' vol. ii, June, 1916.

9. Regaud, Cl.—"Étude sur la structure des tubes séminifères et sur la spermatogenèse chez les Mammifères," 'Arch. d'Anat. Micr.,' vol. xi.

10. Hermann.—"Beiträge zur Kenntniss der Spermatogenese," 'Arch. f. Mikr. Anat.,' Bd. l.

11. Bonnevie, K.—"Zur Kenntniss der Spermiogenese bei den Gastropoden Enteroxenos östergreni," 'Biol. Centralbl.' Bd. xxiv.

12. Duesberg.—"Recherches cytologiques sur la fécondation des Ascidiens et sur leur développment," 'Contributions to Embryology,' Carnegie Inst., Wash., vol. iii, Nos. 7, 8, 9.

13. Conklin, E. G.—"Organ-Forming Substances in the Eggs of Ascidians," 'Biol. Bull.,' vol. viii.

——— "Mosaic Development in Ascidian Eggs," 'Journ. Exp. Zool.,' vol. ii.

——— "The Organisation of the Egg, etc., of Phallusia mamillata," ibid., vol. x.

14. Benda, C.—"Weitere Beobachtungen über die Mitochondria und ihr Verhältnis zu Sekretgranulationen, nebst Kritischen Bemerkungen," 'Verh. der Phys. Gesellsch.,' Berlin.

——— "Die Mitochondria," 'Ergebnisse der Anat. und Entwickl.,' Bd. xii.

15. Meves.—"Uber den von v. La Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Gamenzellen," 'Arch. f. Mikr. Anat., lvi.

15a. Broman, I.—"Normale und abnorme Entwicklung des Menchen." J. F. Bergmann, Wiesbaden.

16. Minchin.—"Report of British Assoc. Dundee," 1912, p. 510.

17. Duesberg.—"Nouvelles recherches sur l'appareil mitochondrial des cellules séminales," 'Arch. f. Zellforch.,' Bd. vi, 1911.

18. Montgomery, Th.—"Complete Discharge of the Mitochondria from the Spermatozoon of Peripatus," 'Biol. Bull.,' vol. xxii.

19. Meves, T.—"Über das Verhalten des Plastomatischen Bestandteiles des Spermiums bei der Befruchtung des Eies von Phallusia mamillata," 'Arch. f. Mikr. Anat.,' vol. lxxxii, 1913.

20. Shaffer, E. L.—"Mitochondria and Other Cytoplasmic Structures in the Spermatogenesis of Passalus cornutus," 'Biol. Bull.,' vol. xxxii, 1917.

21. Just, E. E.—"Fertilisation in Platynereis megalops," 'Journ. Morph.,' xxvi, 1915. This author gives a Bibliography on the Middlepiece.

EXPLANATION OF PLATES 16, 17, AND 18,

Illustrating Mr. J. Bronté Gatenby's paper on the "Germ-Cells of Some Other Pulmonates."

LETTERING.

A. Acrosome. *A.F.* Axial filament. *C*, C^1, C^2. Centrosome. *C.H.* Stray chromosomes. *C.R.* Central rod (mitochondrial). *G.E.* Germinal epithelium. *M.* Mitochondria. M^1. Micromitochondria. M^2. Macromitochondria. *M.X.* Upper mitochondria. *M.Y.* Lower mitochondria. *N.* Nucleus. *N.C.* Nurse-cell. *N.K.* Chondrioplast apparatus. *OOTE.* Oocyte. *P.N.G.* Post-nuclear granules. *P.N.A.* Post-nuclear apparatus. *R.* Residual mass. *S.P.* Outer spiral. *SPYTE.* Spermatocyte. *T.G.* Terminal granule (probably mitochondrial).

In all the plates the figures have been drawn with the aid of a camera lucida, using a $\frac{1}{15}$th semi-apochromatic Koritska oil immersion and compensating eye-pieces. Except where otherwise expressly stated all figures are multiplied circa 4000 diameters. With the exception of Pl. 18, all the figures were drawn from material preserved in either Champy's fluid (*Ch.*) or Flemming-without-acetic acid (*F.W.A.*) and stained in iron-alum hæmatoxylin.

PLATE 16.

Fig. 1.—Nearly full-grown spermatocyte of Limax agrestis. The archoplasm is surrounded by eight chondrioplast rods. *Ch.*

Fig. 2.—Full-grown spermatocyte of L. maximus. The chondrioplasts are more numerous than in the previous figure. *Ch.*

Fig. 3.—The bead of protoplasm which is finally sloughed off the ripening spermatozoon in later stages of spermateleosis (see Fig. 9 R). The mitochondria chondrioplasts and archoplasm generally stain badly, indicating change. *Ch.*

Fig. 4.—First maturation division of L. agrestis. Short chromatisation (see p. 222) so that chondrioplasts are not seen. Mitochondria pale, hollow, and have changed. *Ch.*

Fig. 5.—Spermatocyte of L. maximus in the prophases of the first maturation division. This cell is abnormally small, having been starved because of the propinquity of a large mass of later spermatids and spermatozoa. The chondrioplasts' rods are getting pale preparatory to disappearing (see p. 222), they have become disarranged, and the centrosome (*c.*) has divided. *Ch.*

Fig. 6.—Late spermatid of L. agrestis ($\frac{1}{15}$ 0 × 12 C eye-piece, reduced by half), showing formation of tail envelope (mitochondrial sheath) and the strewing of the mitochondria and archoplasm + chondrioplasts (*N.K.*). *F.W.A.*

Fig. 7.—Spermatid of L. agrestis, showing curiously compact grouping of mitochondria (*M.*) behind the post-nuclear apparatus (*P.N.G.*). *Ch.*

Fig. 8—Later spermatid of L. agrestis. The axial filament, where it emerges from the mass of mitochondrial grains (*M.*), is seen to be covered by a granular coat (M^2.), which may be the micromitochondria. *Ch.*

Fig. 9.—Part of the ovotestis of L. agrestis × 660, to show the spermatids in three stages (1, 2, 3) with different propensity for the staining of their tail-sheaths (mitochondrial apparatus) at different stages. A group of spermatocytes (*SPYTE*) and a part of a nearly ripe egg (*OOTE.*) are also shown. *Ch.*

Fig. 10.—Young spermatid of L. maximus, showing the grouping of the mitochondria and the appearance of the chondrioplasts and archoplasm. The post-nuclear apparatus is also seen (*P.N.G.*).

Fig. 11.—Arion hortensis, spermatocyte at end of prophases of the heterotypic division and at a time when the post-nuclear granules are most conspicuous (*P.N.G.*). *F.W.A.*

Fig. 12.—Arion ater, full-grown spermatocyte showing post-nuclear granules (*P.N.G.*), chondrioplasts, and mitochondria. *Ch.*

Fig. 13.—Early spermatid of A. hortensis to show post-nuclear granules collected together into a mass near the chondrioplasts and archoplasm. *F.W.A.*

Fig. 14.—Spermatid of A. hortensis at a little later stage; the post-nuclear granules have become grouped to form a wall around one hemisphere of the nucleus. The acroblast is at *A.* *F.W.A.*

Fig. 15.—Spermatid of A. ater at a later stage; the post-nuclear granules have begun to fuse to form the post-nuclear plate. The mitochondria have become grouped behind this plate. *F.W.A.*

Fig. 16.—Later stage in A. ater; the post-nuclear plate has swollen to form a most conspicuous structure. The mitochondria are becoming grouped in the region of the axial filament. *F.W.A.*

Fig. 17.—Much later stage in A. ater; the mitochondria are grouped in the manner characteristic for A. ater. In some forms the axial filament appeared to be invested with a powdery coat (micromitochondria ?).

PLATE 17.

Fig. 18.—Arion hortensis, later spermatid showing beginning of the sorting out of the mitochondria into two parts (see Fig. 22). At *X.* the archoplasm and the chondrioplasts mark the position at which the division starts. *F.W.A.*

Fig. 19.—Spermatid of Helix aspersa; the cell has been differentiated till the micromitochondria are pale grey, the macromitochondria still dense black. The division line betwixt micro- and macromitochondria is quite sharp. At *X.* is a micromitochondrium which is as large as the macromitochondrium at *Y.*; the latter stains densely black, the form is grey. *F.W.A.* diluted $\frac{1}{6}$.

Fig. 20.—Spermatid of Helix nemoralis; no micromitochondria could be found. At *P.N.A.* is the post-nuclear apparatus. The shape of the nucleus is very characteristic for this stage. The archoplasm and chondrioplasts are not yet in their usual position behind the nucleus. At *T.G.* is the terminal granule above the second centrosome. The former may be a mitochondrium. Dilute *F.W.A.*

Fig. 21.—Spermatid of H. nemoralis at a later stage; there is a group of mitochondria (*M.X.*) behind the post-nuclear apparatus (*P.N.A.*), then a clear space near which lie the archoplasm and chondrioplasts (*N.K.*), and behind this is the bulk of the mitochondria (*M.Y.*).

Fig. 22.—Group of spermatids of A. hortensis showing characteristic arrangement of mitochondria into two groups, *M.X.*, *M.Y.*, with archoplasm and chondrioplasts intermediate in position (*N.K.*). The heads of the spermatids (*N.*) are attached to a yolk- or nurse-cell (*N.C.*). At *SPYTE.* is a group of spermatocyte; *A.L.* is the outer or Ancel's layer of the ovotestis, at *G.E.* a germinal epithelial cell. × 1070. *F.W.A.*

Fig. 23.—Young spermatid of Helix rufescens, to show at M^1 what may be a collar of micromitochondria. Dilute *F.W.A.*

Fig. 24.—Second spermatocyte metaphase of Testacella haliotoïdes, showing mitochondria hollow spheres; the chondrioplast rods

are not to be seen as the material was not treated to show them. Champy $\frac{1}{4}$ dilute.

Fig. 25.—Head and upper part of the nearly ripe sperm of Testacella; At C^1. the first centrosome, at *P.N.A.* the small post-nuclear plate, at *S.P.* the outer spiral, left-handed coil, and at *C.R.* the central rod. Champy dilute.

Fig. 26.—Nearly ripe spermatozoon of Testacella to show at *N.*, nucleus, at *P.N.A.*, post-nuclear plate, at *S.P.*, spiral coil, and at *R.*, 1. 2. 3. and 4. several protoplasmic beads containing mitochondria, archoplasm, and chondrioplasts (*N.K.*). $\frac{1}{4}$ diluted, *Ch.* × 510.

Fig. 27.—Advanced spermatid of Testacella showing the fine mitochondria fairly evenly grouped and strewn behind the post-nuclear apparatus. The large size of centrosome at C^2. is noteworthy. $\frac{1}{4}$ dilute, *Ch.*

Fig. 28.—Spermatocyte of Testacella in prophases of mitosis. The chondrioplasts have already become sorted out into two groups each around the centrosome; the chromosomes are appearing and the cell would shortly have divided. Later the chondrioplasts lose their staining power.

Fig. 29.—Young spermatid of Testacella to show post-nuclear granules and acroblast. $\frac{1}{4}$ dilute, *Ch.*

Fig. 30.—Spermatid at a time when the spiral is being formed. This process is taking place between the regions *X.–X.* and *Y.–Y.* At the letters *Z.*, *Z.*, the inner rod drawn in Fig. 25 (*C.R.*) is being formed. × 830 dilute, *Ch.*

Fig. 31.—Four regions of the tail of the sperm of Testacella; *C.* is the oldest, *A.* the next, while *B.* and *D.* are at the lower region of the tail of a fairly unripe spermatozoon (see text, p. 217). Not drawn to special scale.

PLATE 18.

All figures drawn from same section and cyst from a Benda Alizarin crystal violet preparation.

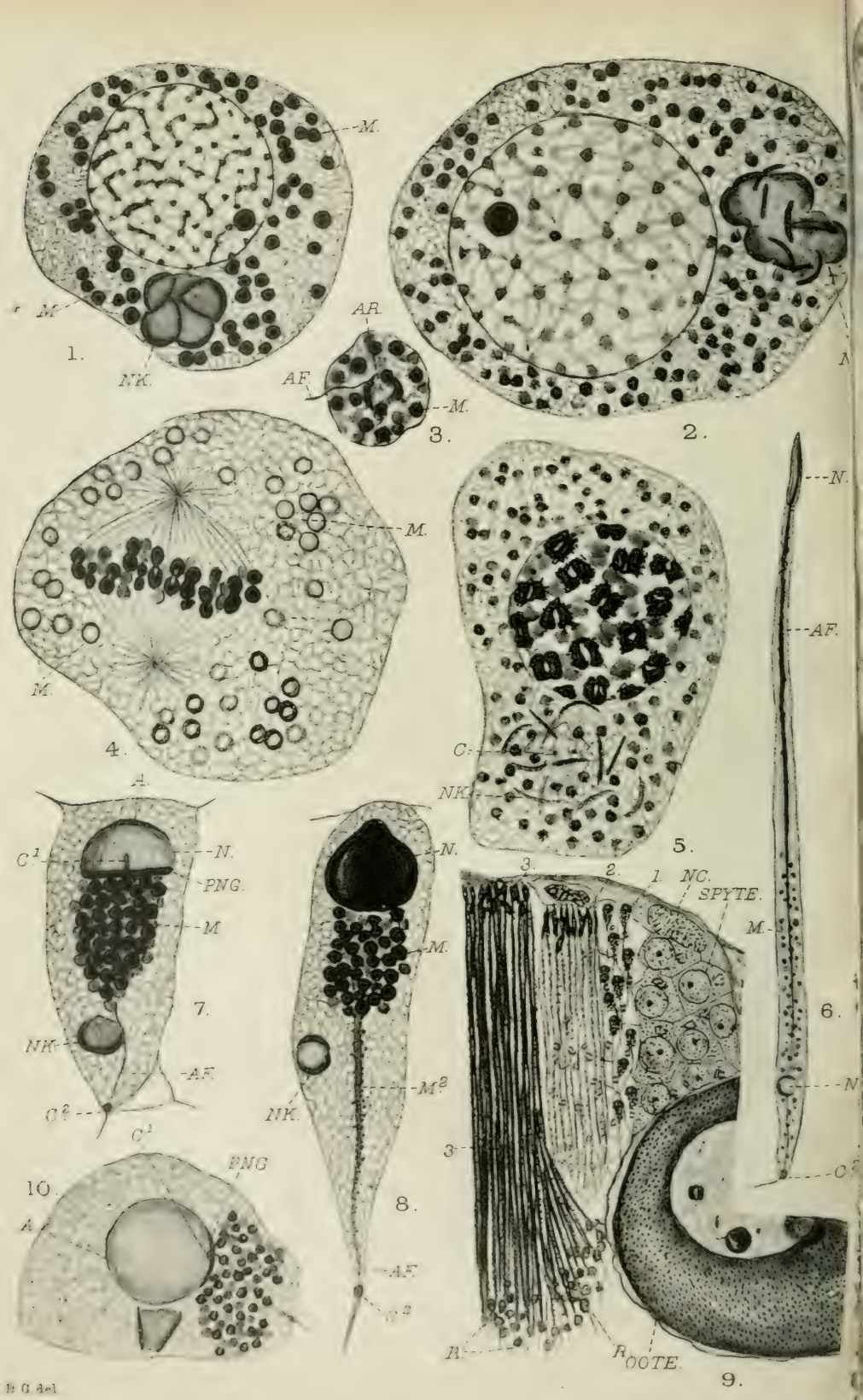
Fig. 34, × 8000, other figures about half this magnification.

Fig. 32.—Bouquet stage, showing chromatin reddish-brown, cytoplasm light brown, chondrioplasts and mitochondria violet.

Fig. 33.—Head of ripe sperm; nucleus yellow-brown, tail-sheath (*T.S.*) violet.

Fig. 34.—Magnified up twice from camera lucida drawing; shows micromitochondria brownish-violet, macromitochondria usual violet shade (see text, p. 226).

Fig. 35.—Tail bead just before sloughing off, containing mitochondria.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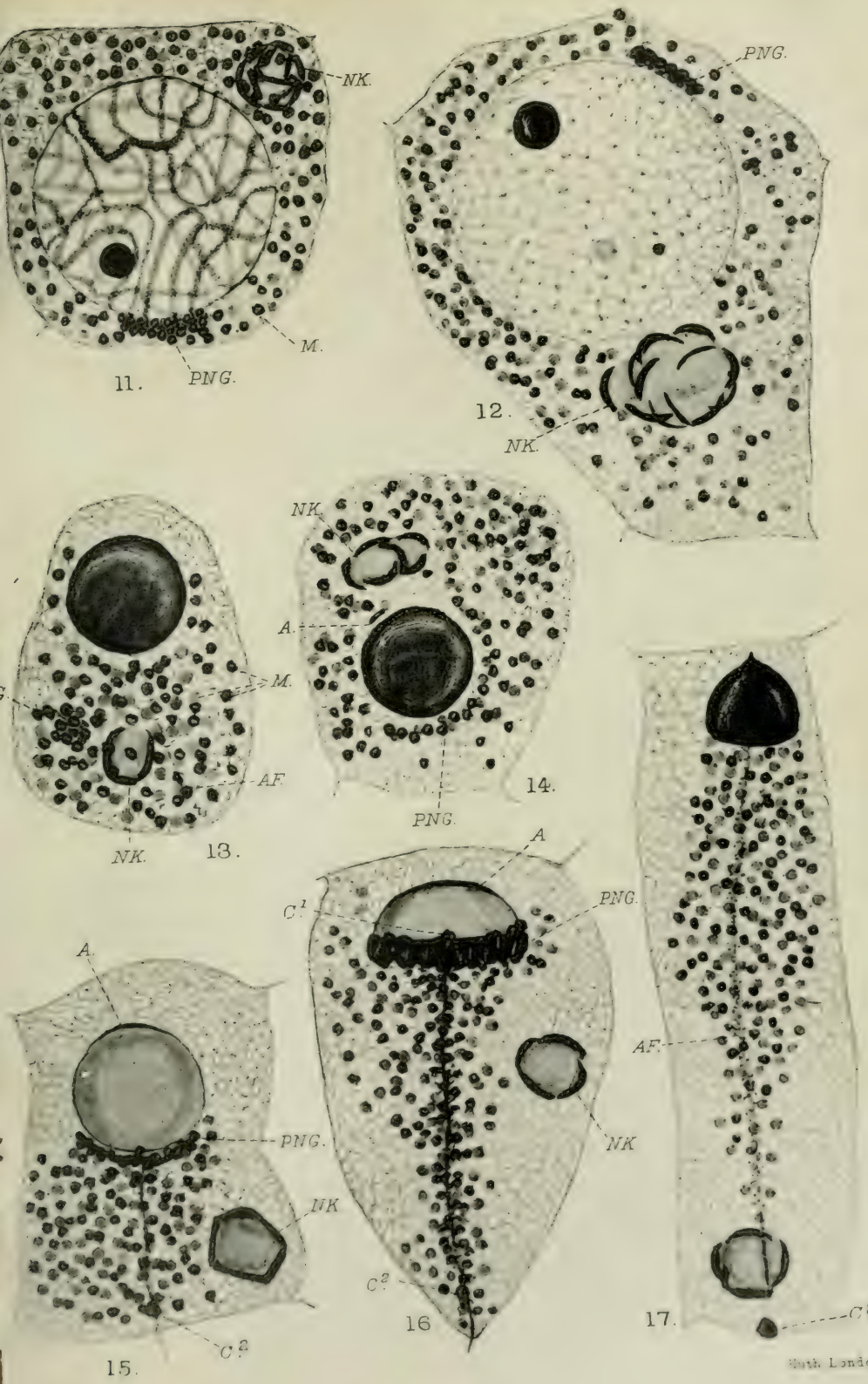
GATENBY—L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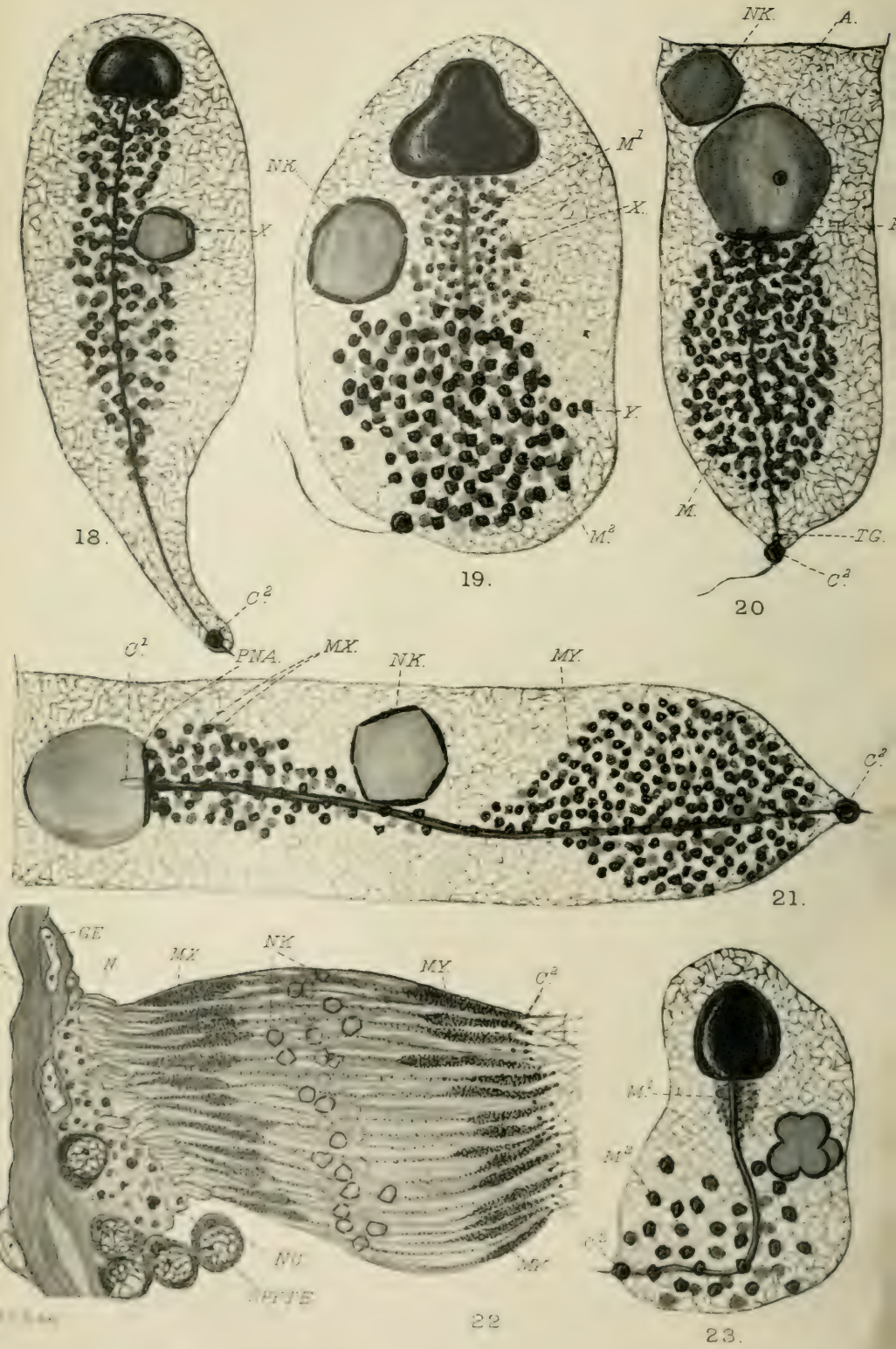
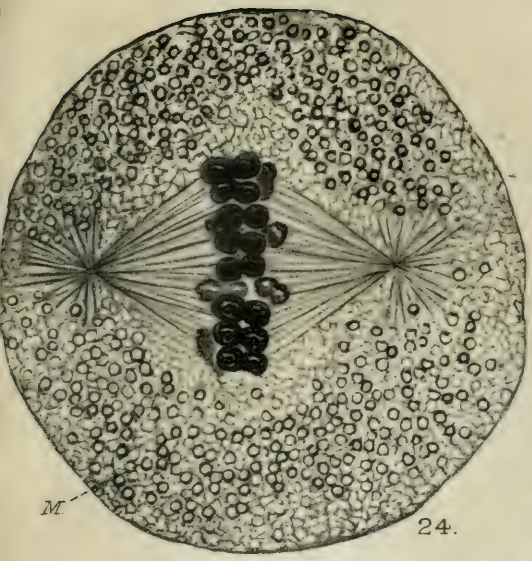
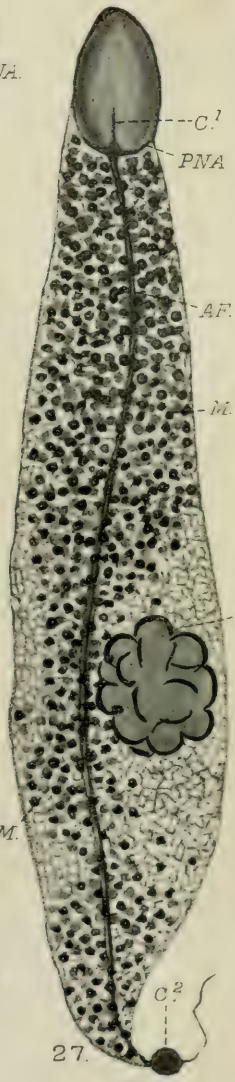
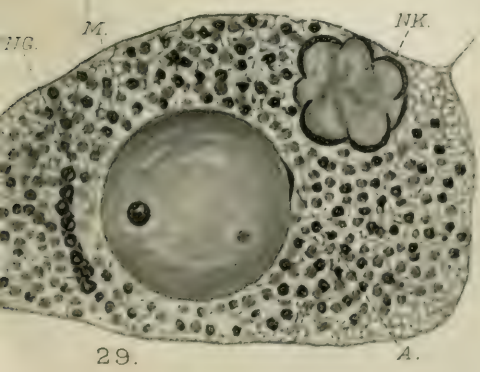
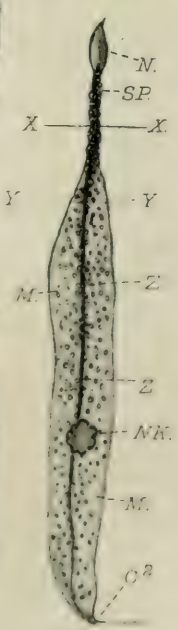


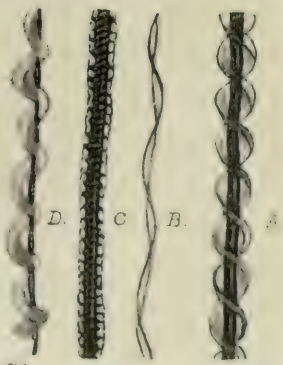
GATENBY—HELIX



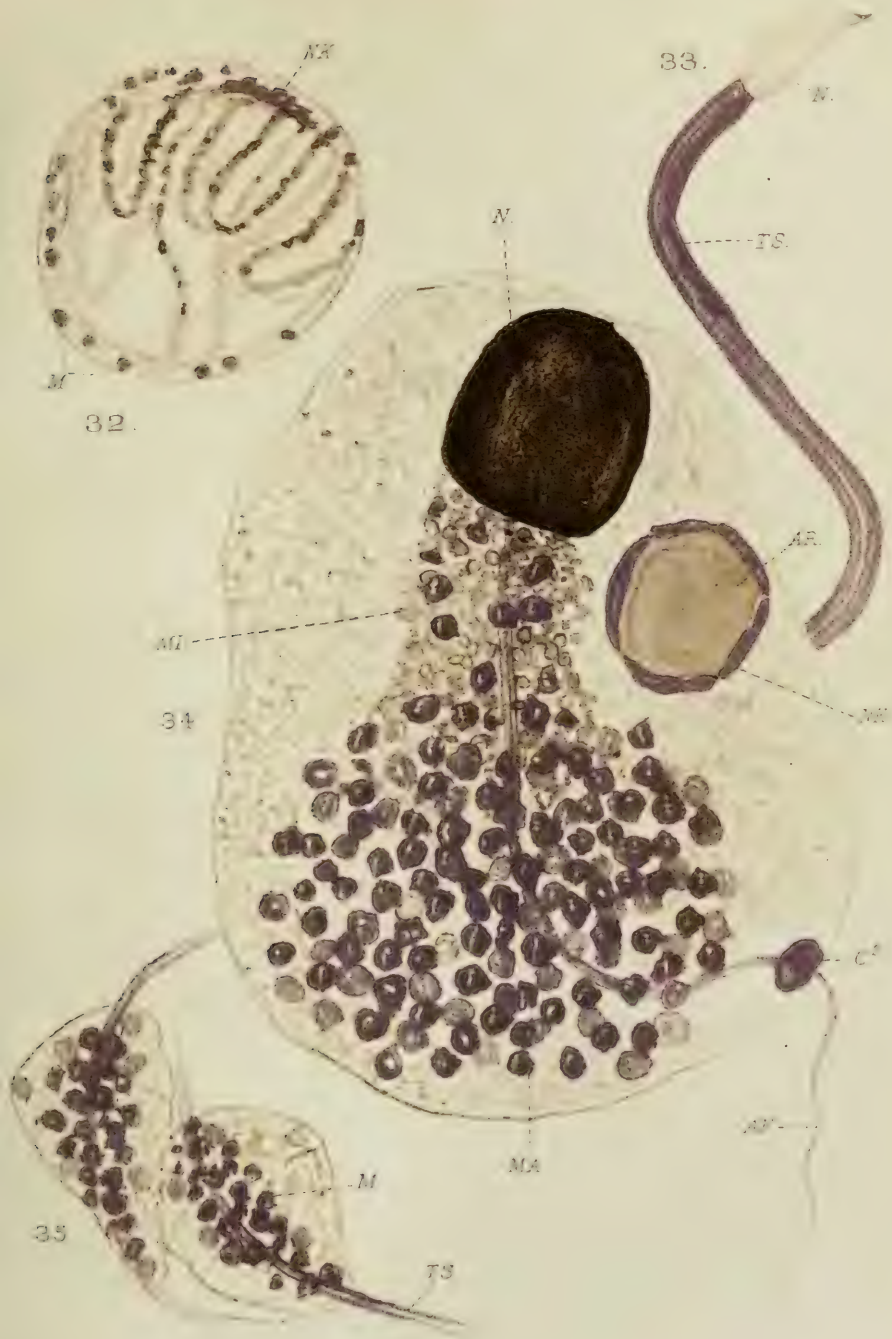








D TESTACELLA.



GATENBY—HELIX ASPERSA.

The Development of Echinocardium cordatum.

Part II.—The Development of the Internal Organs.

By

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With Plate 19.

In a former number of this Journal (**10**) I gave an account of the development of Echinocardium cordatum, including in it a description of the living larvæ and of the external features of the young urchins just after their metamorphosis. Since then I have been engaged (with frequent interruptions) in exploring the development of the internal organs by means of sections, and the facts recorded in the present paper, taken together with what was communicated in the earlier paper, constitute the first complete account of the development of a Spatangoid irregular urchin which has been published.

The methods of preservation and staining employed were substantially the same as those which I used when I was engaged in working out the development of the regular urchin Echinus esculentus, and are fully described in my paper on that subject (**8**). I need only recall to the reader's memory that dilute osmic acid, followed by Müller's fluid was employed for fixation, and that Delafield's hæmatoxylin followed by alcoholic eosin was used for staining. The larvæ were doubly embedded in celloidin and paraffin, but whereas

in dealing with the larvæ of Echinus esculentus, I orientated most of the specimens in such a way that the sections were cut parallel to the frontal plane containing the two antero-lateral ciliated arms, I found it more advantageous to cut sections of the larvæ of Echinocardium cordatum parallel to a plane, cutting the antero-lateral arms at right angles. A few larvæ were sectioned parallel to the frontal plane and a few parallel to the median sagittal plane, but all the sections of larvæ figured in this paper are parallel to the transverse plane.

When the mass of adult species and tube-feet which constitutes the "Echinus-rudiment" makes its appearance, it becomes obvious that in accordance with the oval outline of the adult, one axis of this rudiment is longer than the other; and it is then seen that the longer axis of the future sea-urchin is directed perpendicularly to the antero-lateral and post-oral larval arms. It follows that when one cuts transverse sections of the larva one cuts longitudinal sagittal sections of the Echinus-rudiment, and it is, therefore, far easier to correlate sections of the larva and of the young urchin in the case of Echinocardium than it is in the case of Echinus.

The earliest stages of development: segmentation of the egg, gastrulation, formation of the cœlom, and completion of the alimentary canal are very similar to the corresponding stages in the development of Echinus esculentus, and have been adequately described in Part I of this paper (**10**). We shall, therefore, begin our account of the development of the internal organs with the formation of the madreporic pore. When the larva has attained the age of five days the cœlom has the form of two sacs lying one on each side of the œsophagus (Pl. 19, fig. 1). From the left of these a tube grows up vertically (*p.c.*, Pl. 19, fig., 2) and meets a very slight indentation of the dorsal ectoderm (*m.p.*, Pl. 19, fig. 3). The fusion of this indentation with the tube completes the PRIMARY MADREPORIC PORE (Pl. 19, fig. 4), and the tube is, of course, the PRIMARY PORE-CANAL. In Part I of this paper (**10**) I stated that the appearance of a pore and pore-canal on the

right side of the œsophagus as well as on the left, was a frequent phenomenon. This was an error based on appearances in the living larva and in frontal sections, the reason for which will be explained later. I have never found more than one pore-canal in all the larvæ which I have sectioned.

Muscle-fibres embracing the ventral side of the œsophagus are formed as outgrowths from the cœlomic sacs, and constitute a constrictor of the larval œsophagus (*l.musc*, Pl. 19, fig. 4).

When the larvæ have attained an age of about seven days the cœlomic sacs have grown backwards, so that their posterior portions lie against the larval stomach, and constrictions appear, of which the left one is more pronounced, tending to separate the posterior portions of these sacs from the anterior portions at the sides of the œsophagus (Pl. 19, fig. 4). When the larva has reached the age of nine days the posterior portions of these sacs have been completely separated from the anterior portions; and the single sac on each side of the œsophagus has thus been replaced by two sacs, to which the names ANTERIOR and POSTERIOR CŒLOM may be given.

A new constriction now appears in the left anterior cœlom by which an anterior portion into which the pore-canal opens is delimited from a posterior portion. The hinder end of this anterior portion becomes dilated, and is termed the AXIAL SINUS (*ax.*, Pl. 19, fig. 5 B), and the posterior portion is the HYDROCELE or rudiment of the water-vascular system, whilst the neck connecting the two becomes the STONE-CANAL. The ectoderm adjacent to the hydrocele becomes invaginated so as to form a small thick-walled sac which becomes pressed against the hydrocele. This sac is the AMNIOTIC INVAGINATION, and its floor, together with the underlying hydrocele, constitutes the ECHINUS-RUDIMENT (Pl. 19, fig. 5A.)

At the same time as the hydrocele is formed on the left side, an outgrowth of the right anterior cœlom is formed, which, however, is not directed posteriorly but dorsally, and extends round the larval œsophagus until it nearly reaches the mid-dorsal line. It then becomes hollowed out, and forms

a sac termed the MADREPORIC VESICLE (*mv.*, Pl. 19, fig. 5c.), which in Echinocardium is unusually large. It was this vesicle, as it appeared in surface views of the living larva and in some frontal sections, which I mistook for a second pore-canal, and which I so described in Part I of this paper.

This vesicle is found in the larvæ and adults of Asteroidea, Ophinroidea, and Echinoidea. Its development was first described by me in Asterina gibbosa (**7**). In the larva of this species it arises as a bud on the posterior wall of the single anterior cœlom a little to the right of the mid-dorsal line. Masterman described its development in the Asterid Cribrella oculata, where he found it originating much in the same way as it does in Asterina gibbosa; only he maintains that its point of origin is exactly median (**12**). In view of the fact that the single anterior cœlom both in Cribrella and Asterina represents the result of the fusion of two anterior cœlomic sacs of probably unequal size, the difference between Masterman and myself in this respect is trivial. Gemmill found in Solaster endeca that the sac originated in the same way as it does in Asternia (**2**), but a subsequent examination of the larva of Solaster papposus and a re-examination of the larva of Solaster endeca led him to the extraordinary view that in both these species the sac arose as an ectodermal invagination.

When studying the development of Echinus esculentus (**8**) I found the origin of this sac no easy matter to trace. With great difficulty I detected growing out from the dorsal surface of the right anterior cœlom a single cell which remained connected with the cœlomic sac by a slender string of protoplasm. This cell multiplied and formed a solid cellular knob, which soon became hollowed out, and so gave rise to the madreporic vesicle. Gemmill (**3**) described a similar cell as the earliest distinguishable rudiment of the sac in Asterias rubens; but he thought it probable that this cell, whose origin he could not trace, had been budded from the wall of the œsophagus or of the pore-canal. Now in Echinocardium cordatum the matter is perfectly clear;

the madreporic vesicle develops as an outgrowth from the right anterior cœlom. The clearness with which its origin can be made out in this species is due to the circumstance that it is unusually large. It is exceedingly unlikely that a homologous organ develops in a totally different way in different groups of Echinoderms; it is much more probable that when the rudiment of the vesicle is small and resembles a mesenchyme cell, it is easy to be mistaken about its place of origin. Gemmill's latest statement about the mode of origin of the vesicle in the two species of Solaster is so revolutionary and opposed to the results of other workers, that before taking it into account it would be desirable to await its confirmation.

I formerly regarded this vesicle as a right antimere of the hydrocele and termed it the RIGHT HYDROCELE (7). My justication for doing this was afforded by certain abnormal larvæ of Asterina gibbosa, in which a right hydrocele was present exhibiting the same characteristic lobes as the normal hydrocele on the left side. In these larvæ the madreporic vesicle appeared to be absent, and I naturally concluded that it was represented by the right hydrocele. Subsequently, larvæ of Asterias rubens, in which both a madreporic vesicle and two hydroceles were present, turned up in Dr. Gemmill's cultures and were described by him (3), and similar larvæ were found by me amongst cultures of the larvæ of Echinus miliaris. The existence of such larvæ renders my former view untenable; and it seems to me, therefore, that the view put forward by Bury (1) and endorsed by Gemmill (3) that the madreporic vesicle is the homologue of the pericardial vesicle of Balanoglossus has the greater weight of evidence in its favour. Both Bury and Gemmill have seen the madreporic vesicle pulsate in the larva of Asterias. Its floor is raised into a protrusion containing blastocelic fluid which probably represents the heart of Balanoglossus. This protrusion can be clearly seen in the older larvæ of Echinocardium (Pl. 19, fig. 10 A).

When the larva has attained an age of twelve days the

hydrocele has grown out into five lobes which are the rudiments of the RADIAL CANALS OF THE WATER-VASCULAR SYSTEM. In Echinus the tips of these canals, covered with protrusions of ectoderm, project into the amniotic space and constitute the five primary unpaired tentacles of the young urchin; but in Echinocardium the tips of these canals form only vestigial projections.

From the interspaces between these canals there now arise (as in the larva of Echinus) a series of T-shaped ridges (*ep.f.*, Pl. 19, fig. 6), which I have termed the EPINEURAL RIDGES; the edges of adjacent ridges meet one another and form a series of arches covering in the radial regions of the amniotic floor and so constituting the ambulacral areas of the test of the adult.

The Heart-urchins or Spatangoidea are distinguished from other groups of Echinoidea by the loss of the teeth, and it therefore becomes a matter of interest to find out whether any traces of teeth are to be found in the larval heart-urchin. Now, the teeth are developed in Echinus as outgrowths from the walls of the dental sacs, and these walls also give rise to that complex series of ossicles known as Aristotle's lantern. The five dental sacs arise as evaginations of the walls of the left posterior cœlom.

In Echinocardium five dental sacs (*d.s.*, Pl. 19, fig. 7) arise in the same way as in Echinus, but their cavities remain very much narrower than in Echinus. The subsequent fate of these sacs will be discussed later.

When the larva has attained the age of sixteen days there arise from the floor of the amniotic cavity, i. e. from the surface of the future test, the rudiments of numerous spines (*sp.*, Pl. 19, fig. 8), and the five primary tube-feet (*bucc.t.*, Pl. 19, fig. 8). These tube-feet do not correspond to the primary tube-feet of Echinus (*az.t.*, Pl. 19, fig. 11), which are the protuberant tips of the radial canals. On the contrary, they are basal outgrowths of these canals and become converted into buccal tube-feet in the adult. They thus correspond to the first paired tube-feet of Echinus,

which in E. miliaris are formed before metamorphosis (*bucc.t.*, Pl. 19, fig. 11), but in E. esculentus only after this event. The primary tube-feet of Echinus are represented in Echinocardium by slight cushion-like bulges of the tips of the radial canals (*az.t.*, Pl. 19, fig. 8); these obviously become the so-called ocular tentacles or eye-spots of the adult; and it is to be remembered that even in Echinus as the adult stage or "imago" grows in size, the freely projecting primary tube-feet are reduced to ocular cushions, like those of the young Echinocardium.

When the larva has attained an age of from eighteen to twenty days the Echinus-rudiment attains its maximum development and shortly afterwards metamorphosis occurs. As pointed out in Part I of this paper this process takes place with great rapidity and is complete in fifteen to twenty minutes. Pl. 19, figs. 10 A and 10 B represent two sections through a larva which is about to metamorphose. It will be noticed that the rudiments of the adult spines have greatly progressed in their development. These spines are formed as outgrowths of the ectoderm forming the floor of the amniotic invagination. They become filled with mesenchyme cells budded off from the outer wall of the left posterior cœlom, and in the stage which we are describing these cells become arranged in two masses, one representing the boss with which the movable spine articulates and the other the movable spine itself. Calcareous matter is secreted by these cells, and as this is dissolved away by the fixative it leaves clear spaces (*calc.*, Pl. 19, fig. 10 A). The longitudinal muscles which connect the spine with its boss have already been developed (*sp.musc.*, Pl. 19, fig. 10 A).

We may further notice the development which the dental sacs have undergone. These have now become completely closed off from the left posterior cœlom; from the apex of each an outgrowth of cells has been formed which fills a small triangular outgrowth of the test which I shall term the DENTAL SPINE (*d.sp.*). In Pl. 19, fig. 11, a section of a larva of Echinus miliaris is represented in order to facilitate

comparison with the larva of Echinocardium. This larva is just about to metamorphose, as is clearly evident from the fact that the roof of the amniotic cavity has broken through. The rudiment of the tooth (*T.*, Pl. 19, fig. 11) can be seen growing out from the apex of the dental sac just as the dental spine does in Echinocardium, but it does not as yet project beyond the test. We may, therefore, provisionally assume that the tooth and the dental spine are homologous.

From the base of each dental sac in Echinocardium an outgrowth is given off, which, joining with its fellows, gives rise to a PERIHÆMAL RING-CANAL (*ph.r.c.*, Pl. 19, fig. 10), which in the adult forms the only relic of Aristotle's lantern.

In Pl. 19, fig. 10 B, an invagination of the ectoderm in the centre of the Echinus-rudiment can be seen. This is the ADULT STOMODÆUM (*a.st.*, Pl. 19, fig. 10 B), which, after metamorphosis by union with an outgrowth of the stomach, will form the adult mouth. It is to be noticed that it is still covered by the conjoined edges of the epineural ridges, to which I have given the name of EPINEURAL VEIL (*ep.v.*, Pl. 19. fig. 10 B). If we compare it with the adult stomodæum (*a.st.*, Pl. 19, fig. 11) in the larva of Echinus we shall notice that in the latter larva this stomodæum is very much deeper and better developed. This is because in the adult Echinus the stomodæum forms that part of the canal which traverses Aristotle's lantern, and, as all are aware, Aristotle's lantern is a comparatively high structure in regular Echinids.

In the late larva of Echinocardium the development of the ADULT NERVOUS SYSTEM is well advanced. The radial nerves are formed as thickenings of the ectoderm overlying the radial canals of the water-vascular system. The nuclei become arranged in many layers, and beneath a great tangle of fine fibres makes its appearance (*r.n.*, Pl. 19, fig. 10 B). At the same time a LARVAL BRAIN has become developed. This organ was first described by me in the larva of Echinus esculentus [8], where it forms a pit on the dorsal surface between the bases of the antero-lateral anus. In Echinocardium it is found to be in the same position (Pl. 19, fig. 9), but it

does not form a pit. The ectoderm in this region is thickened, and at the bases of the cells a thick felt-work of nerve fibrils can be seen.

In the stage which we are considering the primary buccal tube-feet have greatly progressed in development, and each is now provided with a well-marked sucking disc (*bucc.t.*, Pl. 19, fig. 10 B), and its base has grown out into a flattened ampulla projecting into the body-cavity (*amp.*, Pl. 19, fig. 10 B).

The axial sinus (*ax.*, Pl. 19, fig. 10 A) into which the stone-canal opens remains small, whereas the madreporic vesicle (*mv.*, Pl. 19, fig. 10) has greatly increased in dimensions, and the protrusion of the basal wall, which we have compared to the heart of Balanoglossus (Pl. 19, fig. 10 A), is very prominent. On the visceral wall of the left posterior cœlom, a pocket-like evagination of cells with large round nuclei can be seen (*germ.*, Pl. 19, fig. 10 A). This is almost certainly the first rudiment of the genital cells (compare Pl. 19, fig. 14 B). Finally, we may call attention to the great development of the Echinus-rudiment in the larva of Echinocardium. If we compare Pl. 19, figs. 10 and 11, we can see that, whereas in Echinus the Echinus-rudiment only occupies a portion of the left side, in Echinocardium it not only occupies the whole of the left side, but sweeps round both before and behind on to the right side. We may further notice that in Echinocardium no spines or other appendages are developed from the right side outside the limits of the Echinus-rudiment, whereas in Echinus, as I have already described (**8**), two pedicellariæ (*ped.*, Pl. 19, fig. 11) are developed in this situation.

We may now turn our attention to the young urchin, our "imago" just after it has undergone metamorphosis. I have already described the external appearance of this stage in Part I, and need only recall to the reader's memory that the urchin is already oval in outline when viewed from above, that the test is thickly covered with spines, except for a comparatively large area in the centre of the dorsal surface, and a smaller area in the centre of the ventral

surface. The latter area corresponds to the region of the adult mouth, but this aperture is still covered by the epineural veil. It is surrounded by a circle of five buccal tube-feet, five dental spines, and five sphæridia, or spines modified in connection with the balancing function, the spine being represented by an oval transparent knob. These spines can be detected in the later larva (*sph.*, Pl. 19, fig. 10 B). At one end of the imago a circle of flattened spines can be seen, but, with the exception of these and of the sphæridia, all the other spines are cylindrical pointed "radioles" like the spines of an ordinary regular Echinid. As previously mentioned, transverse sections of the larva are equivalent to longitudinal sagittal sections of the imago. In addition to many series of such sections, I possess several series cut parallel to the disc, i.e. perpendicular to the oro-apical axis as well as a number of whole mounts.

Pl. 19, fig. 12, represents a sagittal section of a young imago immediately after the process of metamorphosis has been completed. The larval anus has disappeared, but a cord of deeply-staining cells (*l.an.*) embedded in the test, seems to represent the last trace of it. The adult œsophagus (*a.œs.*) has arisen as an outgrowth from the stomach, but it has not yet fused with the adult stomodæum. The stone-canal is cut in three places; below it appears as a thin membranous outgrowth of the hydrocele extending upwards; at the side of the stomach a portion lined with columnar cells is seen, whilst above stomach another portion can be seen opening into a thin-walled sac, which is the axial sinus (*ax.*). or left anterior cœlom. The outer opening of the adult stomodæum has become narrowed, so that its edges form projecting lips. The dental spines can be seen projecting freely at the sides of the oral area of the epineural veil, and at the base of each a small thin-walled cavity can be made out, which is the remnant of the dental sac.

The next stage at my disposal is represented in Pl. 19, fig. 13. The feature which calls for most remark is the appearance of the ADULT ANUS (*a.an.*). This is situated

in the centre of the dorsal surface in the position in which it is found in a regular sea-urchin. It is, therefore, obvious that the dorsal region of the test which is devoid of spines in the young imago corresponds to the PERIPROCT of a regular urchin. If one makes the reasonable assumption that in the history of the race the old anus was not lost and a new one subsequently formed, one may infer that the disappearance of the larval anus and the formation of the adult anus are a shortened record of a shift in position of the anus, and that this opening has remained functional throughout the whole of the change. If we compare the position of the anus in the late larva represented in Pl. 19, fig. 10, with its position in the imago which we are discussing, it will be seen that this shift has taken place along the line of attachment separating the small right posterior cœlom from the large left posterior cœlom.

The adult œsophagus and stomodæum are in close contact, but the adult mouth is still covered in by the epineural veil.

In addition to giving rise to the œsophagus the gut has undergone great changes. The larval epithelium (*l.ep.*), which is distinguished by the possession of pale nuclei which do not stain well is being pushed towards the lumen of the gut, whilst further out a layer of flattened cells with deeply-staining nuclei can be seen. This layer is the adult epithelium (*a.ep.*), and it appears to be regenerated from isolated pockets of embryonic cells. There is little doubt that a similar process takes place in the development of the regular urchin, but in describing the development of Echinus (**8**) I did not succeed in unravelling the process. I spoke of a process of "histolysis accompanied by a great multiplication of nuclei," and of a "clearing up of histolysis" when an orderly epithelium reappeared. In Echinocardium the nature of the process is quite clear. At the same time, the gut undergoes a great shrinkage in volume; this is shown not only by the decrease in diameter of its lumen, but by the great thickening of its peritoneal covering (*per.*). This

tissue, which in the larva formed a thin flattened layer of cells, now becomes many-layered. This change argues a sudden decrease in tension and bears witness to the fact that one main factor in the changes which bring about the metamorphosis is an increase in the permeability of the cells forming the wall of the gut. As a consequence, a large amount of water is transferred from the cavity of the gut to the cœlom, and the cœlomic cavities, which up till the time of metamorphosis were slit-like, now become swollen and enlarged.

The siphon (*siph.*) can now be made out as a narrow tube opening into the stomach at both ends.

The oldest stage in my possession is represented by the two specimens shown in Pl. 19, figs. 14 and 15. In these the epineural veil is beginning to show a minute perforation in the centre, which is the first indication of the opening of the adult mouth (*a.m.*, Pl. 19, fig. 14 A). In the regular urchin the adult mouth is formed long before the adult anus, but in Echinocardium the reverse is the case. It follows that I did not succeed in rearing the urchins up to a point where they had begun to feed. The adult stomodæum and the adult œsophagus are now in open communication, but the adult stomodæum has become, so to say, shallowed out until it forms only an insignificant rim round the mouth—a fact which is to be correlated with the vestigial character of the representative of Aristotle's lantern (*a.st.*, Pl. 19, figs. 14 A and 15). The gut epithelium is now reconstituted.

In Pl. 19, fig. 15, the siphon is cut twice; one section shows its wall consisting of flattened cells, the other of more columnar cells.

Köhler (**6**), in his description of the siphon of the adult Spatangoid, mentions that it can be divided into two regions showing different histological features. The thin-walled division is the initial portion which opens into the top of the adult œsophagus, whereas the thick-walled portion is the terminal part which opens into the stomach where this joins the intestine. It is interesting to find that these two divisions

of the siphon can be clearly discriminated before the young imago has begun to feed.

In Pl. 19, fig. 15, the adult anus is seen. As compared with its condition in the preceding stage it is to be noticed that it is now provided with dilator (*a.dil.*) and sphincter (*a.sph.*) muscles. The mesentery dividing the right posterior from the left posterior cœlom has broken down. In other sections belonging to the same series, it can be seen as a thin lamina of cells projecting freely into the cœlom. By good luck in the section figured a radial nerve is cut longitudinally and the sensory cushion (*az.t.*) in which it terminates is well seen. This cushion represents the terminal unpaired tentacle in an Asterid, which carries the eye-pits and the long unpaired tentacle on which the young imago of Echinus esculentus walks about. It may, therefore, be said that whereas a regular urchin starts its post-larval existence in an asteroid condition, a heart-urchin begins its post-larval existence as a regular urchin. This conclusion is further supported by the whole mount shown in Pl. 19, fig. 16. This figure shows that the intestine exhibits a reversed coil similar to that exhibited by the intestine of a regular urchin.

The structure of the stone-canal and its annexed glands in the adult Spatangoid is a subject on which much obscurity and difference of opinion has prevailed. Köhler (**6**), who worked principally with injections and dissections, described two ring-shaped vessels surrounding the adult œsophagus. From each of these there arose a vertical vessel, one being provided with a cubical epithelium and one with a flattened epithelium. These two vessels, after ascending a certain distance, became fused into one, and this one led into a series of irregular canals surrounded by the tissue of the "ovoid gland" or "glande madreporique," as Köhler terms it. From this gland above there issued two canals, one of which led straight to the exterior through the pores of the madreporite, whilst the other ended blindly beneath it. It is implied that both of these canals were prolongations of the irregular canals forming the cavities of the ovoid gland.

Prouho, who published a note on the vascular system of Spatangus (13), asserts that the "cavity" of the gland is part of the stone-canal, and that this canal, although covered with branches of the blood-vessel, does not communicate with it.

Hamann (5), who made more extensive use of sections, agrees with Köhler in some points but contradicts him in others.

According to Hamann, of the two rings which surround the œsophagus, one is a blood-ring; that is to say, a space devoid of epithelial walls. We may remind the reader that the cavities recognised as blood-spaces in Echinodermata are really hæmal strands—i. e. tracts of modified connective-tissue in which the amœbocytes have multiplied, whilst the fibres have become sparse, and the ground substance has acquired the property of being stained with neutral dyes. From the blood-ring as well as from the water-vascular ring a vertical canal is given off, but the vertical blood-vessel is, of course, like the ring devoid of an epithelium. The epithelium covering the water-vessel is low. As one proceeds upwards the single water-vessel enters the ovoid gland and here breaks up into a network of vessels, whose lumina are partly obstructed by pigmented cells. The blood-vessel also breaks up in the gland into a network of spaces which communicate with the network of water-vessel, but Hamann gives no proof of this assertion and no figure illustrating it. The vessels within the gland terminate above in the stone-canal (recognisable at once by its ciliated epithelium), which opens widely into them, and, in addition, the end of the gland projects into a space which ends blindly below the madreporite. [This space obviously corresponds to the second efferent canal of the gland described by Köhler.]

When I wrote the account of the structure and habits of the Echinodermata which is embodied in the Cambridge Natural History (9), I summarised the position of our knowledge as to the structure of the stone-canal and its adjacent sinuses in regular Echinoidea as follows: (1) The "cavity"

of the gland is the axial sinus or left anterior cœlom of the larva. (2) The madreporite is traversed by ciliated pores which open into a collecting space or ampulla beneath it; from this ampulla the stone-canal originates and the axial sinus also opens into it. (3) The closed space (Köhler's efferent canal, which ends blindly) beneath the madreporite is the madreporic vesicle; this in some genera extends downwards alongside the ovoid gland, but never communicates with the axial sinus. (4) There is a blood-ring, from which branches are given off, which ramify on the exterior of the ovoid gland, but these branches naturally communicate neither with the axial sinus nor the madreporic vesicle. (5) The ovoid gland or dorsal organ is developed as a thickening or fold in the wall of the axial sinus; into this fold a cord of cells penetrates, which is derived from an invagination of the cells forming the outer peritoneal covering of the axial sinus; from these cells is also derived the genital rudiment.

Interpreting Hamann's results in the light of what I had found in regular Echinoidea, I suggested that Hamann's "stone-canal" into which the pore-canals open really represents the ampulla of regular Echinoidea, and that the membranous space or spaces into which this opens below represents an enlarged axial sinus, whilst the opening of this space below into the water-vascular ring would be all that remained of the true stone-canal.

But the arrangement of parts found in the young imago of Echinocardium throws a new light on the question. If we examine Pl. 19, figs. 12, 14A, and 14B, we see that the true axial sinus remains vestigial. The opening of the stone-canal into it remains so contracted that it appears quite probable that in the full-grown adult this opening would become completely closed, and that the stone-canal would then communicate directly with the pore-canals. The thin-walled spaces into which the stone-canal opens below are, as Pl. 19, fig. 12, shows, nothing but modified portions of the stone-canal itself, as, indeed, Prouho had asserted (13).

The primitive germinal involution (*pr. germ*) from which

the characteristic cells of the ovoid gland and also the genital cells arise is well shown in Pl. 19, fig. 14 B.

The reason for the loss of the ciliated epithelium over such a great extent of the stone-canal in Echinocardium is the diminished function of the organ as compared with its function in the regular urchins. I have suggested (**9**) that all the available evidence points to the conclusion that the principal part played by the stone-canal in the economy of the animal is the maintenance of the turgidity of the water-vascular system; and that there is also evidence that the necessity of maintaining this turgidity principally arises when there are extensive movements of the tube-feet; these movements leading to a wastage of fluid by osmosis and a consequent loss of turgidity. In Echinocardium a few of the dorsal tube-feet in the anterior ambulacrum are capable of enormous extension, but the remaining tube-feet are largely immobile, whereas in Echinus all the tube-feet except the buccal tube-feet, are capable of very great extension.

The dental spines have undergone great development by the time that the imago has reached the stage represented in Pl. 19, fig. 15. Unfortunately the section represented in this figure does not pass through any of them. They grow long, and each develops a joint, and so comes to resemble an ordinary spine. This raises the question whether they are directly homologous to teeth or whether, perhaps, they represent new formations developed in the places where true teeth have been lost. It is to be noted that these spines are rudimentary at a time when all the other spines of the imago are fully developed and functional, and their fate in the later history of the imago would be an interesting point to determine.

When in Part I I described the external appearance of the imago, I stated that the only hint of its Spatangoid affinities was to be found in the presence of a "crest" of flattened spines at one end. I regarded these spines as the first formed of the spade-like spines with which the adult Spatangoid slowly ploughs its way through the sand. But

the examination of sections renders this point of view untenable. These spines (*fasc.* Pl. 19, figs. 13 and 14), are devoid of any joints, and are covered with cilia. They are clearly the beginning of the FASCIOLE of ciliated spines characteristic of the genus Echinocardium, which is situated just below the anus. This fasciole enables us to discriminate the future position of the anus in the adult even when the anus is still central—for this fasciole is in the anal inter-radius.

We may now return to the question of the buccal tube-feet. In Echinus there is one pair of these in each radius, in the adult Echinocardium several pairs; but in the young imago only a single tube-foot is developed in connection with each radial canal of the water-vascular system, and there must be, therefore, an asymmetrical arrangement of these.

I examined by means of horizontal sections the arrangement of these tube-feet in two imagines and to my surprise I found it different in each case. The two arrangements which I found are represented in Pl. 19, fig. 17; the position of each tube-foot being indicated by its ampulla, which, after all, is only the enlarged basal end of the tube-foot. It will be seen that in each case two ampullæ are situated in the anal inter-radius, but that in one urchin there are two ampullæ in the right anterior inter-radius, which is the inter-radius containing the madreporite, whereas in the other no ampullæ are to be found in that inter-radius, but two are situated in the left anterior inter-radius. This variability in the development of these tube-feet raises several interesting points. It suggests strongly that neither the number nor the exact positions of the tube-feet which are developed is inherited, but that the position and number of those which do appear is determined by the amount of space available.

Embryologists comparing organs developed in series in larvæ and in adults are struck with the fact that the number of these organs is apt to undergo drastic reduction in the larva, and that this reduction cannot possibly be accounted for on the theory that it is a representation of the smaller

number of such organs present in a primitive ancestor which the larval stage represents. The reduction in number, as I have pointed out, is rather to be regarded as a consequence of the reduction of larval size which is so marked a feature in most larvæ (11).

The complete working out of the development of two such divergent types as Echinus and Echinocardium, which are, nevertheless, members of the same order, puts us in a position to institute interesting comparisons. As all are agreed that the regular urchin is the more primitive type, it becomes an interesting matter to see how far the modification which the adult has undergone has affected the ontogeny. The facts recorded in this paper enable us to give a clear answer to that question. As compared with the regular sea-urchin, Echinocardium discards the larval appendages and relinquishes its free-swimming life at a far more advanced stage of development than Echinus, yet, nevertheless, the time occupied in reaching this more advanced stage is far shorter than the free-swimming life of the regular urchin.

The young Echinus requires from six to eight weeks to reach the period of metamorphosis, whereas the young Echinocardium accomplishes the same task in eighteen or twenty days.

The young Echinus, with its horizontal radial canals ending in unpaired tentacles is really an Asterid, whereas the young Echinocardium, with its meridional radial canals terminating in sensory knobs and its central anus is really a regular sea-urchin. This hurrying over of the earlier stages of development in the more modified type, is the phenomenon described by the American authors as "TACHYGENESIS," which has played such a large part in blurring the embryological record.

It follows that almost the whole history of the changes which converted a regular urchin into a Spatangoid is contained in the post-larval development of Echinocardium. I made some efforts to determine the arrangement of the plates in the young imago, but these attempts were unfor-

tunately unsuccessful. The spines are so numerous that they completely obscure the arrangement of the plates in a dried specimen, but if alkalies be employed to make the spines fall off the connection of the plates with one another is loosened and the whole of the little test falls to pieces. I hope to be able at some future time to overcome this difficulty and resume the study of the subject.

The principal results recorded in this paper are as follows:

(1) Only one pore-canal is developed in Echinocardium, and this is formed as a vertical outgrowth from the left anterior cœlom which meets a shallow indentation of the dorsal ectoderm.

(2) Although Echinocardium is devoid of teeth, a series of five dental sacs is formed in the larva. From the walls of these sacs outgrowths are formed which rise above the test and develop into spines. These dental spines may possibly represent the teeth of a regular urchin, but they resemble ordinary spines in possessing a ball and socket joint.

(3) The left anterior cœlom becomes modified into an axial sinus, but this—in contrast to what occurs in regular urchins—remains small and becomes vestigial in the imago.

(4) A large madreporic vesicle is formed and can be clearly seen to originate as a dorsal outgrowth of the right anterior cœlom.

(5) The hydrocele sends out five lobes which become the radial canals of the water-vascular system; the tips of these canals terminate in sensory knobs, but they never project freely as unpaired tube-feet as they do in the larvæ of regular urchin.

(6) From the basal portions of the radial canals there arise a series of five freely projecting tube-feet, one from each canal. These tube-feet are the only ones which the young imago possesses and they become converted into buccal tube-feet when growth is complete. As they are lateral outgrowths of the radial canals their arrangement is necessarily asymmetrical and this arrangement varies from specimen to specimen.

(7) The young imago possesses an anus situated in the

centre of the dorsal surface, and the intestine is curved in a reversed coil like that of a regular urchin.

(8) All the spines of the young imago are similar to those of a regular urchin, except the spines of the sub-anal fasciole, which are precociously developed.

(9) The epithelium of the larval gut undergoes ecdysis after metamorphosis has taken place, just as is the case with the larval gut cells of one of the higher insects at the time of metamorphosis. The epithelium of the adult gut is regenerated from pockets of cells which remain in the embryonic condition. At the same time, the gut shrinks greatly in diameter and the volume of the cœlomic cavity increases; it seems, therefore, that one important factor in metamorphosis is alteration in the permeability of the gut-wall, which allows of the transference of fluid from the gut to the cœlom.

Imperial College of Science and Technology;
April 23rd, 1918.

List of Works referred to in this Paper.

1. Bury, H.—"The Metamorphosis of Echinoderms," 'Quart. Journ. Micr. Sci.,' vol. 38, 1895.
2. Gemmill, J. F.—"The Development of the Starfish Solaster endeca," 'Trans. Zool. Soc. (Lond.),' vol. xx, 1912.
3. ——— "The Development and Certain Points in the Adult Structure of the Starfish Asterias rubens," 'Phil. Trans. Roy. Soc. (Lond.),' Series B, vol. ccv, 1914.
4. ——— 'Annual Report of the Scottish Marine Biological Association,' 1917.
5. Hamann, O. v.—"Beiträge zur Histologie der Echinodermen," 'Jen. Zeit. Naturges,' vol. xxi, 1887.
6. Köhler, R.—"Recherches sur les Echinides des Cotes de Provence," 'Annales du Musée d'histoire naturelle de Marseille,' vol. i, 1883.
7. MacBride, E. W.—"The Development of Asterina gibbosa," 'Quart. Journ. Micr. Sci.,' vol. 38, 1896.
8. ——— "The Development of Echinus esculentus together with Some Points in the Development of E. miliaris and E. acutus," 'Phil. Trans. Roy. Soc. (Lond.),' Series B, vol. cxcv, 1903.

9. MacBride, E. W.—Article, "Echinodermata," 'Cambridge Text-book of Natural History,' vol. i, 1906.

10. ——— "The Development of Echinocardium cordatum. Part I, The External Features of the Development," 'Quart. Journ. Micr. Sci.,' vol. 59, 1914.

11. ——— 'Text-book of Embryology,' London: Macmillan & Co., 1914.

12. Masterman, A. T.—"The Early Development of Cribrella oculata (Forbes), with Remarks on Echinoderm Development," 'Trans. Roy. Soc. (Edin.),' vol. xl, 1902.

13. Prouho.—"Sur le système vasculaire de Spatangus purpureus," 'Comptes Rendues,' Tome cii, 1886.

EXPLANATION OF PLATE 19,

Illustrating Prof. E. W. MacBride's paper on the "Development of Echinocardium cordatum."

List of Abbreviations Employed.

a.an. Adult anus. *a.dil.* Dilator muscle of the anus. *a.ep.* Adult epithelium of the gut-wall. *a.int.* Adult intestine. *a.m.* Adult mouth. *amn.* Amniotic invagination and amniotic cavity. *amp.* Ampulla of buccal tube-foot. *a.œs.* Adult œsophagus. *ap.* Aperture in the web of tissue at the base of the post-oral arm. *a.st.* Adult stomodæum. *ax.* Axial sinus. *az.t.* Vestigial unpaired tentacle at the end of the radius in Echinocardium, and well developed tentacle in the same position in Echinus. *bucc.ect.* Ciliated ectoderm of the buccal cavity of the larva. *bucc.t.* buccal tube-foot. *calc.* Empty spaces left by solution of calcareous matter. *d.s.* Dental sac. *d.sp.* dental spine. *ep.* Epineural space. *ep.f.* epineural fold. *ep.v.* Epineural veil. *fasc.* Ciliated spines of the sub-anal fasciole. *germ.* Primary germinal involution from which germ-cells and characteristic cells of the ovoid gland originate. *H.* Heart. *hy.* Hydrocele and water-vascular ring. *int.* Adult intestine. *l.a.c.* Left anterior cœlom. *l.an.* Larval anus. *l.cœ.* Left cœlomic sac (undivided). *l.ep.* Larval epithelium of the gut-wall. *l.int.* Larval intestine. *l.musc.* Larval constrictor muscle of the œsophagus. *l.œs.* Larval œsophagus. *l.p.c.* Left posterior cœlom. *m.p.* Madreporic pore. *m.v.* Madreporic vesicle. *n.ect.* Nervous ectoderm. *n.f.* Nerve fibres. *ov.g.* Rudiment of ovoid gland. *p.c.* Pore-canal. *ped.* Pedicellaria of Echinus. *per.* Peritoneum. *ph.r.c.* Perihæmal ring-canal (representing Aristotle's lantern in Echinus). *r.a.c.* Right anterior cœlom. *r.n.* Radial nerve-cord. *r.p.c.* Right posterior cœlom. *r.w.v.c.* Radial

canal of the water-vascular system. *sp.* Adult spine. *sp.musc.* Muscles which move the adult spine. *st.c.* Stone-canal. *syn.* Synovial cavity between boss and shaft of an adult spine. *T.* Tooth rudiment of Echinus. *v.con.* Ventral concavity of the larva cut tangentially.

[All the figures, except fig. 16, which is an optical section, and fig. 17, which consists of diagrams, represent sections cut transverse to the plane containing the antero-lateral arms of the larva and parallel to the median sagittal plane of the imago. Except where otherwise stated, all are magnified 190 diameters.]

PLATE 19.

Fig. 1.—Section of a larva about four days old. *r.cœ.*, *l.cœ.* Right and left cœlomic sacs respectively, as yet undivided.

Fig. 2.—Somewhat oblique section of a larva about five days old, showing the pore-canal (*p.c.*) as an outgrowth of the left cœlomic sac.

Fig. 3.—Section of a larva of about the same age as that shown in fig. 2, showing the formation of the madreporic pore (*m.p.*). *v.con.* Ventral concavity of the larva cut tangentially.

Fig. 4.—Section of a larva about seven days old, showing the beginning of the segmentation of the cœlom. *l.a.c.*, *l.p.c.* Anterior and posterior divisions of the left cœlomic sac. *l.musc.* Constrictor muscle of the larval œsophagus.

Figs. 5 A, B and C.—Three consecutive sections of a larva about nine days old, showing the formation of the hydrocele, amniotic invagination, and madreporic vesicle. The right cœlomic sac as well as the left is now completely divided into anterior and posterior portions. *a.inv.* Amniotic invagination. *hy.* Hydrocele. *l.an.* Larval anus. *m.v.* Madreporic vesicle. *r.a.c.* Right anterior cœlom. *stom.* Stomach cut tangentially.

Fig. 6.—Section through a larva about twelve days old, showing the formation of the epineural ridges (*ep.f.*). *amn.* Amniotic cavity. *ap.* Aperture in the web of tissue at the base of the post-oral arms cut tangentially.

Fig. 7.—Section through a larva about fourteen days old, showing the formation of the dental sacs (*d.s.*). *r.n.* Radial nerve-cord. *sp.* Rudiment of adult spine. *st.c.* Stone-canal cut in two places: above it opens into the axial sinus (*ax.*), below it opens into the hydrocele.

Fig. 8.—Section through a larva about sixteen days old, showing the formation of the primary buccal tube-feet (*bucc.t.*). *az.t.* Vestigial unpaired terminal tentacle. *ph.rc.* Perihæmal ring-canal formed as an outgrowth from the dental sac. *r.n.* Radial nerve cut longitudinally. *r.w.v.r.* Radial water-vascular vessel.

Fig. 9.—Portion of a section through a larva about eighteen days old, showing the larval brain. Magnification about 800 diameters. *bucc.ect.* Ciliated ectoderm of the larval buccal cavity. *n.ect.* Ectoderm which forms the brain. *n.f.* Nerve-fibres.

Figs. 10 A and B.—Two sections through a larva about twenty-one days old which is just on the point of metamorphosis. The Echinus-rudiment has reached its maximum extension. In fig. 10 A a dental spine (*d.sp.*) is seen to originate as an outgrowth from a dental sac. A trace (*germ*) of the invagination which ultimately gives rise to the germ-cells is also seen. *H* is the heart-cavity, i. e. the cavity of the protrusion of the floor of the madreporic vesicle; *l.an.* the larval anus, which is just grazed. In fig. 10 B the adult stomodæum (*a.st.*) is seen beneath the epineural veil; also portions of two buccal tube-feet and the ampulla (*amp.*) of one of these. A sphæridium (*sph.*) has also been grazed.

Fig. 11.—Section through a larva of Echinus miliaris which is just about to metamorphose. Magnification 105 diameters. As contrasted with fig. 10, the Echinus-rudiment in this genus can be seen to be much smaller than it is in Echinocardium. *T.* Rudiment of a tooth growing out from the dental sac, but not as yet projecting to the exterior. *az.t.* Large unpaired tube-foot. *bucc.t.* Small tube-foot, one of the first pair, cut tangentially. *ped.* Rudiment of pedicellaria on the right side.

Fig. 12.—Longitudinal sagittal section of a young imago of Echinocardium immediately after the metamorphosis. *a.œs.* The adult œsophagus, a new outgrowth from the stomach. *a.st.* The diminishing adult stomodæum. *fasc.* Ciliated spines of the sub-anal fasciole. The stone-canal (*st.c.*) is cut in three places—below a membranous section opens into the hydrocele; above this a section lined by ciliated epithelium is cut; whilst still higher can be seen the opening of the canal into the reduced axial sinus (*ax.*). *l.an.* Possible vestige of larval anus.

Fig. 13.—Longitudinal sagittal section of a young imago, slightly older than that shown in fig. 12. *a.an.* Adult anus widely open. *a.ep.* Adult epithelium of the gut. *l.ep.* Larval epithelium of the gut in the act of being thrown off. *siph.* The siphon, now distinct from the rest of the alimentary canal. *sp.musc.* Muscles which move the adult spines.

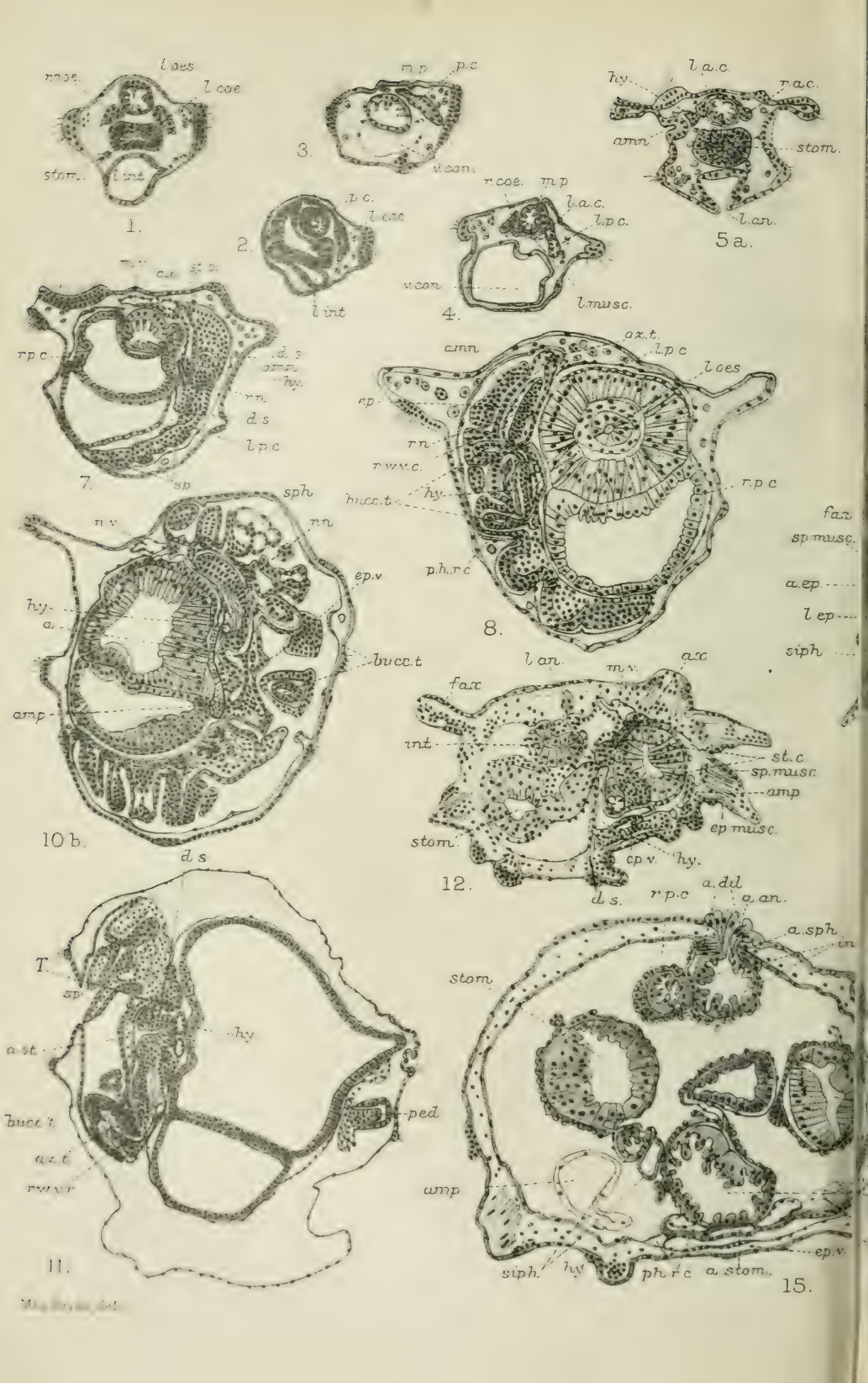
Fig. 14 A.—Longitudinal sagittal section of an imago somewhat older than that shown in fig. 13. *a.m.* Minute pore in the centre of the epineural veil, which is the first trace of the adult mouth. *germ.* The invagination which gives rise to the germ-cells. *per.* The thickened peritoneum enveloping the larval gut. *synov.* The joint cavity in one of the adult spines.

Fig. 14 B.—Portion of another section belonging to the same series as that from which the preceding figure was taken The stone-canal (*st.c.*) is shown opening by a very contracted aperture into the axial sinus (*ax*). *m.v.* The madreporic vesicle. *m.p.* The madreporic pore grazed. *ov.g.* The ovoid gland.

Fig. 15.—Longitudinal sagittal section of the oldest imago in my collection. The anus is provided with dilator (*a.dil.*) and sphincter (*a.sph.*) muscles. The septum dividing the right posterior cœlom (*r.p.c.*) from the left posterior cœlom has broken down. A radial nerve is cut longitudinally, and is seen to end in a sensory cushion (*az.t*). The mouth is open, but the section does not pass through it. The adult stomodæum (*a.st.*) has become almost vestigial.

Fig. 16.—An optical section of a young imago of about the same age as that represented in the preceding figure. taken parallel to the disc at right-angles to the oro-apical axis. Magnification 90 diameters. *a.int.* Adult intestine, showing the reversed coil.

Fig. 17.—Two diagrams showing the relation of the buccal tube-feet to the radii in two young imagines.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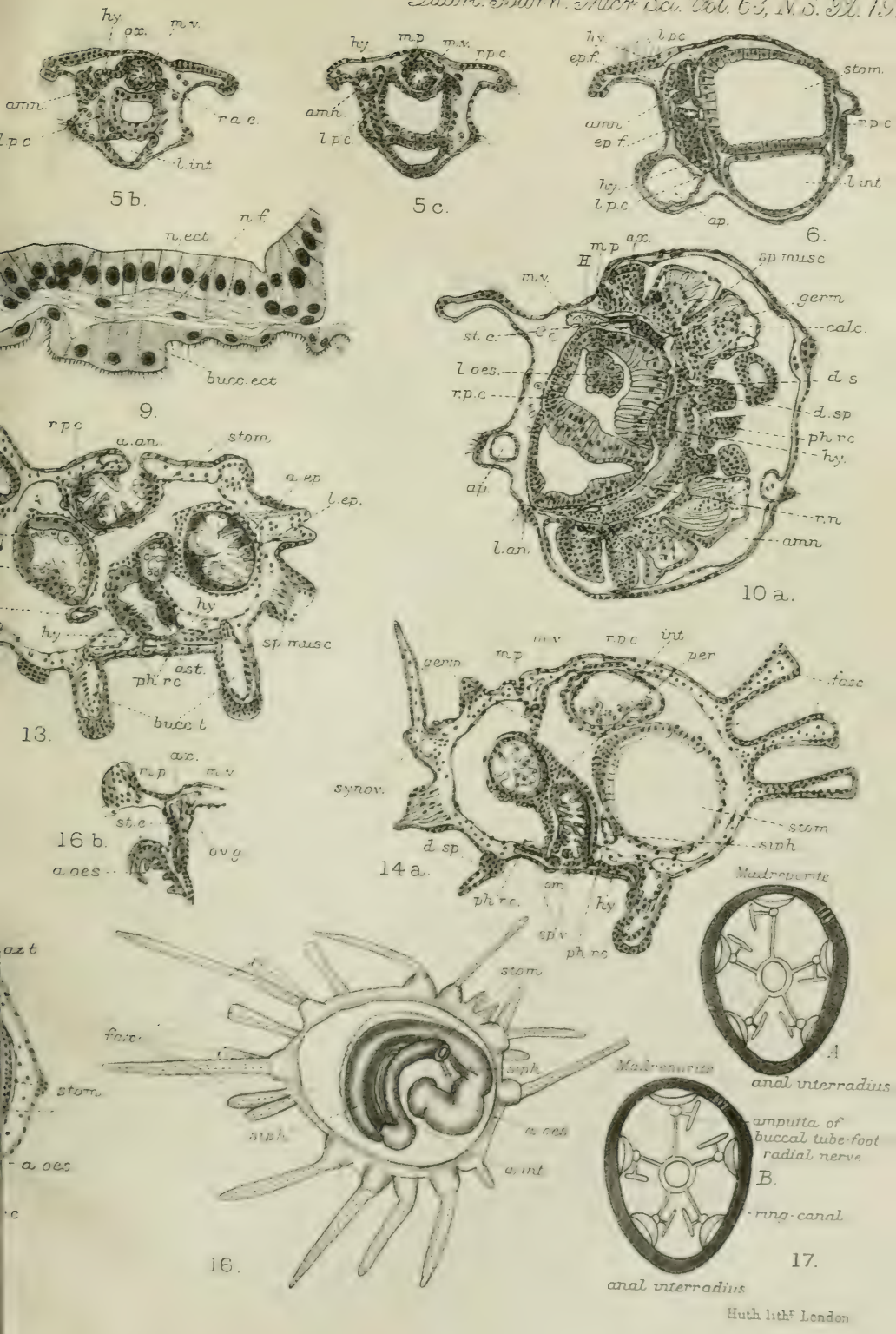
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A Revision of Certain Points in the Early Development of Peripatus capensis.

By

Edith H. Glen, B.Sc.

With Plate 20.

Some valuable material of Peripatus capensis and Peripatus Balfouri was very kindly presented to the Zoological Laboratory of the Imperial College of Science by the widow of the late Prof. Adam Sedgwick, and at the suggestion of Prof. MacBride, who handed over the material to me, I have revised two points in Prof. Sedgwick's "Monograph of the Development of Peripatus capensis," which had raised a great deal of controversy when the paper was published.

I wish to express my most sincere thanks to Prof. MacBride for placing this material at my disposal and also for the kind assistance and very valuable advice which he has given me during my work.

General Remarks.

The first point I shall deal with is the theory propounded by Sedgwick that, in the early stages of Peripatus capensis and even as late as stage B, there are no cell limits (**10**).

This revolutionary statement caused a great deal of comment at the time, threatening to overthrow the prevailing idea that the ancestral Metazoon was a colonial Protozoon, and suggesting instead that it was a multinucleated Infusorian-like animal. It influenced all Sedgwick's later work, and in his

paper on the "Inadequacy of the Cellular Theory of Development" (11) some years later, he says that his subsequent work has amply confirmed his earlier view that embryonic development "must be regarded as a multiplication of nuclei and a specialisation of tracts and vacuoles in a continuous mass of vacuolated protoplasm" (1883).

In this paper he deals with two tissues of the vertebrate embryo—the mesenchyme and the system of peripheral nerve trunks. The mesenchyme has always been described as consisting of branched cells lying between the ectoderm and endoderm. Sedgwick rejects the idea of cells here and affirms that the so-called mesenchyme consists of a reticulum with nuclei at its nodes, and that these nodes are what have always been described as cells. He also comes to the conclusion that nervous and muscular tissues are special developments of the same primitive reticulum, and, in fact, that all tissues are merely modifications of a reticulum.

The second point in Sedgwick's paper, which was responsible for a large amount of controversy, was with regard to the nephridium. He maintains that it is of a purely mesodermal nature, and that it opens directly to the exterior.

There have been repeated misunderstandings and misinterpretations of the term "nephridium." This name was given to the structures previously called "segmental organs," by Sir E. Ray Lankester in 1877. We may quote his original definition:

". . . in Rotifera, Flatworms, Gephyræa (not the genital ducts), Mollusca, in the metameres of Chætopoda, in the Vertebrata, and even in some Arthropoda we have evidence of the existence of a single pair of canals, more or less highly modified by glandular developments, which usually open by ciliated funnel-like mouths into the cœlome at one end and directly to the exterior in the neighbourhood of the anus, or into a cloacal chamber at their other end, thus placing the cœlome in communication with the exterior.

"This pair of ciliated funnels appears to be the same organ in all cases. Primarily it develops like the stomo-

dæum by an ingrowth of the ectoderm or deron. At present no name is in use for this important pair of organs; they are spoken of as 'segmental organs' in some groups, as 'primitive excretory organs' in others. Since very usually these canals acquire an excretory function and give rise to kidneys, though they may also serve as genital ducts, I propose to call them by the diminutive of the Greek word for a kidney—namely, 'nephridium'" (**8**).

From this it will be seen that Lankester believed that the nephridia were most decidedly of ectodermal origin, and this view was generally accepted.

Meyer, who in 1886–7 studied the development of the Annelida carefully, came to the same conclusion with regard to the development of the nephridium (**9**). On the other hand, Bergh (1899) (**1**) and Bürger (1902) (**2**) maintained that the nephridium of the Oligochæta was of cœlomic origin. They said it developed as an outgrowth of the cœlome which fused with the ectoderm, and finally became hollowed out. But in 1895 Goodrich (**3**) proved that the nephridia of Oligochæts were of ectodermal origin.

They develop from large outer layer cells (funnel cells) and pass through a pronephridial stage.

"In the first (most forms) and sometimes in the trunk segments (Chætogaster) they never develop beyond that stage. In the other segments the nephridia grow towards and open into the cœlome by means of a funnel formed from the original 'funnel cell.'"

Goodrich also (1897–8) (**4**) studied the development of some marine Annelids and found that in all those which he examined the nephridia were of essentially ectodermal origin, consisting of blind tubes which are ectodermal ingrowths, and later on, acquire a communication with the cœlome. So-called nephridia, which develop from the cœlome, as in Terebellidæ and Mollusca, he terms "cœlomoducts," and he draws attention to the presence of both a cœlomoduct (the genital duct) and a nephridium in the same somite of Lumbricus, hence the two structures cannot be homologous.

The development of the Alciopinæ, also worked out by Goodrich in 1900 (**4**), is extremely interesting. In these worms the nephridia are closed internally, and are provided at the inner end with well-developed solenocytes. The genital funnel (cœlomostome) develops later, and, growing down, becomes grafted on to the nephridial duct, and, finally, an opening is formed at the point of junction. Hence the genital products pass down the nephridial duct to the exterior. This development was investigated in more detail by Goodrich in 1912 (**5**), and he found that the nephridium is a slender tube, and that the cœlomostome grows back as a pocket from the septum near the nephridium and unites with the nephridium at maturity.

Again, Staff in 1910 (**12**) found that in the Oligochæt Criodrilus the mother-cells of the nephridium appear in the ectoderm.

The nephridia of Sipunculus also have been found to appear as solid ectodermal ingrowths in which a cavity appears at a later stage. They eventually open into the cœlome, and the cells at the point of junction give rise to the funnel (**6**).

This overwhelming mass of evidence in favour of the ectodermal origin of the nephridium casts considerable doubt on the correctness of Sedgwick's conclusions, especially as at about the same time that Sedgwick was working out the development of P. capensis (**10**), Kennel was doing the same with P. Edwardsii (**7**), and he did not agree with Sedgwick in regard to the origin of the nephridium.

He declared that there was an ectodermal inpushing which ultimately became a canal, and that the funnel only of the nephridium was mesodermal. He also said that the funnel was in direct communication with the body cavity, but it is quite probable that this was due to the fact that the vesicle is very thin-walled and easily broken.

Sedgwick denied the existence of an ectodermal ingrowth of any importance.

"I have only to say that the ectodermal ingrowth at the

opening of the nephridium is extremely inconspicuous, and that at the early stage immediately before and after the establishment of the external opening, an ectodermal part such as Kennel describes can only be made out with difficulty" (**10**, p. 81), and holds that the cœlome opens directly to the exterior at the base of the leg and just external to the nerve cord.

(i) Presence of Cell Limits in Peripatus capensis at Stage A.

The following statements are taken from Sedgwick's paper and relate to stage A of the embryo:

"The ectodermal part of the embryo consists of a closely-reticulated protoplasm which contains a single layer of oval nuclei of fairly uniform size" (**10**, p. 52).

"During stages A and B the ectoderm on the dorsal and ventral surfaces is composed of what may be called cubical cells, . . . but these cells are not isolated from one another or from the endoderm" (**10**, p. 53).

"With regard to the internal boundary of the ectoderm in the gastrula stage, there was no line of demarcation between it and the endoderm. In stage B the mesoderm appears, but causes no break in the continuity" (**10**, p. 55).

"We cannot speak of cells till . . . stage B" (**10**, p. 106).

He describes the endoderm as the "inner portion of the vacuolated wall of the embryo. The two are perfectly continuous" (**10**, p. 65), and as being, at the close of stage E, "a layer of vacuolated protoplasm with nuclei of irregular size" (**10**, p. 66).

Considering these statements as a whole, I take it that Sedgwick denies the presence of cells altogether in the endoderm at this stage, but admits that there are "what may be called cubical cells" in the ectoderm. But he also says (**10**, p. 106) that we cannot speak of cells till stage B. By this I suppose he means that there are no cell-walls visible till that stage.

In examining sections of stage A, I found the general appearance practically that described by Sedgwick—the ectodermal nuclei oval and rather crowded together, the endodermal nuclei larger, very irregular in outline, and scattered in the protoplasm, which was extremely vacuolated, particularly on the dorsal side. I must admit that when one first sees sections of the early stages of P. capensis Sedgwick's theory seems quite probable.

But careful examination with a $\frac{1}{12}$-in. oil-immersion objective showed that in the ectoderm one could distinguish a certain number of undoubted cell-walls.[1] In some parts it was impossible to make out the cell-walls with certainty, partly owing to the crowding of the nuclei, and partly owing to the extremely large vacuoles on the dorsal side, which made it almost impossible definitely to distinguish cell-walls from strands of protoplasm. But those that could be distinguished were quite undoubtedly cell-walls. The existence of even a certain number of cell-walls makes it impossible for the ectoderm at this stage to be a "closely reticulated protoplasm with a layer of nuclei."

The cell-walls of the endoderm are much more difficult to distinguish, as they are very irregular and might be confused with protoplasmic strands, but, on very careful examination, one can make them out almost without exception.

Sometimes one finds what appears to be a cell without a nucleus, but this is explained by the large size of the cells, which makes it possible for a section to pass through the cell and yet not through the nucleus. Pl. 20, figs. 3–5 represent three sections drawn in series, and, allowing for the irregularity of the walls, one can see that they correspond with one another in all three sections. This would be rather unlikely if they were simply protoplasmic strands.

[1] By cell-wall I mean the layer of non-protoplasmic substance which is formed as a secretion and which separates the cells from one another. In preparations preserved and stained by the usual methods and examined under a high power of the microscope, it appears as a thin dark line.

The dividing line between ectoderm and endoderm is quite a well-defined thing, and can be seen relatively easily.

There seems to me to be not the slightest doubt of the presence of cell-walls at this stage of the development of P. capensis, although I do not mean to infer by this that I consider that each cell in a multicellular organism is absolutely independent of every other cell. There must be some connection, and that probably by protoplasmic strands. But to deny that there are cell-walls and cells, in the accepted sense of the word, in the earlier embryonic stages of Peripatus seems to me entirely wrong.

The difficulty in seeing them is due to the extremely large size and irregular shape of the cells. This is, no doubt, caused by the fact that the ovum of P. capensis is passing from the yolked to the non-yolked condition, and the yolk is bound to have distended the cells.

Two things, in all probability, accounted for the fact that Sedgwick did not admit the existence of cell-walls. One must take into account, first, the extremely large size and irregularity of the endodermal cells and the presence of numerous vacuoles; and second, the very poor preservatives that were available in his time.

(ii) The Origin of the Nephridium.

According to Sedgwick, the whole of the nephridium is mesodermal.

Each somite becomes divided into a dorsal and ventral part by a septum. The dorsal part eventually disappears, except in the posterior segments of the body, where it gives rise to the generative organs, but the ventral part gives rise to the nephridium. At stage F this consists of "(1) . . . a vesicular internal part extending to the hind end of the appendage and forwards as a blind diverticulum, and opening into (2) a tubular part projecting ventrally and opening to the exterior" (**10**, p. 76). "The tubular part is the nephridium and its opening into the vesicle is the funnel" (**10**, p. 79).

Kennel maintains that the tubular part which opens to the exterior is ectodermal and that the funnel only is mesodermal. But Sedgwick's reply to this was that the ectodermal ingrowth was too inconspicuous to be taken into account.

Kennel partly bases his views on the difference in the appearance of the ectodermal and mesodermal nuclei. The ectodermal nucleus he describes as "rundlich, bei Sublimatbehandlung homogen und färben sich nicht sehr intensiv. Die Kerne der mesodermzellen sind länglich, mehr körnig, stehen dichtgedrängt und färben sich in Pikrocarmin und Boraxcarmin sehr intensiv" (7, p. 39).

This differentiation of the nuclei is very well seen at an early stage, where the cavity of the somite is just being divided into a dorsal and a ventral part. The mesodermal nuclei look almost like a string of beads inside the ectoderm. The difference is not quite so marked in the later stages, but it is still evident. (I had some difficulty in staining these sections sufficiently without obliterating all the detail, and the result was that they were rather faintly stained. This is probably the reason why the difference between the two kinds of nuclei is not so clear as in the earlier stages.)

The ectodermal ingrowth described by Kennel is, without doubt, a very conspicuous thing and forms a part of the nephridium, as will be clearly seen from Pl. 20, fig. 8. The distinction between its nuclei and those of the funnel or the vesicle is well marked.

Pl. 20, fig. 6, shows the beginning of the ectodermal ingrowth and the ventral part of the somite. Pl. 20, fig. 7, is a later stage, probably just before the opening of the ectodermal canal into the cœlome, and Pl. 20, fig. 8, shows a much later stage in which one can distinguish the ectodermal part which has grown in, from the mesodermal part which originated from the ventral part of the somite.

The internal vesicle seems to me to be larger than is shown in Sedgwick's diagrams, and fills almost the whole cavity of the leg. Part of it is seen in surface view in Pl. 20, fig. 8, but the nuclei which I consider mesodermal extend farther

down the leg, hence the vesicle also must extend farther down, although one cannot see the whole of its boundary.

Pl. 20, fig. 7, in particular seems to me to prove the existence of an ectodermal ingrowth without the slightest doubt. The ingrowth there is very conspicuous and it is most certainly ectodermal, even without the evidence of the nuclei.

If this ingrowth only formed part of the tube it would not be possible to say that it was a real homologue of the Annelid nephridium, as it might be merely a "gateway" to a cœlomiduct. But when, as in this case, the whole tube is ectodermal with the exception of the funnel, which is mesodermal, and when one considers that Peripatus is the most primitive Arthropod and most nearly allied to the worms, in which, as we have seen, the true nephridia are ectodermal, then I think one may say that the "nephridium" of Peripatus is not a cœlomiduct, but that it is homologous with the true nephridium of worms.

Bibliography.

1. Bergh.—"Nochmals über die Entwichelung der Segmentalorgane." 'Zeit. für. wiss. Zool.,' vol. lxvi, 1899.
2. Bürger.—"Weitere Beiträge zur Entwickelungogeschichte der Hirudineen: Zur Embryologie von Clepsine," ibid., vol. lxxii, 1902.
3. Goodrich.—"On the Cœlome, Genital Ducts, and Nephridia." 'Quart. Journ. Micr. Sci.,' vol. 37, 1895.
4. ——— "On the Nephridia of the Polychæta," Part I, ibid., vol. 40, 1897; Part II, ibid., vol. 41, 1898; Part III, ibid., vol. 43, 1900.
5. ——— "Observations on the Nephridia of the Alciopinæ," 'Zool. Jahrbücher,' Suppl. 15, Bd. 2, 1912.
6. Hatschek.—"Über die Entwickelung von Sipunculus nudus." 'Arb. Zool. Inst. Wien.,' vol. v, 1883.
7. Kennel, J.—"Entwicklungsgesch. v. P. edwardsii," Th. ii, 'Arb. a. d. Zool. Inst.,' Würzburg, Bd. viii.
8. Lankester, E. Ray.—"Notes on Embryology and Classification." 'Quart. Journ. Micr. Sci.,' vol. 17, 1877.
9. Meyer, E.—"Studien über den Körperbau der Anneliden." 'Mitt. aus der Zool. Stat. Neapel,' vols. vii and viii, 1886–7.

10. Sedgwick, A.—"The Development of the Cape Species of Peripatus." 'Studies from the Morphological Laboratory of the University of Cambridge,' vol. iv.

11. ——— "The Inadequacy of the Cellular Theory of Development," 'Quart. Journ. Micr. Sci.,' 1894.

12. Staff.—"Organogenetische Untersuchungen über Criodrilus lacuum," 'Arb. aus. dem. Zool. Inst.,' Wien, vol. xviii, 1910.

EXPLANATION OF PLATE 20,

Illustrating Miss E. H. Glen's paper on "A Revision of Certain Points in the Early Development of Peripatus capensis."

List of Abbreviations.

app. Leg. *cav.app.* Cavity of leg. *c.b.s.* Central blood space. *ect.* Ectoderm. *ect.in.* Ectodermal ingrowth. *end.* Endoderm. *f.* Funnel. *l.b.s.* Lateral blood space. *neph.* Nephridium. *v.som.* Ventral part of somite. *v.n.c.* Ventral nerve cord. *w.v.som.* Wall of ventral part of somite grazed by razor.

[All the drawings were made with the aid of a Zeiss-Abbé drawing apparatus.]

Fig. 1.—Transverse section of an embryo of stage A, showing nuclei and cell-walls of ectoderm and endoderm.

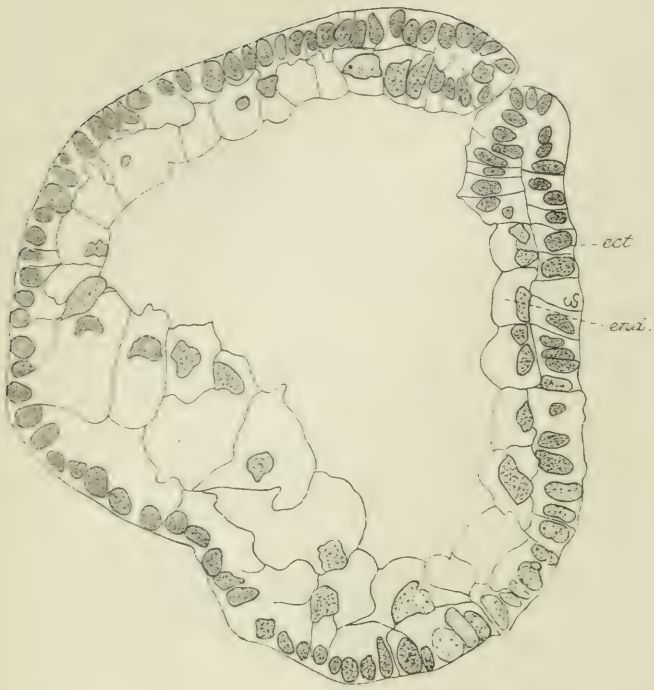
Fig. 2.—Part of the same section as seen with a $\frac{1}{12}$-in. oil immersion objective.

Figs. 3–5.—A series of transverse sections of the same embryo, to show that the cell-walls occur in approximately the same position in all three sections.

Fig. 6.—Transverse section of an embryo of late spiral stage, showing the beginning of the ectodermal ingrowth and the slight down-growth of the somite.

Fig. 7.—A later stage where the ectodermal ingrowth is extending inwards, but does not yet open into the cœlome.

Fig. 8.—A still later stage where the ectodermal ingrowth has become a canal and now communicates with the cœlome.



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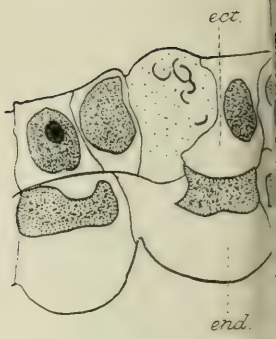

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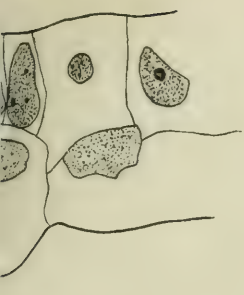


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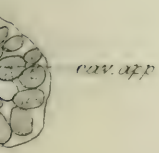
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Observations on the Insect Parasites of Some Coccidæ.

By

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Reader in Agricultural Entomology in the Victoria University of Manchester.

II. On Chalcid Parasites of Lecanium capreæ.

With 35 Text-figures.

CONTENTS.

I. Introduction.

In the first paper of this series (Imms, 1916) a tolerably full account was given of the life-history of Aphelinus mytilaspidis, Le Baron, the chief parasite of the Mussel Scale (Lepidosaphes ulmi). The present contribution deals with certain features in the structure and life-economy of two abundant Chalcid parasites of Lecanium capreæ. Although the genus Lecanium comprises certain highly destructive insects, the species under consideration is not often of direct economic significance. An investigation of its insect enemies, however, has been prompted by the following considerations. Firstly, Lecanium capreæ is one of the most heavily parasitised of our indigenous Coccidæ, and a plentiful supply of material is readily available. Secondly, the parasitism is effectual to an exceptional degree in checking any undue abundance of the host. Thirdly, a study of the problems associated therewith, appeared to afford an exceptionally favourable opportunity for investigating the factors which contribute towards determining the efficiency of Hymenopterous parasites, as natural controlling agents. This research has been carried out in the Department of Agricultural Entomology, Manchester University, and at various localities in the field. I am again indebted to Prof. S. J. Hickson, F.R.S., for affording various facilities for prosecuting these investigations. Mr. A. A. Girault, of the U.S. Bureau of Entomology, has kindly examined the parasites in

question and identified them as Aphycus melanostomatus, Timberlake, and Blastothrix brittanica, Girault, sp. nov. Mr. J. T. Wadsworth, my research assistant, has rendered help in collecting material of the Lecanium from various localities, and in cutting serial sections for histological purposes.

II. Observations on the Biology of the Host.

According to Newstead (1903, p. 112), Lecanium capreæ is abundantly and generally distributed in suitable localities throughout England, but appears to be local in Scotland and Wales. I have met with it less plentifully in the southern counties of England than in the north midlands. It is common on the Continent of Europe (Marchal, 1908, p. 302), and also occurs in North America. Its principal food-plant is the hawthorn (Cratægus oxycantha), but I have also found it on lime (Tilia europea) and hazel (Corylus avellana). A few examples were also obtained from Horse Chestnut (Æsculus hippocastanum), sweet gale (Myrica galeæ), and apple (Pyrus malus). In addition to these host-plants others are recorded by Newstead (loc. cit.) and Marchal (loc. cit.).

Unless stated to the contrary, all observations mentioned in this paper were made upon material obtained from hawthorn.

From numerous observations, conducted in Cheshire, Lancashire, and Derbyshire during 1914–16, it was found that the young Lecanium larvæ commence to emerge from the eggs during the end of June. In 1914, from material obtained from Northenden (Cheshire), the larvæ commenced to appear in a field insectary on June 26th; they were very abundant on June 30th, and continued emerging until the middle of July. During 1915, ova obtained from the same locality commenced to hatch in a cool laboratory on June 17th. Shortly after emergence from the egg, the larvæ crawl freely over the twigs of the host-plant. They eventually reach the

leaves and take up positions near one of the principal veins on the under surface. The proboscis is inserted into the tissues, and the insect subsists upon the juices which it extracts therefrom. Shortly before the annual leaf-fall, the

TEXT-FIG. A.

A young twig of hawthorn (Cratægus oxycantha) bearing ten fully-grown examples of Lecanium capreæ; slightly larger than natural size.

larvæ migrate from the leaves on to the twigs. In Cheshire, this migration was found in 1915 to be most active during the second and third weeks in October. In 1916 the autumn was markedly warmer and the migration commenced during the fourth week in October. Upon reaching the twigs, the larvæ

usually establish themselves in or near the axils of the buds or bases of the thorns. On young and succulent twigs they are sometimes found scattered along the general surface of the shoot. Old and resistant shoots and branches are seldom utilised, and the positions which the larvæ take up during the autumn remain permanent, no further migration taking place. In almost all cases the ventral aspect of a twig is chosen. Where the branches or twigs are more or less vertically directed, no particular aspect appears to be selected. During the winter very little growth in size takes place, and unparasitised larvæ, measured on December 21st, 1915, averaged 1·1 to 1·3 mm. long and ·7 mm. broad. On April 14th of the following year, larvæ from the same locality measured 2·1 to 2·5 mm. in length, and about 1·2 mm. in greatest breadth. After fertilisation, the females grow much more rapidly, and this goes on until the insect assumes the rounded, berry-like form shown in Fig. A. When fully grown, towards the end of May, the adult female scale insects measure from 5 to over 6 mm. along the antero-posterior axis, and approximately 3·75 mm. in transverse diameter. In the vertical plane they vary from 3 to about 5 mm. Considerably smaller examples, however, are not infrequently met with. The male covering scales are much smaller, elongate and flattened in form, and measure approximately 2·5 mm. in length. The winged males were observed to first appear during 1915 on May 5th, and in 1917 on May 15th. Females preponderate very greatly in number over the males, and were found at Knutsford (Cheshire) in the proportion of 86 : 9. In two localities near Northenden (also in Cheshire) the proportions were 77 : 4 and 5 : 1 respectively. Oviposition takes place at the end of May, or the first week in June, according to the prevailing climatic conditions. The number of eggs laid varies considerably and is to some extent proportional to the size of the parent insect. Counts were made on five average-size examples and the number varied from 1372 to 1919. According to Newstead (1903, p. 111) the number laid by a healthy female averages about 2000.

Tullgren (1906, p. 90) gives a similar estimate. After oviposition is fulfilled, the female rapidly declines and dies. Her shrivelled remains, invested by the scaly covering, function as a kind of brood-pouch, protecting the eggs until they hatch. Empty scales of the previous year are not infrequently found still adhering to the host-plant.

III. Parasites previously recorded from Lecanium capreæ.

Various Chalcid parasites have been recorded from Lecanium capreæ (Morley, 1910, p. 30), though none of them have been met with during the present investigations, and the majority appear to be infrequent in this country. With the exception of Eunotus, which belongs to the Pteromalidæ, all are members of the Encyrtidæ. In the accompanying list it will be observed that the host has been recorded under various specific designations, all of which, however, are now regarded as being synonyms of one and the same insect.

Parasite.	Authority.	Name under which host is recorded.
Eunotus cretaceus, Walk.	De Gaulle (1908, p. 103)	Lecanium capreæ, Linn.
Eucomys obscura, Dalm.	De Gaulle (1908, p. 98)	Coccid on Tilia.
E. scutellata, Swed. .	Kawall (1855, p. 231)	L. tiliæ, Linn.
Aphycus punctipes, Dalm.	De Gaulle (1908, p. 99)	L. alni, Modeer.
,, ,,	Ratzeburg (1848, p. 146)	L. alni, Modeer.
Blastothrix sericea, Dalm.	Ashmead (1900, p. 390)	L. æsculi, Kol. L. tiliæ, Linn.
,, ,,	Newstead (1903, p. iii)	L. capreæ, Linn.
B. schonherri, Westw.	Mayr (1875, p. 700)	L. æsculi, Kol.
Microterys chalcostomus, Dalm.	Mayr (1875, p. 719)	L. æsculi, Kol.
M. sylvius, Dalm. .	Mayr (1875, p. 719)	L. æsculi, Kol.

IV. Observations on Blastothrix britannica, Gir.

(a) Systematic Position.

The genus Blastothrix, like Aphycus, belongs to the sub-family Encyrtinæ of the Encyrtidæ.[1] It was likewise described by Mayr in the same memoir (1875, p. 697), in which he erected the latter genus. The various species may be readily distinguished by the fact that they are metallic or sub-metallic in appearance. In the females the joints of the funicle of the antennæ are longer than thick, and the marginal vein is present. According to Howard (1881, p. 365), the males are characterised by having the scape of the antenna less compressed than in the female; the pedical is scarcely longer than thick, and is much shorter than the following joint; the joints between the pedical and the club are strongly incised above at the articulations, and each joint bears upon its upper side two whorls of long erect hair.

Schmiedenknecht (1909, p. 241) catalogues nine species of Blastothrix, although eleven are quoted by Dalla Torre (1898, pp. 252–253). By the former authority Blastothrix longipennis, How., is regarded as a synonym of B. sericea (Dalm.); and, furthermore, he transfers B. rosæ, Ashm. to the genus Aphidencyrtus, Ashm.

(b) Habits and Distribution of the Genus Blastothrix.

Members of this genus are internal parasites during the larval stage, and attack Coccidæ of the genus Lecanium, living on various plant hosts, and also Pulvinaria. Two species, however, are apparently exceptions to this rule, i.e. B. bifasciata, which is recorded by Mayr (1875, p. 701), as follows: "Aus einer Zucht von Gallen der Cynips cerricola[2] erhielt ich im Mai zwei Weibchen, welche wahrscheinlich

[1] For the characters of the Encyrtidæ see p. 341.

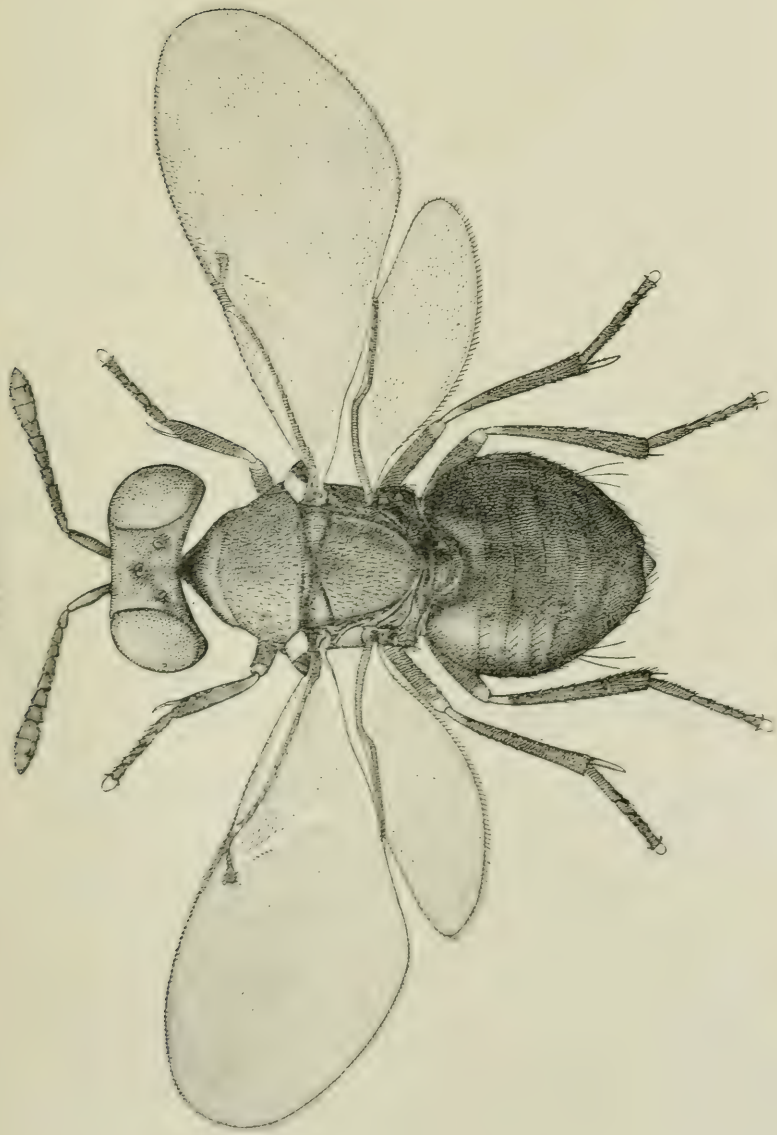
[2] Hymenoptera fam. Cynipidæ.

in Schildlausen auf Zerreichen lebten"; and B. bohemani, Westw., concerning which Mayr remarks (p. 700): "Diese Art lebt wahrscheinlich von Schildlausen an Rubus cæsiusi da ich aus siner grosseren Zucht von Gallen der Lasioptera rubi[1] im Mai des v J. II Exemplare erhalten habe und es nicht wahrscheinlich ist, dass sie aus den Gallen selbst gekommen seien."

The only published observations, known to me, dealing with the biology of any member of this genus are those of Newstead (1891, p. 31; 1893, pp. 66–67, 108–111, and 251), which relate to B. sericea. This author describes the effect which the parasite has upon the general form of its Coccid host, together with observations as to its method of oviposition and the larval feeding habits.

The genus Blastothrix is widely distributed in Central and Western Europe, and occurs also in North America, from Florida and the West Indies across to California. Of the European forms, two species are catalogued by Morley (1910, p. 22) as being British, viz. B. bohemani, Westw. and B. sericea, Dalm. The former is recorded by Walker (1838, p. 111) as being found on grass beneath trees near London, and the same writer (p. 106) records B. sericea from lime and oak trees near London. He also mentions North Wales, Ireland, and Scotland, while Newstead (1901, p. 1) states that it is the most widely distributed and the commonest of the British Coccid parasites. To these I am now able to add B. britannica, a species shortly to be described by Mr. A. A. Girault, from material which I sent originally to the United States National Museum for identification. B. sericea, Dalm is the only species listed by de Gaulle (1908, p. 99) from France, and its range also extends into Sweden, Germany, Austria, and North America. B. bifasciata Mayr occurs in Germany and Austria, B. erythrostethus (Walk.), in Austria, and B. schonherri (Westw.), in Germany. The remaining species catalogued by Schmiedenknecht are recorded from North America. B. britannica is an abundant species,

[1] Diptera fam. Cecidomyidæ.



TEXT-FIG. 1.

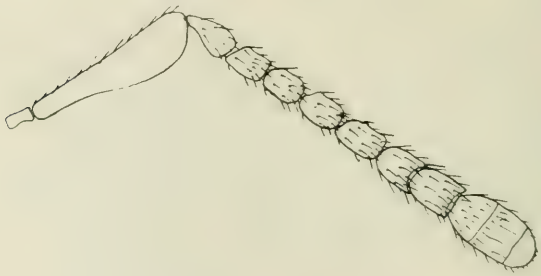
Blastothrix britannica, Gir., female. × circa 44.

and it is remarkable that it should have remained undiscovered for so long. It has been found plentifully in the following localities during the years 1914–17. Lancashire: West Didsbury near Manchester, and Fallowfield. Cheshire: Northenden, Knutsford, and Lymm. Derbyshire: Edale, Whaley Bridge, Marple, and Lathkil Dale.

(c) The Female.

Coloration.—Dark metallic, blue-green, inclining in many specimens to blue-black ventrally; the hairs investing

TEXT-FIG. 2.

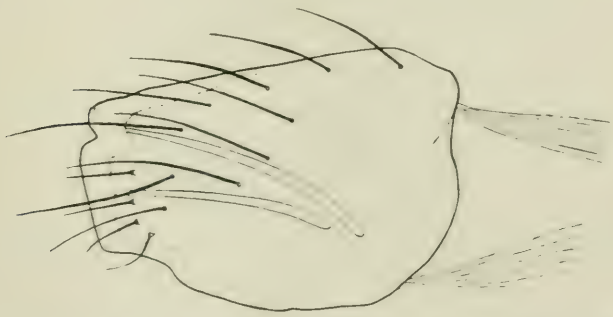


Blastothrix britannica, Gir., female. Left antenna, viewed laterally.

the head and trunk white. The eyes are pruinose, and the ocelli vary from deep crimson to almost black. The scape of the antenna is brown-black with metallic reflections; the remaining joints are smoky brown, those forming the pedicel and club being the darkest; frequently the last joint of the funiculus is paler than the preceding joints. The tegulæ are dirty white basally and dark brown distally. The legs are dark, smoky brown; the distal ends of the femora and the proximal and distal ends of the tibiæ of the fore and middle legs, the distal ends of the femora, and the proximal ends of the tibiæ of the hind legs, and the tibial spurs and first tarsal joints of the middle legs are dirty white.

The Head.—The head is as broad as the maximum width of the thorax, and is much compressed in the antero-posterior plane; when viewed from its anterior aspect it is shaped like an equilateral triangle with the angles greatly rounded. The eyes are distinctly hairy, and the ocelli are disposed in such a manner that the posterior pair are situated individually nearer to the eyes, and to the hind margin of the head, than to the median unpaired ocellus (Fig. 1). The antennæ (Fig. 2) measure 1 mm. long, and are 2-jointed, the joints being mutually related in length in the following proportions:

TEXT-FIG. 3.



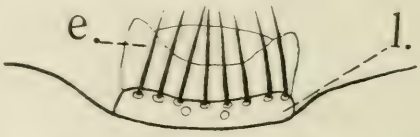
Blastothrix britannica, Gir., female. Right mandible. × 325.

Basal joint.	Scape.	Pedicel.	Funiculus.	Club.
3	19	6	4 : 4 : 5 : 5 : 4 (2–3) (3–4) (6–7) (6–7)	5 : 4 : 4 (10–12) (12–13) (7–8)

On the 5th to 11th joints elongate rod-like sensoria are evident, and their average numbers are indicated above in brackets, placed beneath the respective joints. The mandibles (Fig. 3) are closely similar to each other, somewhat quadrangular in form, with the points of muscular insertion and the articular surfaces but little emphasised. Each mandible measures ·17 × ·1 mm., it is bluntly and

unequally bi-dentate, and about 14–16 slender bristles are present on its upper surface. Situated dorsally to the mandibles is a stout transverse chitinised bar, as is frequent among Chalcids; one of its functions appears to be that of affording support to the anterior margin of the mouth. The labrum (labrum superiore of some authorities) is extremely short, and extends between the bases of the mandibles; it measures ·008 mm. long, and has a breadth of ·07 mm.; its anterior edge is slightly concave, and is armed with 7–8 stout marginal setæ (Fig. 4). The epipharynx (*e.*) is a broad membranous lip-like structure projecting from beneath the labrum; its surface is clothed with minute hairs, and its antero-dorsal margin is deeply incised to form a well-marked sinus.

TEXT-FIG. 4.

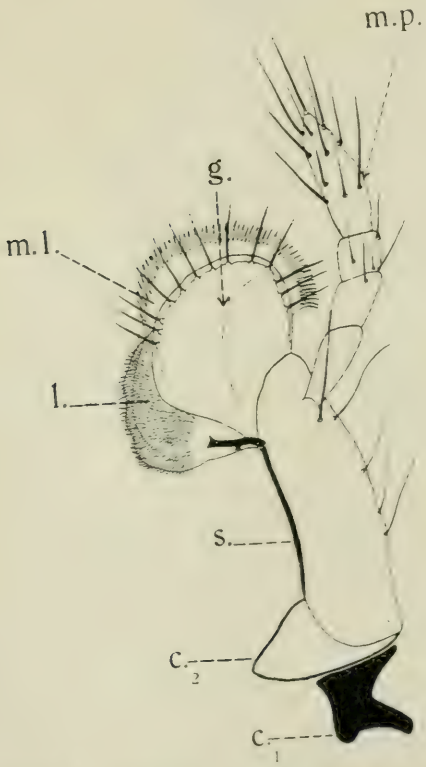


Blastothrix britannica, Gir., female. Labrum seen from above. *l.* labrum. *e.* Epipharynx. × 250.

The first maxillæ (Fig. 5) are somewhat complex in structure, and each measures ·30 mm. in length. The cardo appears to be represented morphologically by two separate elements—a proximal and a distal. The proximal sclerite ($c._1$) is densely chitinised, almost black in colour, and somewhat boot-like in form, with the toe of the boot directed outwards. The distal sclerite ($c._2$) is triangular, with its apex directed inwards and in contact with that of its fellow of the opposite side. What may be regarded as the base of the triangle articulates with the stipes, and does not exhibit any special chitinisation; the two sides, on the other hand, are markedly thickened. The stipes (*s.*) is the largest element in the maxilla, and attains a length of ·17 mm., with an average breadth of ·05 mm. Its inner side is strengthened by a chitinous rod, which articulates at its distal extremity

with the postero-lateral angle of the mentum; its proximal extremity is in contact with the cardo (c_2). The maxillary palpi are well developed and measure ·17 mm. long; each palp is four-jointed, with the joints related to one another in

TEXT-FIG. 5.

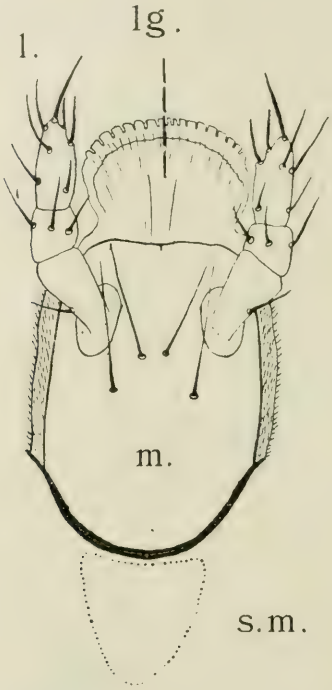


Blastothrix britannica, Gir., female. First maxilla seen dorsally. c_1, c_2. Cardo. *g.* Galea. *l.* Lacinia. *m.l.* Membranous lobe of lacinia. *m.p.* Maxillary palp. *s.* Stipes. × 250.

the approximate ratio of 3 : 2 : 2 : 5. At its apical extremity the stipes carries two much-flattened lamellate lobes, which, however, are closely adpressed and incompletely separated from each other. The outer and ventral lobe is the stouter of the two, and very possibly is to be regarded as being the

galea (*g.*); its free margin and outer surface are provided with stout elongate setæ. The inner lobe or lacinia (*l.*) is membranous and finely hairy. Attached thereto is an extremely thin and transparent membrane with a deeply

TEXT-FIG. 6.



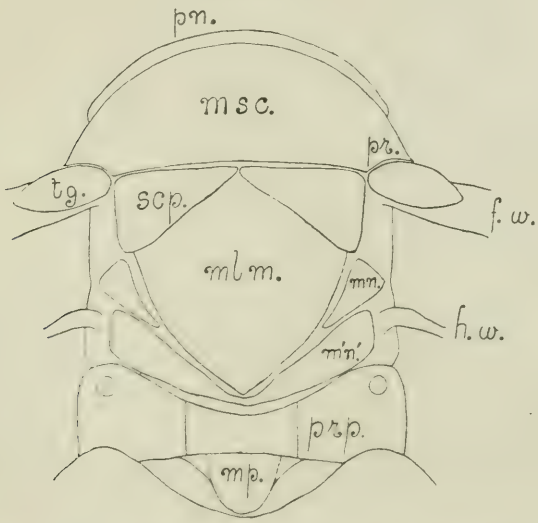
Blastothrix britannica, Gir., female. Labium (second maxillæ) seen ventrally. *l.* Labial palp (right). *lg.* Ligula. *m.* Mentum. *s.m.* Sub-mentum. × 270.

pectinate margin (*m.l.*); on account of its remarkable delicacy and transparency this structure is liable to be overlooked.

The labium (second maxillæ) is embraced, as it were, by the first maxillæ and very largely maintained in position by means of its attachments to the latter. The median unpaired portion of the labium (Fig. 6) measures ·16 mm. long

and ·076 mm. in maximum breadth; it is divisible into a semi-transparent distal element or ligula (*lg.*), and a more firmly chitinised basal element or mentum (*m.*). The posterior border of the mentum is strengthened by means of an edge or band of dark chitin; the extremities of this band articulate with the chitinous inner margin of the first maxilla of either side. On the pharyngeal aspect, its sides are

TEXT-FIG. 7.



Blastothrix britannica, Gir., female. Thorax viewed from above (diagrammatic). *f.w.* Base of fore wing. *h.w.* Base of hind wing. *mlm.* Mesoscutellum. *mn.*, *m'n'.* Metanotum. *mp.* Mesophragma. *msc.* Mesoscutum. *pn.* Pronotum. *pr.* Parapsides. *prp.* Propodeum. *tg.* Tegula.

supported by firm pads densely clothed with fine hairs. Situated behind the posterior border of the mentum is a small triangular membranous area, which is probably to be interpreted as being a vestigal sub-méntum (*s.m.*). The base of the ligula is slightly overlapped on its ventral aspect by the anterior margin of the mentum. The free distal edge of the ligula is crenated, and its inner or pharyngeal surface is markedly convex and beset with 6–8 regularly arranged rows

of chitinous papillæ. Within the mouth-cavity, and situated at the base of the ligula on either side, is a chitinous pad or sclerite bearing a large number of slender bristles; this structure appears to subserve some function in relation with swallowing the food. The labial palpi (*l.*) measure ·087 mm. in length, and are three-jointed, the joints being related to one another in the proportion of 7 : 3 : 5.

The Thorax (Fig. 7).—In dealing with the thorax I have adhered to the same nomenclature of the parts as was used in my previous paper in this series (Imms, 1916, p. 227). The whole of the surface of the cuticle of the mesothorax bears a uniform, finely reticulated pattern; on the tegulæ the cellular pattern assumes a more elongate form than elsewhere.

The pronotum (*pn.* in Fig. 7) is of the usual narrow, band-like character; both it and the mesonotum are invested dorsally with numerous short, uniform, adpressed hairs. The mesoscutum (*msc.*) and its parapsides (*pr.*) form an extensive continuous area, sutures separating the former from the latter being wanting. The tegulæ (*tg.*) are very large and conspicuous. The mesoscutellum (*mlm.*) is extensive, longer than the mesoscutum, and separated from the latter by the well-developed scapulæ (*scp.*). The mesophragma (*mp.*) is less developed than in Aphelinus, but is clearly visible dorsally as a mammiliform tubercle fitting into the deeply formed sinus of the first segment of the abdomen. The metanotum is represented by the triangular sclerites (*mn.*), one on either side of the mesoscutellum, and by a narrow, band-like portion, unpaired and crescentic in form (*m′n′.*). The propodeum (*prp.*) is likewise band-like in character, constricted in the median line, but notably expanded on either side; it bears the usual pair of large circular spiracles.

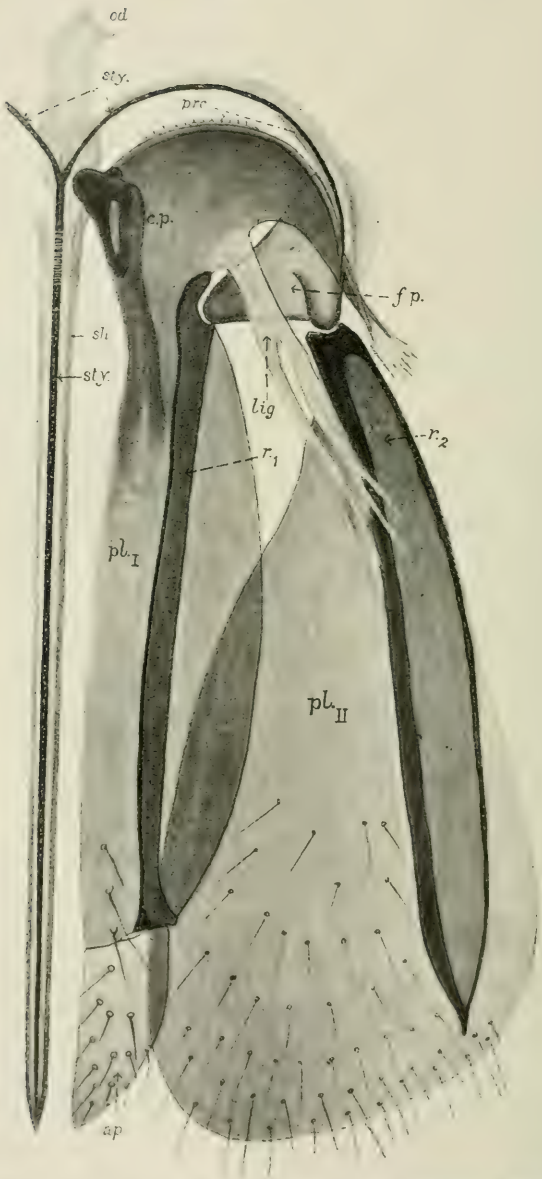
The fore-wings (Fig. 1) are finely pilose except for a small hairless tract extending obliquely backwards from a point near the stigmal vein. This area is clearly the homologue of the well-defined hairless belt, which is a

characteristic feature of the wings of Aphelinus. In the present species it is imperfect and does not, as a rule, extend to the posterior margin of the wing. In this respect, however, it is somewhat variable, certain individuals having the hairless tract extending right across the wing, while in others the area is more limited in extent. At its point of origin, near the root of the stigmal vein, the proximal edge of the clear tract is demarcated by 4–6 setæ rather larger and more conspicuous than those found elsewhere on the wing membranes; opposite these, on the distal margin of the same area, is a row of six peg-like processes. The sub-marginal, marginal, post-marginal, and stigmal (or radial) veins are related to one another in length in the proportion of 38 : 4 : 8 : 7. A well-marked clear area or "break" is present at the junction of the sub-marginal and marginal veins. The stigmal vein is slightly bifurcate at its apex and bears four rounded annuli or "cells"; these latter objects are frequent among Chalcids, and are possibly sensory organs fulfilling some obscure function. Marginal hairs commence at a point close to the base of the wing and extend around the latter until they reach the chitinous edge or rim on the hind margin, where they appear.

In the hind-wings, the single vein is confluent with the anterior margin for the first half of its course after which it becomes sub-marginal in position; its apex is somewhat expanded and bears three hook-like setæ which fit into the chitinous rim (already referred to on the fore-wing), thereby retaining the two wings together when the insect is in flight.

In the three pairs of legs the ratio of the length to the maximum breadth of the femora is as follows: 4 : 1, 6 : 1, 4 : 1; and of the tibiæ, 4 : 1, 25 : 3, 8 : 1. The tarsal joints measured along the dorsal edge (excluding the onychia and claws) are related to one another in length in the proportion of 12 : 8 : 7 : 7 : 12, 24 : 11 : 7 : 7 : 13, 19 : 11 : 9 : 7 : 14. The tibial comb of the fore-legs is composed of six slender bristles; the apical spur is long and slender, measuring

TEXT-FIG. 8.



Ovipositor and sheath of B. britannica, together with their associated parts of the left side, seen from the ventral (external)

·13 mm. long, unequally bifid, hairy on its ventral aspect and curved inwards. In the middle legs the tibial comb consists of six or seven short, thick, peg-like teeth; the spur measures ·13 mm. long and ·02 mm. in thickness across the middle region, and is closely invested with fine hairs. Short teeth, similar to those of the tibial comb, are present on the plantar surface of the first four tarsal joints. The tibial comb of the third pair of legs consists of about twenty slender bristles and the spur measures ·07 to ·09 mm. in length.

The Abdomen.—The abdomen is broad and slightly flattened, and consists of seven visible tergites (Fig. 1). The anterior margin of the first tergite (morphologically the second tergite) is very deeply incised, forming a prominent sinus receiving the mesophragma; the form and disposition of the remaining tergites are shown sufficiently clearly in Text-fig. 1 to render further description unnecessary. On the ventral aspect five sternities are evident, and what appears to be a divided sixth sternite is represented by a pair of plates on either side of the outer plates of the ovipositor. These plates are the "Ecailles chitineuses" of Bugnion (1890, p. 514 and Pl. 25, fig. 52), and are apparently wanting in Aphelinus (Imms, p. 231).

The ovipositor (Fig. 8), in the retracted condition, does not project beyond the apex of the abdomen. It is composed of the same chitinous parts which occur in Aphelinus, but, owing to their greater size and stouter nature, they are more easily studied than in the latter genus. The stylets (*sty.*) measure ·40 mm. in length, and each has an average diameter of ·007 mm., becoming narrower and obliquely pointed at its apex. The sheath (*sh.*) is as nearly as possible of the same length as the stylets which it protects.

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surface. The stylets have been separated anteriorly from the corresponding portions of the sheath. *ap.* Palp-like appendage of inner plate. *f.p.* Fulcral plate. *lig.* Ligament. *od.* Common oviduct. pl_1, pl_2. Inner and outer plates. *prc.* Splint-like process of fulcral plate. r_1 and r_2. Median ribs of outer and inner plates. *sh.* Sheath of ovipositor. *sty.* Stylets. × 360.

It consists of a pair of elongate pieces completely fused together to form a median groove. The edges of the groove are thicker than the remainder of the sheath and appear as a pair of sharply defined refractive lines. Basally, the paired nature of the sheath becomes evident, as its component parts are no longer fused, but take the form of a pair of diverging arms which curve upwards in a manner similar to the corresponding parts of the stylets. At its apex the sheath is pointed, and armed with three minute teeth on each side. The sheath and its curved arms function as guides to the stylets of the ovipositor, which are closely associated with them. On either side of the sheath are two conspicuous lamellæ—the outer and inner plates. The inner plates ($pl._1$) are elongate structures, ·43 mm. in length with an average breadth of about ·04 mm. Anteriorly, each inner plate expands in the vertical plane, and has a crescentic margin of such a shape that it closely fits into a groove in the arm of the ovipositor sheath. Unless carefully disarticulated after maceration in weak potash, the arm of the ovipositor sheath is so closely attached to the margin of the inner plate that it appears as if it were merely the thickened rim of the latter (vide Fig. 8). At its apex, each plate bears a moveably articulated palp-like appendage (*ap.*) possibly tactile in its function. Towards its base the inner plate presents a powerful chitinised process (*c.p.*), which is closely related to its counterpart of the opposite side, though not in actual contact with it. The main function of this process seems to be to help to maintain the sheath of the ovipositor in position. Passing down the inner plate is a prominent median rib ($r._1$) of chitin; this structure imparts rigidity, and, at the same time, affords a firm basis for articulation with the fulcral plate. The outer plates ($pl._2$) are considerably wider and shorter than the inner plates, which they dorsally overlap. They measure ·35 mm × ·19 mm., and are similarly strengthened by a median rib ($r._2$) in each case. The plates are held in position by means of stout ligaments, each arising from the anterior portion of the inner plate of

its side (*lig.* in Fig. 8). In addition to these elements, a triangular fulcral plate (termed the supporting plate in Aphelinus) is present on each side. This structure has its two posterior "angles" rounded so as to form condylar surfaces which articulate with the outer and inner plates of its side. Its remaining "angle" is directed forwards and prolonged into a narrow splint-like process (*prc.*), which is closely attached to the corresponding arm of the stylet. The outer and inner plates of the ovipositor function largely as levers, which work upon the fulcral plate and govern the movements of the stylets and their sheath. A backwardly directed pull exerted by the muscles attached to the inner plate would have the effect of protruding the ovipositor sheath for the purpose of perforating the tissues of the host. By means of a forwardly directed pull, exerted by the muscles inserted on the outer plates of the ovipositor, these lamellæ are brought forwards and downwards. At the same time, this has the effect of tilting the outer angle of the fulcral plate, in such a manner, that the stylets are produced from their sheath, and carried downwards, until they assume a vertical position at right angles to the long axis of the body.

Comparison of the Structure of the Ovipositor with the Sting of the Honey Bee.

If we closely examine the sting of the honey bee, or consult the excellent accounts of the mechanism of the organ given by Cheshire, Packard, Kraeplin, and other writers, it will be observed that its morphology closely agrees with that of the ovipositor of Blastothrix and Aphelinus. We can, without any hesitation, readily homologise the various elements which together make up the completed organ in the two cases. Furthermore, the method of action of the sting, as interpreted by Cheshire, shows a close agreement with what I believe to be the correct physiological interpretation of the function of the various elements which compose the ovipositor in Blastothrix. In the honey bee the so-called palpi or

sting-feelers are represented by the palp-like appendages in Blastothrix, and probably most other Chalcids. The inner plates, which basally support these organs, are the homologues of the oblong plates in the honey bee, and similarly the outer plates are homologous with the quadrate plates in the latter insect. The elements which I have termed the fulcral plates in Blastothrix perform a similar function to the angular plates of the bee, and the resemblance in form in the two cases is also very close. It is, furthermore, noteworthy that the extremity of the arm of each stylet in both the bee and the Chalcid is closely attached to a process of the angular or fulcral plate of its side. Similarly the sheath or darts are intimately connected with the oblong or inner plate. The general morphology and relations of the sheath and the stylets also agree in their essential features in the two cases.

Size.—Individuals pertaining to the first generation exhibit a comparatively slight range of variation in size. Those of the second generation, however, vary between wide limits. They are, as a general rule, markedly larger than examples belonging to the preceding generation, although in some cases extremely small specimens have been bred out. These, without exception, were obtained from heavily parasitised hosts. The first generation of this Chalcid issues from the larval Coccids when the latter are small, and consequently afford only a restricted amount of nourishment. The imagines of the second generation, on the other hand, have been nourished upon adult hosts, which, unless very heavily parasitised, afford the larval Chalcids very ample supplies of food. It is noteworthy that I have never bred out small examples of the second generation from scantily parasitised hosts. In the light of these observations there is reason to believe that the ruling factor determining size may possibly be the amount of food supply available. In this connection I might recall my previous observations upon Aphelinus mytilaspidis, a Chalcid which similarly passes through two annual generations. On p. 255 of my previous paper I men-

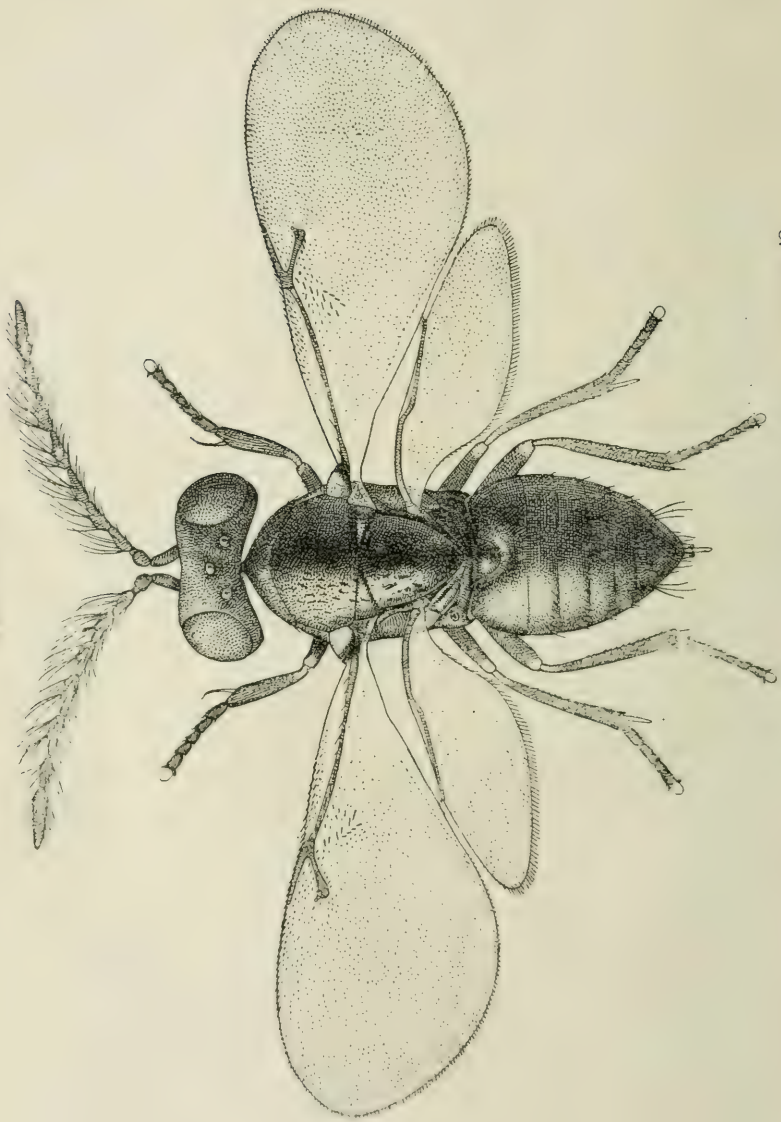
tioned that the first generation of Aphelinus spends some eight months in the larval stage, and subsists upon the ample food supply afforded by the full-grown adult hosts. In the second generation, however, only three weeks to one month is spent in the larval condition, and each larva has for its sustenance a single immature host averaging 1 mm. long. In Aphelinus, therefore, the largest individuals belong to the first generation, while in Blastothrix britannica and, I may add, in Aphycus melanostomatus also, the largest examples are found in the second generation. These three instances all support the conclusion that food is an important factor determining size. There may be germinal factors also, which are capable of transmission by heredity, but this aspect of the subject is beyond the purport of the present investigation.

Measurements:

	First generation.	Second generation.	
	mm.	mm.	mm.
Length to apex of abdomen	1·84	1·55	2·68
Length of head and thorax	1·04	0·96	1·48
Length of abdomen	0·8	0·59	1·2
Breadth of thorax	0·64	0·56	0·8
Length of fore-wing	1·68	1·44	1·92
Breadth of fore-wing	0·8	0·72	1·04
Length of hind-wing	1·12	0·96	1·44
Breadth of hind-wing	0·33	0·36	0·6
Expanse of fore-wings from tip to tip	4·0	3·44	4·64

(*d*) The Male.

Observations conducted during 1915 and 1916 showed that the sexes may occur in nearly equal proportions. Thus, out of 44 examples of the second generation bred during 1915 from hosts obtained from Northenden (Cheshire), males and females were reared in equal numbers. In 1916, out of 99 examples of the first generation, bred from material obtained in the same locality, 44 were females and 46 were males. Out of 606 examples of both sexes, obtained from



TEXT-FIG. 9.

Blastothrix britannica, Gir., male. × circa 48.

various localities during 1915 and 1916, 348 were females and 258 were males.

The following are the principal sexual differences in this species: (1) The males are, as a rule, markedly smaller than the females, although occasionally females are met with which are smaller than exceptionally large males. (2) The antennæ of the males are much paler coloured, and the metallic blue-green reflections are much less evident in the male on the dorsal aspect of the abdomen and on the whole of the ventral surface of the body. (3) The abdomen is smaller and more slender, and it further differs from that of the female in the

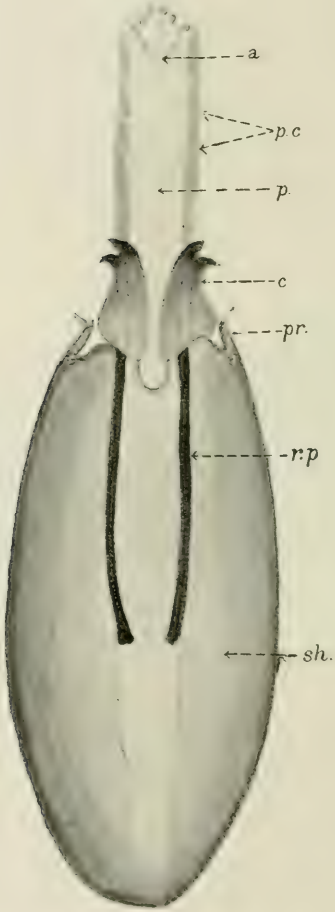
TEXT-FIG. 10.

Blastothrix britannica, Gir., male. Right antenna, viewed laterally.

form of the last three segments. (4) The antennæ of the male (Figs. 9 and 10) are composed of 10 joints related to one another in length in the proportions of 2 : 12 : 4 : 6–7 : 8 : 10 : 10 : 10 : 8 : 13–14. The joints between the pedicel and club are deeply incised, and each bears on its dorsal side long, erect, sensory (?) hairs. (5) The external genitalia (Fig. 11). They consist of (*a*) a cylindrical tubular penis (*p.*) tapering at the apex, and measuring ·22–·26 mm. in length and ·026–·030 mm. in maximum breadth. Basally it is continuous with its sheath (*sh.*), into which it is capable of being retracted very much after the fashion of the finger of a glove. Basally the side walls of the penis are strengthened by a pair of chitinous rods (*r.p.*). That these elements are

actually parts of the penis and not of its sheath is evident from the fact that their position within the latter alters in

TEXT-FIG. 11.

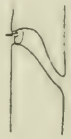


Male genital armature of B. britannica seen ventrally. *c.* Clasper (left). *p.* Penis. *p.c.* Pore canals. *pr.* Lateral process. *r.p.* Chitinous rod. *sh.* Sheath of penis. × 340.

accordance with the amount of protrusion or retraction exhibited by the penis. At the apex of the latter organ are

five pairs of minute genital papillæ, which are most probably tactile in function. The tubular investment of the penis is perforated distally by three pairs of pore canals (*p.c.*) having an average width of ·0027 mm. Each is closed externally by means of an exceedingly minute spinose papilla (Fig. 12), possibly also of a sensory nature. Arising from the hinder border of the penis sheath, and ventral to the penis itself, is a pair of short claspers (*c.*). These elements are bidentate and freely moveable in the horizontal plane. They measure from ·035 to ·048 mm. long with an average breadth of ·016 mm. Situated externally to the base of each clasper is a small lateral process (*pr.*) which is surmounted by an obliquely and outwardly directed seta. In close association

TEXT-FIG. 12.



Lateral pore canal of the penis of B. britannica, highly magnified.

with each process is a minute ventral papilla homologous with a similar structure found in Aphelinus.

The following measurements are based upon an examination of half a dozen examples selected from each generation; those concerning the wings include the marginal fringe of hairs:

	First generation.	Second generation (two examples).	
	mm.	mm.	mm.
Length to apex of abdomen	1·55	1·04	2·24
Length of head and thorax	1·04	0·6	1·28
Length of abdomen	0·51	0·44	0·96
Breadth of thorax	0·56	0·35	0·60
Length of fore-wing	1·52	1·04	1·72
Breadth of fore-wing	0·72	0·51	0·86
Length of hind-wing	0·92	0·64	1·24
Breadth of hind-wing	0·35	0·24	0·48
Expanse of fore-wings from tip to tip	3·56	2·39	4·04

(*e*) Oviposition.

Oviposition was observed in the second generation of this parasite at various dates during the end of August and beginning of September, 1916. The observations were conducted

TEXT-FIG. 13.

An egg of Blastothrix britannica, Gir., seen in situ beneath the cuticle of its larval host. *m*. Lateral margin of host. *o*. Ovum seen beneath the cuticle of the host. *p*. Pedicel of ovum protruding through the cuticle to the exterior. ×250.

both under laboratory conditions and out in the field. In the laboratory, captured females were transferred to glass cylinders, in which were placed freshly-cut twigs of hawthorn, bearing young Lecanium larvæ on the leaves. The cylinders were closed at either end by means of fine bolting-silk, and

the process of oviposition noted under a binocular microscope. The Chalcid first surveys the surface of a Lecanium larva by means of the antennæ, and when the ovipositor is brought into position it is inserted into the tissues of the host, only for a relatively short distance. The egg is then placed obliquely into the perforation, with its long axis lying just beneath the body-wall and about four-fifths of the pedicel protruding freely to the exterior (Fig. 13). Parasitised hosts at this stage measured, on an average, ·8 mm. × ·5 mm., and are consequently very small. They may be easily recognised by the protruding pedicel just referred to; when viewed by transmitted light the egg of the Blastothrix can be recognised beneath the body-wall of the host. The actual position of the egg is tolerably constant, and was specially noted in thirty instances. In all cases the dorsal body-wall of the host was perforated, and in twenty-two examples the egg was placed near the anterior end of the Coccid, close to the margin of the body (Fig. 13). In the remaining cases, it was placed either near the posterior end of the host or between the two extremities of the latter. As a general rule, only one egg is deposited in each host, and, as will be referred to on a later page, the Coccid only affords sufficient nutriment to admit of the development of a single parasite. It was only rarely that two eggs were found in the same individual Lecanium, and it is probable that these were laid by two separate females. On still rarer occasions three eggs were present in a single host. What the essential factors are which determine the selection of any particular Lecanium larva by this Chalcid I am unable to say. Parasitised hosts appeared to exhibit at this stage no appreciable differences either in size, form, or colour from those which were unattacked.

Females pertaining to the first generation of the Blastothrix frequently lay in a similar manner three to nine or more eggs in a single host. This is accountable from the fact that the Lecanium is, at that period (May), nearly fully grown, and consequently is able to support numerous parasites.

The significance of this remarkable method of oviposition, which results in the greater part of the pedicel of the egg protruding from the host, is discussed on a subsequent page. Only two other species of Chalcidoidea appear to have been recorded as exhibiting a similar method of egg-laying. Howard and Fiske (1911, p. 180) mention that the long-stalked eggs of Schedius kuvanæ, How., have their pedicels protruding through the chorion of the eggs of the gipsy moth (Liparis dispar), which serve as the host for this species. Furthermore, Timberlake (1913, p. 297) states that Microterys flavus, How., lays its eggs in Coccus hesperidum, leaving the stalk-like portion projecting through the integument into the outside air.

(*f*) The Egg.

If examined under a binocular microscope, the eggs of this species may be observed in situ beneath the body-wall of the living host. Owing to the fact that their pedicels protrude to the exterior through the body-wall of the scale-insects, it is a difficult matter to dissect out uninjured eggs. When about one day old[1] the egg is elongate-oval with a smooth, glistening surface, and measures, on an average, ·18 × ·08 mm. (Fig. 13). At its posterior extremity it is prolonged into a pedicel ·05–·06 mm. long. This structure soon shrivels at the apex, and its cavity becomes in communication with the outside air—an important fact which is related to the larval respiratory process.

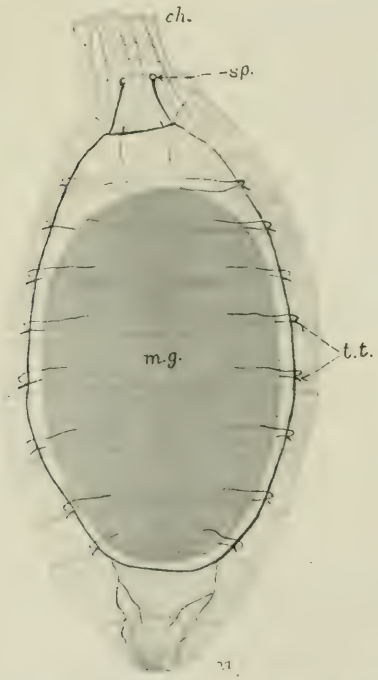
The chorion of the egg admits of a certain amount of extension, and eggs, which contain larvæ nearing the time of emergence, are larger than those freshly laid. In the latter cases the chorion becomes extremely thin, and invests the young larva so closely, that it is often a matter of some difficulty to ascertain whether one is dealing with a newly-emerged larva, or one still enclosed within the egg-shell.

[1] The eggs were dissected out from the body of the host.

(*g*) The Young Larva.

The smallest larva met with measured ·25 mm. long, and differs from the fully-grown larva in its more elongate form, and the rudimentary condition of the tracheal system. It is, moreover, attached by means of the persistent chorion of the

TEXT-FIG. 14.



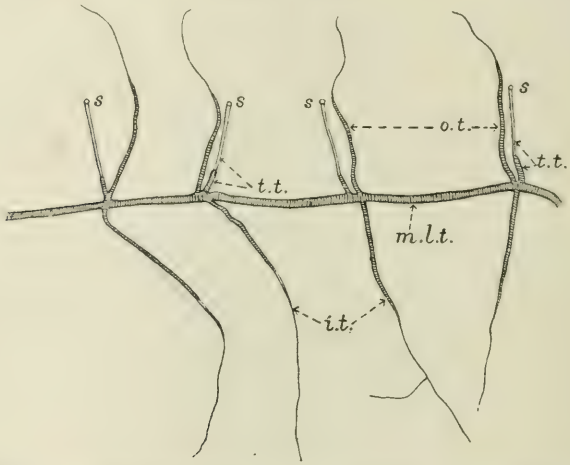
A young larva of Blastothrix britannica attached by its caudal extremity to the chorion of the egg; February 15th, 1916. *ch.* Chorion. *hd.* Head. *m.g.* Mid-gut. *sp.* Posterior spiracles. *t.t.* Developing transverse tracheal branches to the spiracles. × 110.

egg to the body-wall of its host. Thirteen segments can be readily recognised, and the tracheal system consists of two thread-like longitudinal trunks united by an anterior and posterior commissure; lateral tracheæ are, as yet, undeveloped.

Larvæ, after the first ecdysis (Fig. 14), measure about

·35 mm. and more in length, and are still attached to the host's body-wall. Their most striking difference from newly-hatched larva is seen in the tracheal system, developing lateral tracheal branches and stigmatic branches being evident (Figs. 14 and 15). A single pair of open spiracles (the ninth) are present. The mandibles are very similar in form to those of the fully-grown larva, but are correspondingly smaller in size. The fat-body is very little developed, and

TEXT-FIG. 15.



Portion of the tracheal system of a living larva of B. britannica, ·44 mm. long, showing the first four pairs of segmental tracheæ of the right side; March 14th, 1916. *i.t.* Inner segmental tracheal branches. *m.l.t.* Main lateral tracheal trunk of the right side. *o.t.* Outer (dorsal) segmental tracheal branches. *s.* Developing spiracles. *t.t.* Developing transverse tracheal branches to the spiracles. × 280.

the larva is consequently more transparent than it is at a later stage.

(*h*) The Fully-grown Larva.

Coloration.—Uniformly pearly-white to greyish-white with the food contents of the mesenteron showing through the body-wall as an ovoid lemon yellow mass. The cuticle is colourless, smooth, and shining.

Size.—Measurements were made upon twenty-eight larvæ in the last stage of development, preceding the prepupal condition. The smallest larvæ of the first generation, shortly after moulting, attains an average length of ·8 mm.; fully-fed larvæ vary from 1·04 to 1·25 mm., with a maximum breadth of ·64 mm. Larvæ of the second generation are, as a rule, noticeably larger, sometimes reaching a length of 2·8 mm.; the smallest larvæ of this generation measured ·96 mm.

General Morphology.—In shape the larva does not materially differ from the preceding stage. It may, however, be easily separated from the young larvæ firstly, from the fact that it is no longer associated with the body-wall, but lies

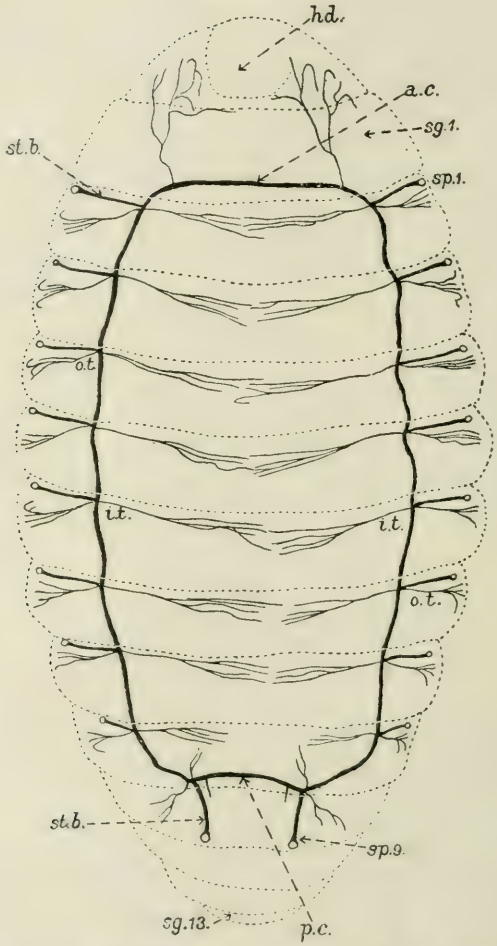
TEXT-FIG. 16.

Mandibles of a larva of B. britannica, ·8 mm. long; March 14th, 1916. Ventral aspect. × 670.

in the body-cavity of its host; secondly, the principal tracheal trunks are of wider calibre, the lateral tracheæ are much more extensively branched, and nine pairs of open spiracles are present.

The larva is divisible into a reduced head region, followed by thirteen trunk segments. The first segment is the largest, the succeeding nine segments are smaller and sub-equal in size, while the remaining segments are much contracted and difficult to make out individually in the living insect. The mandibles (Fig. 16) closely agree in shape with those of younger larvæ; they are, however, rather more strongly chitinised, and measure about ·05 × ·03 mm. The tracheal system (Fig. 17) consists of a pair of main lateral longitudinal trunks, united in the first and ninth segments by

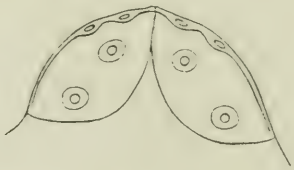
TEXT-FIG. 17.



Tracheal system of a fully-grown larva of B. britannica; July 6th, 1916. *a.c.* Anterior commissure. *hd.* Head. *i.t.* Inner segmental trachea. *o.t.* Outer ditto. *p.c.* Posterior commissure. *sg.* 1. First trunk segment. *s.g.* 13. Thirteenth ditto. *s.p.* 1. First spiracle (right). *sp.* 9. Ninth ditto. *st.b.* Spiracular or stigmatic branch.

means of anterior and posterior commissures respectively (*a.c.* and *p.c.*). From the anterior commissure a pair of tracheal branches takes its origin; these tracheæ bifurcate in the first segment, and ultimately subdivide into a series of smaller branches, which are mainly distributed in the head. Nine pairs of spiracles are present, and are situated on the second to tenth segments inclusive. Those of the ninth pair are somewhat larger than the preceding spiracles; they are, moreover, dorsal rather than lateral in position, and open into rather longer stigmatic branches (*st.b.*). In each spiracle-bearing segment a pair of lateral tracheæ are given off on either side. One branch (*i.t.*) arises from the main lateral

TEXT-FIG. 18.



Oral lobes and papillæ of a larva of B. britannica in the peripneustic stage; April 4th, 1916. × circa 500.

trunk of its side opposite the point of junction with the segmental stigmatic branch; this branch is directed inwards, and its tracheoles are mainly ventral in distribution. The other branch (*o.b.*) takes its origin either from the base of the stigmatic branch of its side or at the point of union of that branch with the main lateral trunk. It breaks up into tracheoles, which are mainly dorsal in their distribution.

The fully-grown larva of this species may be separated from that of Aphycus melanostomatus by the following among other characters: (1) Its more elongate form; (2) the lemon yellow colour of the contents of the mid-gut; (3) the mandibles being more curved at their apices; (4) the hind-most segments of the body are contracted, and not easily separable individually; (5) the larger size of the ninth pair of spiracles and its more dorsal situation on its segment.

(*i*) The Pupa.

Coloration.—When newly formed the pupa is glistening white, but the various parts assume a smoky-grey colour very early in pupal life. This suffusion involves the dorsal surface first, apparently owing to its being nearest the light. Later, the grey coloration extends to the ventral surface also. The intersegmental, and other feebly chitinised areas, remain white until near the close of the instar. The eyes are dull brown, darkening gradually until they become almost black. About eight days prior to the emergence of the adult Chalcid, the pupa becomes almost entirely black with the exception of greenish reflections on the head; the wing sheaths, however, are whitish, with basal and apical suffusions (Fig. 19).

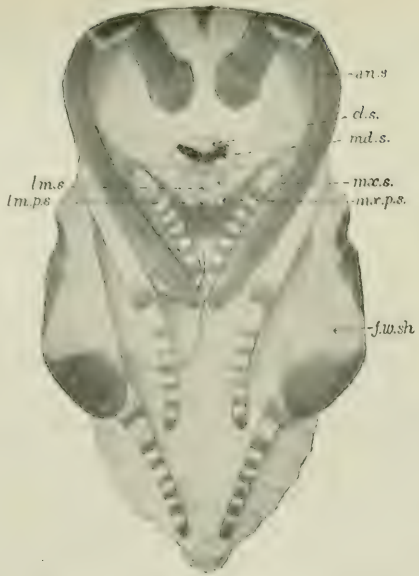
Size.—Pupæ of the first generation measure from 1·5 to 1·7 mm. in length and ·7 to ·9 mm. in breadth across the wing sheaths. Those of the second generation are often distinctly larger and more variable in size, male pupæ measuring from 1·6 to 2 mm. in length and from ·7 to 1·1 mm. in breadth across the wing cases. Female pupæ measure from 1·7 to 2·5 mm. long and ·9 to 1·3 mm. in breadth.

Morphology.—The sex of an individual pupa can be readily determined by the fact that in the male the antennal sheaths are longer and somewhat more slender than those of the female (Figs. 19 and 20). The genitalia also offer a second though less obvious criterion. Seen from the dorsal aspect (Fig. 21) the head, thorax, and abdomen are mutually related in length in the approximate proportion of 2 : 9 : 9. The antennal sheaths (*an.s.*) are partially visible laterally in the female; in the male they project forwards in front of the eyes and curve round the sides of the head, thus exhibiting a greater portion of their length to view. The thorax is divided into three conspicuous areas—the pronotum, mesoscutum, and mesoscutellum. The sheaths of the fore-wings (*f.w.sh.*) are visible laterally to as far back as the commencement of the second abdominal segment. In the abdomen seven segments are clearly distinguishable. On the

TEXT-FIG. 19.

Female pupa of B. britannica, ventral aspect. Reference lettering as in Text-fig. 21. × 55.

TEXT-FIG. 20.



Male pupa of the same species, ventral aspect. *an.s.* Antennal sheath. *cl.s.* Sheath of clypeus and labrum. *f.w.sh.* Sheath of fore-wing. *lm.s.* Labial sheath. *lm.p.s.* Sheath of labial palp. *md.s.* Sheath of mandible. *mx.s.* Maxillary sheath. *mx.p.s.* Sheath of maxillary palp. × 55.

TEXT-FIG. 21.

Female pupa of the same species, dorsal aspect. *an.s.* Right antennal sheath. *f.w.sh.* Sheath of the left fore-wing. × 55.

ventral aspect (Figs. 19 and 20) the following features are most evident. The antennal sheaths (*an.s.*), as is usual among Chalcids, are markedly elbowed. Their basal or scape portions are inclined upwards and outwards, and their points of insertion on the head are separated by an interval equal in width to the scape at its broadest point. The flagellum is inclined backwards and inwards; in the female it is swollen terminally and does not reach the apices of the sheaths of the first pair of legs. In the male the antennal sheaths are longer and more slender than in the female, and, moreover, they extend backwards to the apices of the first pair of legs. The mandibular sheaths (*md.s.*) are situated a short distance behind the bases of those of the antennæ. That of the clypeus (*cl.s.*) is sub-triangular and partially overlies the mandibular sheaths. Two maxillary sheaths are present on either side. One, a broad common sheath (*mx.s.*), encloses the body of the maxilla of its side. The other (*mx.p.s.*) encloses the maxillary palp; it has a broad flattened apex and is external in position. The labial sheath (*im.s.*) is quadrangular and lies between the bases of the maxillary sheaths (*mx.s.*). At its apex is a pair of short broad sheaths (*lm.p.s.*), enclosing the labial palpi.

(*j*) Life-history.

Methods of Investigation.—For the purposes of this research three methods were resorted to: (1) Material consisting of shoots and young branches of hawthorn, bearing the host Lecanium, was collected from numerous localities, and any parasites present were bred out. The hawthorn shoots were cut up into convenient lengths and the cut ends sealed up by dipping into molten paraffin wax. They were then placed in the same type of breeding-cage as was utilised in my previous study of Aphelinus (Imms, 1916, p. 248, text-fig. 2). The parasite breeding-cages were placed in the open insectary which has already been described and figured (loc. cit., p. 247, text-fig. 1). By this means the parasites were reared as far as possible under out-of-door conditions. (2)

Over 600 examples of the host Coccid were collected at frequent intervals from various localities in the field and dissected under a binocular microscope in the laboratory. By this means a tolerably complete series of stages in the life-history of the Blastothrix was obtained. (3) Nine young, well-grown bushes of hawthorn (Cratægus oxycantha) were planted in the University of Manchester Biological Experiment Ground at Fallowfield. These were thoroughly searched for the presence of any examples of the Lecanium, but were found to be quite free from that Coccid. A wooden framework was erected over these plants and ordinary netting, of small mesh, similar to that used to protect fruit bushes from the attacks of birds, was fastened over the sides and roof; entry to the interior of the cage was gained through a door situated in the middle of one side. By means of this cage tits and other birds which prey upon Coccidæ were excluded. The hawthorn bushes were subsequently experimentally infected with Lecanium and its parasites.

The First Generation.—The first generation of adult Blastothrix is derived from larvæ which have passed the winter within the bodies of the larval Lecania. When the time arrives for the emergence of the parasites, the hosts are small, measuring on an average from 1·5 to 2 mm. in length and from ·9 to 1·25 mm. broad. They are invariably dead, and all that remains of the former insect consists of the dried integument together with its dorsal scaly covering. When about to emerge, the Blastothrix cuts a neat hole by means of its mandibles through the scales of its defunct host. The aperture is median and dorsal in position (Fig. 22), though usually situated nearer to the posterior than the anterior extremity of the scale, and may be readily seen with the unaided eye. When the parasite issues through the emergence hole its wings and other appendages are fully formed, and, after devoting a short interval to preening itself, the insect flies away to seek its impressions of the outer world. The earliest observed date of emergence was May 3rd, and the insects continued appearing in the breeding-cages until

June 2nd. As a general rule, males appear at first in greater abundance than the females, the latter subsequently increasing in numbers. Sexual reproduction was found to invariably occur, no instance of parthenogenesis being observed. The eggs are laid during the latter half of May and the beginning of June, and the general details of oviposition have already been described (p. 320). At this period the hosts have assumed their globular berry-like form and are fully grown. The young parasitic larva upon hatching out from the egg remains in close association with the ruptured chorion of the

TEXT-FIG. 22.

A young larva of Lecanium capreæ, showing the emergence hole made by Blastothrix britannica (first generation). × circa 12.

latter, its posterior extremity lying at the base of the pedicel. By this means it is enabled to freely breathe the atmospheric air through the open apex of the pedicel, and the significance of its metapneustic tracheal system therefore becomes evident. As regards feeding habits, the larva is at first largely hæmophagous, feeding freely upon the blood plasma of its host. From a study of the serial sections, made through the larva in situ within the Coccid, it is evident that it subsequently devours as much of the surrounding fat-body as lies within its reach. Two ecdyses have been observed, and the process of casting the skin is an extremely gradual one. During

growth the original cuticle becomes more and more stretched until it ruptures near the anterior extremity of the body, The old skin is gradually sloughed off backwards, until its shrivelled remains are to be found around the tail end of the larva. In almost all cases, the cast-off mandibles may be found after a careful search among the remains of the old cuticle.

The ecdysis having been passed through, the larva is still attached to the chorion of the egg, and, although the tracheal tubes are now of larger calibre, and exhibit more extensive ramification of the tracheoles, only a single pair of metapneustic spiracles are present. Subsequently the larva loses its attachment to the chorion and comes to lie free in the body-cavity of its host. In feeding habits it is steatophagous. devouring the fat-body of the Coccid. At this stage it varies very greatly in size, some larvæ measuring ·9 mm. long, while others may exceed a length of 2 mm. The latter are usually about to undergo a further ecdysis, which takes place in a similar manner to that undergone previously. The remains of this later moult are to be detected, along with the cast-off covering of the jaws, in close contact with the previous skin at the hinder end of the body. It appears, therefore, that the old larval skins are not completely cast off. In this connection it is noteworthy that Newport (1855, p. 73, pl. viii, fig. 16) detected three successive cast skins at the posterior end of the body in the larva of the Ichneumon Paniscus virgatus, Fourc. After this latter moult, the larva of the Blastothrix becomes peripneustic (Fig. 17), with nine pairs of spiracles. This latter condition is a further adaptation to changed circumstances. The host at this stage contains a good deal of free air among its tissues, and probably air is liberated by the laceration of the smaller tracheal branches by the mandibles of the parasite during its search for food. When the fat-body is entirely devoured, the larva becomes sarcophagous, and commences to devour the various internal organs, ultimately bringing about the death of its host.

The number of parasites harboured by any individual

example of Lecanium capreæ is subject to a wide range of variation. Hosts containing a single parasitic larva are frequent; but, on the other hand, large numbers of the latter may often be present within a single Coccid. The greatest number of the Blastothrix larvæ found in any one individual host was forty-two, all of which were fully fed, and some had already assumed the prepupal condition.

Not infrequently the older larvæ in the body-cavity of the host are enclosed in a kind of enveloping sheath or envelope. In other instances, the larvæ are entirely free, no such investment being present. From an examination of numerous serial sections, it is evident that the sheath is composed entirely of host tissue. When stained with hæmatoxylin and eosin it is markedly eosinophilous, and is seen to be composed very largely of dead, ill-defined tissue devoid of definite cell-boundaries, and with nuclei only very occasionally evident. Tracheæ are frequent, and fine branches pass from neighbouring tracheal vessels and terminate in the walls of the sheath. Scattered through the substance of the latter, are numerous conidia of a fungoid nature, similar in every way to those which are constantly present in the fat-body and other tissues of the host. The pupæ are not infrequently found enclosed in a membranous cocoon-like structure which appears to be nothing more than the dried remains of the sheath within the dead host.

When fully fed, the larva discharges the contents of the alimentary canal, which are usually observable as small ovoid brown pellets, clustered around the posterior region of the pupa; no excretory matter was voided earlier in life, and, in fact, its presence would scarcely fail to act deleteriously on its host, and possibly involve both the death of the latter and its parasite. The time spent as a larva varies from about three to four and a half weeks. The earliest pupæ were met with on June 23rd, and they may be found within the hosts up to about the middle of August. The pupal stage lasts from twelve to twenty-three days, the length of time spent in this stage depending upon current climatic conditions.

Parasitised hosts cannot be recognised by any invariable symptoms; when only a single parasite or a very small number are present, within any individual host, it is often impossible to detect their presence unless the latter is carefully dissected. On the other hand, certain unmistakable indications of parasitism are frequently evident. The hosts very often lose some of their rounded berry-like form, and have a less regular and somewhat humped appearance. This is due to the presence of parasites near the surface of the body-wall, which causes the latter to bulge externally. The Lecanium (Coccus) gibberum of Dalman is only a parasitised Lecanium capreæ, characterised by the presence of two large mammiform swellings on the sub-dorsal region. The swellings are very irregular in size, some being scarcely visible, while others are very pronounced (Newstead, 1893, p. 108). Frequently parasitised hosts are much paler in colour than unaffected examples, and markedly translucent, with the integument harder and more brittle. This change in appearance is due to the excavation of the underlying tissues by the larval parasites, thus rendering the integument more transparent than it would otherwise be.

It is a remarkable fact that the first generation of this parasite exercises very little effect upon the fecundity of its host, death of the latter seldom occurring until after it has deposited its ova. Some 225 female hosts, of as nearly as possible uniform size, were collected at Northenden (Cheshire), a few days before the larval Lecania commenced to issue from the egg. The eggs were carefully removed from beneath each scale, and dropped into a fine glass tube graduated into millimetres and filled with alcohol. As soon as all the ova had settled down their quantity was judged by the graduated scale. This method gave approximately accurate results, but it involved a great deal of time transferring the eggs to the tube by means of a camel-hair brush. Subsequent dissection of the hosts proved that 132 were parasitised; of these no less than 94 examples (71·9 per cent.) laid what was estimated to be approximately the normal quantity of eggs;

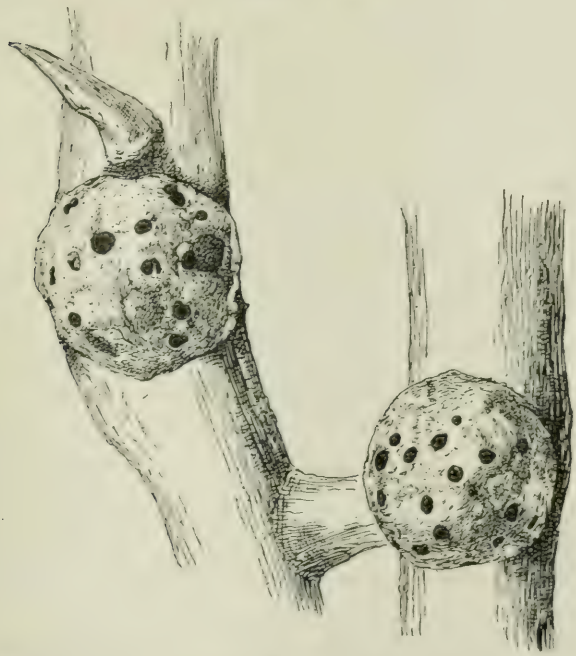
29 (21·9 per cent.) individuals laid no eggs at all, and 9 (6·8 per cent.) individuals laid less than the normal quantity of eggs. From among the ninty-three unparasitised hosts, 47 (50·5 per cent.) laid about the normal quota of eggs; 39 examples (41·9 per cent.) laid no eggs at all, and 7 examples (7·5 per cent.) laid less than the usual quantity of eggs. It is further noteworthy that 15 out of the 94 parasitised hosts, which laid the normal quantity of eggs, supported no less than 18 to 42 parasites apiece. It is remarkable that in spite of being so heavily parasitised oviposition was carried out without interruption. A number of the eggs were kept under observation and hatched into larvæ in the usual time. The fact that twenty-nine parasitised hosts deposited no eggs at all, affords no certain evidence that the presence of the Blastothrix larva exercises an inhibiting effect upon oviposition. This conclusion is supported by evidence derived from an examination of unparasitised hosts, 42 per cent. of the latter failing to lay any eggs. It is difficult to account for these facts except upon the supposition that they were unfertilised individuals, as the male Lecanium capreæ is much scarcer than the female.

In the vast majority of instances the Lecanium is attacked by the first generation of the parasite so late in life that little or no interference with egg production occurs. The Blastothrix larvæ only assumes the sarcophagous habit at a time when their hosts have laid the greater number of their ova.

The Second Generation.—On June 30th, 1914, while examining some parasitised Lecania I observed movement beneath the integument of one of the Coccids. It proved to be due to an example of the Blastothrix which was endeavouring to effect its entry to the exterior. In a short time it was seen to perforate the covering of the scale insect by means of its mandibles (at 11.10 a.m.). By cutting a successive series of sausage-shaped pieces from the body-wall it succeeded in making a round and clean emergence hole through which it issued just thirty minutes later (Fig. 23). It immediately crawled away, making no attempt to fly. The

earliest date of emergence was June 23rd, and the last male appeared on July 30th; females continued to emerge until August 25th. At first males appear in greater abundance than the females; out of 481 bred males, 67 (13·9 per cent.) emerged between June 23rd and 30th; 240 (49·8 per cent.)

TEXT-FIG. 23.



Two fully-grown Scale insects (Lecanium capreæ) with numerous emergence holes made by the parasitic Chalcid dealt with in this paper. The two larger holes in the upper specimen were made by Blastothrix britannica; the remaining smaller holes are due to Aphycus melanostomatus. × 8.

between July 1st and 7th; 108 (22·4 per cent.) between July 8th and 14th; 50 (10·3 per cent.) between July 15th and 21st; 16 (3·3 per cent.) between July 22nd and 30th. From among 435 bred females, 21 (4·8 per cent.) emerged between June 23rd and 30th; 92 (21·1 per cent.) between July 1st and 7th; 104 (24·1 per cent.) between July 8th and 14th; 156

(35·8 per cent.) between July 15th and 21st; 53 (12·1 per cent.) between July 22nd and 31st; 9 (2 per cent.) between August 1st and 25th.

An important factor in the life-history of the two generations of the parasite is the size of the host. The first generation lays its eggs in the fully-grown host, which may support upwards of fifty individual parasites. When the second generation deposits its eggs the host is in the condition of a very young larva, attached to the underside of the leaves of its food-plant, and never finally supports more than a single parasite. If more than one egg is laid therein, only one of the parasitic larvæ ever comes to maturity. Oviposition takes place over a lengthy period, during a prevalence of warm and sunny weather. It was observed at the end of July, and females have been noticed laying their eggs as late as the first week in September. Unhatched eggs have been found in the host up to November 7th. The young larvæ upon emerging remain associated with the chorion of the egg, respiring through the pedicel of the latter throughout the winter months. Its habits and metamorphosis are very similar to those already described with reference to the first generation. Towards the end of the winter the larvæ attain an average length of ·7 to 1·2 mm., lose this attachment, and come to lie free in the body space of their hosts. The earliest larva in this condition was observed on February 15th, 1916, but it was not until the middle of March that free larvæ became numerous. When fully fed the larva voids the contents of the alimentary canal in the form of light brown pellets, which are discharged in such a manner as to form a chain on either side of the body, and are clearly visible through the integument of the host (Fig. 24). It is about this time that the parasite turns completely round inside the host. Hitherto, in the majority of cases, the head of the larva was directed towards the anterior extremity of the host, but its position now becomes reversed—a curious fact which has also been noted by Miss Embleton (1904, p. 243) in larva of Comys infelix which parasitises

Lecanium hemisphæricum. Pupæ were first observed in 1915 on April 19th, and in 1916 on May 1st, and commence to occur abundantly about the middle of the latter month. The pupa fills the greater part of the body space of its host (Fig. 24), and lies with its dorsal surface upwards and its head end directed toward the posterior extremity of the Coccid; occasionally, however, pupæ are found with the head pointing in the opposite direction. The period spent in the pupal stage varies approximately from four to seven weeks, but it is very dependent upon prevailing climatic

TEXT-FIG. 24.

Enlarged figure of a young larva of Lecanium capreæ containing a pupa of Blastothrix britannica. The anterior extremity of the latter is directly toward the anal end of the host. The ovoid opaque bodies are excrementa ejected by the larval parasite prior to pupation.

conditions and may be prolonged or curtailed accordingly. Examples which pupate early in May were observed to spend a much longer period in that condition than larvæ which delayed pupation until June.

The effects of the second generation of parasitisim upon the host always lead to the death of the latter. When first parasitised the larval Coccids betray no symptoms of being affected and continue to grow normally. Apart from actual dissection, the presence of the parasite at this stage can only be detected by searching for the pedicel of the egg, which protrudes through the body-wall. After the winter the parasites have grown con-

siderably, and the older they become the clearer do their hosts exhibit symptoms of parasitism. By the month of April affected hosts are, as a rule, smaller than unattacked examples, and may usually be readily recognised by their uniform yellowish-brown or brown colour. The presence of the parasite causes the Coccid to become swollen and somewhat distended, and, by examining the host from the ventral surface, the parasitic larva can be frequently detected through the thin membranous body-wall. Occasionally the hosts are distorted in shape, or exhibit a mammiform bulging, owing to the pressure exerted by the parasite while it was still attached to the chorion and lying close beneath the body-wall.

The most important biological feature in the life-history of this Chalcid is the presence of a metapneustic tracheal system in the young larva, which is intimately correlated with direct respiration of the atmosphere through the medium of the pedicel of the egg. This species affords the first described case of the occurrence of a metapneustic larva among the Hymenoptera. It, furthermore, presents an interesting example of convergence towards a condition which is very general among parasitic larvæ belonging to the Diptera Cyclorrhapha. Keilin (1915) has discussed at length the essential differences between the endoparasitic larvæ of the Diptera and Hymenoptera. In an extremely able essay this observer takes into consideration both biological and morphological characters, and emphasises the fact that the primary larvæ of parasitic Diptera are metapneustic, with their spiracles in free communication with the air. He contrasts this condition with that found among Hymenoptera, and points out that Seurat (1899) has shown that the young parasitic larvæ of that order are apneustic. The cuticle investing the general surface of the body is extremely thin, and it is by means of cutaneous respiration that those larvæ derive their oxygen from that dissolved in the blood-tissue of their hosts. It is evident, therefore, that the life-history of Blastothrix presents an exception to this generalisation, and that other metapneustic hymenopterous parasites remain to be dis-

covered in the future. The method by which the larva of Blastothrix gains communication with the free air is, however, totally different to that which obtains among the "larves entomobies" of those Diptera studied by Pantel and others. Among the latter insects their larvæ gain access to the air either by perforating the skin of the host, or by boring through the wall of a tracheal trunk, and acquiring an intimate secondary connection therewith.

Observations on Aphycus melanostomatus, Timb.

(a) Systematic Position.

Aphycus melanostomatus is a member of the Chalcid sub-family Encyrtinæ, which is included within the extensive family of the Encyrtidæ. The Encyrtidæ may be readily recognised by the stout saltatorial spur to the middle tibiæ and the large non-impressed mesopleura. The sub-family Encyrtinæ is distinguished according to Ashmead (1904, pp. 286–87) by the following characters: The mesonotum is entire, convex or sub-convex, and the parapsidal furrows are totally absent. The marginal vein is rarely very long, often punctiform, and always much shorter than the sub-marginal or sub-costal vein; the stigmal vein is usually short, rarely long. The scutellum is never short or transversely linear, and the middle tibiæ are devoid of lateral spines.

The genus Aphycus was erected by Mayr in 1875 (p. 695), who also pointed out the essential differences between the males and females. The characteristics of the females as enumerated by Howard (1881, p. 364) are as follows: The antennæ are short, two-jointed, and inserted close to the mouth; the scape is broadened or cylindrical, with the pedicel just about twice as long as thick; the joints which follow the pedicel gradually increase in thickness, and, moreover, are thicker than long; the club is about equal in length to the three preceding joints, obliquely rounded, and often flattened. The face, vertex, and dorsum of the thorax are devoid of lustre, finely punctured, and are frequently clothed with

yellowish hair. The marginal vein is undeveloped, and the stigmal vein is given off at the junction of the sub-costal with the costa. A more detailed diagnosis of the genus will be found in the recent memoir by Timberlake (1916, p. 587).

The males may be distinguished by their more slender antennæ, with the scape less widely dilated below, the flagellum more pubescent, and the club devoid of any traces of jointing.

According to Schmiedenknecht (1909, p. 240), thirty-four species of Aphycus were known at the time of his generic monograph. Richardson (1916) brings the total up to forty-three, but of these four or five species are somewhat doubtfully included within the genus.

(*b*) Habits and Distribution of the Genus Aphycus.

The vast assemblage of Chalcids which are included in the family Encyrtidæ live as parasites of the ova, larvæ, or pupæ of other insects, and hardly a single order of that group of animals is immune from their attacks. As Ashmead remarks (1900, p. 323): "In this family, and more especially in the sub-family Encyrtinæ, the species are of more than ordinary interest and importance, since so many of them are found attacking and destroying the scale- and bark-lice (Coccidæ and Aleyrodidæ) and the plant-lice (Aphididæ and Psyllidæ), containing some of the most destructive and troublesome pests with which fruit-growers, agriculturists, and florists have to contend."

The various species of Aphycus are parasites of Coccidæ (scale insects), and chiefly attack members of the genus Lecanium. Several species also parasitise Pulvinaria, Ceroplastes, and Coccus; other forms have been occasionally bred out from species of Saissetia, Physokermes, Lichtensia, Eriococcus, Filippia, and Tourmeyella.

The genus Tachardia has also been recorded as a host, but this statement should be received with reservation. It is, furthermore, noteworthy that Aphycus annulipes

(Ashm.) has been recorded by Ashmead as a parasite of the coleopterous insect Attelabus bipustulatus. I am informed, however, by Dr. L. O. Howard that this record is a very doubtful one, and it is impossible now to ascertain the conditions under which the late W. H. Ashmead reared the species. Aphycus chrysopæ, Ashm., which was bred out from an undetermined species of the neuropterous genus Chrysopa, is placed by Schmiedenknecht (1909, p. 236) in the allied genus Isodromus. There are consequently no undoubted records of a species of Aphycus utilising any insects other than Coccidæ for their hosts. No detailed study of the biology of any species of the genus has so far been published, but it is evident that the latter includes both ecto- and endo-parasites. According to Mercet (1916, p. 776), Aphycus hesperidum, Mer., is an ectophagous parasite, while the species dealt with in the present paper lives within its host. The occurrence of two different modes of larval life among parasites of the same genus is by no means unique, since it is also exhibited, for example, among species of Aphelinus.

Aphycus is a genus whose species are known from many regions of the world, including most countries of Europe, also North and South America, Sandwich Islands, Philippines, South Africa, Australia, New Zealand, Ceylon, China, Japan, and the West Indies. Although the greater number of the known forms have been recorded from North America, this fact is mainly due to the importance attached to the study of parasites in the United States. When the European forms have been collected equally assiduously, it is probable that they will not fall far short in point of numbers.

Only two species, viz. A. hederaceus (Westw.) and A. pappus (Walk.), are listed by Morley (1910, p. 22) as being British insects. A. pappus, however, is retained by Schmiedenknecht (1909, p. 245) in its original genus Encyrtus, to which it was relegated by Walker. This species is also not included by Timberlake in his recent revision of Aphycus and closely allied genera. Walker

(1838, p. 108) records A. hederaceus as being found on grass beneath trees near London, and at Holywood, near Belfast. A. melanostomatus is the only other British species, and has been recently described by Timberlake[1] (1916, p. 608), who mentions (p. 610) that it occurs in Denmark and England. Danish examples were reared by Kryger from a species of Lecanium growing on lime at Ermelunden and on oak at Dyrehaven. The English specimens were reared by Douglas in 1890 from Lecanium fuscum[2] (Gmelin) growing on oak. I know of no further records of this insect. During the years 1914–17 I have bred out this Chalcid from examples of Lecanium capreæ obtained from the following localities: Lancashire: Fallowfield and West Didsbury, near Manchester. Cheshire: Northenden, Ashley, Knutsford, and Lymm. Derbyshire: Edale, Castleton, Whaley Bridge, and Lathkil Dale. In each locality the species occurred plentifully, and did not appear to be at all local in distribution.

(c) The Female.

Coloration.—Black, extensively marked with orange-brown and yellowish-white. The scape of the antenna black, with a narrow line along its upper margin, and a rounded spot on the outer surface at the proximal extremity, yellowish-white; the proximal half of the pedicel, the first three joints of the funiculus and the club black; the distal portion of the pedicel and the remaining joints of the funiculus yellowish-white, the funicular joints usually with a variable amount of smoky suffusion. The head between the eyes orange-yellow, with the occipital region black, which extends laterally until it expands into a conspicuous area at the lower angle of the cheeks on

[1] Timberlake (1916, p. 608) gives the following synonyms for this species:

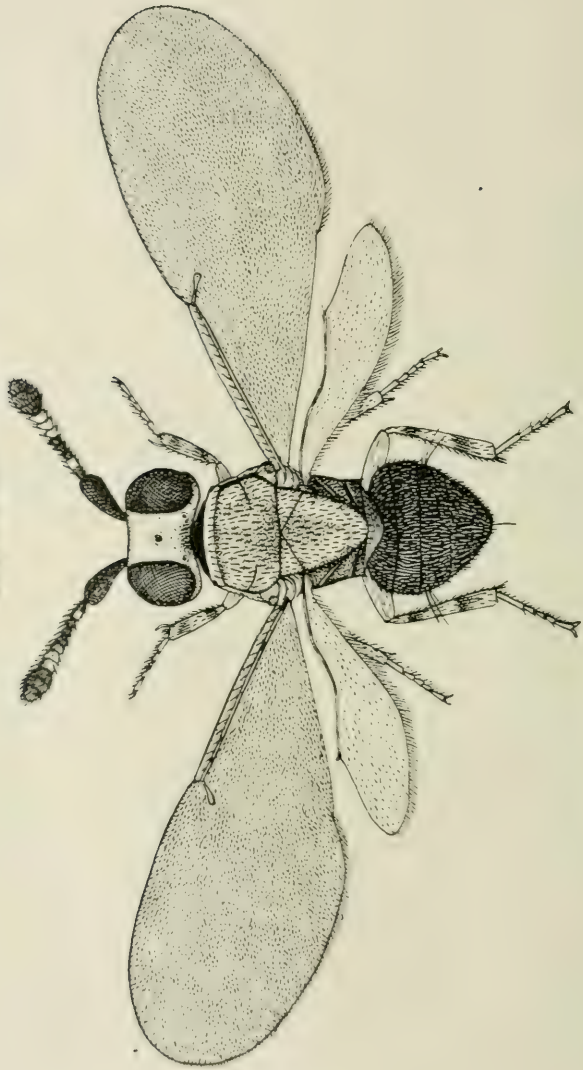
Encyrtus punctipes, Dalman (part), Svensk. 'Vet.-Akad. Handl.,' vol. xli, 1820, p. 371.

Encyrtus punctipes, var. 2, Nees, 'Hym. Ichn. affin. Monogr.,' vol. ii, 1834, p. 202.

[2] A synonym of L. capreæ.

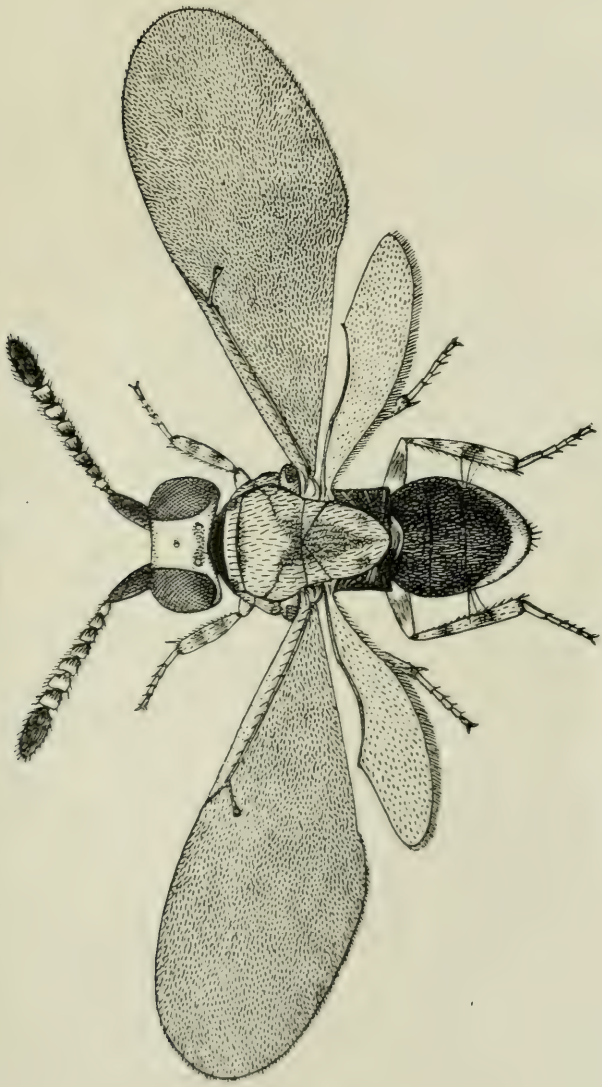
each side; the remainder of the head yellowish-white faintly tinged with pink; the eyes leaden-coloured or primrose when fully pigmented. The concealed portion of the pronotum black, the remainder whitish with a small dark-brown spot near its outer edge on each side. The mesonotum orange-yellow, with the anterior margin deeply suffused with black. The metanotum, propodeum, and the dorsal surface of the abdomen black. The legs yellowish-white or pale straw-coloured, the tibiæ with two to three smoky annulations and the last tarsal joint also smoky. The wings hyaline with the nervures pale yellowish-brown. Ventrally the insect is mostly yellowish-white.

Individuals agreeing with the above description (and also with Timberlake's diagnosis) are to be regarded as being typical for the species. During the present investigations, however, three well-marked, colour variations were frequently met with and have not hitherto been described. These may be most conveniently referred to as follows:—Variety α: In this variety the whole or the greater portion of the bright orange coloration of the mesonotum is replaced by dark brown, and the antennal joints beyond the pedicel are uniformly black. Variety β: This agrees with the preceding variety, with the exception of the antennæ, which exhibit the normal typical coloration. Variety γ: In this variety the normal coloration of the species is retained, with the sole exception that the antennal joints beyond the pedicel are uniformly black as in variety α. Although these varieties are, for the most part, sharply defined, occasional individuals intermediate in colour between the vars. α and β and the type were found, and also examples intermediate between the vars. β and γ. This fact, together with the absence of any structural differences, removes, in my opinion, any valid reason for elevating them to specific rank. It is, furthermore, noteworthy that typical females, together with individuals pertaining to these three varieties, have been repeatedly bred out from hosts obtained from various localities. None of these varieties are confined to any one locality or generation.



TEXT-FIG. 25.

Aphycus melanostomatus, Timb., female. × circa 45.



TEXT-FIG. 26.

Aphycus melanostomatus, Timb., male. × circa 46.

From among 151 examples of this parasite which were specially examined with reference to their coloration, only 44 (or 29 per cent.) were typical in every respect; 30 (or 19 per cent.) belonged to the variety α; 51 (or 33 per cent.) were of the variety β, and 26 (or 17 per cent.) belonged to the variety γ.

The accompanying measurements were taken from an average-sized example selected from each generation:

	First generation.	Second generation.
	mm.	mm.
Length to apex of abdomen . .	1·12	1·51
Length of head and thorax . .	0·68	0·8
Length of abdomen . . .	0·41	0·71
Breadth of thorax	0·42	0·55
Length of fore-wing . . .	1·31	1·56
Breadth of fore-wing . . .	0·56	0·7
Length of hind-wing . . .	0·88	0·96
Breadth of hind-wing . . .	0·25	0·32
Expanse of wings from tip to tip .	3·04	3·6

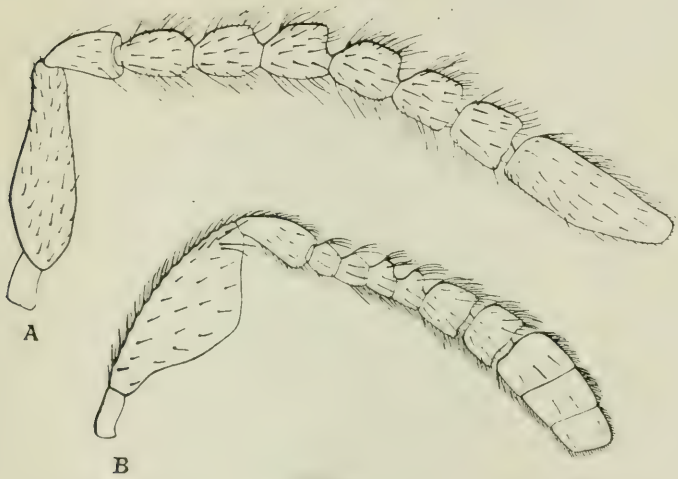
(*d*) The Male.

The males are less numerous than the females: out of 49 examples of the first generation bred from two localities 8 were males and 41 females. From among 549 specimens of the second generation 131 were males and 418 females. The proportions of the sexes differed from different localities as follows: Northenden, 36 males, 238 females; Ashley, 63 males, 94 females; Marple, 20 males, 35 females—the approximate proportion of males to females in this generation being 1 : 3.

The male insect may be readily distinguished from the female by the following characters: The front and vertex of the head are wider than long; in the female these parts are distinctly longer than broad (Figs. 25 and 26). The antennæ (Figs. 27A and 27B) are pale yellow-brown and rather longer than the female. The scape is much narrower than in the latter sex, being about three times as long as broad; the pedicel is a trifle shorter than the first joint of the funiculus; the funicular joints are all sub-equal in length, and

the club is unjointed and longer than in the female. In the latter sex the scape is approximately one-half as wide as long and the pedicel more than twice the length of the first joint of the funiculus; the first four funicular joints are sub-equal in length, but the remaining two are distinctly larger; the club is three-jointed and shorter and more globular than in the male.

TEXT-FIG. 27.



Aphycus melanostomatus, Timb. A. Antenna of male. B. Antenna of female.

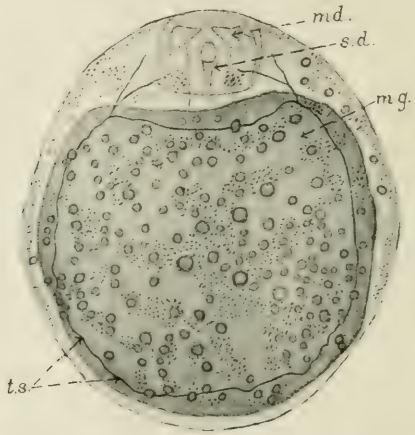
The following measurements were made from average-sized examples, one selected from each generation; those relating to the wings include the marginal fringe of hairs:

	First generation.	Second generation.
	mm.	mm.
Length to apex of abdomen . .	1·04	1·36
Length of head and thorax . .	0·65	0·77
Length of abdomen . . .	0·39	0·59
Breadth of thorax	0·42	0·51
Length of fore-wing . . .	1·23	1·31
Breadth of fore-wing . . .	0·59	0·6
Length of hind-wing . . .	0·81	0·8
Breadth of hind-wing . . .	0·25	0·24
Expanse of wings from tip to tip .	2·88	3·13

(*e*) The Egg.

The eggs of this species lie free in the body-cavity of the hosts. They are rounded oval in form, devoid of a pedicel, and measure ·17 mm. through the long axis. The chorion is smooth and very transparent, clearly revealing the contained larva within (Fig. 28). When near to the time for hatching, the egg undergoes a certain amount of distension and becomes almost spherical.

TEXT-FIG. 28.

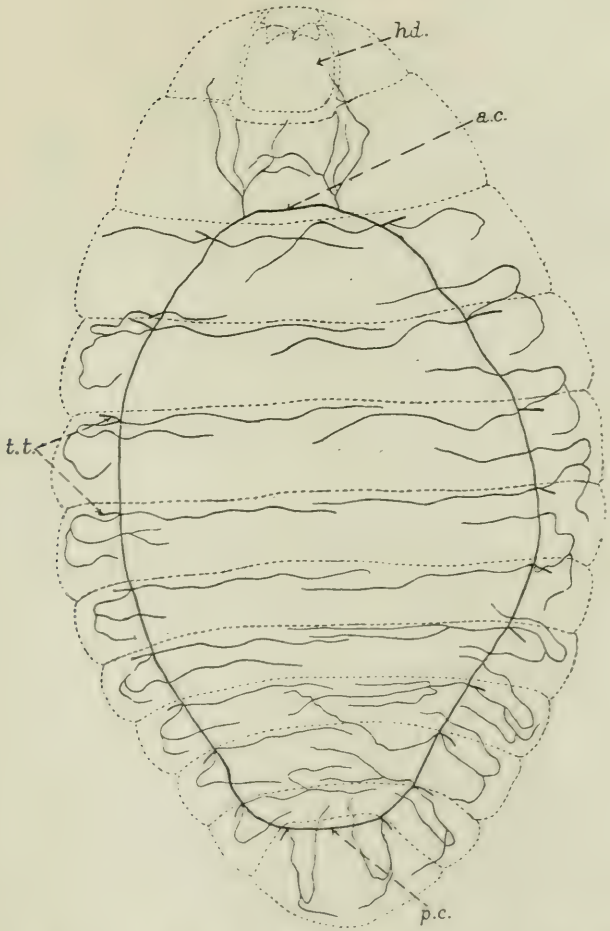


Egg of Aphycus melanostomatus dissected from a young larva of Lecanium capreæ December 21st, 1915. The mandibles (*md.*), median salivary duct (*s.d.*), mid-gut (*m.g.*), and tracheal system (*t.s.*) are clearly visible beneath the chorion. × 200.

(*f*) The Larva.

Stage I.—When newly hatched from the egg the larva is globular in form, and presents a nearly spherical outline when viewed from above. It is, furthermore, slightly flattened in the dorso-ventral plane. On an average it measures ·2 mm. in length and ·19 mm. in a transverse direction across the widest region. In appearance the larva is whitish and divisible into a small head region and thirteen segments. Of these, the anal or terminal segment is very small and papilla-

TEXT-FIG. 29.



Tracheal system of a larva of A. melanostomatus, ·45 mm. long; February 22nd, 1916. The preparation was treated with dilute KOH in order to render the branches more distinct; the reagent had the effect of obscuring the boundaries between the two hindmost segments. *a.c.* Anterior commissure. *hd.* Head. *p.c.* Posterior commissure. *t.t.* Developing transverse tracheal branches to the spiracles. × 160.

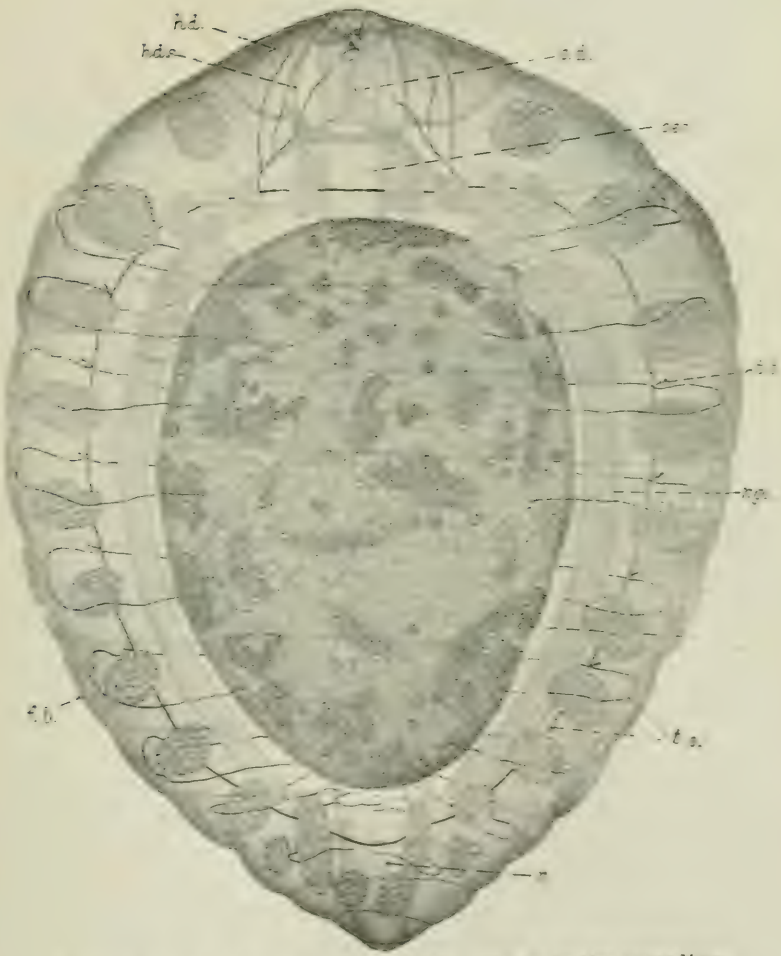
like. The mid-gut forms a conspicuous central area, and is very evident in virtue of its brownish contents. Spiracles are absent, and the tracheal system is extremely simple, and its tubes of very narrow calibre. It consists of a main lateral trunk passing down each side of the body, and the two trunks are united by means of a pair of transverse commissures, one of which being anterior and the other posterior in position. With the exception of a single branch, which passes to the head region on either side, lateral tracheæ are undeveloped. The mandibles measure ·02 mm. from the apex to the outer angle of the base, and are very similar in form, though much less chitinised than those of the older larvæ. The fat-body is represented by scattered globules, and is very little in evidence. In other essential features the newly-hatched larva differs very little from individuals in later stages of development.

Stage II.—(Figs. 29 and 30.) In this stage the larva is more oval in form, pearly-white in colour, and rather denser in appearance than the first stage larva. The mid-gut or stomach is very conspicuous, and its orange-brown contents show clearly through the body-wall. The cuticle is colourless, smooth, and shining.

Size.—The larva varies in length from ·4 mm. up to about ·6 mm., according to the age of the individual. In greatest breadth it measures from ·3 to ·46 mm.

External Morphology.—It is broadly oval in form and somewhat flattened in the vertical direction. It is divisible into a head and thirteen segments, and attains the greatest breadth across the third and fourth segments. The last five segments are narrow and more annular than the preceding ones, while the terminal segment is vestigial and papilla-like. The head is devoid of antennæ, and has a well-defined and endo-skeleton of the usual type met with among Chalcid larvæ. The mandibles are curved and sharply pointed at their apices. They are minute structures, measuring ·038 to ·041 mm. from apex to base, and ·035 across the base at its widest part. They are adapted for piercing the soft tissues

TEXT-FIG. 30.



A larva in the same stage of development as in the preceding figure, showing general anatomy: February 22nd, 1916. *f.l.* Lobe of fat-body. *h.d.* Head. *h.d.s.* Endo-skeleton of head. *m.g.* Mid-gut. *oe.* Œsophagus. *r.* Rectum. *s.gl.* Salivary gland. *s.d.* Median salivary duct. *t.s.* Trachea. *t.t.* Developing transverse tracheal branches to the spiracles. × 160.

of the host and maintaining a hold thereon. Spiracles, at this stage, are absent.

Internal Morphology.—The tracheal system (Fig. 29) differs from that of the first stage larva in that the main longitudinal trunks are of larger calibre and lateral segmental branches are evident, eleven pairs being present. The first pair arises from the anterior commissure (*a.c.*), and subdivides into fine tracheoles distributed to the head and first body-segments. The following nine pairs of tracheæ consist of an outer or dorsal branch and an inner or ventrical branch on each side of the body. In the tenth pair the branches are less completely developed. Ten pairs of transverse stigmatic branches are present, though incompletely formed. As yet they have no open connection with the exterior through the agency of spiracles. Only at their points of origin from the main lateral trunks, and for a short distance outwards, they are rendered evident through the refractivity of the air which they contain (*t.t.* in Figs. 29 and 30). The digestive system is of the very simplest type, and closely resembles that already described in Aphelinus mytilaspidis (Imms, 1915, p. 243). The mouth leads into a short œsophagus, which communicates with a very large sac-like stomach (Fig. 30). The hind-gut is still in the condition of a proctodæal invagination, and there is no communication with the mid-gut. A pair of long tubular salivary glands lie one on each side of the alimentary canal; their ducts converge and fuse in the hinder region of the head to form a median common salivary duct (*s.d.*). The aperture of the latter is situated in the floor of the mouth.

Stage III.—(The fully-grown larva.) When fully grown the larva frequently assumes a pinkish tinge, and, on account of the greater development of the fat-body, is rather more opaque than previously.

Size.—Shortly after ecdysis it measures from ·57 to ·9 mm. in length, and when complete growth is attained prior to pupation the average length is from 1·6 to 1·9 mm., with a maximum breadth of about ·7 mm. (Fig. 31). In one excep-

tional case a larva examined on April 28th, 1915, measured 2·7 mm. long. The host also was exceptionally large, and possibly the size of the larva was due to its having developed from

TEXT-FIG. 31.

Fully-grown larva of A. melanostomatus seen from the left side; from a living specimen; May 6th, 1915. The head, thirteen trunk segments, and nine spiracles are clearly visible. × 40.

a late deposited egg at a time when the host was larger than when oviposition usually takes place. Larvæ of the second generation are noticeably larger than most examples of the

TEXT-FIG. 32.

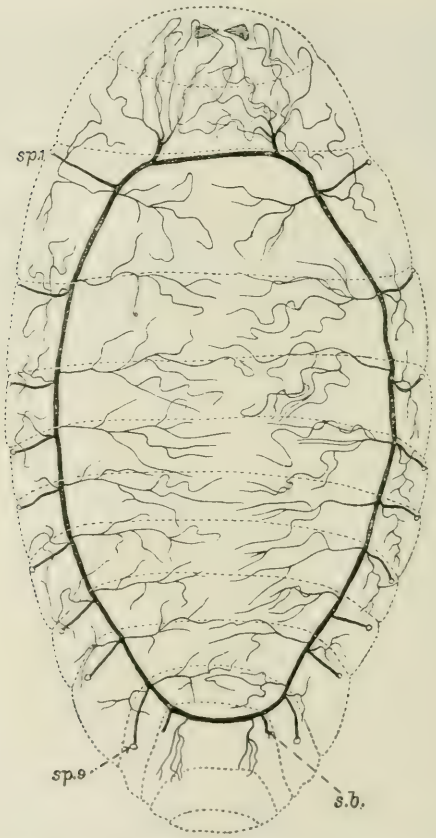
Right and left mandibles of a larva of A. melanostomatus. ·8 mm. long, with peripneustic tracheal system; March 25th, 1916. × 480.

earlier brood, and their length when completely grown varies from 2·1 to 2·5 mm.

General Morphology.—The general structure of the larva offers few features requiring comment beyond those afforded by the tracheal system. The size of the mandibles

varies considerably, but their general form is constant. In a larva measuring ·8 mm. × ·5 mm. belonging to the first generation, the mandibles (Fig. 32) measured ·04 ×

TEXT-FIG. 33.



Tracheal system of the same species in the peripneustic stage; February 8th, 1916. *sp.* 1. First spiracle (left). *sp.* 9. Ninth ditto. *s.b.* Vestigial branch related to a former tenth spiracle (right).

·05 mm.; in an example measuring 1·5 × 6 mm. pertaining to the second generation their size was ·05 × ·03 mm. The tracheal system (Fig. 33) is characterised by the

presence of nine pairs of open spiracles situated on segments 2–10. The vestigial stigmatic branches (*s.b*). of the eleventh segment are still evident, although spiracles are never developed in connection therewith. The retention of this pair of branches is most probably a case of persistence of an ancestral character suggesting that the progenitors of the Chalcididæ possessed ten pairs of larval spiracles. In this connection it is noteworthy that ten pairs of spiracles are present on identical segments in the newly-hatched larva of the honey bee (Nelson, 1915). The remaining features in the tracheal system are (1) the increase in the calibre of the principal tracheal trunks and (2) the greatly increased branching and ramification of the smaller tracheæ.

(*g*) Pupa.

The pupæ of this species very closely resemble those of Blastothrix. They may, nevertheless, be separated therefrom by the fact that the head region is proportionately somewhat longer, the points of insertion of the antennal sheaths are slightly wider apart, and the pronotal region is rather less strongly arched. In length the pupa varies from 1 to 1·25 mm., and has a breadth across the wing-sheaths of from ·5 to ·6 mm.

(*h*) Life-History.

In studying the life-history of this species the methods of investigation adopted were similar to those detailed in the case of Blastothrix parasite. The biology of the two species was followed concurrently, and in their second generation both may be present in one and the same individual host.

The First Generation.—Individuals belonging to this generation are derived from larvæ which have over-wintered within the bodies of the larval Coccids. When the time for emergence arrives, the adult Aphycus cuts a round hole through the dorsal body-wall of the host by means of its

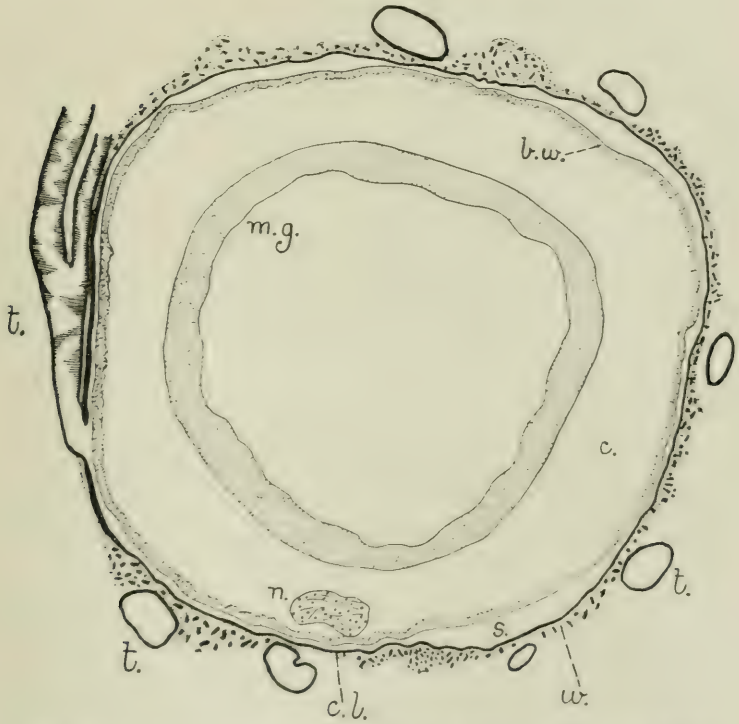
mandibles. The aperture is similar in position to that made by Blastothrix though slightly smaller. The first generation of adults all emerged between May 1st and June 15th. Oviposition was observed at the end of the former month, and the eggs are deposited within the body-cavity of the young adult hosts. The larvæ upon hatching are apneustic, respiration taking place through the skin. In feeding habits they are hæmophagous, subsisting upon the blood-tissue of the Coccid. Later on they devour the fat-body, together with vast numbers of the conidia of a fungoid organism (Blastomycetes?) which always abound in that tissue in Lecanium capreæ. Two ecdyses were observed during the life-history of the larva. After the first moult, the latter still remains in the apneustic condition, open spiracles not being present until after the succeeding moult, which usually occurs early in June. At this stage the larva becomes sarcophagous, and gradually brings about the death of its host. The greatest number of the Aphycus parasite found within an individual host was 48, all of which were in the pupal stage; hosts containing 15–20 parasites were plentiful.

Most of the older larvæ were enclosed in a curious sheath-like investment (Fig. 34), although in some instances no traces of the structure were to be found. The sheath invests the whole of the parasite with the exception of the head, and is intimately connected with branches of the tracheal system of the host. If one examines a series of sections taken through a nearly full-grown larva, along with the sheath, the latter is seen to consist of an inner layer of chitin (*c.l.*) which, for the most part, appears to be colourless and structureless. In places, however, it may assume a brown or yellowish colour where the secretion of chitin has been profuse. External to the chitinoid layer is a stratum composed for the most part of ill-defined and apparently dead tissue. Enclosed in the matrix of the latter are numerous conidia of a fungoid nature which have already been referred to on a previous page. Numerous tracheæ,

and often lobes of the fat-body, adhere to the outer layer of the sheath.

When stained with hæmatoxylin and eosin, the sheath is markedly eosinophilous, and, by means of this reaction,

TEXT-FIG. 34.



Diagrammatic transverse section across a nearly full-grown larva of Aphycus melanostomatus enclosed within its sheath-like investment. *b.w.* Body-wall of larva. *c.* Body-cavity of larva. *c.l.* Chitinous lining of sheath. *m.g.* Wall of larval mid-gut. *n.* Nervous system. *s.* Space between the body-wall of the larva and the sheath. *t.* Trachea. *w.* Outer layer of the sheath (the small dark bodies enclosed therein are the conidia of a fungoid organism).

is readily distinguishable from the parasitic larva within. Notwithstanding an examination of a large number of microtome sections, made through larvæ of various ages,

it has not been possible to arrive at any certain conclusion with regard to the origin of this sheath. From a study of both dissections and serial sections I am of opinion that it arises as a proliferation of the chitin-forming cells of a trachea, most probably as a result of injury or perforation caused by the parasite. It is evident that the secretion of a chitinous membrane takes place, which gradually extends around and almost encloses the parasitic larva. In support of the tracheal origin of the sheath is the remarkable fact that tracheæ can be seen intimately connected therewith, and directly opening into the space between the innermost layer of the sheath and the body-wall of the parasite within (Fig. 34). If a parasite be dissected from the host along with the sheath, these tracheæ have the appearance, in a surface view, of arising from the body of the larva and growing outwards into the tissues of the host. It is only after a study of serial sections that the true relations of these tracheæ can be ascertained.

A search through the extensive literature dealing with the parasitic Hymenoptera has failed to bring to light any previous observations of a kindred nature. It is noteworthy, however, that Miss Embleton (1904, p. 241) has described and figured a somewhat similar condition in the third stage larva of the Chalcid, Comys infelix. She states that a pair of tracheal branches are connected with each of the four functional spiracles of the parasite. The branches ramify and subdivide into five tracheæ distributed among the tissues of the host. The authoress, however, is quite uncertain as to whether the tracheæ take their origin from the respiratory system of the host or its parasite. On the other hand, among certain of the parasitic Diptera the relations between the latter and their hosts bear a very considerable likeness to those just described in the case of the Aphycus larva and Lecanium capreæ. In his remarkable researches on the biology of the Tachinidæ, Pantel (1910) has shown that, in certain species, the larvæ perforate a tracheal trunk of the host, and subsequently

become enclosed in a chitinous membrane derived from the trachea. In this manner the parasite acquires an intimate, though secondary, connection with the respiratory system of its host. Pantel describes his observations as follows (p. 122):

"Le processus de perforation et de fixation, d'après un ensemble concordant d'observations, parait être le suivant. Le parasite se loge tout d'abord dans un lobe adipeux, et le pousse dans un mouvement de recul contre la trachée. Celle-ci s'imprime plus ou moins dans la masse molle et se trouve immobilisée, tandis que, sous l'action des accidents chitineux péristigmatiques, le lobe adipeux d'abord et ensuite la paroi trachéenne finissent par être perforés. Le lobe dégénère et se transforme en une poche membraneuse affaissée sur le parasite; l'épithélium trachéen réagit à la manière de l'épithélium cutané, en développant une gaine de fixation qui s'insinue entre le parasite et la poche adipeuse, et constitue comme une doublure de celle-ci. L'orifice peut être plus étroit que la trachée."

After reading Pantel's memoir one is naturally led to the conclusion that very possibly the enveloping sheath of the Aphycus larva and the tracheæ attached thereto will prove to be subservient to a similar explanation. Further research, however, is necessary before coming to any definite conclusion on the matter.

When fully fed the Aphycus larva discharges the contents of the alimentary canal, in the form of small ovoid pellets, and passes into the pupal stage. The latter occurs within the interior of the host, which at this period is usually dead.

The Second Generation.—The adult insects almost all emerge during the month of July; less than 4 per cent. were reared in June, and only one example appeared in August. Males commence to emerge a little earlier than the females.

In all essential features the general life-history is similar to that described in the case of the first generation. Oviposition takes place within the young larval Coccids, which occur on the undersides of the leaves of their food-plant. The eggs

may be found within the larval Scale insects up to the end of the autumn, and I have never found the newly-hatched larval parasite earlier than October 15th. It seems probable, therefore, that several months elapse between the time of oviposition and that of the first appearance of the larva. Furthermore, larvæ of this generation were never found until after the hosts have deserted the leaves of their food-plant and taken up positions on the smaller branches and twigs of the latter. Throughout the winter months growth is extremely slow, the parasites seldom exceeding a length of ·3 mm. before February. During this month many of the larvæ were found enclosed in the developing sheath and had acquired attachment with the host's tracheal system. On February 8th, the first larvæ (·7 mm. long) with an open peripneustic tracheal system were met with. Contemporaneously with them, in other examples of the host, were larvæ no larger than ·2 mm., with only a rudimentary tracheal system. There is, therefore, considerable overlapping of the larval stages. Early in April, the majority of the parasites have a well-developed tracheal system, with nine pairs of open spiracles. This stage lasts approximately for one month. pupæ not usually being found until the first week in May, The life-cycle is completed by the emergence of the adult insects during the next and succeeding months.

During the prevalence of a warm autumn in 1915 a partial third generation of this species was observed. Eggs laid by the second generation of adults developed rapidly, and the young parasitic larvæ emerged during the earlier part of August while the larval hosts were still feeding upon the leaves of the hawthorn. The parasitised Lecania grew more rapidly in size than those unattacked, as if to accommodate themselves to the growing requirements for food and space demanded by the larval Aphycus. By the end of September the life-history was complete, and five female examples emerged on the 29th of that month. The hosts on that date were quite dead.

VI. Economic Status of the Two Parasites.

The criterion, which has been applied for the purpose of ascertaining the economic value of the two species of parasites dealt with in this paper, consists in estimating the extent by which they reduce the normal rate of increase of their host. Of the two species under consideration, Blastothrix britannica is by far the most abundant, 83 per cent. of the total number reared belonging to that species. It has already been pointed out that the effects of the two generations of each species upon the host are similar. The first generation of both species has, for practicable purposes, a negligible effect. During three years' investigation, I have not come across a single indubitable instance in which either parasite killed its host before the latter had deposited ova. Furthermore, no conclusive evidence was discovered which might indicate any inhibitory action on the part of the parasite in relation to egg production by the host. On the other hand, the effects of the second generation of parasitism is complete, all parasitised hosts succumbing during the following spring. It is noteworthy that both sexes of the Coccid were attacked, and it is reasonable to conclude that for every male destroyed, at least a corresponding number of females remain unfertilised. The fact that the proportion of males to females in Lecanium capreæ varies considerably has been clearly pointed out (p. 315). If we accept an average of one male to twelve females, it indicates that each male must either fertilise a considerable number of females or many of the latter remain unfertilised or are parthenogenetic. Of parthenogenesis in this species we have no evidence, although Mr. E. E. Green informs me that the closely allied L. persicæ appears to depend entirely upon this method of reproduction in this country. On one occasion I was sufficiently fortunate to observe a male Lecanium capreæ copulate with two females in the course of a few minutes. It appears likely, therefore, that in the normal course of events the male fertilises several females. Mr. E. E. Green further informs me that he has watched a newly-

emerged male of Tachardia albizziæ in Ceylon fertilise five females in quick succession. The destruction of the male Lecanium capreæ, therefore, is a fact of economic significance, and, if we assume that for every member of this sex destroyed, only two females remain unfertilised, we obtain a reduction of some 3600[1] ova in each instance.

TABLE I.—Percentage of Parasitism entailed by the Two Species of Chalcids under Consideration, during the Years 1914–1917 in Various Localities.

Locality.	Dates of examination.	Number of hosts examined.	Number of hosts containing parasites.	Percentage of parasitism.
Second Generation of Parasites of 1914.				
West Didsbury (Lancashire)	March 3rd to April 14th, 1915	153	75	49
Northenden (Cheshire)	April 18th to May 4th, 1915	191	51	27
Edale (North Derbyshire)	April 26th to May 23rd, 1915	330	139	42
First Generation of Parasites of 1915.				
Northenden (Cheshire)	June 2nd to June 14th, 1915	131	87	66
Whaley Bridge (North Derbyshire)	June 18th to July 8th, 1915	44	24	54
Edale (North Derbyshire)	June 9th, 1915	65	16	24
Second Generation of Parasites of 1915.				
Northenden (Cheshire)	February 8th to May 18th, 1916	634	233	36
Edale (North Derbyshire)	April 29th, 1916	70	31	44
Knutsford (Cheshire)	May 20th, 1916	173	68	39
First Generation of Parasites of 1916.				
Northenden .	June 1st to July 1st, 1916	470	331	70
Second Generation of Parasites of 1916.				
Northenden .	January 7th, 1917	102	46	45

Average parasitism: First generation of parasites, 53 per cent.; second generation of parasites, 40 per cent.

[1] Allowing 1800 as the average number of eggs laid per female.

For purposes of determining the extent to which parasitism obtains in Lecanium capreæ, the figures given in Table I relate to the combined efforts of the two species of Chalcids, in so far as the female host is concerned. Among male hosts parasitism is less evident; 70 males were examined from three different localities during the year 1915, and 14 (or 20 per cent.) thereof were found to be parasitised. According to observations already referred to, male and female hosts occur on an average in the proportion of one male to about twelve females. Consequently, in every 1000 examples of the Lecanium, we may expect to find about 83 males and 917 females. If reference be made to Table I, it will be seen that the percentage of parasitism by the second generation of the Chalcids varied greatly in different years and also in different localities. An average of 40 per cent. is the nearest estimate it is possible to arrive at from the data available. On this basis, therefore, among every 917 females 366 (or 40 per cent.) will be destroyed by parasites. At the same time it is necessary to take into account the fact that out of every 83 males 16 will succumb to the effects of parasitism. Assuming that each male normally fertilises only two females, the destruction of these 16 males implies that at least 32 females will remain unfertilised, and, from the purely economic standpoint, may be regarded as having been destroyed. Consequently, out of every 1000 examples of the host Coccid, 398 (366+32) are destroyed, or rendered infertile, through the direct or indirect action of parasitism, and thereby entailing a reduction of 716,400 eggs. It is evident, therefore, that the second generation of parasitism is a most important factor in limiting the rate of increase of Lecanium capreæ. All evidence points to the conclusion that the two species of Chalcids under consideration are efficient natural enemies. They fly readily, have considerable powers of migration, and appear to be little affected by other than the most adverse climatic conditions. They occur at all elevations up to over 1000 ft. in North Derbyshire, wherever their host flourishes. Observations conducted during 1914 showed that

the parasites could be readily established if desired under experimental conditions. Some nine hawthorn bushes about 4 ft. high and entirely free from scale insects were planted in the autumn of 1913. On each plant twigs bearing heavily parasitised Lecania, containing abundant ova, were securely tied on to the younger branches. The eggs hatched in due course, and the young larval Coccids established themselves in large numbers on the undersides of the leaves of their food-plant. In the spring of 1914 over 80 per cent. of the scale insects were found to be parasitised, and only a very small number of the remainder succeeded in attaining the adult condition. In the following year no scale insects were discoverable on any of the bushes. The primary cause of their disappearance was parasitism, but as the experiments were conducted within the limits of the city boundaries of Manchester, local conditions were not entirely favourable to the host Coccid, although these same conditions seemingly exercised no apparent ill-effects upon the Chalcid parasites. I may add that the observations were carried out in experimental cages, which precluded the entry of tits and other birds, which might have otherwise greatly reduced the number of scale insects present.

With reference to the influence of parasites upon Lecanium capreæ, it is noteworthy that Newstead remarks (1903, p. 111) that this Coccid is sometimes extremely abundant in sheltered hedges, so much so that he has known it to kill large patches of a hawthorn hedge skirting the borders of the city of Chester. Its ravages were particularly noticeable in the year 1890, when they reached the maximum. Since that time the insect has gradually decreased through the attacks of natural enemies—both of birds and insects, most especially the latter. Carpenter (1914, p. 157) records this Coccid as being present in co. Waterford in such numbers as to constitute a pest. In cases where this species gains a temporary ascendency its increase in numbers is probably always traceable to factors which inhibit the activity of its insect enemies. Miss Embleton (1904, p. 235) has emphasised

the great value of Comys infelix in controlling the closely allied Coccid Lecanium hemisphericum.

It is significant to compare the effects of parasitism upon Lecanium capreæ with those upon the "Mussel Scale" Lepidosaphes ulmi. In the former species we are concerned with a Coccid endowed with a high rate of fecundity, since each female lays, on an average, about 1800 eggs. The combined effects of parasitism, by the two species of Chalcids under consideration, entailed a destruction of about 40 per cent. of their hosts. In the case of the Mussel Scale, each female lays on an average about thirty-seven eggs, while the net result of the activities of its principal parasite (Aphelinus mytilaspidis) brings about a reduction of only 7 per cent. of the eggs of its host. Notwithstanding the fact that Lecanium capreæ has a fecundity more than forty-eight times greater than L. ulmi, I have never succeeded in obtaining more than 196 examples of the former species in the course of a day's collecting in a favourable locality. On the other hand, it is a well-known fact that many thousands of the Mussel Scale may often be observed on a single tree. In the one case we have a species with a high rate of fecundity, but its abundance is limited through the agency of parasites to such a degree that it seldom becomes sufficiently plentiful to be of economic importance. In the second instance we are dealing with a Coccid of very low fecundity, but so little affected by parasites, that it is a universally common pest to the fruit grower.

VII. Summary of Conclusions.

(1) Blastothrix britannica, Gir., and Aphycus melanostomatus, Timb., are two important Chalcid parasites of the Scale Insect Lecanium capreæ.

(2) B. britannica passes through two generations in the year, and both males and females occur in approximately equal numbers. The first generation of adults are derived from hibernated larvæ, and emerge during May and early June. The female lays one or several eggs in the young fully-

grown host, only perforating the body-wall of the latter with her ovipositor and leaving the pedicel of the egg protruding to the exterior. The newly-hatched larva is unique among Hymenoptera in being metapneustic, and its spiracular extremity remains attached to the chorion of the egg. By this means the parasite respires free air through the open apex of the pedicel. Subsequently it loses its attachment, becomes peripneustic with nine pairs of open spiracles, and lies free in the body-cavity of the Coccid. At this stage it frequently becomes enclosed in a phagocytic sheath formed by the host. Pupation takes place within the body of the latter, and occurs towards the end of June; as many as forty-two pupæ were found within a single Lecanium.

The second generation of adults emerge in greatest numbers during the first three weeks of July. The females utilise the very young larval hosts for purposes of oviposition, and lay a single egg within each Coccid selected. The resulting larvæ pass through changes similar to those undergone in the first generation, but remain throughout the winter within the bodies of their hosts, and pupate, as a rule, during the following April. The Chalcids which emerge therefrom constitute the first generation of adults for that year.

(3) A. melanostomatus similarly passes through two annual generations, and the various stages of its life-history occur almost contemporaneously with those of the preceding species. Males, however, are less abundant than females, and occur in the approximate proportion of 1 : 3. The first generation of adults emerges between the beginning of May and the middle of June. The eggs are devoid of a pedicel, and are deposited within the body-cavity of the young adult hosts. The larvæ upon hatching are apneustic, respiration taking place through the skin. They subsequently become peripneustic with nine pairs of open spiracles, and are usually enclosed in a sheath or cyst. Pupation takes place within the host, and from one to forty-eight pupæ were found in a single example of the latter. The second generation of adults emerge about the same time as those of the previous

species, and, similarly to the latter, they utilise the very young larval hosts for purposes of oviposition. The eggs are laid singly, a female never depositing more than one egg in an individual Coccid. The larval parasites over-winter in the apneustic condition, and give rise to the first generation of adults of the following year. A partial third generation of adults has been observed.

(4) The results of the first generation of parasitism upon the host are similar in both species of Chalcids. From the purely economic standpoint they are negligible. An average of about 53 per cent. of the Lecanium are attacked, but the latter do not succumb to the effects thereof until after they have deposited their ova. Furthermore, no conclusive evidence was discovered which might indicate any inhibitory action on the part of the parasitism in relation to egg production by the host. On the other hand, the effects of the second generation of parasitism are complete; about 40 per cent. of the hosts are attacked and destroyed a long period before attaining sexual maturity.

(5) The second generation of parasitism is of great importance in limiting the abundance of the host, which, in consequence, seldom occurs in sufficient numbers to constitute a pest, notwithstanding its high fecundity.

MANCHESTER,
January, 1918.

ERRATA.

The following corrections should be made in my previous paper in this series—"On Aphelinus mytilaspidis, Le Baron, a Chalcid Parasite of the Mussel Scale (Lepidosaphes ulmi)," 'Quart. Journ. Micr. Sci.,' 1916, vol. 61, pp. 217–274, pls. 19 and 20.

P. 242, 17th line from foot of page, for "twelve" read "thirteen."

P. 243, top line, for "first" read "second."
2nd line, for "second" read "third."
15th line, for "first" read "second."

P. 243, 16th line, for "ninth" read "tenth."

P. 264, 6th line from foot of page, for "234" read "254."

Literature.

Ashmead, W. H. (1900).—"On the Genera of Chalcid-flies belonging to the Sub-family Encyrtinæ," 'Proc. U.S. Nat. Mus.,' vol. xxii, pp. 323–412, pl. 12.

Dalla Torre, C. G. De (1898).—'Catalogus Hymenopterorum,' vol. v, "Chalcididæ et Proctotrupidæ," Lipsiæ: Sumptibus G. Engelmann, pp. viii–598.

Embleton, A. L. (1904).—"On the Anatomy and Development of Comys infelix, Embleton, a Hymenopterous Parasite of Lecanium hemisphæricum," 'Trans. Linn. Soc. Zool.,' 2nd Ser., vol. ix, pp. 231–254, pls. 11 and 12.

Gaulle, J. de (1908).—'Catalogue systématique et biologique des Hyménoptères de France,' Paris: Paul Klincksieck, 171 pp. (extrait de la 'Feuille des Jeunes Nat.,' ann. 1906–08).

Howard, L. O. (1899).—"On Some New Parasitic Insects of the Sub-family Encyrtinæ," 'Proc. U.S. Nat. Mus.,' vol. xxi, pp. 231–248.

——— and Fiske, W. F. (1911).—"The Importation into the United States of the Parasites of the Gipsy Moth and the Brown-tail Moth," 'U.S. Dept. Agr., Bur. Entom., Bull.,' xci, 312 pp., 74 text-figs. and 28 pls.

Imms, A. D. (1916).—"Observations on the Insect Parasites of some Coccidæ. I. On Aphelinus mytilaspidis, Le Baron, a Chalcid Parasite of the Mussel Scale (Lepidosaphes ulmi, L.)," 'Quart. Journ. Micr. Sci.,' vol. 61, pp. 217–274, pls. 19 and 20, and 5 text-figs.

Kawall, H. (1855).—"Entomologische Notizen aus Kurland," 'Stett. Entom. Zeit.,' t. 16, pp. 227–232.

Keilin, D. (1915).—"Recherches sur les Larves de Diptères Cyclorhaphes," 'Bull. Sci. Fr. et Belge,' 7e ser., t. xlix, 198 pp., 16 pls. and 27 text-figs.

Marchal, P. (1908).—"Notes sur les Cochenilles de l'Europe et du Nord de l'Afrique," 'Ann. Soc. Ent. Fr.,' t. lxxvii, pp. 223–309, pl. 3 and 46 text-figs.

Mayr, G. (1875).—"Die Europäischen Encyrtiden," 'Verh. Zool.-bot. ges. Wein,' vol. xxv, pp. 675–778.

Mercet, R. G. (1916).—"Aphycus hesperidum, n. sp., an Ectophagous Parasite on the Cochineal of Citrus Fruits Chrysom-

phalus dictyospermi in Spain," 'Mthly. Bull. Agric. Intell. and Pl. Dis.,' Rome, vii, no. 10, p. 1555 (abstract from 'Rev. Real. Acad. de Ciencias exactas fisicas, y naturales de Madrid,' xiv, pp. 776–788, 5 figs.).

Morley, C. (1910).—"On the Hymenopterous Parasites of Coccidæ," 'Entom.,' vol. xliii, pp. 27–31.

Nelson, J. A. (1915).—"The Embryology of the Honey Bee," 'Princetown Univ. Press,' pp. iii + 282 and numerous figs.

Newport, G. (1855).—"The Anatomy and Development of certain Chalcididæ and Ichneumonidæ, compared with their Special Economy and Instincts, with Descriptions of a New Genus and Species of Bee Parasites," 'Trans. Linn. Soc.,' vol. xxi, pp. 61–77, pl. viii, and pp. 85–93, pl. ix.

Newstead, R. (1903).—"Monograph of the Coccidæ of the British Isles." London: 'Ray Society,' vol. ii, pp. viii + 270, with 41 pls. and 7 text-figs.

Pantel, J. (1912).—"Recherches sur les Diptères à larves entomobies. I, Caractères parasitiques aux points de vue biologique, éthologique et histologique," 'La Cellule,' t. xxvi, pp. 27–216, 5 pls. and 26 text-figs.

Ratzeburg, J. T. C. (1844–52).—'Die Ichneumonen der Forstinsecten.' Berlin, Bd. i, 1844; ii, 1848; iii, 1852.

Seurat, L. G. (1899).—"Contribution à l'étude des Hyménoptères Entomophages," 'Ann. Sci. Nat. Zool.,' ser. 8, t. x, pp. 1–159.

Timberlake, P. H. (1913).—"Preliminary Report on the Parasites of Coccus hesperidum in California," 'Journ. Econ. Entom.,' vol. vi, pp. 293–303.

——— (1916).—"Revision of the Parasitic Hymenopterous Insects of the Genus Aphycus, Mayr., with Notice of some Related Genera," 'Proc. U.S. Nat. Mus.,' vol. l, pp. 561–640, pls. 26–31.

Tullgren, A. (1906).—"Om sköldlöss," 'Entom. Tidskr.,' 1906, vol. xxvii, pp. 69–95, 17 text-figs.

Walker, F. (1838).—"Monographia Chalcitum," 'Entom. Mag.,' vol. v, pp. 102–118.

List of Figures illustrating Dr. A. D. Imms's Paper on "Observations on the Insect Parasites of some Coccidæ."

Fig. A.—A young twig of hawthorn (Cratægus oxycantha) bearing ten fully-grown examples of Lecanium capreæ; natural size.

Fig. 1.—Blastothrix britannica, Gir., female. × circa 44.

Fig. 2.—Blastothrix britannica, Gir., female. Left antenna, viewed laterally.

Fig. 3.—Blastothrix britannica, Gir., female. Right mandible. × 325.

Fig. 4.—Blastothrix britannica, Gir., female. Labrum seen from above. *l.* Labrum. *e.* Epipharynx. × 250.

Fig. 5.—Blastothrix britannica, Gir., female. First maxilla seen dorsally. c_1, c_2. Cardo. *g.* Galea. *l.* Lacinia. *m.l.* Membranous lobe of lacinia. *m.p.* Maxillary palp. *s.* Stipes. × 250.

Fig. 6.—Blastothrix britannica, Gir., female. Labium (second maxillæ) seen ventrally. *l.* Labial palp (right). *lg.* Ligula. *m.* Mentum. *sm.* Sub-mentum. × 270.

Fig. 7.—Blastothrix britannica, Gir., female. Thorax viewed from above (diagrammatic). *f.w.* Base of fore-wing. *h.w.* Base of hind-wing. *mlm.* Mesoscutellum. *mn.*, *m'n'*. Metanotum. *mp.* Mesophragma. *msc.* Mesoscutum. *pn.* Pronotum. *pr.* Parapsides. *prp.* Propodeum. *tg.* Tegula.

Fig. 8.—Ovipositor and sheath of B. britannica, together with their associated parts of the left side, seen from the ventral (external) surface. The stylets have been separated anteriorly from the corresponding portions of the sheath. *ap.* Palp-like appendage of inner plate. *f.p.* Fulcral plate. *lig.* Ligament. *od.* Common viaduct. pl_1, pl_2. Inner and outer plates. *prc.* Splint-like process of fulcral plate r_1 and r_2. Median ribs of outer and inner plates. *sh.* Sheath of ovipositor. *sty.* Stylets. × 360.

Fig. 9.—Blastothrix britannica, Gir., male. × circa 48.

Fig. 10.—Blastothrix britannica, Gir., male. Right antenna, viewed laterally.

Fig. 11.—Male genital armature of B. britannica seen ventrally. *c.* Clasper (left). *p.* Penis. *p.c.* Pore canals. *pr.* Lateral process. *r.p.* Chitinous rod. *sh.* Sheath of penis. × 340.

Fig. 12.—Lateral pore canal of the penis of B. britannica, highly magnified.

Fig. 13.—An egg of Blastothrix britannica, Gir., seen in situ beneath the cuticle of its larval host. *m.* Lateral margin of host. *o.* Ovum seen beneath the cuticle of the host. *p.* Pedicel of ovum protruding through the cuticle to the exterior. × 225.

Fig. 14.—A young larva of Blastothrix britannica attached by its caudal extremity to the chorion of the egg; February 15th, 1916. *ch.* Chorion. *hd.* Head. *m.g.* Mid-gut. *sp.* Posterior spiracles. *t.t.* Developing transverse tracheal branches to the spiracles. × 110.

Fig. 15.—Portion of the tracheal system of a living larva of B. britannica, ·44 mm. long, showing the first four pairs of segmental tracheæ of the right side; March 14th, 1916. *i.t.* Inner segmental tracheal branches. *m.l.t.* Main lateral tracheal trunk of the right side. *o.t.* Outer (dorsal) segmental tracheal branches. *s.* Developing spiracles. *t.t.* Developing transverse tracheal branches to the spiracles. × 280.

Fig. 16.—Mandibles of a larva of B. britannica, ·8 mm. long; March 14th, 1916. Ventral aspect. × 670.

Fig. 17.—Tracheal system of a fully-grown larva of B. britannica; July 6th, 1916. *a c.* Anterior commissure. *hd.* Head. *i.t.* Inner segmental trachea. *o.t.* Outer ditto. *p.c.* Posterior commissure. *sg.* 1. First trunk segment. *sg.* 13. Thirteenth ditto. *sp.* 1. First spiracle (right). *sp.* 9. Ninth ditto. *st.b.* Spiracular or stigmatic branch.

Fig. 18.—Oral lobes and papillæ of a larva of B. britannica in the peripneustic stage; April 4th, 1916. × circa 500.

Fig. 19.—Female pupa of B. britannica, ventral aspect. Reference lettering as in Text-fig. 21. × 60.

Fig. 20.—Male pupa of the same species, ventral aspect. *an.s.* Antennal sheath. *cl.s.* Sheath of clypeus and labrum. *f.w.sh.* Sheath of fore-wing. *lm.s.* Labial sheath. *lm.p.s.* Sheath of labial palp. *md.s.* Sheath of mandible. *mx.s.* Maxillary sheath. *mx.p.s.* Sheath of maxillary palp. × 60.

Fig. 21.—Female pupa of the same species, dorsal aspect. *an.s.* Right antennal sheath. *f.w.s.* Sheath of the left fore-wing. × 60.

Fig. 22.—A young larva of Lecanium capreæ, showing the emergence hole made by Blastothrix britannica (first generation). × circa 12.

Fig. 23.—Two fully-grown Scale insects (Lecanium capreæ) with numerous emergence holes made by the parasitic Chalcid dealt with in this paper. The two larger holes in the upper specimen were made by Blastothrix britannica; the remaining smaller holes are due to Aphycus melanostomatus. × 4.

Fig. 24.—Enlarged figure of a young larva of Lecanium capreæ containing a pupa of Blastothrix britannica. The anterior extremity of the latter is directly toward the anal end of the host. The ovoid opaque bodies are excrementa ejected by the larval parasite prior to pupation.

Fig. 25.—Aphycus melanostomatus, Timb., female. × circa 45.

Fig. 26.—Aphycus melanostomatus, Timb., male. × circa 46.

Fig. 27.—Aphycus melanostomatus, Timb. A. Antenna of male. B. Antenna of female.

Fig. 28.—Egg of Aphycus melanostomatus dissected from a young larva of Lecanium capreæ; December 21st, 1915. The mandibles (*md.*), median salivary duct (*s.d.*), mid-gut (*m.g.*), and tracheal system (*t.s.*) are clearly visible beneath the chorion. × 200.

Fig. 29.—Tracheal system of a larva of A. melanostomatus, 45 mm. long; February 22nd, 1916. The preparation was treated with dilute KOH in order to render the branches more distinct; the reagent had the effect of obscuring the boundaries between the two hindmost segments. *a.c.* Anterior commissure. *hd.* Head. *p.c.* Posterior commissure. *t.t.* Developing transverse tracheal branches to the spiracles. × 160.

Fig. 30.—A larva in the same stage of development as in the preceding figure, showing general anatomy; February 22nd, 1916. *f.b.* Lobe of fat-body. *hd.* Head. *hd.s.* Endo-skeleton of head. *m.g.* Mid-gut. *œs.* Œsophagus. *r.* Rectum. *r.gl.* Salivary gland. *s.d.* Median salivary duct. *t.s.* Tracheæ. *t.t.* Developing transverse tracheal branches to the spiracles. × 160.

Fig. 31.—Fully-grown larva of A. melanostomatus seen from the left side; from a living specimen; May 6th, 1915. The head, thirteen trunk segments, and nine spiracles are clearly visible. × 40.

Fig. 32.—Right and left mandibles of a larva of A. melanostomatus, ·8 mm. long, with peripneustic tracheal system; March 25th, 1916. × 480.

Fig. 33.—Tracheal system of the same species in the peripneustic stage; February 8th, 1916. *sp.* 1. First spiracle (left). *sp.* 9. Ninth ditto. *s.b.* Vestigial branch related to a former tenth spiracle (right).

Fig. 34.—Diagrammatic transverse section across a nearly full-grown larva of Aphycus melanostomatus enclosed within its sheath-like investment. *b.w.* Body-wall of larva. *c.* Body-cavity of larva. *c.l.* Chitinous lining of sheath. *m.g.* Wall of larval mid-gut. *n.* Nervous system. *s.* Space between the body-wall of the larva and the sheath. *t.* Trachea. *w.* Outer layer of the sheath (the small dark bodies enclosed therein are the conidia of a fungoid organism).

The Somatic Mitosis of Stegomyia fasciata.

By

Lucy A. Carter, S.N.D., B.Sc.

With Plate 21.

Introduction and Material.

In the summer of 1917 Dr. Ashworth sent some eggs of Stegomyia fasciata to Prof. Graham Kerr, of Glasgow University, for museum purposes. These eggs had been obtained from females of a strain brought from Freetown, West Africa, by Mr. A. Bacot, F.E.S., Entomologist to the Lister Institute, London, successfully bred by him in London since his return.

Some of the eggs were incubated in the Laboratory of the College of Notre Dame where Sister Monica Taylor (D.Sc.) found their development to be unusually rapid, the fulfilment of a condition necessary for the successful study of somatic mitosis, in order that a copious supply of dividing nuclei may be secured in a single specimen—thus rendering the seriation of stages possible.

While awaiting the discovery of a technique which will overcome the difficulties of sectioning later stages of the eggs of Culex pipiens, in order to furnish material for tracing out the gradual evolution of parasyndetic conditions in Culex, she had suggested that I should investigate the somatic mitosis in another mosquito.

The arrival of the rapidly developing Stegomyia

fasciata furnished material admirably suited for the proposed study.

Prof. Graham Kerr gave up his surplus supply and Dr. Ashworth very courteously added to his original gift by sending fresh batches direct to the College. I take this opportunity of thanking the donors for their kindness and liberality, and also record my thanks to Sister Monica for her advice and helpful criticism throughout.

This short investigation has, therefore, been made with a view to determining whether the pairing of the Chromosomes in the somatic tissues exists, and, if so, of possibly linking up by means of another species, with a less pronounced condition of parasyndesis, the egg of Culex pipiens with the later stages of development.

Very much work remains to be done on the passage of the telophase nucleus into the resting nucleus, and since in C. pipiens the telophases of the later spermatogonial divisions were observed to pass directly into the synizetic nuclei, it seemed probable, as already mentioned, that mosquito material would be favourable for such a study, as it has so proved.

Abundant cases of dividing nuclei were found in the body-wall cells, particularly in larvæ just about to undergo ecdysis. Tracheal tube cells, as well as cells of other tissues also showed occasionally many cases.

The methods used were the same as those employed for C. pipiens, i.e. small portions of the mosquito were fixed in the fluids known as Flemming, Gilson-Petrunkewitsch, in warm solution of corrosive sublimate in alcohol. (The fixative used for any particular study is indicated in the explanation of plates.) Sagittal sections 8μ thick were used exclusively, and all nuclei described and figured were wholly within one section. This precaution is necessary where the chromosomes are long as in this case.

The Flemming fixation has the effect of making the nuclei look larger. It does not, however, so clearly preserve the spindle apparatus, which appears to greater advantage in

other fixatives where, at the same time, the chromosomes also stand out just as sharply defined and are as readily counted as in the Flemming. The stain was Heidenhain's hæmatoxylin throughout.

The gonad having the same topographical relations as in Culex, the third segment from the hind end of the abdomen was cut out and sectioned in order that the spermatogonial and oogonial mitosis might be investigated.

Both the ovary and testis are similar in structure to those of Culex pipiens, and need not be described in detail. The testis is divided up into a number of compartments, all the cells of one compartment being approximately at the same stage of division. As might be expected in the more rapidly developing species, a greater range of "spermatogenesis" stages is obtainable in one specimen—large early spermatogonia, smaller spermatogonia of the multiplication zone, primary and secondary spermatocytes, and complete spermatozoa often being visible in a single section.

The later stages of spermatogenesis follow quite closely those described for C. pipiens (**3**) (Pl. 21, fig. 29), and throw no special light on parasyndesis, hence they have not been included in this account.

Most probably because of its rapid development—the imago in some cases emerging from the pupal case seven days after the larva hatched—the synizetic nucleus which occurs so abundantly in the C. pipiens testis is not nearly so plentiful in Stegomyia, although, as will be shown later, the synizetic stages are analogous in the two mosquitoes. Thus the assumption that the synizetic nucleus of the male gonad functions as a sort of inactive phase in C. pipiens seems justified. Later it will be shown that the so-called synizetic nucleus in Stegomyia is really the stage characteristic of the nuclei of the two newly-formed daughter-cells, i.e. the stage following immediately on the telophase.

A synizetic-like stage in somatic—apart from spermatogonial and oogonial—mitosis was noticed in C. pipiens, but its connection with the telophase of a dividing nucleus could

not be demonstrated. The ovary in the larva and pupa of Stegomyia is quite immature. It apparently grows rapidly and attains its full development when the imago has been suitably fed. Synizetic-like nuclei are much more numerous in the tissues of the immature ovary than in those of the testis. In the former the growth is relatively slower, and may be suspended altogether until the suitable diet has been supplied to the imago. Hence here the synizetic-like nucleus seems to function as a "waiting" nucleus.

There is no difficulty in determining four as the diploid number of chromosomes.

Somatic Mitosis. (Passage of the telophase nucleus into the resting nucleus.)

I. Synizesis. (Pl. 21, figs. 1, 2, 3*a*.)

As already indicated, the two masses of chromatin formed from four daughter-chromosomes which cap the pole of the spindle in late telophase become surrounded by a clear liquid as the nuclear membrane is completed round the daughter-products.

(N.B.—The nuclear membrane persists during mitosis as in C. pipiens.)

The spindle-fibres either disappear altogether or are drawn into the chromatin masses and the synizetic nucleus is to be seen in each of the two daughter-cells. These two nuclei become gradually separated one from the other by the growth of the cytoplasm surrounding them, but they can easily be detected until they have almost reached the resting condition. This account holds good for all the fixatives employed.

II. Synizesis (i.e. late Telophase) to Resting Stage (Pl. 21, figs. 3–6).

The telophasic mass becomes gradually invaded by the surrounding nuclear sap, and assumes a vacuolated appearance.

Strands of pale staining substance are now seen to connect the chromosomic mass with the nuclear membrane, and there is a tendency for the chromatin to be drawn towards the membrane.

In favourable specimens at this stage a condition similar in form to that of an early prophase can be detected (Pl. 21, figs. 3*c* and 4)—i.e. the chromatin has the appearance of two pairs of chromosomes—the members of the pairs being very much twisted one around the other. A comparison of Pl. 21, fig. 17 (an early prophase in a young testis where the number of compartments making up the whole gonad is twelve) with Pl. 21, figs. 3*c* and 4 brings out the likeness between a very late telophase and an early prophase, these interesting figures (3*c* and 4) illustrating undoubted cases of the telophase. There is, however, a difference in the staining capacity of the two stages, the early prophase showing a freshness and limpidity which is absent in the later stage of telophase.

Pegs of chromatin now begin to extend from the chromosomes, to lengthen and to anastomose, and as the chromosomes become more vacuolated the nucleus presents the appearance of a crown (Pl. 21, fig. 5), the nucleolus being conspicuous. Eventually the chromatin is fairly well distributed in blotches and patches, and a typical resting nucleus is the result (Pl. 21, fig. 6).

From the above description it would seem that there is a marked tendency for the chromosomes to emerge from the telophasic mass in two pairs, the outline of these two pairs being gradually obscured as the vacuolisation of the chromatin proceeds.

III. Resting Stage to Prophase.

At the outset of mitosis the chromatin assumes the character of a densely matted mass of very fine threads—difficult to stain—surrounding a characteristic deeply stained nucleolus (Pl. 21, fig. 7). In fact, this latter structure often looks like an irregular blob of chromatin; and since it gradually disappears as the new chromosomes become recognisable, it

would seem as though it had acted as a storehouse of chromatin ready to furnish the prophase chromosomes.

The incipient chromosomes now begin to thicken up in places and to contract. Liquid collects in the nucleus, which becomes more distended in consequence, and this liquid apparently pushes out the newly-forming chromosomes towards the nuclear membrane (Pl. 21, fig. 8). In this, the early prophase condition, two very long chromosomes can be distinguished (Pl. 21, fig. 9). They are double, the two membranes of the pair being twisted about each other (Pl. 21, fig. 9). Gradually a condensation proceeds, the pairs untwist, but the degree of untwisting varies greatly. When the untwisting has been slight—i. e. when the full condensation of the chromosomes has been effected before the component chromosomes of the pair have succeeded in extricating themselves from their close approximation—then, in full prophase, there are apparently only two chromosomes (Pl. 21, figs. 11, 12, 13.)

When the untwisting process is completed simultaneously with the full condensation of the chromosomes, then the members of the pairs lie side by side (Pl. 21, fig. 14). Sometimes the untwisting is completed before full condensation, when four chromosomes showing only slight parasyndesis are the result (Pl. 21, figs. 15, 16).

That faulty fixation has nothing to do with the varying appearance of two or four chromosomes is evident from the fact that the same phenomena occur whatever the fixatives employed, and in all the tissues—parasyndesis not being confined to any one or other of the somatic tissues.

There is no method of distinguishing the spermatogonial prophase from that of the primary spermatocyte. The degree of parasyndesis is always great in the gonad cells—the fully condensed chromosome always giving the impression of the haploid number. Studies made from the youngest compartment in young gonads composed of few compartments show a meiotic-like character of chromosome like that occurring in meiosis ring, S-shaped and 8-shaped figures being common (Pl. 21, figs. 17, 18).

These appearances in prophase are, however, followed by an anaphase which is typically somatic, and the nuclei can, therefore, be distinguished as spermatogonial in anaphase (Pl. 21, fig. 28). As in C. pipiens, the primary spermatocyte cells are spindle-shaped, whole chromosomes being separated in the first meiotic division (Pl. 21, fig. 29).

IV. Metaphase and Anaphase.

The two pairs of long chromosomes arrange themselves towards the centre of the nucleus, parallel to the plane of the future equator of the spindle (Pl. 21, fig. 19).

In the large cells, where the chromosomes are very long, i.e. longer than the equator, they accommodate themselves by twisting up in a zig-zag fashion. As a consequence of this they are often difficult to count. In favourable specimens of small cells, however, they can be distinguished, and the longditudinal splitting can be observed. Most probably because of this great length the usual V-shaped chromosome of anaphase is often replaced in Stegomyia by a W-shape (Pl. 21, fig. 25).

That there is a tendency for the homologous chromosomes to become clearly associated in their passage to the poles can be demonstrated in many cases of uncut nuclei where this association can in no wise be attributed to bad fixation. As a result, only two chromosomes can in such cases be detected at the poles (Pl. 21, figs. 23, 24, 27).

In other cases, however, the four chromosomes at the poles can be counted quite readily (Pl. 21, fig. 26), and that the members of homologous pairs are sometimes separated is evident from a study of the sizes of the chromosomes (Pl. 21, fig. 25).

As explained above, the long chromosome has a W-shape in anaphase, the short chromosome either a or rod-shape. This alternate arrangement of W, with a rod at each pole of the spindle, must betoken a separation of the members of the pairs. In cases such as Pl. 21, fig. 25, the repairing of

the homologous chromosomes would appear to take place in the synizetic mass, since as explained above, two pairs of chromosomes can often be detected in late telophase.

From the foregoing observations it will be seen that Stegomyia fasciata offers no exception to the parasyndetic condition, as worked out by Metz (**2**), for other Diptera. In fact, the varying degrees in which that condition exists in this mosquito may be considered to place Stegomyia in a median position, acting as a link between the extremes of little or no parasyndesis in the segmenting-egg stage of Culex pipiens, and the complete parasyndesis as found in the later stages of development of the same animal.

Summary.

(1) The diploid number of chromosomes in Stegomyia fasciata is four.

(2) A varying degree of parasyndesis (pairing of the chromosomes) is exhibited in the somatic cells, extreme parasyndesis giving a haploid count.

(3) Each telophasic mass of chromatin gives rise directly to a synizetic nucleus instead of the usual "resting" nucleus.

(4) The nuclear membrane persists throughout mitosis.

(5) The homologous chromosomes pair either in anaphase or in telophase.

Note.—At the Editor's request I append the following short list of certain terms used in this paper, together with the name of the author of each and the publication in which it appeared.

Anaphase (G. ana, back or again), introduced by Strasburger, 1884, in his paper, "Die Controversen der indirecten Kerntheilung" ('Arch. für Micr. Anat.,' xxiii, p. 260). His own definition translated runs thus: "The phases passed through (by the nucleus) from the complete separation of the daughter-segments to the final establishment of the daughter-nuclei may be known as the anaphases of the division," i.e. the phases during which the nucleus returns to its original condition.

As used by Strasburger, it will be seen that his term "Anaphasen" includes the more modern "telophase." The restricted definition of anaphase is now "the later period of mitosis during which the divergence of the chromosomes takes place," i. e. the passage of the chromosomes from the equatorial plate to the poles, when the telophases now begin.

Diploid (G. diploos, double), as opposed to haploid, q. v.

It is employed to indicate the normal number of chromosomes present in the nucleus of a somatic cell (Strasburger, 1906).

Haploid (G. haploos, single or simple), indicating the number of chromosomes present in the nucleus at the end of the "reduction" period, that is, the number in each ultimate germ-cell. On the conjugation of the male and female germ-nuclei a nucleus is produced containing double this "haploid" number, or what is known as the "diploid" number of chromosomes.

Both words haploid and diploid were introduced by Strasburger in his paper, "Typische und allotypische Kernteilung" ('Jahr. f. wiss. Bot.,' vol. xlii, p. 62, 1906), thus:

"Finally, it is desirable that the terms Gametophyte and Sporophyte, at present confined to plants with the single and double chromosome number respectively, should be discarded when the distinction is extended to the animal kingdom. To this end I propose the words Haploid and Diploid, or Haploid and Diploid generation."

Meiosis (G. meiosis, lessening), first used by J. B. Farmer and J. E. S. Moore in 1905 in their paper, "On the Maiotic Phase (Reduction Divisions) in Animals and Plants" ('Quart. Journ. Micr. Sci.,' vol. 48, N.S.).

It is the period during which the reduction in the number of chromosomes takes place, including both maturation divisions.

Metaphase (G. meta, between), introduced by Strasburger with Prophase and Anaphase, q. v., the "middle stage" of the mitosis. He says: "The stages from the beginning

of the breaking asunder of the daughter-segments to their complete separation and arrangement in a ring (round the spindle axis) I would term the Metaphases."

This definition still holds good. It will be noted that Strasburger employs these terms Anaphase, etc., in the plural —Anaphasen, Metaphasen, etc.—thus indicating a series of changes in each case.

Parasyndesis (para, beside). The longitudinal pairing of the chromosomes—as opposed to Metasyndesis—introduced by Häcker, 1909, in "Die Chromosomen als Angenommene Vererbungsträger" ('Ergeb. u. Fortsch. der Zool.,' vol. i, p. 74). Thus: "For the pairing of the chromosomes I would, as already mentioned, keep the term Syndesis. When, in order to effect this union, the chromosomes arrange themselves (in pairs) with their long axes parallel, I propose to call this arrangement a Parasyndese (Paradese), or paradetic union. When the arrangement of the chromosomes is "end to end," the term metasyndese (metadese) or metadetic coupling would be more appropriate.

Prophase (pro, first). Introduced by Strasburger with Anaphase, q. v., thus: "The introductory phases of the nuclear division which I shall call, collectively, Prophases, begin with the appearance of the filament-skein (spireme). . . . Finally, the nuclear plate is ready to form, and herewith the prophases of the division terminate."

Resting Nucleus.—A nucleus in a state of vegetative activity, in which the chromatin is not arranged in the form of compact chromosomes; a nucleus "nesting" from actual mitosis—the period between two successive nuclear divisions.

Synizesis (G. syn, with; hizo, place), introduced by McClung, 1905, in "The Chromosome Complex of Orthopteran Spermatocytes" ('Biol. Bull.,' ix, 1905).

It indicates the clumping together of the chromatin often observed in the meiotic prophase. It was included in Moore's earlier term, "synapsis."

Telophases (telos, end), first used by Heidenhain, 1894 in the "Neue Untersuchungen über die Centralkörper und ihre Beziehungen zum Kern und Zellenprotoplasma" ('A. m. A.,' xliii).

The closing phases of mitosis, from the massing together of the chromosomes at the two poles of the nuclear spindle up to the final establishment of the daughter-nuclei—i. e. to the end of the mitosis.

LITERATURE.

1. Metz, C. W. (1914).—"Chromosome Studies in the Diptera. I. A Preliminary Survey of Five Different Types of Chromosome Groups in the Genus Drosophila," 'Journ. Exper. Zool.,' xvii.

2. ——— (1916).—"Chromosome Studies in the Diptera. II. The Paired Association of Chromosomes in the Diptera and its Significance," ibid., xxi.

3. Taylor, Monica (1914).—"The Chromosome Complex of Culex pipiens," 'Quart. Journ. Micr. Sci.' (N.S.), vol. 60.

4. ——— (1917).—"The Chromosome Complex of Culex pipiens," ibid., (N.S.), vol. 62.

EXPLANATION OF PLATE 21,

Illustrating Miss Lucy A. Carter's paper on "The Somatic Mitosis of Stegomyia fasciata."

[All figures were drawn with the Abbe camera under Leitz' $\frac{1}{12}$ in. oil immersion objective and Zeiss' compensating ocular 12. Magnification of 3500 diameters. The magnification of the figures as reproduced is shown by scale. Fixatives indicated: Gilson Petrunkewitsch, G.P. Flemming, F. Corrosive sublimate in alcohol (warm), C.A. All sections stained with Heidenhain's hæmatoxylin.]

Fig. 1.—Cell from gonad. Telophase passing into resting stage. G.P.

Fig. 2.—Nucleus passing into synizesis. Cell from body-wall. G.P.

Fig. 3.—Telophase—three stages *a*, *b*, *c*, from group of nerve cells. In *c*, two pairs of chromosomes coming out of the mass. G.P.

Fig. 4.—Interesting stage of somatic telophase, showing paired character of threads coming out of the telophasic-synizetic mass. Cell from the body-wall of fairly old larva. F.

Fig. 5.—Somatic. Synizetic mass unfolding; gradual conversion into resting nucleus; paired character of threads. F.

Fig. 6.—Two resting nuclei from body-wall. F.

Fig. 7.—Somatic. Very early prophase showing chromatin passing to chromosomes from nucleolus; from larva. C.A.

Fig. 8.—Early prophase from Spermatocyte I. Imago ♂. C.A.

Fig. 9.—Gonad cell showing double character of chromosomes. G.P.

Fig. 10.—Early prophase. Somatic cell from tracheal tube. G.P.

Fig. 11.—Prophase; two chromosomes—from ovary. G.P.

Fig. 12.—Somatic from body-wall. ♀. F.

Fig. 13.—Early full prophase in somatic cell from body-wall; chromosomes long and lying in the nuclear membrane. F.

Fig. 14.—Prophase; four chromosomes in two groups; cell from body-wall of fairly old larva. F.

Fig. 15.—As fig. 14.

Fig. 16.—Late full prophase; somatic; four chromosomes. F.

Fig. 17.—Cell from youngest compartment in a gonad consisting of twelve compartments; probably spermatogonial. F.

Fig. 18.—As in fig. 17. ♂.

Fig. 19.—Very early metaphase. Two long and two short chromosomes nearing the future equator. F.

Fig. 20.—Metaphase; somatic ♂; gonad has twenty-five compartments. F.

Fig. 20*a*.—Somatic from larva. C.A.

Fig. 21.—Metaphase; spindle threads well differentiated; apparently four chromosomes; from gonad ♀. G.P.

Fig. 22.—Early metaphase. Four chromosomes splitting longitudinally from body-wall of pupa. G.P. Faintly stained.

Fig. 23.—Metaphase showing chromosomes in groups of two; somatic cell from body-wall. ♀. F.

Fig. 24.—From gonad ♀, looks like two chromosomes. G.P.

Fig. 25.—Telophase. Somatic from fairly old larva. F. Two long daughter-chromosomes separated by the two short chromosomes.

Fig. 26.—Telophase. Somatic from body-wall of larva. C.A.

Fig. 27.—Telophase. Two somatic cells from alimentary canal wall of larva. C.A. The two long chromosomes have reached the poles, the two short ones are still at the equator.

Fig. 28.—Spermatogonial telophase from gonad (fifteen to eighteen compartments) of larva. G.P.

Fig. 29.—First meiotic division from pupa. F.

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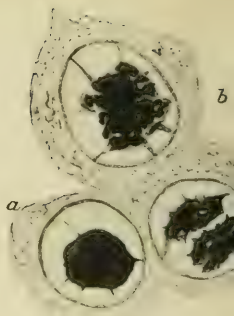

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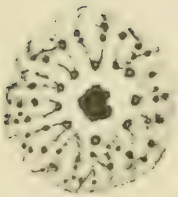

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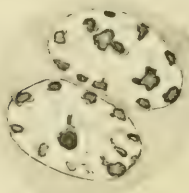

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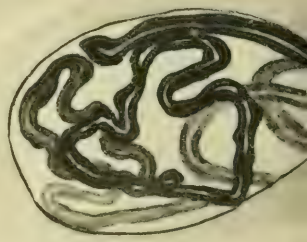

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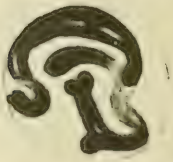

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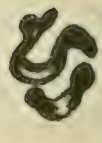

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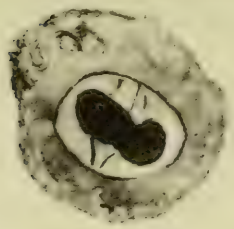

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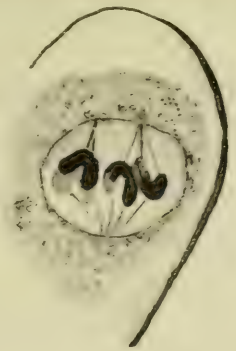

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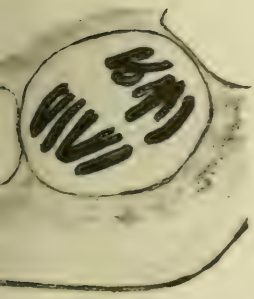

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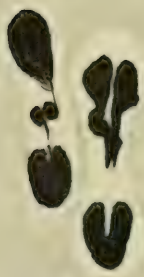

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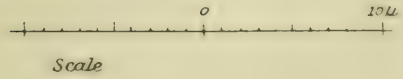

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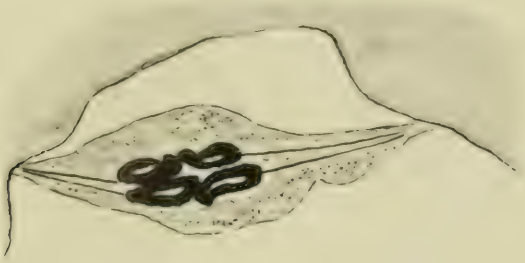


29.

Huth, London

GOMYIA.

On the Cytomorphosis of the Enamel Organ in the Hake.

By

J. Thornton Carter,

Department of Zoology, University of London, University College.

With Plates 22, 23 and 24.

In the Gadidæ each tooth is surmounted by a pointed cap of enamel which rests on a platform of dentine, whose central area extends into the enamel cap, thus affording a firm support without increasing the outside dimensions of the tooth over this area. The development of this enamel cap is associated with certain marked changes in the enamel cells investing it.

So far as I am aware there is but one published paper dealing with the formation of enamel in the Gadidæ—"Upon the Development of the Enamel in Certain Osseous Fish," C. S. Tomes, 'Phil. Trans.,' cxciii, B. 186, 1900—and the cytological conclusions advanced therein were so much at variance with the appearances found in a number of fishes and other vertebrates in which I had followed out a complete cytomorphosis of the enamel cells, and also with isolated sections of developing teeth of Gadidæ in my possession that I have worked out the full life-history of the cells forming the enamel cap in the Hake (Merluccius vulgaris), as this was the creature principally employed by Tomes.

Through the kindness of Prof. Meek one or two heads were obtained at sea and immediately fixed in corrosive-formalin-acetic mixture. In one of these heads the fixation is very

fine and from it about fifty teeth and developing tooth-germs have been isolated, cut into complete series of sections of 10 μ, and stained with iron hæmatoxylin, followed by a counter-stain. Serial sections are a necessity when endeavouring to obtain a complete cytomorphosis where it is imperative to know the exact plane of a section for comparison with other stages.

The origin of the tooth-germ is as in Mammals, there being an ingrowth of the deeper layer of the oral epithelium and the growth of a dentine papilla which becomes invested by the epithelium except at its base.

The epithelial enamel organ consists of two layers of cells, the one lying in apposition to the dentine, consisting of columnar ameloblasts with well-defined cell outlines and the nuclei lying about the centres of the cells (Pl. 22, fig. 1, *a.*). Immediately external, separating the ameloblasts from the surrounding connective tissues lies a layer of polygonal cells usually two or three deep, constituting the external epithelium of the enamel organ (*e.e.*). Throughout the whole life-history of the enamel organ these two layers of cells remain in contact, there being no such differentiation as is seen in Mammals, where the ameloblasts and external epithelium become separated by the modified cells known as the stellate reticulum, which act as a storehouse for the materials to be elaborated by the ameloblasts into the secretion which gives origin to the enamel. The absence of such provision is associated with a marked modification of the ameloblasts.

With the appearance of a very thin layer of dentine over the apex of the dentine papilla the columnar ameloblasts undergo a marked change (Pl. 22, fig. 2). Their nuclei are seen to have receded somewhat towards the bases of the cells, the cytoplasm about them still preserving the outlines of the individual cells, but between the nuclei and the secreting surface—i. e. toward the dentine—the individual outlines of the cells are lost owing to a very rapid formation of metaplasm in this area which lies in vacuoles (*vac.*) separated by a fibrillar cytomitoplasm (*c.m.t.*) whose fibrils run fairly parallel

to the long axis of the cell. The contents of these vacuoles, when not washed away, appear faintly coagulated and tinged with the counter-stain, whilst the fibrils of the cytomitoplasm are basophile.

This process of vacuolation or accumulation of metaplasm progresses towards the cell base and is associated with a considerable increase in the length of the cells (Pl. 22, figs. 3 and 4). The nuclei become considerably elongated and are situate at various levels in different cells, more frequently lying somewhat towards the secreting surface. At this stage a considerable amount of ameloblastic secretion has been deposited on the surface of the dentine (Pl. 22, fig. 6, *a.s.*), and there is no sign of any merging of the cells into the secretion, such as one would find did the ameloblasts themselves become transformed into a stroma which became incorporated into the enamel. Though the outlines of the individual cells are lost, the secreting surface presents a well-defined regular continuous margin, a sharp line in longitudinal section, and a finely granular lamina in oblique section, in strong contrast to the vacuolated cytoplasm (Pl. 22, figs. 4, 5, 6, *i.a.m.*). This secreting surface of the cell, the inner ameloblastic membrane, is not a metaplasmic product but is a specialised area of the cytoplasm, showing traces of a fine polygonal structure corresponding to individual cell areas, and the fibrils of the cytomitoplasm appear to terminate in certain granules lying immediately below the surface. When this stage is reached, the shedding of the ameloblastic secretion is very active, leading to a marked diminution in the size of the vacuoles and decrease in their number (Pl. 22, fig. 5), and this passage of the contents of the vacuoles carries with it the great majority of the nuclei until they appear to rest almost in contact with the inner surface of the inner ameloblastic membrane. The fibrils of the cytomitoplasm (*c.m.t.*) now become arranged in more or less straight lines running parallel with the long axes of the enamel cells.

When this stage in enamel formation is reached there is usually a considerable amount of dentine formed below the

shoulder on which the enamel cap rests. This dentine also is invested by elongated enamel cells (Pl. 23, fig. 8), which, however, do not increase in length very greatly, and do not undergo the changes which take place in those cells corresponding to the area occupied by the enamel cap. They are usually separated from the cells of the external epithelium by a sharply defined line, and the portion of their cytoplasm between this line and the nuclei appears almost clear, whilst the surface of the nuclei towards it is concave. This is probably due to some metabolic activity, for with the complete formation of the enamel cap and of the dentine of the body of the tooth this appearance of the cell is lost, the nucleus becomes (Pl. 23, fig. 9) of a rounded oval form, and the cytoplasm throughout the whole cell becomes faintly granular.

Except for the enamel cap, I do not think any enamel is formed over the surface of the tooth, for all teeth composed entirely of vaso-dentine exhibit a considerable degree of flexibility, and with carefully ground sections of fresh teeth, I have not been able to demonstrate the existence of even the thinnest layer of any highly refractile substance, the dentine ending sharply in a well-defined margin, as seen in Pl. 23, fig. 9, at *d*.

When the tooth has erupted and become functional these cells rapidly lose their columnar form and assume a flattened polygonal shape.

The restoration of the outlines of individual cells foreshadowed at the stage shown in Pl. 22, fig. 5, becomes well marked in the next (Pl. 22, fig. 7), where the distance between cell base and secreting surface has diminished greatly, the nuclei (*n*.), now deeply chromatic and much elongated, have receded towards the centres of their respective cells, and the individuality of each cell has again become apparent. This recovery of the cell usually progresses until the nuclei have become of an oval shape and the chromatin aggregated into two or three masses (Pl. 23, fig. 10, *a*.).

The formation of the enamel cap now appears to be complete, since if germs at this stage of development are

dissected out, the transparent caps of enamel are seen resting on their shoulders of dentine. The enamel appears to be fully calcified, and may be ground down into very thin sections without disclosing any trace of structure, but on washing such a section with an acid, a faint prismatic structure is revealed.

With the full development of the enamel cap the process of eruption of the tooth proceeds rapidly, and is accompanied by certain marked changes in the enamel cells, whose function has now been accomplished. An ingrowth of the cytoplasm of certain cells of the external epithelium takes place which, inserting itself between the ameloblasts, divides them up into groups or nests, frequently with no definite arrangement of the latter, but often causing them to become arranged in a series of loops as seen in Pl. 23, fig. 10, where the ameloblasts (*a.*) are seen arranged regularly about these ingrowths of the external epithelium (*e.e.*), whilst the inner ameloblastic membrane (*i.a.m.*) preserves an even contour towards the formed enamel. In all my preparations of germs which had completed the formation of the enamel cap, the cells of the external epithelium invade the ameloblasts, and this invasion is most marked along a line corresponding to that area where the enamel cap will emerge in eruption. When the apex of the tooth is to be the point first to emerge, the cells of the external epithelium insert themselves between the bases of the ameloblasts over the whole vertex of the enamel organ forming a deep furrow. Sometimes there are two furrows running almost parallel, as in Pl. 23, fig. 12 (*e.e.*), and the ameloblasts (*a.*) lying beneath become changed, so that their nuclei stain with difficulty, and their cytoplasm disappears in a sort of coarse vacuolation.

The apex of the tooth, however, is not invariably the point first to emerge from its investing epithelium, and when the tooth is going to erupt sideways the cells of the external epithelium (*e.e.*) traverse a small sector opposite which the rarefaction of the ameloblasts proceeds to permit of its exit (Pl. 23, fig. 11).

Associated with these regressive changes there is an

ingrowth of the oral epithelium, which on reaching the enamel organ appears to extend about it for some distance, inserting itself between the enamel cells and the tissues of the tooth sac. This is particularly well seen in Pl. 23, fig. 14, where the ingrowth of the cells (*o.e.*) extends down to the very limits of the cells responsible for the enamel cap. In Pl. 24, fig. 15, is shown a somewhat greater magnification of a similar condition in which it is seen that the ingrowth where close to the tooth-germ consists in section of a double row of cells (*o.e.*).

This ingrowth rapidly increases in size until it forms a large mass of epithelial cells (Pl. 24, fig. 16, *o.e.*), in contact at one end with the enamel organ over the enamel cap of the tooth and at the other end continuous with the cells of the oral mucous membrane. In Pl. 24, fig. 17, is shown a transverse section through such an ingrowth taken about midway between the tooth-germ and the surface. It is seen to be merely a mass of epithelial cells with no sign of any lumen or other evidences of glandular structure and seems to be solely a provision for the eruption of the tooth similar to the ingrowth of oral epithelium which Profs. Wilson and Hill have shown to obtain in Marsupials.

The cells overlying the formed enamel cap now consist of groups or nests of elongated ameloblasts interspersed with cells of the external epithelium (Pl. 24, fig. 16). With the emergence of the tooth the columnar form of the ameloblasts is rapidly lost (Pl. 24, fig. 18, *a.*) and they become rounded cells undergoing degenerative changes which lead to their rapid disappearance (Pl. 24, fig. 19). The epithelial cells which invest the remainder of the tooth and which were continuous with the ameloblasts forming the enamel cap (Pl. 23, figs. 8 and 9) rapidly lose their columnar form so that the erupted functional tooth is invested by a layer of flattened polygonal cells.

These changes which I have described are so much at variance with the appearances described by Tomes in the paper mentioned at the commencement of this paper that it is necessary to quote from it at some length.

He writes:

"In its earliest stages the tooth-germ of the Hake does not present any marked peculiarities and resembles a mammalian tooth-germ . . . there is an enamel organ which consists of the usual double row of cells, the inner of which (ameloblasts) form the internal epithelium of the enamel organ and are elongated columnar cells 19 μ in length.

"At the next stage which it is necessary to describe, a very thin skin of dentine has calcified. . . . The space above the top and sides of the dentine germ is occupied by a delicate tissue which has a reticulated appearance and reaches quite out to the walls of the tooth sac, thus occupying the position of an enamel organ. But in it none of the usual constituents of an enamel organ can be recognised; there are no ameloblasts, no stellate reticulum, nor external epithelium of the enamel organ, but in their place and in the position but a short time before occupied by the ameloblasts is this reticulated stroma. . . .

"This stroma has a general appearance of fibrillation in a direction at right angles to the dentine surface . . . its outermost portion always stains much more deeply than the rest and rounded forms are there seen which at first I was inclined to regard as nuclei, the nuclei of the transformed ameloblast cells. But in sections which lie at right angles to the long axis of the tooth-germ . . . it is found that though the stained areas are circular, their outer borders are indefinite, and that they surround sharply-defined circular areas which are less deeply or not at all stained; this seems to negative the idea of the stained areas seen in longitudinal section being really nuclei. . . .

"A cross section some distance within the stroma shows nothing but circular areas, lying separated from one another and with a delicately striated tissue intervening between them. The rings vary from 3 μ to 5·5 μ in diameter. . . . When the section is oblique the rings become ovals, sometimes much elongated, so that they appear to be sections of either rods or tubes of considerable length. . . .

"So far the stroma has been shown to consist of two elements, the sharply-defined tubes or rods and a delicately fibrillated tissue which intervenes between them.

"To revert to the rods or tubes . . . it seems hardly possible to doubt that they bear some relation to the forming prisms and are a stage in their development, and the fact that they are not to be distinguished in that portion of the enamel stroma which lies close to the dentine tends to bear out this view.

"Two facts are perfectly clear; the first, that the enamel of these fishes is certainly not an excretion from the ends of the ameloblasts, for they have disappeared long before calcification takes place; the other, that the calcification does take place in the form of a conversion of or a deposition in a pre-existent stroma of definite arrangement."

I have quoted at some length from Tomes since he has employed his conclusions in elaborating a theory of enamel formation which supposes fundamentally different processes in different groups of Vertebrates. Little comment is necessary. The bodies which he was at first inclined to regard as the nuclei of ameloblasts are the cells of the external epithelium (Pl. 23, fig. 13, *e.e.* and *n.*), whilst his "rods or tubes" are the nuclei of the ameloblasts or the vacuoles.

Since this paper was written, Mr. J. H. Mummery has published a paper "On the Structure and Development of the Tubular Enamel of the Sparidæ and Labridæ" ('Phil. Trans.,' B. cccliv, vol. 208), in which he describes certain modifications leading to a definite "conversion of the whole enamel organ into a system of glands and blood-vessels."

His interpretations are based largely on the views of Tomes, quoted above, and he concurs that "the enamel organ is converted into a stroma traversed by tube-like prolongations," the transformed ameloblasts being incorporated into the enamel. With the disappearance of the ameloblasts their function is assumed by an ingrowth of glandular tissue "derived from the deep layer of the submucous tissue of the mouth and pharynx (the italics are mine), which separates "the lime salts from the circulating blood to form the calcified

enamel, the organic matrix of which is formed by the transformed ameloblasts, which have become converted into the stroma."

In a succeeding paragraph Mummery speaks of "these invasions of the enamel organ by prolongations of glandular tissue from the deep surface of the epithelium of the mouth" and "of their direct communication with the unmistakable glandular tissue of the mouth." Thus he would seem to indicate some such ingrowth of the oral epithelium as I have shown to take place after the formation of the enamel cap in the Hake, and which I believe to be connected with the eruption of the tooth.

Certainly, though in Hake the enamel organ of a growing tooth-germ is richly invested by capillaries lying in the surrounding connective tissues there is no sign in my preparations of any vascularity of the enamel organ or of the epithelial ingrowth, nor any evidence of this latter structure having a secretory function.

In Sargus, Mummery writes, "that part of the enamel near the dentine is laid down by true ameloblast cells, the rest of the enamel being formed after these cells have disappeared and the tubes and stroma have taken their place." . . . "From the evolutionary standpoint this seems a very puzzling problem," which it certainly is.

Mummery's figures disclose no cytological detail, but, in so far as his conclusions are based on concurrence with Tomes' deductions from the enamel organ of the Hake, they are negatived by the evidence I have advanced in this communication.[1]

Since the ameloblasts maintain their individuality throughout the whole period of the formation of the enamel cap, it follows that the fully-developed enamel is the product of certain changes taking place in the ameloblastic secretion, which at first occupied the vacuoles in the ameloblasts, and then passed through the inner ameloblastic membrane to be deposited on the surface of the dentine. The substance occupying the vacuoles is clear, taking the acid stain faintly;

[1] See addendum.

the inner ameloblastic membrane persists throughout the whole life-history of these cells, but the ameloblastic secretion having passed through the membrane, is intensely basophile (Pl. 22, fig. 6, *a.s.*). The deposition of this secretion commences immediately after the formation of the first thin layer of dentine and prior to the vacuolation of the ameloblastic layer to form the appearances seen in Pl. 22, figs. 2, 3, and 4, it then goes on continuously and rapidly, so that the fully calcified cap is completed long before the tooth attains its full size and becomes functional.

When but a small amount of ameloblastic secretion has been deposited on the surface of the dentine, as in Pl. 24, fig. 20 (*e*), it forms a sharply-pointed cap, which is easily distorted in shape and easily detached from the dentine. This cap exhibits a spongiform structure in its central area (Pl. 24, fig. 21), the meshes being basophile; towards the periphery the staining takes place much more deeply, and the structure is much more regular, in many areas presenting a regular honeycomb skeleton of organic matrix.

Since these changes take place in a transparent fluid secretion and an organic framework first appears, which eventually undergoes solidification, with the final assumption of a prismatic crystalline structure, it seems reasonable to advance the interpretation that the changes leading to the ultimate solidification and complete calcification of the products of the activity of the ameloblasts are purely physico-chemical changes similar to those known to be passed through by other colloids in their solidification, e.g. a coagulation or gel-formation of the secretion first takes place, the structure of the gel depending on the nature of the protein in the secretion; this precipitation of a large amount of the organic material causes a greater concentration of the lime salts in the fluid occupying the interspaces, and calcification then progresses until the organic matrix is almost, if not entirely, calcified.

Twenty years ago Prof. G. C. Bourne, in his paper "On the Calcareous Skeleton of the Anthozoa," wrote:

"We are ignorant of the laws which govern the formation of these organic crystalline growths as we are of the molecular laws which determine why a given mineral solution shall crystallise out according to a given system."

Much work remains to be done, but to-day, owing to the great advances in our knowledge of colloidal chemistry, we are no longer in complete ignorance of these laws, and with regard to enamel we are in a position to protest yet more strongly "against the discovery of a Deus ex machina in the form of calcified cells."

My thanks are due to Mr. Pittock, of the Department of Zoology, University College, for his kind assistance in the preparation of material, and to Miss M. Rhodes, of the Lister Institute, for the drawings used in illustration of this paper.

Addendum.

I have had no opportunity of examining the genera employed by Dr. Mummery, but, since the proofs of this paper have been in my hands, I have looked over a complete series of sections of the jaws and pharyngeal plates of Pagellus centrodontus and of Labrus bergylta, which I took at sea this autumn and fixed immediately.

My material lends no support to the views put forward by Mummery, for, though the enamel organ becomes richly vascular, the vessels assuming the arrangement figured by him, in place of his "glands with distinct central ducts" and his "tubes," I find that the ameloblasts persist throughout the whole period of enamel formation, separated from the capillaries—which have well-defined endothelial walls—by the cells of the external epithelium. Further, the cytoplasm near the forming enamel becomes modified to constitute the inner ameloblastic membrane, the interspaces between contiguous cells being closed by the development of a structure which, so to speak, cements these areas together, and gives to a section a pattern of well-defined polygonal outlines, each polygon corresponding to an individual ameloblast.

EXPLANATION OF PLATES 22, 23 and 24,

Illustrating Mr. J. Thornton Carter's paper "On the Cytomorphosis of the Enamel Organ in the Hake."

[All the drawings were made direct by means of the Abbe camera lucida.]

The following is a list of the reference letters common to the various figures: *a.* Ameloblasts. *a.s.* Ameloblastic secretion. *c.m.t.* Cytomitoplasm. *d.* Dentine. *d.p.* Dentine papilla. *e.* Enamel. *ee.* External epithelium. *i.a.m.* Inner ameloblastic membrane. *n.* Nucleus. *o.e.* Oral epithelium. *vac.* Vacuoles.

PLATE 22.

Fig. 1.—Longitudinal section through a tooth-germ prior to the formation of the shoulder of dentine on which the base of the enamel cap will rest. The ameloblasts (*a.*) present a columnar form and the outlines of individual cells are quite distinct. × 70.

Fig. 2.—Portion of a longitudinal section through the enamel organ. The outlines of the individual cells have disappeared in the area between the nuclei (*n.*) and the secreting surface (*i.a.m.*), though still visible in the area between the bases of the cells and about the nuclei. × 450.

Fig. 3.—Portion of a longitudinal section through the enamel organ. The outlines of individual ameloblasts have now disappeared due to the formation of vacuoles (*vac.*) in the cytoplasm and the nuclei lie at various levels. × 450.

Fig. 4.—Transverse section through the enamel organ showing the vacuolation of the cytoplasm, the vacuoles (*vac.*) being separated by a cytomitoplasm (*c.m.t.*). Towards the secreting surface the vacuolation disappears and there is a layer, finely granular in structure, in which may be discerned the faint outlines of the ends of the individual ameloblasts. × 450.

Fig. 5.—Portion of a longitudinal section through the enamel organ. The vacuoles seen in the preceding figure are now disappearing owing to the passage of their contents through the inner ameloblastic membrane (*i.a.m.*) and the nuclei (*n.*) have been carried down almost to the secreting surface. The restoration of the outlines of the individual ameloblasts is faintly foreshadowed. × 450.

Fig. 6.—Transverse section through a tooth-germ showing the central core of dentine (*d.*) surrounded by the forming enamel (*a.s.*), beyond which lie the ameloblasts (*a.*) invested by the cells of the external epithelium (*e.e.*). × 70.

Fig. 7.—Longitudinal section through a portion of the enamel organ in which the outlines of individual ameloblasts have become visible again, all trace of vacuolation having disappeared. The nuclei no longer lie at the extreme ends of the cells but are distributed at various levels. × 450.

PLATE 23.

Fig. 8.—Longitudinal section through a portion of the enamel organ showing the transition of the cells responsible for the enamel cap (seen at the stage described in fig. 5.) into the cells which invest the dentine of the body of the tooth. × 300.

Fig. 9.—Longitudinal section through a portion of the enamel organ at a point lower than the enamel cap where no enamel is formed and corresponding to the shorter cells (*a.*) figured in the preceding illustration. A portion of the enamel cap (*e.*) is seen, also fragments of dentine (*d.*) × 400.

Fig 10.—Longitudinal section of the enamel organ showing the ameloblasts (*a.*) arranged in a series of loops with the cells of the external epithelium (*e.e.*) inserting their cytoplasm between these loops. × 250.

Fig. 11.—Transverse section through the enamel organ showing the cells of the external epithelium (*e.e.*) inserting themselves between the ameloblasts (*a.*) and thus forming a furrow. × 300.

Fig. 12.—Longitudinal section of a portion of the enamel organ from over the apex of a tooth showing the cytoplasm of certain cells of the external epithelium (*e.e.*) extending inwards and the ameloblasts beneath undergoing rarefaction. × 450.

Fig. 13.—Transverse section through a portion of the enamel organ stained to show the outlines of the cells of the external epithelium (*e.e.*). The outlines of the ameloblasts are not visible, and the drawing is not continued inwards sufficiently far to show their nuclei. × 450.

Fig. 14.—Longitudinal section through a tooth-germ showing an ingrowth of the oral epithelium (*o.e.*), which, passing over the apex of the tooth extends down to its base, inserting itself between the external epithelium and the surrounding connective tissues. × 50.

PLATE 24.

Fig. 15.—Longitudinal section through the apex of a tooth-germ showing the top of the enamel cap (*e.*) with its investing ameloblasts (*a.*). An ingrowth of the oral epithelium (*o.e.*) is seen which in close proximity to the tooth-germ appears in the drawing as a double row of cells. × 200.

Fig. 16.—Longitudinal section through the enamel organ in the area of the cap. The ameloblasts (*a.*) are seen becoming separated into groups or nests owing to the ingrowth of the cells of the external epithelium (*e.e.*). Extending from the surface into contact with the external epithelium is an ingrowth of the oral epithelium (*o.e.*). × 80.

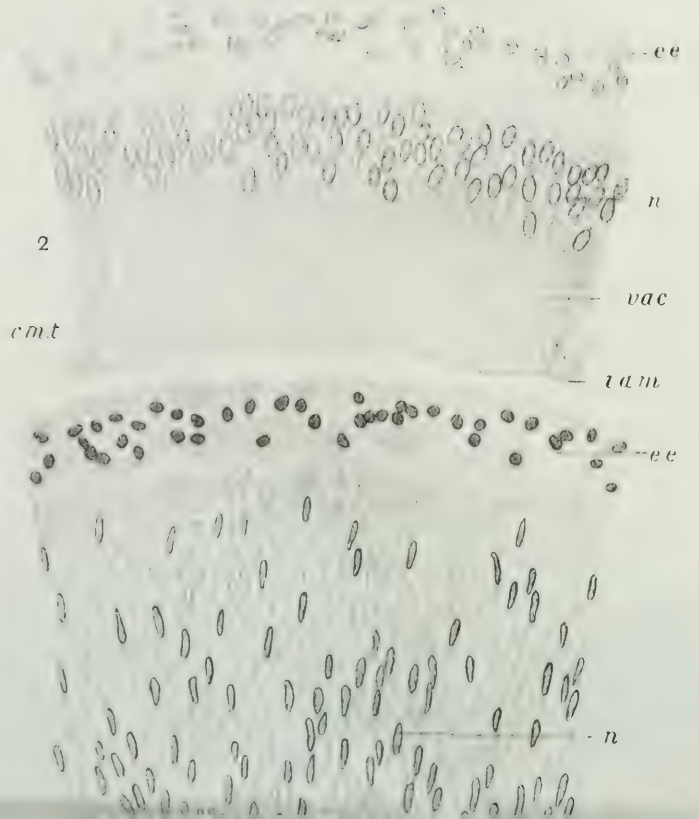
Fig. 17.—Transverse section through an ingrowth of the oral epithelium such as shown at *o.e.* in the preceding figure. The ingrowth is seen to present an irregular outline corresponding to extensions of the investing connective tissues. × 250.

Fig. 18.—Longitudinal section through the apex of a tooth which has just emerged through the overlying tissues, showing the dentine (*d.*) with its shoulder and central core to afford support to the enamel cap which is partly dissolved, its tip being seen at *e*. There is no trace of the columnar ameloblasts seen in preceding figures, but their site is occupied by a mass of rounded cells (*a.*) continuous with the flattened cells investing the dentine below the shoulder. × 70.

Fig. 19.—A portion of the area (*a.*) of the preceding figure showing the rounded cells, which are the original ameloblasts undergoing cytolytic changes. × 450.

Fig. 20.—Longitudinal section through a developing tooth showing the first formed layer of dentine (*d.*) surmounted by the forming enamel cap (*e.*). × 120.

Fig. 21.—A portion of the developing enamel seen in the preceding figure showing its spongiform structure. × 450.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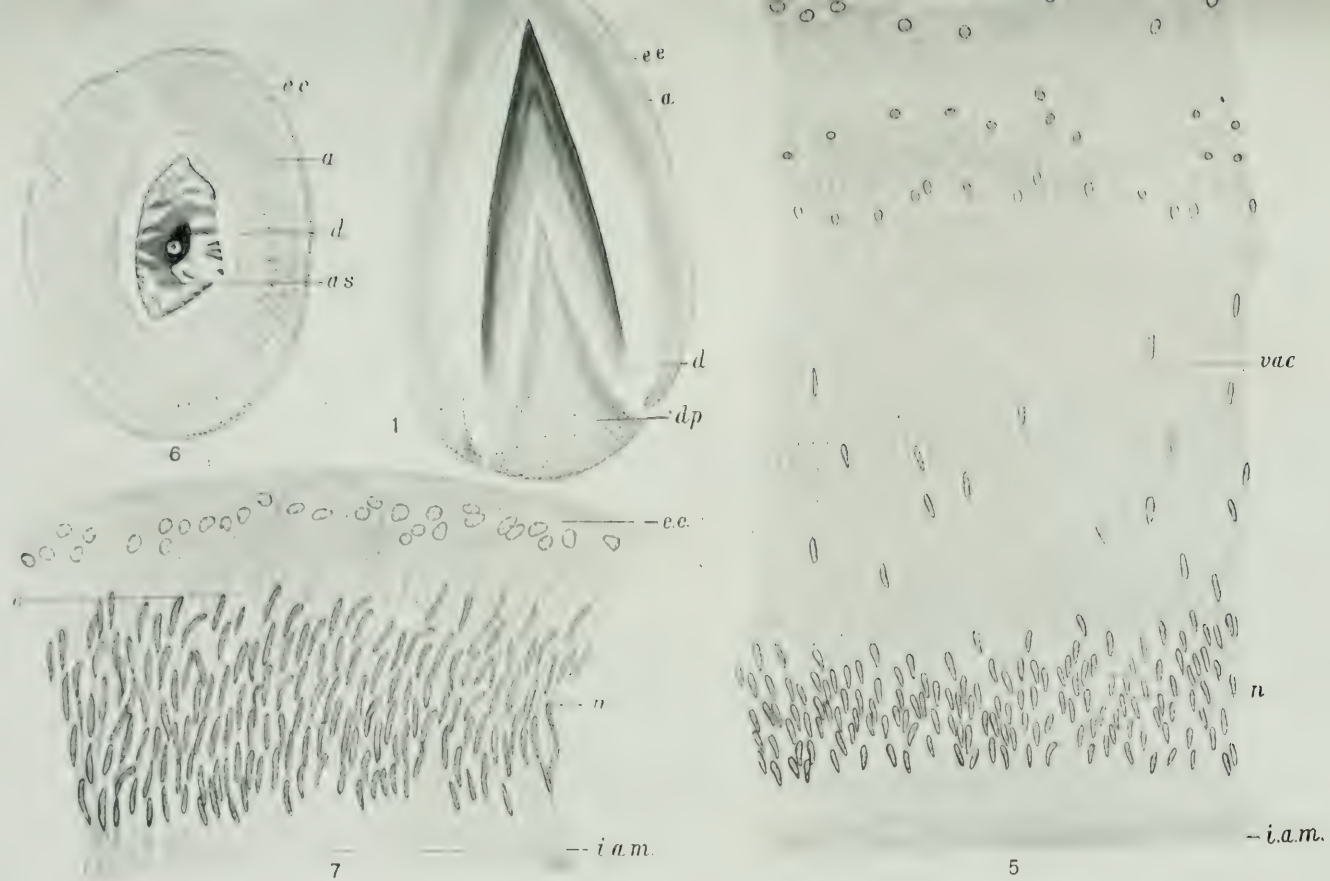
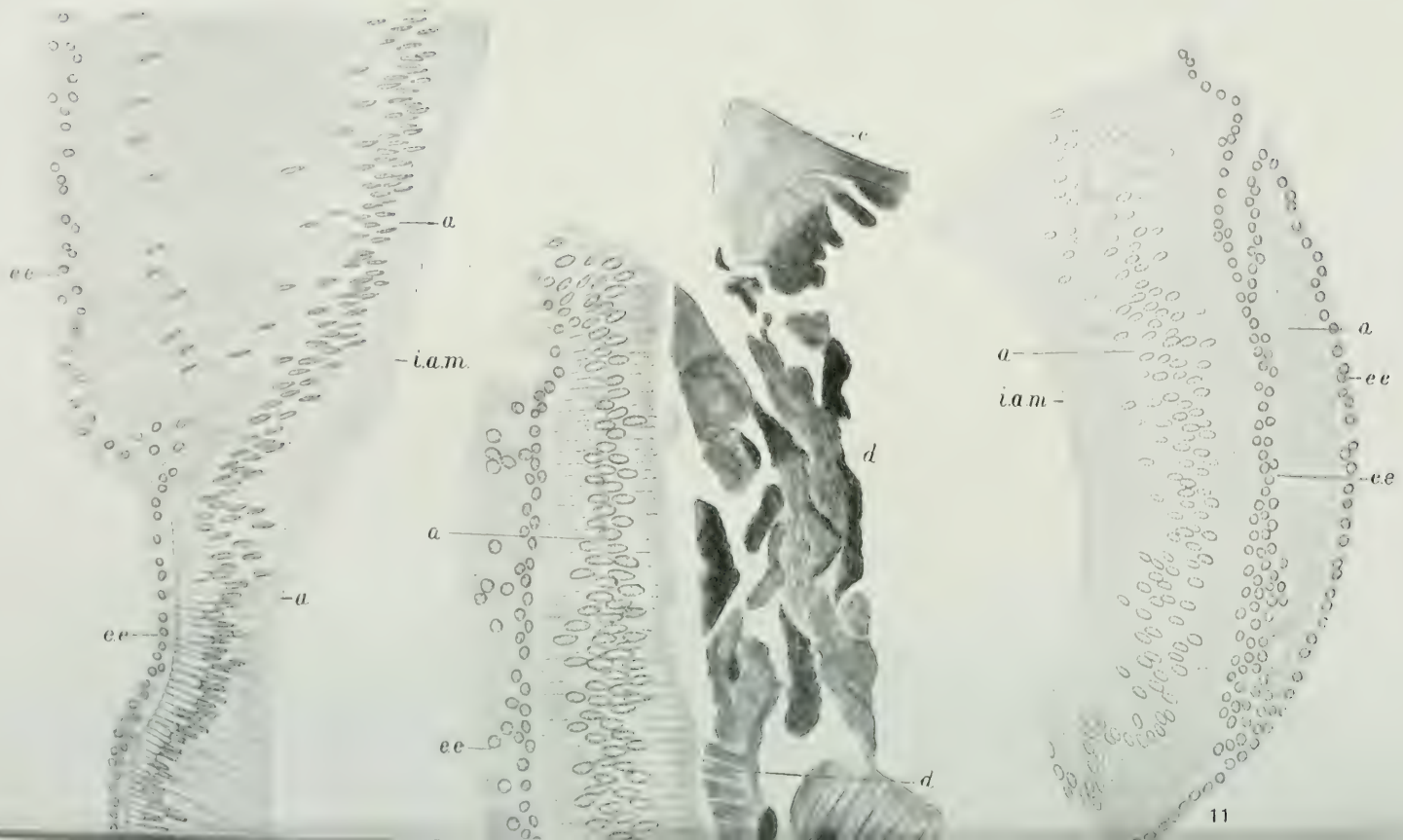


CARTER TOOTH OF HAKE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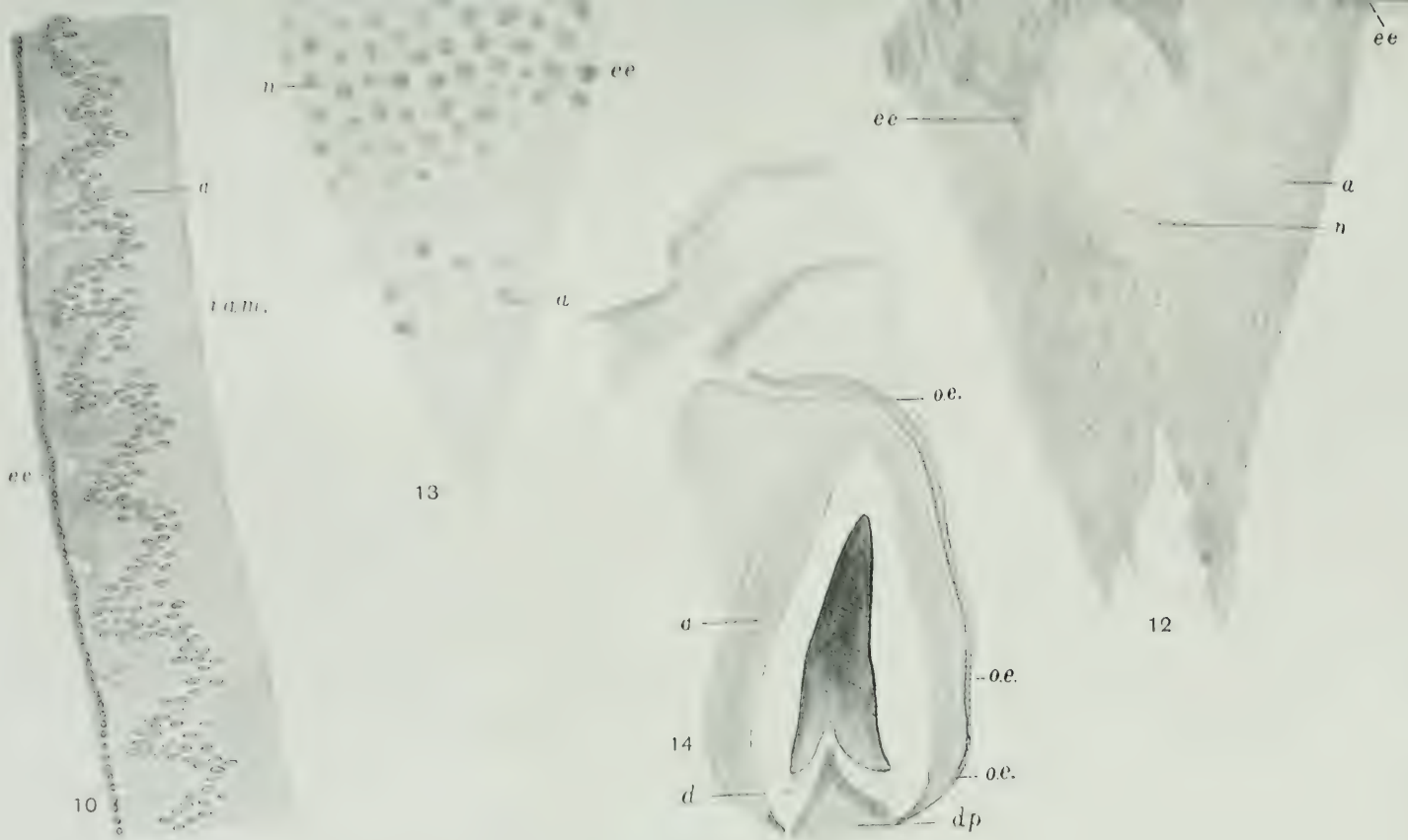

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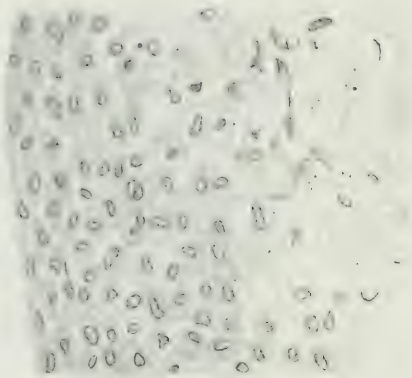

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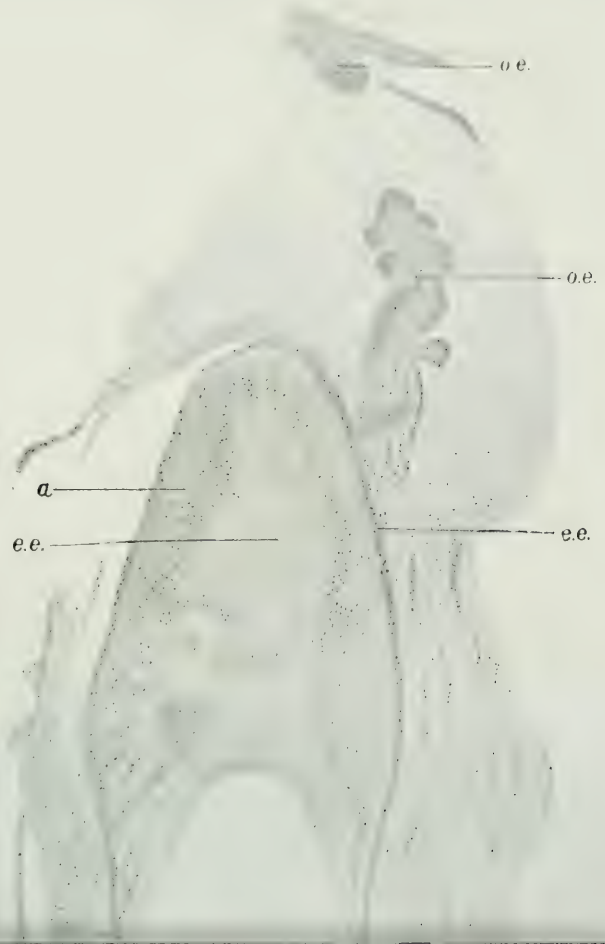

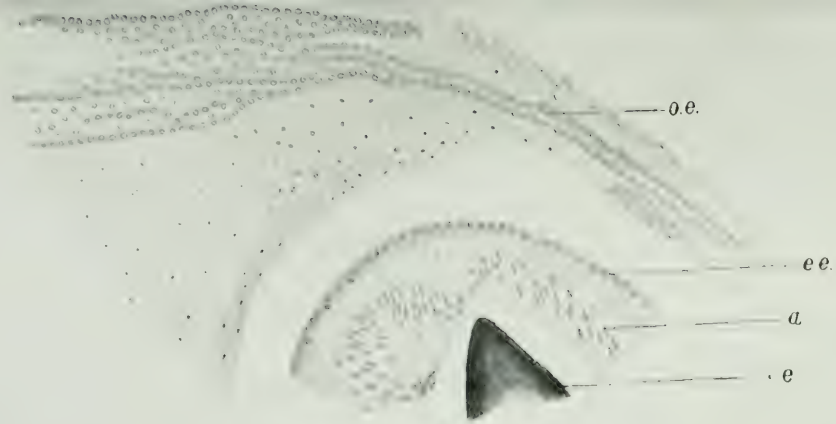

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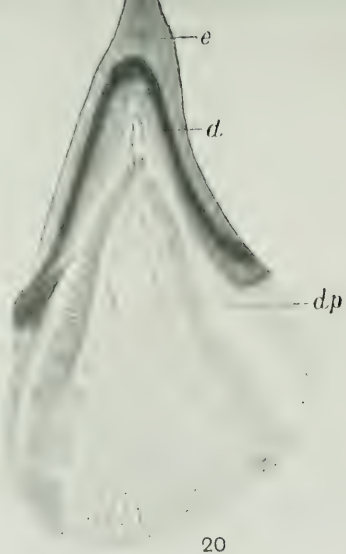

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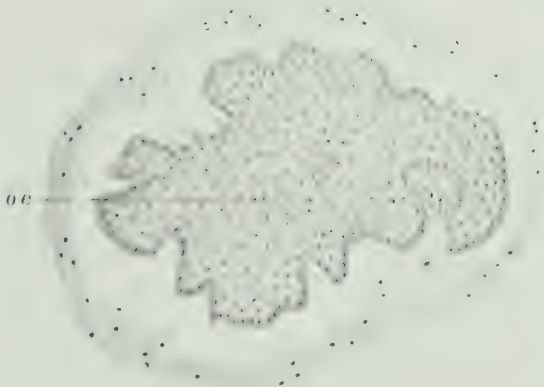

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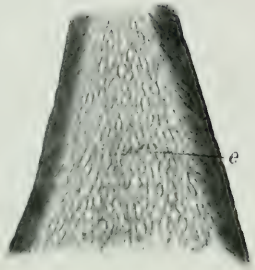

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CARTER—TOOTH OF HAKE

The Cytoplasmic Inclusions of the Germ-Cells.

PART IV. NOTES ON THE DIMORPHIC SPERMATOZOA OF PALUDINA AND THE GIANT GERM-NURSE CELLS OF TESTACELLA AND HELIX.

By

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With Plates 25 and 26 and 21 Text-figures.

TABLE OF CONTENTS.

Introduction.

In this paper, the fourth of this series, I have given an account of the origin of the peculiar atypic sperms of Prosobranchia, and I have also described the remarkable behaviour of the nurse- or yolk-cells of several Pulmonate Mollusca, a peculiar phenomenon hitherto unnoticed, and of importance in any discussion on the nature of the chromatin.

Part I.—On the Recognition of at least Two Separate Categories of Cytoplasmic Elements.

About twenty years ago Golgi (**19**) discovered in the nerve ganglion an intra-cellular network surrounding the nucleus, which he named "apparato interno reticolare." Independently and about the same time the famous Spanish observer Cajal also described this network. Subsequent work showed that this structure was not always in the form of a net, but might consist of branched or unbranched rods, granules, or curved semi-lunar bodies. This in itself did not come very far into the province of the zoologist, but the appli-

cation of Golgi and Cajal's silver nitrate methods and the method of Kopsch to glands and tissues from every part of the body of both vertebrates and invertebrates and to Protozoa showed that this network or its representative was present in every cell; furthermore, modern cytology shows that the Golgi apparatus is, morphologically and chemically, distinct from the mitochondria, and methods which show one category of cell-organs may not and often do not show the other. The technique of the Golgi apparatus has been treated elsewhere. Text-figs. 1–14 give some impression of the universal occurrence of the two categories of cell-bodies —mitochondria and Golgi apparatus; here are drawn nerve, thyroid, spermatogonial, pancreas, hepatic, gastric, protozoan (Monocystis), fat, epithelial (Descemet's) cells of various species, which are partly taken from my own preparations and partly from the figures of other workers. The morphology and histo-chemistry of this apparatus I have studied in another paper. In the oögenesis of several forms properly studied a remarkably developed Golgi apparatus is found; this is the subject of a further communication to be published by me. In the resting cell the Golgi rods or grains may lie upon the archoplasm with which they are generally associated (Text-figs. 1, 3, 4, 5, 6, 11, etc.), but in the prophases of mitosis these rods or grains become sorted out into two groups around the centrosomes as in Text-fig. 12 (upper). In the metaphase and other stages of mitosis the Golgi apparatus keeps around each aster as in Text-fig. 12 (lower), and so each daughter-cell gets one-half approximately of the rods, granules, or branched structures of the mother-cell. Be it noted that the rods do not split like chromosomes, but go over bodily to one or the other daughter-cell. Perroncito (**35**) calls this sorting out at mitosis "dictyokinesis,"[1] the dictyosome or dittosome being a Golgi rod. My chondrioplasts in snails and slugs (**15**, **16**) are evidently Golgi apparatus, as I have been able to show by histo-chemistry and other sources of proof and experiment.

[1] *δικτύον*, a net.

Technique and Material.

Paludina vivipara is quite common around Oxford. The snails were removed from their dish, the operculum drawn open, and the water in the shell shaken out; one drop of chloroform was placed on the head of the animal, and the operculum allowed to close. Immediately afterwards the upper whorls were broken with forceps, and the testis dissected out and cut into pieces about 4 mm. square, if the fixation was to be chrome-osmium.

I used the modification of Flemming without acetic acid, slightly diluted or strong. Also Champy's fluid in the same way just as described in my paper on Helix aspersa ('Quart. Journ. Micr. Sci.,' vol. 62, p. 563, last paragraph).

Besides the methods used in this previous paper, I used especially Kopsch's method (**25**); in this, fresh small pieces of gonad are dropped into about 6 cubic centimetres of 2 per cent. osmic acid in distilled water. They are left here in a dark place for fourteen days, after which they are washed overnight in running water and then through the alcohols—xylol—and embedded. As suggested by Hirschler (**23**), such sections stain well after this method in Altmann's acid

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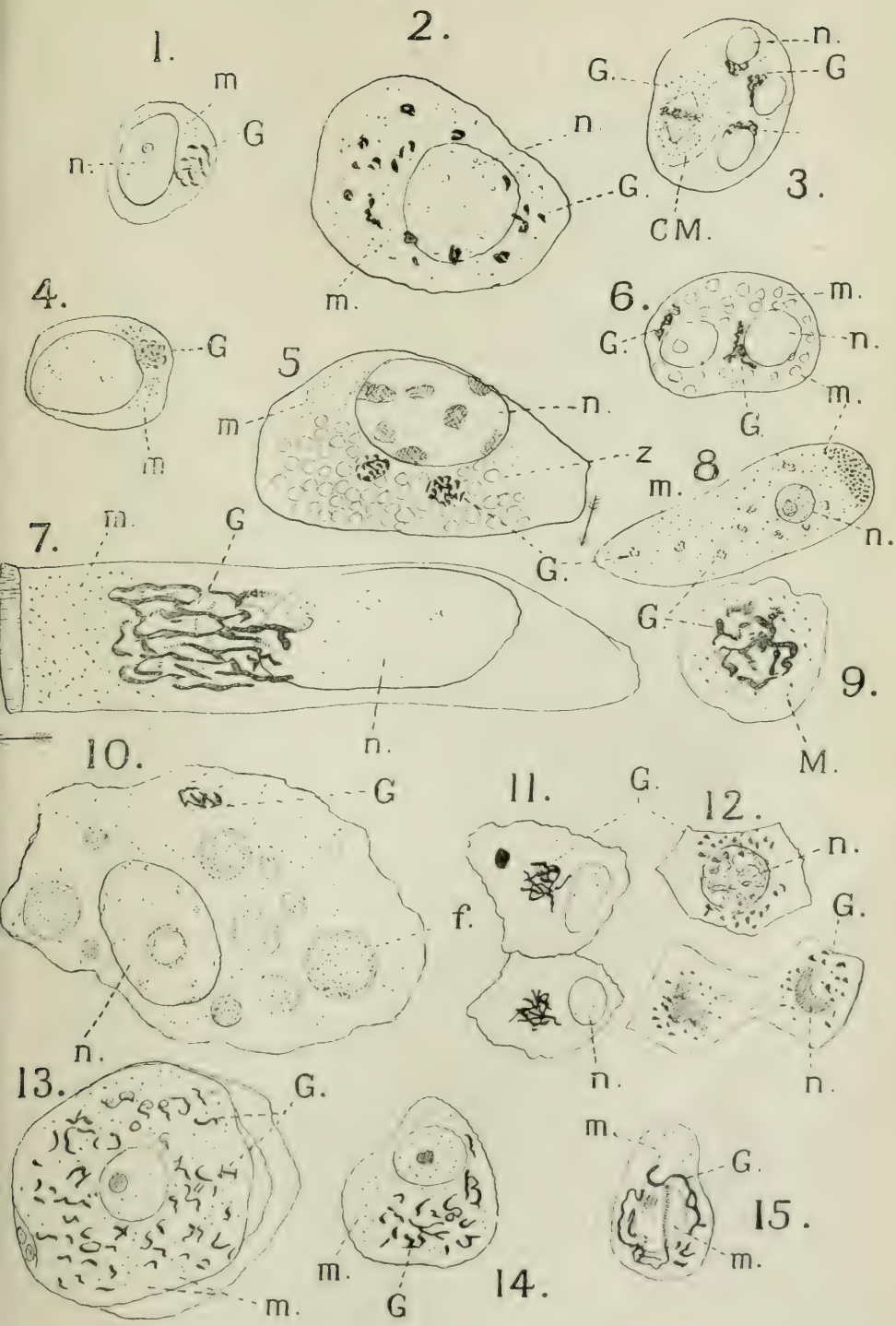
Description of Text-figs. 1–15.

Figs. 1, 2, 4, 5, 7, 8, 9, 10, 13–15, by Kopsch's method; Figs. 3, 6, 11 and 12, by formol silver-nitrate method of Cajal or Golgi; Figs. 1, 2, 4, 5, 7, 9 and 10, original. Fig. 1.—Cerebellar nerve-cell of Bufo vulgaris. Fig. 2.—Spinal ganglion cell of B. vulgaris. Fig. 3.—Thyroid epithelial cell of rat, showing Golgi apparatus in resting cells and one in mitosis (Cajal). Fig. 4.—Spermatogonium of Molge vulgaris. Fig. 5.—Pancreas-cell of M. vulgaris. Arrow points to acinar lumen. Fig. 6.—Liver-cells of rat, after Pappenheimer. Fig. 7.—Intestinal cell of M. vulgaris. Fig. 8.—Monocystis ascidiæ (Hirschler). Fig. 9.—Intestinal cell of Molge, cut transversely across Golgi reticulum. Fig. 10.—Fat-cell of Molge. Fig. 11.—Descemet's membrane cells in rest. Fig. 12.—Same in mitosis (dictyokinesis); compare fig. 3 (Deineka). Figs. 13 to 15.—After Cowdry; nerve-ganglion cells of pigeon, showing diffuse (fig. 13), excentric (fig. 14) and circumnuclear (fig. 15) arrangement of Golgi apparatus. Letters as follows: *G.* = Golgi apparatus. *C. M.* = cell in mitosis. *f.* = fat. *n.* = nucleus. *M.* = mitochondria. *z.* = zymogen granules.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fuchsin-picric acid. The preservation of Kopsch sections is often atrocious, but the method impregnates very intensely the so-called Golgi apparatus of histologists, known to zoologists as "Nebenkern" batonettes, chondrioplasts, dictyosomes, etc. (**2, 4, 9, 13, 15, 33**).

The Dimorphic Spermatozoa of Paludina vivipara.

In the following description I have clearly shown for the first time that the two series of spermatozoa of P. vivipara originate from cells whose mitochondria are either finely granular, or are few and banana-shaped, or more rarely very large and spherical. From the first sort of cell, with finely granular mitochondria, originate giant or atypic sperms; from the second sort, with banana-shaped rods, originate the typic or ordinary spermatozoa. I have identified positively such cell series back to the primary spermatogonia, but, though I have advanced strong evidence for believing that the germinal epithelial cells are also of two sorts, further work is being undertaken on the embryonic gonad in order to settle the question definitely.

Meves, in his latest paper on Paludina, has failed properly to describe the difference between the atypic and typic spermatogonia and spermatocytes. As will be seen, these differences constitute one of the most remarkable facts in our knowledge of the mitochondria in gametogenesis—facts which are all the more noteworthy because we have hardly any clue as to why such differences should exist in cells closely associated in one organ (Meves, 'Arch. f. mikr. Anat.,' Bd. lxi).

The Typic Spermatogenesis of Paludina vivipara.

The germinal epithelium in Paludina is very difficult to understand properly, because, in the differentiated gonad, the epithelium has fewer cells, and regions of proliferation are scantier than in Helix. The yolk or nurse-cells are present, but fairly small, and they are much flattened against the layer

of Ancel; their yolk also is not remarkably well developed. Germinal epithelial cells are few in number, and nearly always covered over by a part of a yolk-cell, or embedded in a yolk-cell (Pl. 25, fig. 13, *Y.*, yolk-cell). The walls of the testis are very scantily covered when compared with the richly-provided alveoli of a Helix or Testacella ovotestis. The germinal epithelium is not a syncytium, its cell elements being discrete.

In Pl. 25, figs. 13 and 14, are drawn two typical germinal epithelial cells; these are not at all easy to find, but they are extremely characteristic, and easily identified when once discovered. Such cells contain a slightly polymorphic nucleus, staining somewhat palely; there are generally two or more net-knots or karyosomes, though the nuclear reticulum is often hardly demonstrable.

In the cytoplasm it is generally possible to identify a mass of mitochondrial matter formed apparently of a number of rods, either separate or closely clumped together.

It is a very difficult matter to get cells in which the rods show so well as in Pl. 25, figs. 13 and 14, these cases being the best fixed and stained in a preparation.

It is quite impossible to count the number of these mitochondrial rods; they are never straight, but are nearly always S-shaped or even more sinuously twisted. In Pl. 25, fig. 14, the rod *M.R.X.* was easily drawn in with a camera lucida, and the presence of these structures I think to be indisputable.

Such primary male cells give rise by indirect division to large bunches of secondary spermatogonia; the diploid chromosome number appears (fourteen, as in Pl. 25, fig. 16), and a series of rapid divisions ensues. If a group of such cells at this stage be examined, it will be found that in the cytoplasm the mitochondrial rods have unravelled, and now form a number of separate, confusedly bent structures, as in Pl. 25, fig. 16, *M.R.* Every cell contains numbers of these rods, and by gently focussing up and down, a rod can be followed at least part of its way through the cytoplasm.

Pl. 25, fig. 16, is very typical, and illustrates how difficult it is to learn anything with regard to the number and mode of disposal of the mitochondrial rods. Such cells, after a series of divisions, enter growth; a reticulum appears in the nucleus, and this soon breaks into a number of faintly-staining chromatin loops; in this state the nucleus grows. After anaphase of the secondary spermatogonial division, the rods become grouped towards one side of the nucleus—as will be seen later, really around an archoplasmic zone, which has embedded on its surface Golgi rods (chondrioplasts, Nebenkern batonettes, dittosomi) (see Pl. 25, figs. 1 and 2, *A.R.*). The mitochondrial rods at this stage are much thicker shorter, and they are crescentic or U-shaped. There appear to be more than six or seven in every case I could see plainly enough to make an estimate. In Pl. 25, fig. 2, is drawn a view of the end of a cell such as that in Pl. 25, fig. 1; the rods are stumpy, all U-shaped, with their free ends generally towards the observer, and I was able to count at least thirteen.

Another element which was quite plain directly after anaphase was the archoplasm and the chondrioplasts; these were possibly eight in number, though it was very difficult to make a certain count. In the growth stage the mitochondrial rods constantly occupied the position indicated in Pl. 25, figs. 1 and 3. As has already been mentioned, the nucleus, from a very early stage, contained a number of loops. Soon these begin to form the synaptene stage, as has already been described by Meves. The loops pairing become resolved into chromosomes, as in Pl. 25, fig. 4, *C.H.* In no case did I find the spermatocyte reach a greater comparative size than that drawn in Pl. 25, fig. 3 (compare the atypic spermatocyte in Pl. 25, fig. 20).

I feel certain that at the prophases of the maturation mitoses the mitochondrial rods lengthen, so as to be as long as those drawn in Pl. 25, fig. 4 (compare this with the previous figure). The first spermatocyte division, seen as a polar view in Pl. 25, fig. 4, is drawn from the side in Pl. 25, fig. 5. The

irregularity of disposition of the mitochondrial rods is very evident; they meander over and around the mitotic figure in such a complicated manner that to count their number accurately was impossible; it is extremely difficult to tell where one rod begins and where it ends. In Pl. 25, fig. 4, there were at least seven rods, in Pl. 25, fig. 5, apparently nine. I am unable positively to say how the rods are disposed in division—that is to say, whether or not they are always divided in half at each division.

In Pl. 25, fig. 6, is a second maturation division at telophase; in all such stages it was impossible to count the rods in a satisfactory manner—I thought there were at least seven in this cell. I feel certain that at this stage they were not disposed regularly, so that one half the rod was in one cell, the other half in the other daughter-cell. In a later stage examination of the testes always revealed that at the latter end of telophase some of the mitochondrial rods were divided, as shown in Pl. 25, fig. 7, *C.M.R.*, by a constriction at the equatorial plate. I found such figures in both first and second spermatocyte divisions. If the cell in telophase was examined from the end as in Pl. 25, fig. 10, it was often possible to count four rods. In this figure some idea of the difficulty of counting the rods can be gleaned; the rods, *M.R.Z.* and *M.R.Y.*, are so twisted that it would be impossible to count them from such a cell viewed from the side; by focussing down one also came to the rod *M.R.X.*, and another above, but it should be pointed out that I was unable to say whether, for example, rod *M.R.Y.* was separate from or intercontinuous with rod *M.R.X.*

In fact I am convinced that to make certain as to the behaviour of these rods one must examine a more favourable species. In the spermatid (Pl. 25, fig. 8) one can often find a cell where the rods are shortening, and, though still bent, can be counted easily. Their number is nearly always four; the archoplasmic mass and the chrondrioplasts become evident after division, and lie beside the nucleus.

Concurrent with changes in the nucleus of the spermatid

heralding the formation of the spermatozoon, the mitochondrial rods become at first banana-shaped, then ovoid, and then quite spherical. Nearly always there are four—rarely five or six. I have never found fewer than four.

Spermateleosis in the Typic Spermatogenesis.

After the contraction of the mitochondrial rods to form the spheres it is very easy to count their number even with a $\frac{1}{6}$th objective. In 99 per cent. of cases the number is four, in 1 per cent. it is either five, six, or seven (Text-fig. 20).

The spheres are arranged almost always as in Pl. 25, fig. 9, and they soon fuse to form a solid mass. This mass elongates as in Pl. 25, figs. 11 and 12, to form the mitochondrial tail of the typic spermatozoon. No portion appears to be sloughed off, but the archoplasm with its two or three chondrioplast rods is rejected. In Pl. 25, fig. 25 A, is drawn the adult typic spermatozoon at half the scale of the other figures of the typic spermatogenesis; from $M.^1$ to $M.^2$ is the mitochondrial part; in front is the spirally twisted nucleus (*N.*); behind the region $M.^2$ the axial flagellum bare of mitochondrial matter.

With regard to the formation of the acrosome I was able to ascertain several facts; in Text-fig. 18 is drawn a part of a group of spermatids just after the fusion of the four or five mitochondrial spheres to form the macromitosome.

It will be seen that in front of each nucleus is adhering the archoplasm + chondrioplast apparatus, and apparently sticking into this is the acrosome. Each archoplasmic mass has, as far as one can see, two chondrioplasts (Golgi rods).

In Text-fig. 19 another drawing of this stage is given at a higher power. Apparently this relationship between nucleus and archoplasm is of short duration; afterwards the archoplasm drifts downwards as shown in Pl. 25, fig. 12 *AR*.

Note on Formation of Acrosome in Paludina and other Forms.

In Lepidopterous insects I showed that certain rods (Golgi apparatus ?) found in the spermatocyte and spermatid swelled out to form spheres, and that it was from the latter that the acrosome appeared to be formed. Re-examination of new and of my old sections reinforces me in my opinion. Apparently the acroblasts of Lepidoptera are homologues of the chondrioplasts of Mollusca, and possibly of the inner Golgi network of the nerve cell.

There is now the evidence of Schitz (**42**) and of myself that in Molluscs the acrosome is formed somehow from the archoplasmic body upon which lie the chondrioplasts. Schitz prefers to believe that the acrosome in Columbella (**42**) is formed from a "graine siderophile" which is really one of the centrosomes.

It is to be noted that in Paludina and Columbella, which are Prosobranchs, there is only one centrosome which has been identified with certainty—that immediately behind the nucleus. In Pulmonata there are two, as explained by me in my work on Helix, but in Pulmonates it seems from certain stages that the acrosome is formed like that of Paludina and Columbella; how, then, could the "graine siderophile" of Columbella be the centrosome, when in Helix and other Pulmonates the two centrosomes have been otherwise accounted for, and yet the acrosome is formed in association with the archoplasm-Golgi apparatus as seems to occur in Paludina and Columbella? It appears that Schitz is wrong in interpreting his "graine siderophile" as a centrosome.

The Atypic Spermatogenesis of Paludina vivipara.

In not all the epithelial cells are the mitochondria rod-shaped. They are generally hard to distinguish in any but the most perfect preparations, but it seems certain that there

are forms of epithelial cells whose mitochondrial apparatus is not formed of rods, but of granules. In such forms the nucleus and other cell elements are similar to those of the cells containing long mitochondria. I consider that the germinal epithelial cells are in the testis of two sorts, one giving rise to typic, the other to atypic spermatozoa.

In Pl. 25, fig. 17, I have drawn what I think is a cell which will ultimately give rise to atypic spermatozoa.

Such cells divide to give rise to secondary cells whose general appearance closely resembles the secondary spermatogonia of Helix (**15**).

In the germinal epithelial cells which I think give rise to the atypic series, there is always to be seen a large archoplasm which is, I believe, never so conspicuous in the cells containing rods. In Pl. 25, fig. 15, is drawn a secondary cell (secondary spermatogonium) containing an archoplasmic mass at *A.M.* with mitochondria on it. From this stage and ever afterwards there can be no doubt as to the differences between the atypic and typic series. In Pl. 25, fig. 18, is a later atypic spermatocyte just after entry into the growth stage. The archoplasm is now seen to be studded over with a number of Golgi rods (chondrioplasts), and around it are very many granular mitochondria (*G.M.*). A later stage is drawn in

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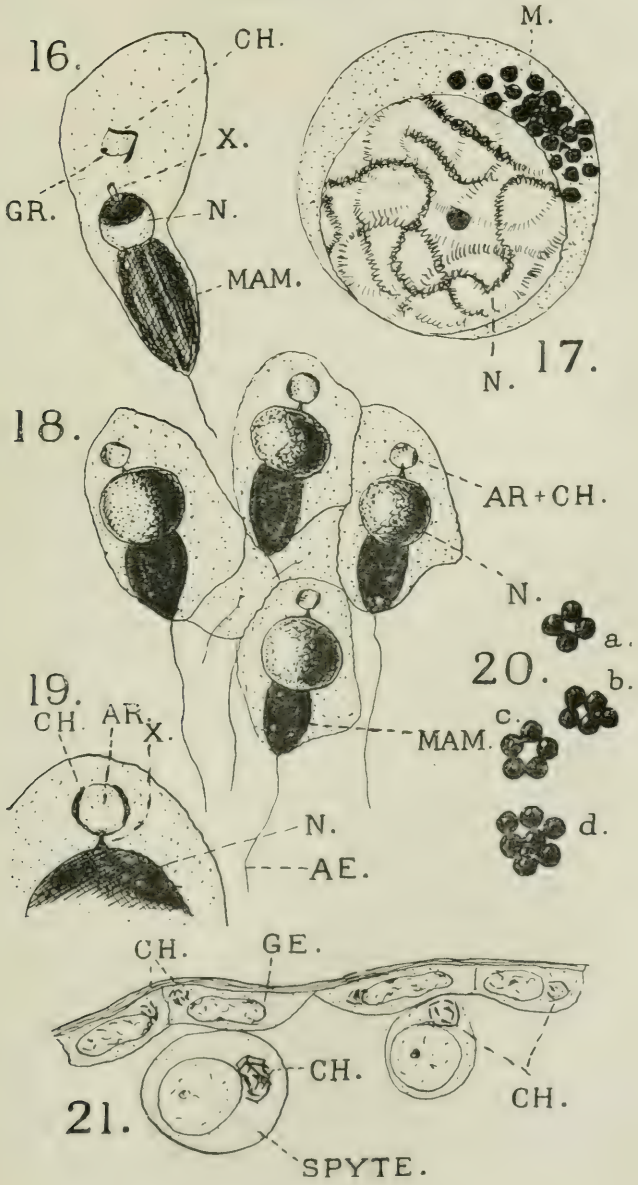
DESCRIPTION OF TEXT-FIGS. 16–21.

Fig. 16.—Spermatid of Columbella, to show formation of acrosome from a grain (*GR.*) embedded in the archoplasm (*CH.*). The grain will become stuck on to the protruding part of the nucleus at *X*. *MAM.* = macromitosome (mitochondrial). After Schitz (**42**). Fig. 17.—Typic spermatocyte of P. vivipara to show another form of mitochondria. Instead of rods the cell contained about twenty-six coarse spheres. Fig. 18.—Formation of the acrosome in P. vivipara. The archoplasm (and its chondrioplasts) is in each case lying in front of the nucleus, and, as is shown in the next figure (fig. 19), appears to be secreting the acrosome at *X*. *N.* = nucleus. Fig. 20, *a*, *b*, *c* and *d*.—Variations found in the mitochondrial spheres of the spermatid, just before they fuse to form the macromitosome, *MAM.* in fig. 18. *AE.* = axial filament. Fig. 21.—Germinal epithelium (*G.E.*) of Helix aspersa, prepared by Kopsch's method, to show Golgi apparatus (*CH.*) or chondrioplasts (Nebenkern) in indifferent cells and in spermatocyte (*SPYTE.*).

TEXT-FIGS. 16–21.



Pl. 25, fig. 19; this is a very characteristic cell; the archoplasm and its rodlets are very dense and around them are grouped the mitochondria, which are just like those of Pulmonates.

From this stage the cell does not generally grow very much, but in Pl. 25, fig. 20, is a larger specimen in the prophases of the first maturation division. The cytoplasmic inclusions have become grouped into two parts, evidently around the two centrosomes. The divisions which take place have been described at length by many authors (**26**, **30**, **39**). Meves may be consulted for a good description of the facts in Paludina, though personally I have not sought to examine thoroughly the nuclear or centrosomic phenomena during maturation of the atypic spermatocyte.

In Pl. 25, fig. 21, is a second maturation division of the atypic series; the mitochondria are scattered here and there in a haphazard manner, just as are the chromosomes.

Before passing on to the spermateleosis of the atypic cell it should be pointed out that the number of chondrioplasts in the typic spermatocyte is smaller than that of the atypic spermatocyte, while, as Meves showed, the archoplasm in the latter is the larger (compare Pl. 25, figs. 2 and 19).

The atypic spermatid contains approximately one-fourth of the mitochondria of the spermatocyte. Directly after telophase of the second maturation division the cytoplasmic inclusions are closely drawn around the centrosomic region to form a thick mass; the chromosomes do not all resume a reticulate shape, some appear to fuse, and generally two or more, rarely three, pale abnormal nuclei result (Pl. 25, fig. 22).

One of these nuclei becomes placed in front of the mitochondrial mass (*G.M.*) and the cell, then begins to metamorphose; the axial filaments (*F.L.*), as described well by Meves and others, now begin to grow out, and by a little later stage are quite long and like a tuft of hairs.

Just after the formation of the spermatid an examination of the cytoplasm reveals the fact that a faint, rather coarse

granulation is becoming evident. This granulation gradually becomes sharper in outline till the individual units are clearly defined. These grains correspond to Perroncito's "Mitochondria of Benda." In Pl. 25, fig. 23, these grains are clearly defined; the ordinary mitochondria at *G.M.* have collected to form a square mass, which later elongates with the growing filaments (*FL.*). In Pl. 25, fig. 24, the mitochondria have seemingly partially sloughed down the filaments in the region *AX*, which is nearly clear of them, to region *BX*, where they are still evident. The main bulk is at *G.M.* Perroncito's "mitochondria of Benda" are now quite evident, and the "nucleus" at *N.* is becoming somewhat shrunken and in parts more darkly staining. At *C.H.* is a mass formed mainly of rejected chromatin and chondrioplasts.

The final stages of spermateleosis in the atypic spermatozoon now take place; the "nucleus" in front shrinks, and if it does not altogether lose its previous character it at least changes greatly in size, shape and stainability.

The mitochondria which surround the multiple axial filamentary apparatus do not apparently slough off; at any rate, they are very difficult to discover in later stages. I can, however, say for certain that they take some part in the formation of the fully-formed atypic sperm, as some authors have already shown.

The axial filaments appear either to fuse, or at least to become so closely applied one to another as to cause the optical effect of a solid structure. In Pl. 25, fig. 25 B, the axial rod is shown stretching from the head to the tail, from which arise the brush of filaments; the so-called "mitochondria of Benda" have become very dense; it should be noticed that Pl. 25, figs. 25 A and B, are drawn at half the scale of the previous figures. Pl. 25, fig. 25 B, is drawn in optical section, the focus being brought on to the edge of the organism.

In Pl. 25, fig. 26, is drawn a part of the upper region of the atypic sperm to show the "Mitochondrialmantel" of Retzius at the same scale as the other figures on the plate.

The Golgi Rods or Chondrioplasts in the Atypic Spermateleosis.

Reference to Pl. 25, figs. 18–25, serves to show the fate of the Golgi apparatus in the atypic series spermateleosis. They finally slough off, as do those of the typic series, and I cannot find that they form part of either typic or atypic spermatozoon. I have examined ripe sperms in Kopsch's method in order to test this question. At no stage did I find an intercontinuous Golgi reticulum as drawn by Perroncito. The latter observer's work on the Golgi apparatus of Paludina is evidently biassed by his knowledge of Cajal's studies on the mammalian Golgi apparatus, where with formol-silver nitrate a proper reticulum seems demonstrable in nerve and other cells.

Possible Intermediate Forms Between Typic and Atypic Cells.

In some cases I have found a group of cells containing about twenty or thirty large spherical mitochondria as drawn in Text-fig. 17. These were much larger and fewer in number than the mitochondria of the atypic series, and I have no doubt that these cells were a variety of the form drawn in Pl. 25, figs. 1 and 2. The cell in Text-fig. 17 is a growing spermatocyte, but I never found round, coarse mitochondria like these in any other stage of spermatogenesis. Such mitochondria might elongate as they grow, but their main importance lies in the fact that they may constitute forms intermediate between atypic and typic spermatogonia and spermatocytes. In the Paludinas I examined such cells were rare.

Discussion.

In this paper I have shown that the well-known dimorphic spermatozoa arise from cells whose mitochondrial apparatus differs very remarkably. Possibly in no other animal is such a remarkable state of affairs existent. It has been established

that these remarkable differences between the cells of the two distinct series of spermatozoa are very early in origin, if not actually already present in the indifferent (undeveloped) germinal epithelial cells.

All the stages in the behaviour of the cytoplasmic elements of both typic and atypic cells have been followed out carefully for the first time. Meves spoilt his work by using a Flemming with acetic acid—a fault followed by Reinke; while Perroncito treats with the Golgi apparatus almost exclusively, and his main object seems to be to establish the fact that Paludina has a distinct Golgi reticulum in its germ-cells. Perroncito does not pay much attention to mitochondria; his claim that a true reticulum exists in Paludina spermatocytes I reject. The Golgi rods or chondrioplasts do not fuse to form a reticulum. Perroncito invents a good word for the sorting out of the Golgi rods between the daughter-cells—"dictyokinesis"—on the analogy of the word "karyokinesis." "Dictyosome" and "Dittosome" are the words used by Italian writers for the Nebenkern or Golgi batonette, but be it noted that Murray (**33**) was the first observer to describe dictyokinesis; he used the snail, and his work is a valuable contribution.

A. The Correct Identity of the "Chondriosomes of Meves" and "Mitochondria of Benda" in Paludina.

What Perroncito calls the "chondriosomes of Meves" are undoubtedly the ordinary mitochondria, and I have called them so throughout this paper. The identity of the "mitochondria of Benda" is a difficult matter. They appear in the cytoplasm, as far as one can make out, pari passu with the spermateleosis stages, from a cytoplasmic condensation. In Pl. 25, fig. 20, and in other such spermatocytes, the cytoplasm appeared smooth. No staining method revealed these granules in spermatocytes or even in young spermatids. The question then arises as to whether these bodies are really mitochondrial; it must certainly be mentioned that they stain

very like mitochondria. They are not, however, destroyed in corrosive acetic acid, and stain very densely with iron-hæmatoxylin after this fixative (**35**).

I am inclined to think that the granular bodies (mitochondria of Benda) are not true mitochondria, but may be in some ways chemically allied to the true mitochondria. More intense fixation and staining methods may possibly reveal them in earlier stages, but there is nothing in the typic series even remotely resembling the "mitochondria of Benda." It is possible that the granular bodies may be homologous with the "albuminous bodies" described by other authors for marine prosobranchs.

B. The Differentiation of the Two Kinds of Spermatozoa of Paludina vivipara.

I have shown that the two sorts of spermatozoa can easily be traced back to two sorts of spermatogonial cells, whose mitochondrial apparatus is quite different. I cannot feel quite so certain as to whether this difference exists among the primordial germinal epithelial cells.

It has been stated that the typic spermatogonia have rod-shaped mitochondria (chondriokonts), while the giant have granular spherical mitochondria (chondriosomes).

A word of warning must be written here with regard to this. We already know of cases where the mitochondria are able to be either granular or rod-shaped (vide Champy (**5**)). It is possible, though I think not very likely, that all germinal epithelial cells in Paludina have chondriokonts or rod-shaped mitochondria, and that this state persists when typic spermatogenesis is followed, and when the cells take the different path leading to the atypic state, the rods fragment and become granular. In the egg the mitochondria are nearly always fine and granular, and in other cases rod-like, but very fine, and this is undoubtedly connected with the metabolic processes carried out by the egg-mitochondria. Maybe, then, the atypic mitochondria become granular because of the peculiar meta-

bolic character of the atypic series; the latter cells become a good deal larger than the typic. It should, however, be mentioned that the main period of growth of the atypic series is during spermateleosis, i.e. the metamorphosis of the spermatid into the spermatozoon. We, however, have seen that at this period the mitochondria become clumped around the filaments, and evidently are not concerned in the growth of the spermatid. I do not mention here Perroncito's "chondriosomes of Meves," because their "mitochondrial" nature is a moot point.

A most remarkable fact which I wish to show here is that the growth-stage of the abnormal or atypic spermatocyte is, in a sense, the normal one; by this I mean to say that the atypic spermatocyte has a mitochondrial apparatus most like that in other animals. It is really the typic spermatocyte of Paludina that possesses the abnormal type—such enormous rods are rare; the vast majority of spermatocytes in the animal groups are characterised by mitochondrial apparatus just like that of the atypic spermatocyte of Paludina. This can easily be proven by reading the literature on the mitochondria (**4**, **24**, **7**, etc.). In the atypic spermatocyte we find the mitochondrial elements closely resembling those of Pulmonate Mollusca. One would hardly venture to conjure up the phylogeny of these molluscs to explain the occurrence of two kinds of sperms, because atypic sperms of this kind are not found elsewhere in the animal kingdom. Those of Lepidoptera are not homologous, as Meves would suggest.

The differences which exist between the atypic and the typic series in Paludina may be best seen in tabular form. It will then be understood that the typic spermatogenesis in so far as comparison with Pulmonate Mollusca goes is really the unusual one, in the early stages especially. Naturally, after the abnormal maturation divisions of the atypic series nothing homologous is to be found in snails of the Helicid type, but the earlier stages are almost exactly like those of Helix aspersa. Did we know the morphology and behaviour of the mitochondria of other groups of molluscs

	Primary spermatogonium.	Spermatocyte size (average).	Maturation prophases (nucleus).	Spermatocyte (full grown).
Paludina (typic) . .	Rest period after division short. Cytoplasm contains a good number of coarse rods near archoplasm	11 μ longest way	Rest, or dictyate stage absent; filaments never break up to form reticulum	Archoplasm about one-quarter size of that of atypic. Chondrioplasts few. Mitochondria rods number apparently about one dozen; more rarely about thirty spheres.
Paludina (atypic) . .	Rest periods after division not short, since cells do not immediately after division proceed to growth. Cytoplasm contains archoplasm with fine granular mitochondria	20 μ longest way	Dictyate stage present for a long time	Archoplasm dense, large, and chondrioplasts numerous. Mitochondria fine spheres; number generally several hundred.
Pulmonate . . .	Cytoplasmic inclusions like those of atypic series, or like those of Mammals, etc.	22 μ (Helix aspersa) longest way	Dictyate stage present as in atypic series	Archoplasm never quite so comparatively large as in atypic series, though chondrioplasts may be as numerous. Mitochondria as in atypic series of Paludina.

some interesting comparisons would be made possible. I only make the above suggestions tentatively, and do not wish them to be construed otherwise.

At the present time examination of the gametogenesis of a number of Molluscan families is being undertaken in order to ascertain, if possible which groups have the large elongate mitochondria of the typic sort in Paludina, and which are like Pulmonates in the possession of the usual granular mitochondria like those of the atypic series of Paludina. It is quite possible that such an organised examination of types related to Paludina and of types which might be within the phylogenetic line of the Prosobranchs might yield important evidence leading to an understanding of the true history of the atypic spermatozoa.

For the present I am uncertain as to what grounds might be brought forward for regarding the atypic spermatozoa as "survivals" of no present function. I am prepared to admit that this attractive view will need strong evidence to support it, and that it may even be quite impossible. At this stage I leave the matter till further researches have been carried out. I may say, however, that I am not attracted by the view that the typic spermatozoa represent the ova in the male Paludina that was once hermaphrodite like Helix.

Summary.

New Facts.—(1) In Paludina vivipara it has been shown that in the case of the well-known dimorphic spermatozoa the atypic (giant) cells have numerous fine granular mitochondria, while the typic cells possess a very few, large, stout, rod-shaped mitochondria.

(2) In the typic divisions it was thought that in some cases these large rods were merely sorted out into two groups, to the daughter-cells, while in other cases it was shown that the rods were divided in the middle.

(3) In the atypic divisions the mitochondria acted like those of Helix aspersa or other pulmonates.

(4) Spermateleosis stages in both atypic and typic spermatogenesis have been carefully followed out; the Golgi apparatus sloughs off in both series.

(5) In rare cases the mitochondria of the typic spermatocytes are very large, coarse granules, quite distinct from the smaller granules of the atypic series.

Note on the Golgi Apparatus ("Nebenkern") of Helix aspersa, etc.

Demoll's (**9**) statement that the appearance of the "Nebenkern" (Golgi apparatus) heralds in some way the differentiation of the indifferent cell into either spermatogonium or oögonium was shown to be wrong in my paper on Helix (**15**). Additional proof of the incorrectness of Demoll's views is provided by preparations of Helix ovotestis made by Kopsch's method. In these the Golgi apparatus or "Nebenkern" is found in the smallest and most indifferent germinal epithelial cells, being stained a dense black. In Text-fig. 21, *CH.*, I have drawn a part of the ovotestis wall from a Kopsch preparation showing the apparatus of Golgi in every cell. Demoll is therefore wrong in considering the Golgi apparatus (his "Nebenkern") has anything to do with the determination of oögonium or spermatogonium. In Pl. 25, fig. 28, a young spermatocyte is drawn from a Kopsch preparation. The Golgi apparatus alone stains black.

I have found this Golgi apparatus in cells of Paludina, Helix, Arion, and Limnæa among Mollusca.

Part II.—The Germ-Nurse Cells of Helix Aspersa, Testacella Haliotoïdes, etc. (Pl. 26.)

In Pulmonate Mollusca it is well known (**2**, **4**) that some of the germinal cells grow to form ova, some spermatozoa, some follicle cells, some sertoli (sperm nurse-cells), and others large hypertrophied nurse-cells especially common near egg-cells, and full of yolk. These large nurse- or yolk-cells are derived from true germ-cells of the original germ rudiment, and belong

to the same series of cells as the other elements in the ovotestis. In my previous paper on Helix (**15**, p. 567), I have given a figure showing the typical nurse-cells of this form. In this animal the nurse-cells are distinctly hypertrophied and hyperchromatic, but rarely more than one and one-half times larger than the full-grown spermatocyte. In the case of Testacella the yolk-cell nucleus is generally at least four and one-half times the size of the nucleus of the full-grown spermatocyte; the same is apparently the case in Limnæa stagnalis, which I have not studied so carefully as Testacella.

In Pl. 26, fig. 29, is drawn a fairly typical nurse-cell containing a large number of yolk spheres at *Y.*, a Golgi apparatus at *G.A.*, and what are possibly to be identified as mitochondria at *M.* In the scheme in Pl. 26, fig. 37, on the bottom right-hand corner at *I.* is a yolk-cell in situ, to which are sticking a large number of unripe spermatozoa (8). At *I.I.*, on the left bottom corner, is another yolk-cell with a partially disintegrated cytoplasm. The size of nucleus of the spermatocytes at 5, in the lower middle region of the scheme, may be compared with that of the yolk-cells. It will be seen that in cases the yolk-cell nucleus may be as large as the entire cell of the spermatocyte; the spermatocytes in Pl. 26, fig. 36, at 5, are drawn to scale exactly, as are all the other elements in this scheme.

In Pl. 26, fig. 28 BIS, is drawn a progerminative germinal epithelial cell—that is, a germinal epithelial cell showing signs of passage to spermatogonium or oögonium; Pl. 26, fig. 29, below, is drawn to the same scale. In Pl. 26, figs. 31 and 33, are drawn two subsequent stages in spermatogenesis —early leptotene, and the bouquet or contraction stage (after synapsis). In Pl. 26, fig. 35, is a spermatid, and in Pl 26, fig. 36, the head of a ripe sperm. In the scheme in Pl. 26, fig. 37, is drawn to a much smaller scale every stage in spermatogenesis, the different stages from spermatogonium to sperm being marked by the Arabic numerals 1 to 9. No. 2 represents the growing spermatogonium, No. 3 the spermato-

cyte half-grown, No. 4 a three-quarter-grown spermatocyte, No. 5 a group of prophases and mitotic figures of the first maturation division, No. 6 second maturation division, No. 7 young spermatids, Nos. 8 and 9 older and nearly ripe spermatozoa. For elaborate drawings of some of these stages see my last paper (**16**). It will be noted, I trust, that all these figures in my scheme are convincing, and correspond with what is already known with regard to the comparative sizes of the elements of spermatogenesis.

At the capital letters, *A*, *B* and *C*, are drawn three oöcytes; the one at *A* is in the contraction figure; compared with the same stage in the sperm series at 3, below, it will be seen that there is a perfect correspondence in size between the two; *B* and *C* are later stages, but the egg grows much larger than that in *C*. All around the wall of the ovotestis (covered by Ancel's layer) are seen the indifferent germinal epithelial cells at *G.E.*

The above-described stages of normal spermatogenesis and oögenesis begin in spring and go on all through the summer and late autumn. Towards autumn the cavities or alveoli in the ovotestis become more or less completely cleared of both ripe eggs and sperms, which pass off to the hermaphrodite duct.

The yolk-cells do not move off, and they lie in the cavities of the ovotestis all through winter without undergoing much change.

Now in spring a wonderful process may begin. The arrival of favourable weather sets in action the factors which cause the new crop of eggs, sperms and nurse-cells to begin developing, and the old yolk-cells are influenced by these factors, and themselves try to develop into germ-cells. A glance at Pl. 26, figs. 30, 32 and 34, will show what remarkable cells are so produced; these are drawn to the same size as all the other elements on the left side of the plate.

Reference to the scheme on Pl. 26, fig. 37, will serve to show these points; all the stages of such cells—I will call them giant germ-nurse cells because they are really only

hypertrophied germ-cells—are marked by roman numerals from *I* to *X*. Such a nurse-cell as at *II*, bottom left side, passes on to a giant leptotene stage as in *III*, at the middle; the latter stage then passes on to a late leptotene as at *IV*, with filaments, which again passes to a peculiarly abnormal synaptene as at *V*; and then one may get such an abnormal form as at *VI* with basophil droplets, to which converge the filaments in their immediate region. Now degeneration rapidly sets in: at *VII* the cell is quite abnormal, and by the stage drawn in Pl. 26, fig. 34, a rupture may appear and the cell contents flows out, and the giant-cell gradually disintegrates. I never found any stage later than synaptene and contraction figure.

Comparisons of the various stages in my scheme on Pl. 26, fig. 37, will serve to show that I have established clearly that what I have described is correct—the three series, egg, sperm and nurse-germ cells are clearly marked, from alpha to omega.

Having given the broad outline of the facts concerning these peculiar cells, I will describe their structure.

By focussing up and down upon a germinal epithelial cell, just pro-germinative, one may see from ten to thirty rough chromatin blocks in the nucleus (Pl. 26, fig. 28 BIS). The nurse-cell nucleus contains blocks of much the same size and appearance, but these are enormously more numerous; the nurse-cell nucleus is in a state known as hyperchromacity (Pl. 26, fig. 29). Of all the elements of the ovotestis the nurse-cell nuclei are most darkly staining and conspicuous. Such nurse-cell nuclei, though quite distinct from the ordinary germinal epithelial cell, are united to the latter by a perfectly graduated series of intermediate stages; the germinal epithelial cell grows quickly, its nucleus becomes filled gradually with more and more chromatin blocks, which often stain more heavily than the original ones in the epithelial nucleus, the yolk in the cytoplasm becomes marked in quantity, till finally one gets the cell as drawn in Pl. 26, fig. 29, or Pl. 26, fig. 37, at *I*.

The above facts are clearly demonstrable, and it is

impossible to recognise when the epithelial germ-cell ends and the nurse-cell begins in the series. This is a very important fact.

In Pl. 26, fig. 37, at the bottom left corner at *II* and on the right at *I* are drawn very typical nuclei of nurse-cells; that in Pl. 26, fig. 29, is not quite typical, as will be noticed later. In the central region of the nucleus one finds a confused conglomeration of chromatin blocks seemingly representing a karyosome.

No sign of a plasmosome could be found. The chromatin blocks otherwise are set apart from one another, as shown in Pl. 26, fig. 29. In the latter the cell is showing the appearance of "pro-germinativeness"—that is, of attempting, at least, to become a developing germ-cell; the cell has broken away from the ovotestis wall (see Pl. 26, fig. 37, at *II*, where this is beginning to take place), there is a large basophil karyosome, and two ring-like bodies at *R.*, whose nature I could not ascertain. They are rings of basophil matter, enclosing a chromophobe material; at *G.A.* in the cytoplasm is a large archoplasm with accompanying Golgi elements (batonettes).

This stage represents for the germ-nurse cells the same stage in the epithelial cell above. Such peculiar cells float out into the liquid inside the ovotestis alveoli, and their yolk soon disappears. In the next stage, drawn in Pl. 26, fig. 30, the chromatin blocks are beginning to run together to form the well-known leptotene stage. In this nucleus are two large abnormal plasmosomes and a large double karyosome; the cytoplasm appeared to be quite clear of yolk, but at one side (below) in a juxta-nuclear position was to be seen a cytoplasmic zone. This cell lay in a region of the ovotestis where the cytoplasmic and deutoplasmic inclusions were not well preserved. Degeneration may also account for the absence of granular bodies in the cytoplasm. The corresponding stage in normal spermatogenesis is drawn in Pl. 26, fig. 31. The mitochondria form a heap in the juxta-nuclear, excentric position, in the same position as the cloud in Pl. 26, fig. 31.

COMPARISON BETWEEN NORMAL AND GIANT-CELLS.

Stage chosen for comparison.	Cell size.	Nuclear size and nuclear condition.	Chromaticity and number of loops.	Cytoplasmic elements.
Normal presynizesis (Pl. 26, fig. 33)—a little later	Circa 16 μ in diameter, longest way	Circa 14 μ	Chromaticity varies somewhat, but may be like that of giant-cell. Loops about 20 in number, fusing to form about 10 pairs	Mitochondria excentric, juxta-nuclear; Golgi apparatus generally, though not always, covered by mitochondria.
Large giant germ-nurse cell in same stage as above (Pl. 26, figs. 32, 34)	Circa 40 μ to 50 μ; in one case 60 μ	Circa 30 μ to 40 μ. Nucleus often contains pathological nucleoli or necrotic droplets	Chromatin, like that of normal stage, varies in staining, according to circumstances of fixation, etc. It is generally like the normal. Number of chromatin filaments or loops seems to exceed 20. Instead of 10 pairs there may be 30. Grouping of filaments abnormal	Mitochondria generally grouped as above. The Golgi apparatus not generally demonstrable, being covered by mitochondria. In cases latter a little larger than normal.

The next stage is one which reveals most remarkable pathological and necrotic forms. Droplets of chromophil matter appear in the nucleus in some cases, and the cells become so unhealthy that one may get the strange appearance as in Pl. 26, fig. 37, at *VI* in the middle of the scheme. Such huge "karyosomes" have radiating from them, like the spokes of a wheel, the chromatin filaments. In Pl. 26, fig. 32, a strange cell is drawn; it is a "paired filaments" stage just before synizesis and contraction, only so abnormal as to be unlike any stage of the normal spermatogenesis; at *X* is a peculiar hub-like body, containing a central slightly chromophil mass, a clear peripheral zone, a ring, and the usual radiating spokes like those of a wheel. There are three or four abnormal "karyosomes" or chromatic droplets in this nucleus. The cytoplasm of the cell contains a large number of clearly-defined mitochondria, somewhat larger than those of the normal cell (see Pl. 26, fig. 35), but disposed mainly in a juxta-nuclear, excentric position, as usual with this stage of the normal spermatogenesis.

In the scheme on Pl. 26, fig. 37, this same cell is drawn at the upper side at *V* for comparison with other cells, and at *IV*, to the right, is a late leptotene stage of the giant germ-nurse cell, the stage just before that in *V* and *VI*. The cell at *IV* is fairly healthy, and would have gone a good distance in its metamorphosis had external conditions been favourable.

The abnormal paired chromatin filaments now fuse, and one has the synaptene stage (Pl. 26, fig. 34). This cell contains a tripartite "karyosome," a meagre cytoplasmic juxta-nuclear cloud as in Pl. 26, fig. 30, and, what is more important, the cell-wall has burst or disintegrated at *B.R.*, and the cell would soon have broken up completely. I have found no giant germ-nurse cell to go much further than this stage, and the majority do not go so far. In Pl. 26, fig. 37, at *VII*, on the left, is drawn another cell with a completely abnormal spireme, and it is possible that this cell represents a pachytene stage just after the bouquet stage. If so, this is the furthest I have traced such cells. Pl. 26, fig. 37, *VII*, has no inclusions

in the cytoplasm. Such is the peculiar history of some nurse-cells.

Golgi Apparatus and General Morphology of the Giant Germ-nurse Cells.

In most cases the Golgi apparatus of the normal spermatogonium, after entry into the prophases of the heterotypic division is covered over by and indistinguishable from a mass of granular mitochondria; in other cases the Golgi apparatus is clearly to be seen. Just the same two conditions appear to old with the giant-cells. Pl. 26, fig. 32, in this way resembles Pl. 26, fig. 33, while the cell in Pl. 26, fig. 29, which has a clearly marked Golgi apparatus, would be like that in Pl. 26, fig. 37, at 3.

Cell size and nuclear size also correspond comparatively in giant- and small cells. Compare Pl. 26, figs. 30 and 31, figs. 32 and 33, and figs. 33 and 34, where it will be noted that a remarkable correspondence in not only size, but in the excentric position of the nucleus in the cell, can be seen to be the case; this, as noted before, applies also to mitochondria. In a few cases the cytoplasm of the giant-cell is too large in comparison with the nucleus (using the normal cell as basis for this comparison), and such cells are found degenerating as well as those in which the nucleo-cytoplasmic ratio is the same as in the normal cell.

Number of Chromosomes in Normal Testacella Germ-cells, compared with the Number of Pachytene Loops in the Giant-cells.

In my material, nearly all of which is fixed in chrome-osmium, it is difficult to count the number of chromosomes. I believe the number of somatic chromosomes is over twenty, the haploid number about ten. In the normal cell at pachytene I count about ten or twelve loops; in the giant-cell, by the same method of counting, I have found from twenty-five to thirty; in one clear case, where a giant-cell and a normal

cell lie side by side in the late bouquet stage, the filaments in each cell are the same thickness, while the nucleus of the giant-cell is literally a dense, tangled mass of filaments; the giant-cell contained twice as many filaments in one section as the normal cell, and the latter only appeared in two sections, while the giant-cell was cut into four sections, and three of the sections contained pieces of nucleus with many tangled filaments. This, however, is not complete proof that were the chromosomes to appear they would be more numerous than in the ordinary germ-cell, for the filaments in the giant-cell might merely be longer than in the normal cell and more coiled therefore. This seems to me to be unlikely, and the evidence seems to show that the filaments are more numerous in the giant-cell than in the other.

Fate of Intermediate Forms between Nurse-cells and Germinal Epithelial Cells.

It has been stated that there is a complete chain of forms intermediate between nurse- and germinal epithelial cell, because the latter gives rise to the former. Every stage between the small germinal epithelial cell and the giant-cells can be found at any time of the year. As has been explained, the larger the nurse-cell the more chromatin lumps there are for the nucleus and the more yolk-disclets in the cytoplasm. There is no true difference between the giant-cell and the small one, as has been shown to be the case in man **31**) (see Montgomery), where a special sertoli or nurse-cell determiner is segregated into those cells destined to form sertoli cells. Careful observation of nurse-cells in Helix and Testacella failed to show any such body in nurse-cells.

Intermediates as above described obviously vary in the distance they have gone on the path of differentiation, and equally, therefore, vary in the capacity they show when they are stimulated to de-differentiate. Many cells somewhat larger than the full-grown spermatocyte appear to succeed completely in de-differentiating to the same size as the spermatocyte

and finally to undergo normal stages in the prophases of the heterotypic division. In Pl. 26, fig. 37, at *IIIA* and *VA* are three cells, all of which were a size which would allow them to recover their equilibrium and so de-differentiate successfully. Compare *IIIA* with the cells at 3, or the right in the same stage.

In some cases these intermediate cells, even though small enough to pass easily to the prophases of the heterotypic division, are abnormal in appearance. This abnormality is seen in the coarseness and number of chromatin filaments in their nuclei. Both *VA* and *IIIA* have nuclei unlike the normal stage.

Germ-nurse Cells in other Molluscs.

Such large cells have been found in profusion in Helix aspersa, and I have little doubt they occur in Limnæa and other molluscs I have studied (**16**). In Helix the yolk-cell nucleus does not grow relatively to the spermatocyte so enormously, and consequently there is a good deal of difficulty in establishing the facts so clearly as for Testacella. Should this description be doubted I will be prepared to publish microphotographs to establish what I claim. No one can study the ovotestis of Helix aspersa without finding large, often naked nuclei lying free in the lumen, mixed up with sperm-cells. These nuclei regain a cytoplasm, and their dense hyperchromatic nuclei gradually change till they resemble those of the ordinary stages of spermatocytes. I have carefully studied them in Helix, and have found that many germ-nurse cells do de-differentiate and finally form spermatozoa.

Germ-nurse Cells—Oöcytes or Spermatocytes?

So far I have compared the giant-cells exclusively with sperm stages. The question then arises: Would the germ-nurse cells have become spermatocytes or oöcytes had they succeeded in developing? Naturally one might consider them

to be likely to become oöcytes because of their yolk, but since they fall into the lumen of the ovotestis and do not stick on to the walls like the oöcytes, they are certainly exposed to two sources of stimuli: besides the yolk-disclets, whose presence must exert some stimulus on the giant-cell, there is the fact that only stages in spermatogenesis are found in the ovotestis lumen, and one might expect that any cell carried into this locality might be affected by the special conditions existing in the lumen of the ovotestis. As has been remarked before (**15**), the cell is outwardly indifferent until it passes beyond the pachytene stage, so that it is not generally possible in Pulmonate Mollusca to identify a cell as spermatocyte or oöcyte during the prophases of the heterotypic division. In the smaller germ-nurse cells, which I believe de-differentiate successfully, the further stages after escape of the cell from the germinal epithelium leads to the formation of spermatocytes. Evidently the cell can be indifferent up to quite a late stage.

Summary on Testacella Germ-nurse Cells.

(1) Germinal epithelial cells, besides producing ova, spermatozoa and follicle cells, may also become much enlarged to form yolk- or nurse-cells, which have very large hyperchromatic nuclei and a cytoplasm full of yolk-discs.

(2) Such cells are easily distinguishable from stages of spermatogenesis, because of their large size, and often because of their abnormal appearance.

(3) In spring and summer normal spermatogenesis and oögenesis goes on, the ova and sperm-cells being nourished by the nurse- or yolk-cells. Towards autumn and winter the cold weather stops such activity, and by this time the ovotestis cavities are nearly vacant, because the ripe products have passed away through the genital duct. The large yolk-cells are left, often exhausted more or less of their yolk-discs, and show signs of falling away from the ovotestis wall to which they previously adhered; they float free in the fluid

contained in the partly empty ovotestis alveoli or lumina, and in many, though not all cases, their cytoplasm partly breaks up.

(4) During winter the Testacella hibernates and all activity is possibly suspended. In the early spring following activity in the ovotestis recommences, and germinal epithelial cells are stimulated to begin proliferating series of egg and sperm-cells. These activating materials (stimuli of some sort) affect not only the above cells but reach to the giant nurse-cells, which begin to undergo the prophases of the heterotypic division, known as leptotene, synaptene, contraction figure, pachytene and diplotene. Such large cells have been found to pass more or less abnormally through all the stages up to pachytene, but about this stage they degenerate. In the majority of cases these nurse-germ cells do not arrive at such a late stage, and many others possibly degenerate very early.

(5) In a scheme on Pl. 26, fig. 37, I have drawn all the elements found in the ovotestis at any time of the year, and in Pl. 26, figs. 28–36, I have drawn carefully examples from both normal male germ-cells and from the giant germ-nurse cells.

(6) The number of chromosomes in Testacella seems to be something over twenty and the haploid number over ten, probably about twelve. The giant germ-nurse cells are found to contain at the synaptene stage too many loops, and this is the case in the pachytene stage; normal pachytenes have some ten loops, the giant-cells apparently as many as twenty-two to thirty, but in no case could I be quite certain as to their number. I feel sure that the giant germ-cells contain an irregular and over numerous series of chromatin loops. Moreover, the nuclei of the giant-cells generally contain many droplets of a chromatoid nature, as well as pale spheres, which are not found in the normal spermatogenesis stages.

Discussion with Regard to the Giant Germ-nurse Cells.

The above summary sufficiently explains the salient points in my researches on the peculiar nurse-germ cells.

The main questions which this part of my paper raises are as follows: What is happening in the germinal epithelial cell as it becomes hyperchromatic? Is the new matter which comes into the nucleus true chromatin? Why cannot the giant-cells succeed in passing through the prophases and form giant chromosomes? What are the chromophil droplets which appear in the nucleus of the giant-cells just before they begin finally to degenerate? If these droplets are chromatin, does such chromatin differ from that still left in the filaments or spireme?

These questions are very difficult to answer, and reach to the root of the various controversies on how. chromatin grows, and what limits the size of the cells and body of any animal or plant. Apart from the nurse-cells of Pulmonate Mollusca, one finds hypertrophied, hyperchromatic cells in many animals: in insects such as Coleoptera and Hymenoptera the ovarioles contain large nurse-cells; in the trophoblast of such a mammal as the mouse and in other cells where there is a storage or constant exchange of food-materials the cells enlarge, and they become very rich in "chromatin." Written in a few words, the whole train of occurrences in the giant-cells of Testacella seems to be that the amount of chromatin increases step by step with the formation of yolk, and when the function of the cell temporarily lapses, the latter tries to recover its equilibrium by shedding the superfluous matter in the form of drops, generally fails, and then undergoes disintegration. Nucleo-cytoplasmic relationship may have something to do with this question, but I have no evidence suggesting that were the cytoplasm of the giant-cell larger the latter would not then lose its equilibrium and perish.

Differentiation may be the key to the problem; differentiated

for nutrition, the giant-cell is stimulated, endeavours to recover the property of metamorphosis into a sex-cell, and fails to do so—not possibly always because its internal condition inhibits this, but because the competition in the ovotestis becomes rapidly great as the new cells develop, and the unwieldy over-differentiated one is choked out by the normal rapidly-growing sperm and egg-cells. To use Child's word, the giant-cell tries to "de-differentiate," but fails (**6**).

It is not desirable to enter seriously at present into the possible view that the binuclearity hypothesis (one of these hypotheses) would serve to explain these phenomena. It might be supposed that the nurse-cell became charged with trophochromatin, and that the appearance of droplets of chromatoid matter in the giant-cell was to be interpreted as an attempt by the latter to throw out its trophochromatin in favour of its idiochromatin. This view, specious as it is, may be worthy of examination and criticism. I neither uphold nor condemn it at present.

Finally, the stimulant which arises in the body of the hibernated animal at spring, and which causes the giant-cell to attempt de-differentiation and metamorphosis, may be identified with some physiological secretion or hormone caused somehow by the changed weather conditions. This phenomenon is no new fact; but that such a hormone should have an effect on a highly differentiated cell like the nurse- or yolk-cell is remarkable, and serves to show that a very highly differentiated cell may, provided certain stimuli be forthcoming, enter upon an attempt to de-differentiate and so prepare tself for a new cellular function.

June, 1918.

Summaries.

(1) Summary to Paludina, p. 421.
(2) Summary to Testacella, p. 432.

Bibliography.

1. d'Agata, G.—"Sur les modifications de l'appareil reticulaire interne dans l'epithélium de la muqueuse gastrique," 'Arch. Ital. de Biol.,' t. liv.

2. Ancel, P.—"Histogenèse et structure de la glande hermaphrodite d'Helix pomatia," 'Arch. de Biol.,' t. xix, 1902.

3. Benda, C.—"Weitere Beobachtungen über die Mitochondria und ihr Verhältnis zu Secretgranulationen, nebs Kritischen Bemerkungen," 'Verh. d. Phys. Ges.,' Berlin.

4. Bolles Lee, A.—"Nouvelles recherches sur le Nebenkern et la régression du fuseau caryocinétique," 'La Cellule,' t. xx.

5. Champy, Chr.—"Recherches sur l'absorption intestinale et le rôle des mitochondries dans l'absorption et la sécrétion," 'Arch. d'Anat. Micr.,' t. xiii, 1911–12.

6. Child, C. M.—'Senescence and Rejuvenescence,' Chicago, 1912.

7. Conklin, E. G.—"Environmental and Sexual Dimorphism in Crepidula," 'Proc. Acad. Sci. Phil.,' 1898.

8. Cowdry, E. V.—"The Relations of Mitochondria and other Cytoplasmic Constituents in Spinal Ganglion Cells of the Pigeon," 'Inter. Monatss. f. Anat. u. Phys.,' Bd. xxix.

9. Demoll, R.—"Über Geschlectsbestimmung im allgemeinen und über die Bestimmung der primären Sexualcharaktere im besonderen," 'Zool. Jahrb.,' Bd. xxxiii, Abt. f. Allg. Zool.

10. Duesberg, J.—"Recherches cytologiques sur la Fécondation des Ascidiens et sur leur Développement," 'Contributions to Embryology,' Carnegie Inst., Wash., D.C., vol. iii.

11. ——— "Plastosomen, 'Apparato reticolare interno,' und Chromidialapparat," 'Ergeb. der Anat. u. Entwickl.,' Bd. xx, 1911, Erste Hälfte.

12. ——— "Der Mitochondrial Apparat in den Zellen der Wirbeltiere und Wirbellosen," 'Arch. f. Mikr. Anat.,' Bd. lxxi.

13. Fauré-Frémiet.—"Etude sur les mitochondries des Protozoaires et des cellules sexuelles," 'Arch. d'Anat. micr.,' t. xi.

14. Gatenby, J. Bronté.—"The Degenerate (Apyrene) Sperm Formation of Moths as an Index to the Inter-relationship of the Various Bodies in the Spermatozoon," 'Quart. Journ. Micr. Sci.,' vol. 62, pt. 3.

15. ——— "The Cytoplasmic Inclusions of the Germ-cells: Part II, Helix aspersa," ibid., vol. 62, pt. 4.

16. Gatenby, J. Bronté.—"Cytoplasmic Inclusions of the Germ-cells: Part III, Other Pulmonates," ibid., vol. 63, pt. 2.

17. Giardina, A.—"Origine dell' oocite e delle cellule nutrici—nel Dytiscus," 'Internat. Monat. Anat. u. Phys.,' Bd. xviii.

18. Goldschmidt, Richard.—"The Function of the Apyrene Spermatozoa," 'Science,' Friday, October 13th, 1916.

19. Golgi, C.—"Intorno alla struttura delle cellule nervose," 'Boll. del. Soc. Med.-Chir. di Pavia,' 1898.

20. ——— "Di un metodo per la facile e pronta dimostrazione dell' apparato reticolare interno delle cellule nervose," ibid., 1908.

21. Gould, H. N.—"Studies on the Sex in the Hermaphrodite Mollusc, Crepidula plana: I, History of the Sexual Cycle," 'Journ. Exp. Zool.,' vol. xxiii, No. 1; II, "Influence of Environment on Sex," ibid., vol. xxiii, No. 2.

22. Granata.—"I mitochondri nelle cellule seminali maschili di Pamphagus marmoratus," 'Biologica,' vol. ii, No. 4.

23. Hirschler, Jan.—"Über die Plasmakomponenten (Golgisher Apparat, Mitochondrien u.a.) der weiblichen Geschlechtszellen (zytologische Untersuchungen am Ascidien-Ovarium)," 'Arch. Mikr. Anat.,' Bd. lxxxix, Heft 1.

24. Jenkinson, J. W.—'Vertebrate Embryology,' Oxford, 1913.

25. Kopsch, Fr.—"Die Darstellung des Binnennetzes in spinalen Ganglienzellen und anderen Körperzellen mittelst Osmiumsäure," 'Sitzungsber. d. k. preuss. Akad. d. Wiss. zu Berlin.'

26. Kuschakéwitsch, S.—"Studien über den Polymorphismus der männlichen Geschlechtselemente bei den Prosobranchia," 'Arch. f. Zellforsch.,' Bd. x, 1913.

27. MacBride, E. W.—"Address to Sect. D., Brit. Assoc., Newcastle-upon-Tyne," 1916.

28. ——— 'Embryology of Invertebrata,' MacMillan & Co., 1914.

29. Meves, F.—"Die Spermatocytenteilungen bei der Honigbiene (A. mellifica)," 'Arch. f. Mikr. Anat.,' Bd. lxx.

30. ——— "Über oligopyrene und apyrene Spermien und über ihre Entstehung, nach Beobachtungen an Paludina und Pygæra," ibid., t. lxi, 1903.

31. Montgomery, T. H.—"Differentiation of the Human Cells of Sertoli," 'Biol. Bull.,' vol. xxii.

32. Morgan, T. H., Sturtevant, Muller and Bridges.—'The Mechanism of Mendelian Heredity,' Henry Holt & Co., 1915.

33. Murray, J. A.—"Contributions to a Knowledge of the Nebenkern in the Spermatogenesis of Pulmonata," 'Zool. Jahrb.,' Bd. xi.

34. Pappenheimer, A. M.—"The Golgi Apparatus: Personal Observations and a Review of the Literature," 'The Anatom. Record,' vol. xi, No. 4, 1916.

35. Perroncito, A.—"Contribution à l'étude de la biologie cellulaire. Mitochondres, chromidies et appareil réticulaire interne dans les cellules spermatiques. Le phénomène de la dictyokinèse," 'Arch. Ital. de Biol.,' t. liv, 1910.

36. Popoff, M.—"Eibildung von Paludina vivipara und Chromidien bei Paludina und Helix," 'Arch. f. mikr. Anat.,' Bd. lxx.

37. Regaud, Cl.—"La Structure des tubes séminifères et la Spermatogénèse chez les Mammifères," 'Arch. d'anat. micr.,' t. xi, 1909–10.

38. Reinke, E. E.—"A Preliminary Account of the Development of the Apyrene Spermatozoa in Strombus and of the Nurse-cells in Littorina," 'Biol. Bull.,' vol. xxii.

39. ——— "The Development of the Apyrene Spermatozoa of Strombus bituberculatus," 'Papers from the Tortugas Laboratory,' v–vi, 1914.

40. Retzius, G.—'Biologische Untersuch.,' Neue Folge xii.

41. Saguchi, S.—"Studies on Ciliated Cells," 'Journ. Morph.,' vol. xxix, 1917.

42. Schitz, V.—"Sur la spermatogénèse chez Columbella rustica, L.," 'Arch. Zool. Exp.,' Notes et Rev. No. 2, t. l.

43. Schäfer, E. A.—'The Endocrine Organs: An Introduction to the Study of the Internal Secretion,' Longmans, Green & Co., London, 1916.

44. Terni, Tullio.—"Condriosomi, idiozoma, e, formazioni periidiozomiche nella spermatogenesi degli Anfibii," 'Arch. f. Zellforch.,' Bd. xii, 1914.

45. Wilson, E. B.—"The Distribution of the Chondriosomes to the Spermatozoa in Scorpions," 'Proc. Nat. Acad. Sci.,' vol. ii, 1916.

DESCRIPTION OF PLATES 25 AND 26.

Illustrating Mr. J. Bronté Gatenby's "Notes on the Dimorphic Spermatozoa of Paludina, and the Giant Germ-nurse Cells of Testacella and Helix."

Lettering to Plate 25.

AF. Axial filament, or filaments. *A.L.* Ancel's layer. *A.M.* Archoplasm with Golgi apparatus (*C.P.*), and sometimes with mitochondria. *A.X.* and *B.X.* Regions in the forming axial rod (*R.D.*). *C.* Centrosome or centrosomes. *CH.* Chromosome. *CP.* Chondrioplast or Golgi (Nebenkern) rod. *G.B.* Granular bodies of unknown nature, "mitochondria of Benda." *G.M.* Granular mitochondria. *H.* Chromatin (?) head of atypic sperm. *M.* Mitochondria. *M.R.* Mitochondrial rod. *M.S.* Mitochondrial sphere. *M.R.s.* Mitochondrial rods tangled. *M.R.x.* Mitochondrial rod especially clear. *N.* Nucleus. *R.D.* Central rod of atypic sperm. *Y.*, *Y.D.* Yolk discs.

Lettering to Plate 26.

B.R. Break in cell wall indicating early disintegration of cell. *G.A.* Golgi apparatus (Nebenkern). *K.* Karyosome. *M.* Mitochondria. *N.* Nucleus. *R.* Chromatin ring. *X.* Abnormal wheel-like structure in giant pathological cell.

For letters in Pl. 26, fig. 37, see description of that figure on page 423.

[Figures drawn either with a 12 or an 18 comp. eyepiece and a $\frac{1}{15}$ semi-apochromatic Koritska oil-immersion objective, and then somewhat reduced. Scale given at middle of plates. In certain spermateleosis stages of the atypic series the chondrioplasts have been added to figures of Flemming-without-acetic iron-hæmatoxylin preparations, from material treated by Kopsch's method.]

PLATE 25.

[With the exception of figs. 25 to 28, all the figures on this plate are drawn to the scale given in the middle of the plate from preparations made in Flemming-without-acetic acid and iron-hæmatoxylin. Figs. 27 and 28 are drawn from Kopsch material. Figs. 25 A and B are at half the magnification of the other figures.]

Fig. 1.—Secondary spermatogonium of typic series just after entry to the growth stage. The archoplasm, chondrioplasts and rod-shaped mitochondria are shown. At least seven mitrochondria could be counted.

Fig. 2.—View of a cell at the same stage as the previous figure, looking down upon the archoplasm and the surrounding mitochondrial rods. There appeared to be at least thirteen of the latter, but it was not possible to say whether or no the individual U-shaped rods were separate.

Fig. 3.—Full-grown typic spermatocyte, before syndesis, and some time before the chromatin filaments condense to form chromosomes. The mitochondrial rods are larger, longer, and in some cases straighter. There were at least nine.

Fig. 4.—Polar view of equatorial chromosome plate (seven chromosomes) of the first maturation division. At least seven mitochondrial rods could be counted. These had become thinner and more elongate, and their enumeration had become very difficult because they were S- and U-shaped, and therefore never quite in the same focus. The cell in fig. 4 is a very clear example.

Fig. 5.—Second maturation division metaphase showing mitochondrial rods. There appeared to be nine.

Fig. 6.—Second maturation division telophase showing characteristic grouping of rods. There were apparently no fewer than seven.

Fig. 7.—Second maturation telophase showing constriction of rods at equator of cells (*C.M.R.*). In each cell there were four rods.

Fig. 8.—Spermatid after re-formation of nucleus. Rods four in number, and are beginning to contract up, preparatory to becoming spheres, as in the next figure.

Fig. 9.—Side and end view of spermatid after the rods have become spheres. In the upper cell the archoplasm and chondrioplasts are clearly seen; the spheres are four in number.

Fig. 10.—End view of a second maturation division telophase to show mitochondrial rods; for a description see page 409.

Fig. 11.—Spermatid after the mitochondrial spheres have fused together to form the macromitosome. The acrosome has just been formed in connection with the archoplasm, which has now drifted aside. (Compare Text-fig. 9.)

Fig. 12.—Archoplasm and part of cytoplasm sloughing off, while macromitosome has elongated.

Figs. 13 and 14.—Two cells from the germinal epithelium to show the rod-shaped mitochondria (*M.R.x.*, *M.R.x.*, *M.R.*). (Compare with the cells in Pl. 25, fig. 17, where there are no rod-shaped mitochondria clearly to be seen.

Fig. 15.—A spermatogonial cell which did not contain elongate mitochondria, and which was supposed to be of the atypic series.

Fig. 16.—A spermatogonial cell in division, polar view, showing rod-shaped mitochondria.

Fig. 17.—Germinal epithelial cells apparently not containing rod-shaped mitochondria, and therefore supposed to belong to the atypic series.

Fig. 18.—Young atypic spermatocyte showing granular mitochondria. Corresponds with the typic cell in fig. 1.

Fig. 19.—Atypic spermatocyte nearly full grown. Archoplasm and chondrioplasts much larger. Latter more numerous than in the corresponding stage of atypic series (fig. 2).

Fig. 20.—Prophase of first maturation mitosis of atypic cell. Mitochondria and chondrioplasts sorted out into two heaps around the two centrosomes. Compare cell bulk with that of the typic series in fig. 4. Cytoplasm smooth.

Fig. 21.—Second maturation division of atypic series. The fourteen chromosomes lie haphazardly generally in a paler median region of the cytoplasm. At *C.P.* are the two groups of chondrioplasts which were added to this and the subsequent figures (21–25) from observations made on Kopsch material. The mitochondria, *G.M.*, are scattered around the paler central region of the cytoplasm. (Compare with the typic second maturation division in fig. 6.)

Fig. 22.—Atypic spermatid. Two nuclei have become re-formed, while at *C.H.* are several of the other chromosomes degenerating; the mitochondria are grouped at *G.M.*, the chondroplasts at *C.P.* In the cytoplasm is gradually appearing definite patchy regions, which later become more darkly staining and form the "mitochondria of Benda" (*G.B.*).

Fig. 23.—A later stage. The axial filaments, some twelve to fourteen in number, which began to grow out in fig. 21, are now quite long, but they grow even longer. In the cell the chondrioplasts are separated into two groups.

Fig. 24.—Atypic spermatid for comparison with the typic spermatid in fig. 11. At *N.* is the modified nucleus, at the back of which is attached the axial filaments. At *C.P.* is a mass formed of chondrioplasts. The granular bodies (*G.B.*), or "mitochondria of Benda," are much clearer. Some (possibly) of the ordinary mitochondria lie in the axial rod (*A.X.*) in front, but most lie behind at *G.M.*

Fig. 25.—Half the magnification of the preceding figures. Shows in A the typic, and in B the atypic spermatozoon; in both cases the sperm is not quite ripe. Before A becomes mature the bodies at *C.H.* and *C.P.* are sloughed off, and the entire length of the sperm becomes nearly of an even bore. The "mitochondria of Benda," or what

preferably are to be called the granular bodies (*G.B.*), are very chromophile.

Fig. 26.—A part of the middle region of a ripe atypic spermatozoon drawn at twice the magnification of the preceding figures to show the granular bodies (*G.B.*) and the axial rod (*R.D.*).

Fig. 27.—The head of a typic sperm to show the spiral twist (Kopsch).

Fig. 28.—Kopsch preparation of an atypic spermatocyte to show the way in which the chondrioplasts (Nebenkern or Golgi-apparatus) alone stain darkly.

PLATE 26. (Lettering, see p. 439.)

[All figures on left side of plate from fig. 28 BIS to fig. 36 drawn to scale indicated in middle of plate. All figures in the scheme in fig. 37 drawn to same scale indicated on right side of plate. Figures drawn from preparations stained and fixed in iron-hæmatoxylin and Flemming without acetic acid or diluted Champy.]

Fig. 28 BIS.—Young progerminative germinal epithelial cell of Testacella.

Fig. 29.—Dislodged slightly abnormal yolk or nurse-cell of Testacella. The dark karyosome is unusually spherical and noticeable; the Golgi apparatus is also very clear, and the cytoplasm is full of yolk-discs (*Y.*).

Fig. 30.—Giant germ-nurse cell in early leptotene stage; contains two large abnormal "plasmosomes," staining palely. Cytoplasm almost clear except for excentric juxta-nuclear cloud marking position of ill-stained or fixed mitochondria.

Fig. 31.—Corresponding stage in normal spermatogenesis.

Fig. 32.—Pathological giant stage of "paired threads" or diplotene stage; the threads converge towards, and are partly covered by, a mass of mitochondria (*R.G.A.*). There are several abnormal karyosomes (*K.*), while at *X.* is a peculiar wheel-like structure of an unusual nature, possibly representing a forming or partially absorbed karyosome (see Pl. 26, fig. 37, *VI*, in middle). The normal stage corresponding has nothing like this body at *X.* Compare for size with normal (slightly later) stage in fig. 33.

Figs. 33 and 34.—Giant and normal bouquet stages. Fig. 33 has some ten or eleven loops, fig. 34 about twenty-six, and this cell was cut into three sections. Karyosome in fig. 34 abnormal, tripartite instead of bipartite. At *B.R.* the cell has begun to disintegrate, and the excentric cytoplasmic mass is abnormal.

Fig. 35.—Early spermatid for comparison with other stages.

Fig. 36.—Head of ripe sperm of Testacella.

Description of Scheme in Fig. 37.

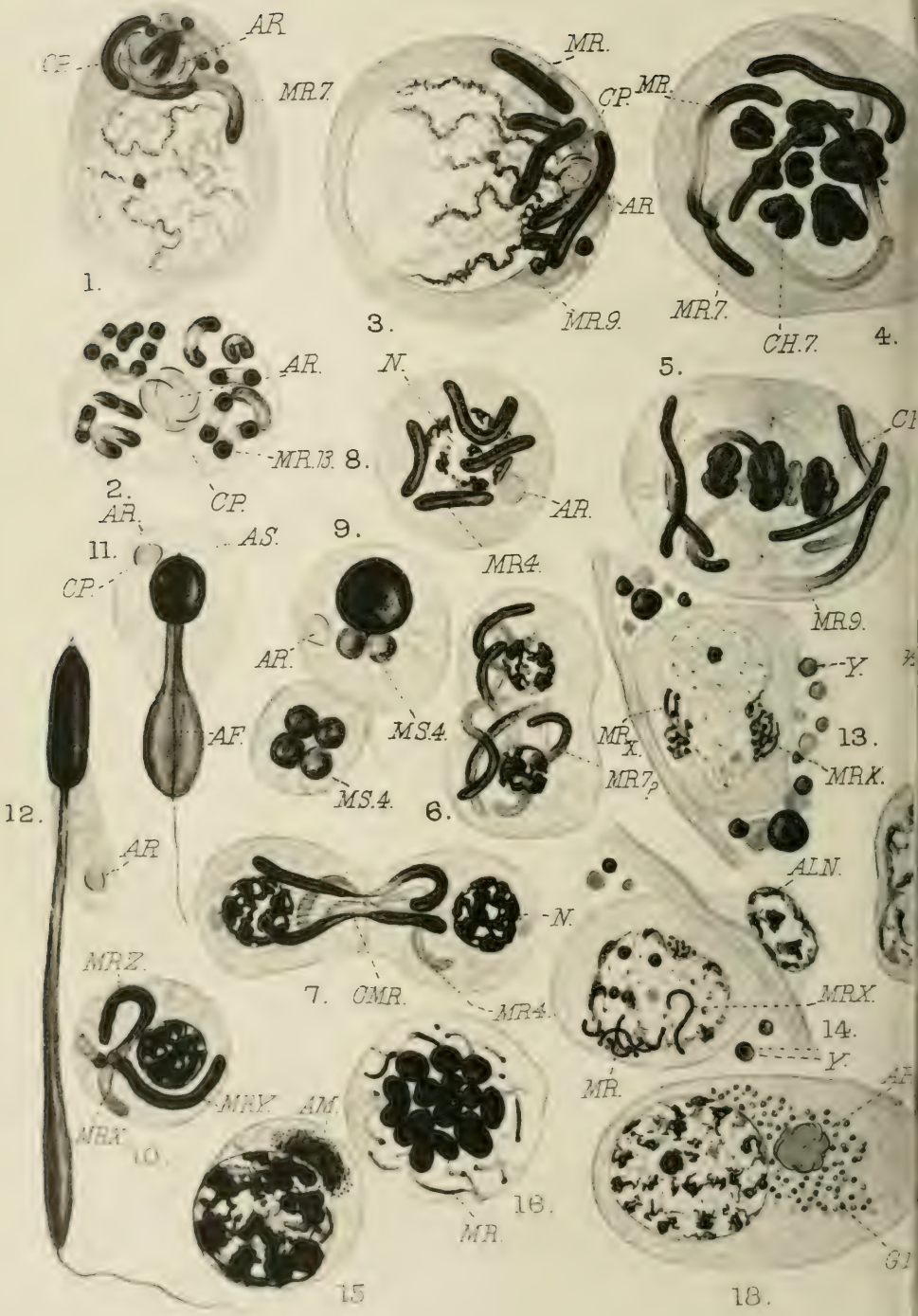
This elaborate figure represents the ovotestis of Testacella haliotoïdes for all times of the year. With the exception of Nos. *I* and *II*, the cells indicated by Roman numerals are never found except in late autumn or early spring. All the other elements are found throughout the year in both winter and summer.

A, *B* and *C* represent three stages of oögenesis, *C* being still small.

The Arabic numerals from 1 to 9 are stages in the normal spermatogenesis described on page 423 and in my previous paper (**16**).

Other letters are: *G.E.* Germinal epithelium. *A.L.* Ancel's layer (fibrous wall of ovotestis). *T.* Trabeculæ or folds in wall of ovotestis, forming pockets. *K.* Rejected karyosome, fig. *VII.*

Roman letters from *II* to *VII* represent stages in metamorphosis of giant germ-nurse cells. (Normal nurse-cell at *I* on right bottom corner.) *X.* represents giant-cells degenerating at an early stage. The cell *I.D.* on the left is degenerating in situ. For more complete description of cells at Roman numerals see pages 425.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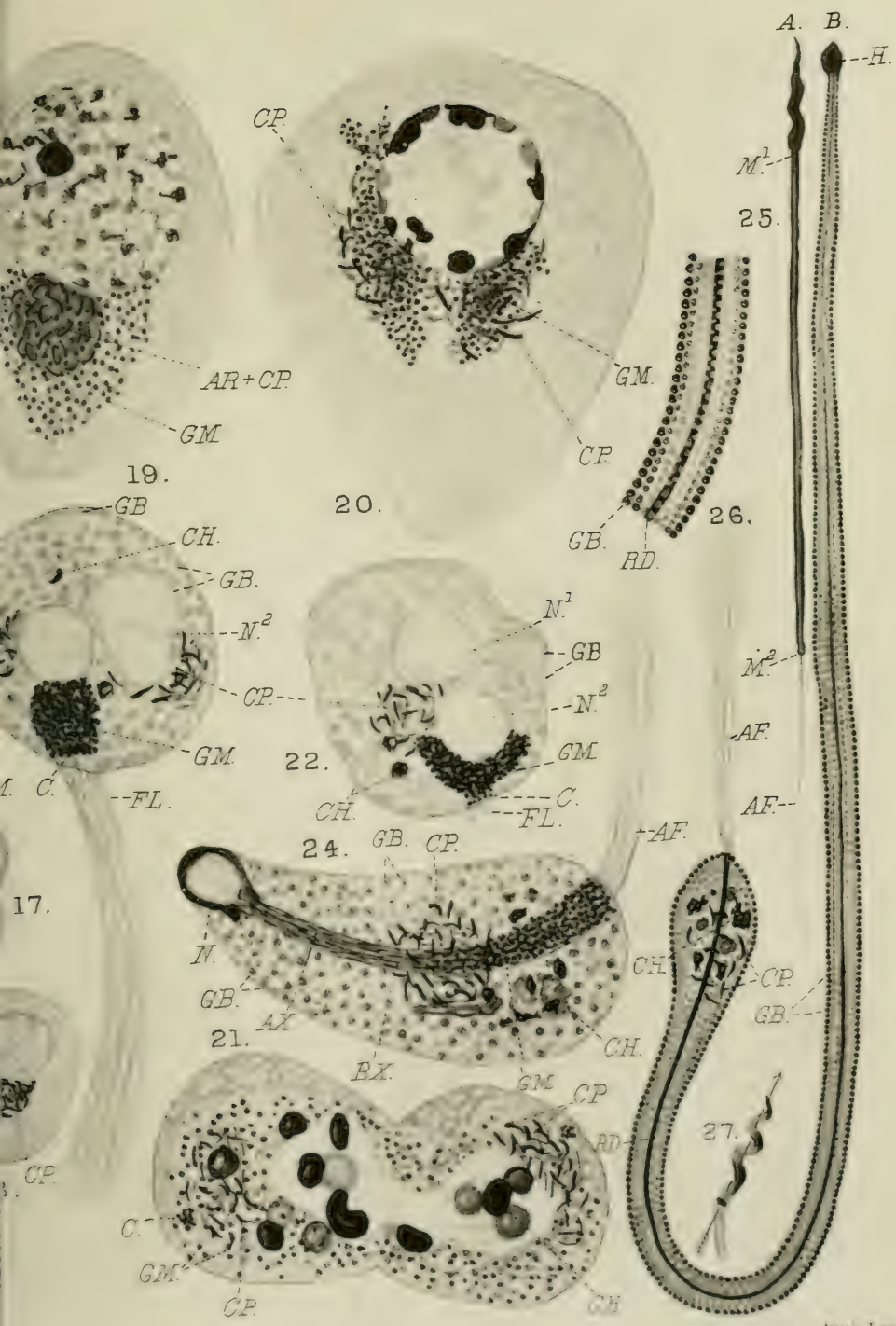
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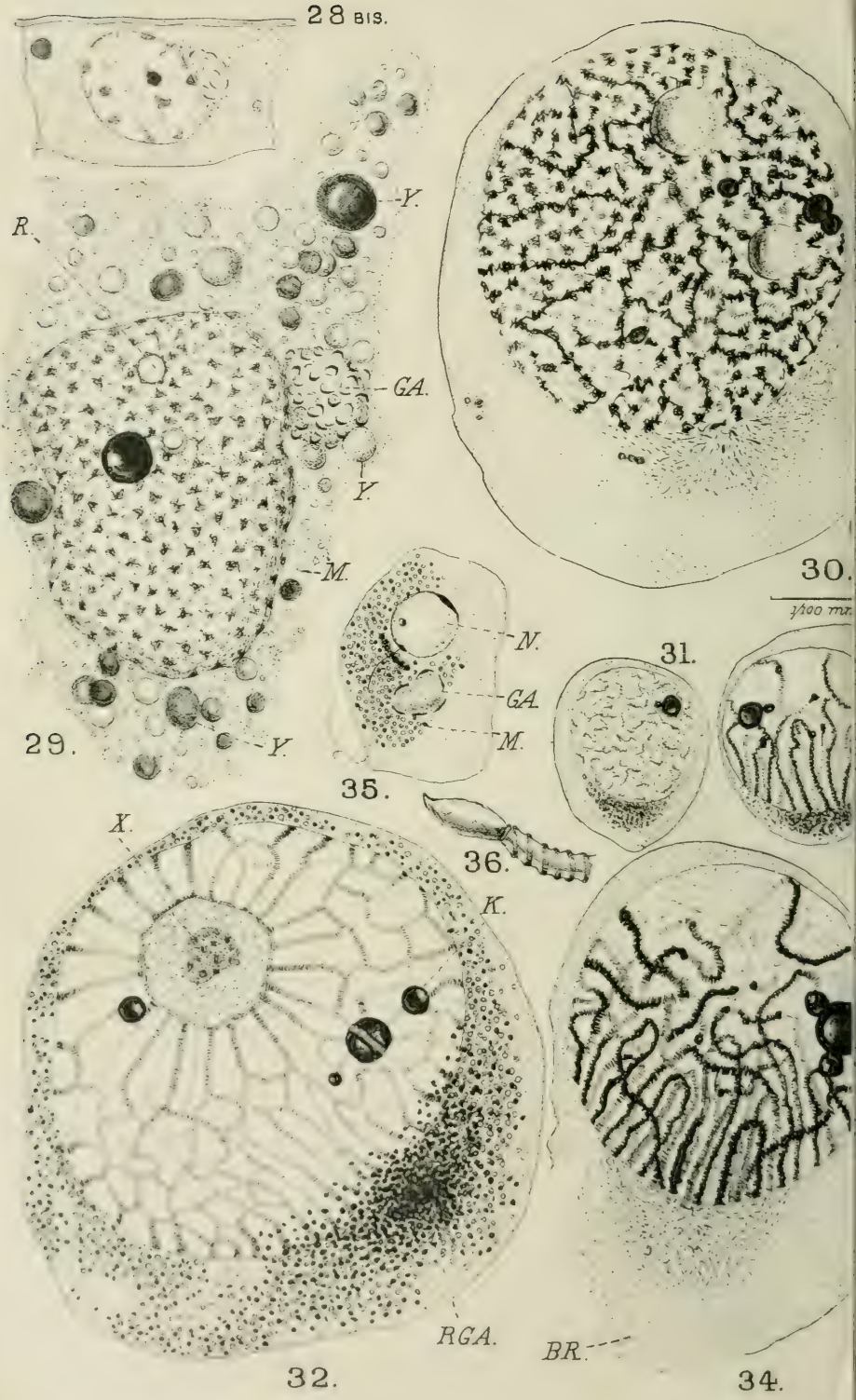

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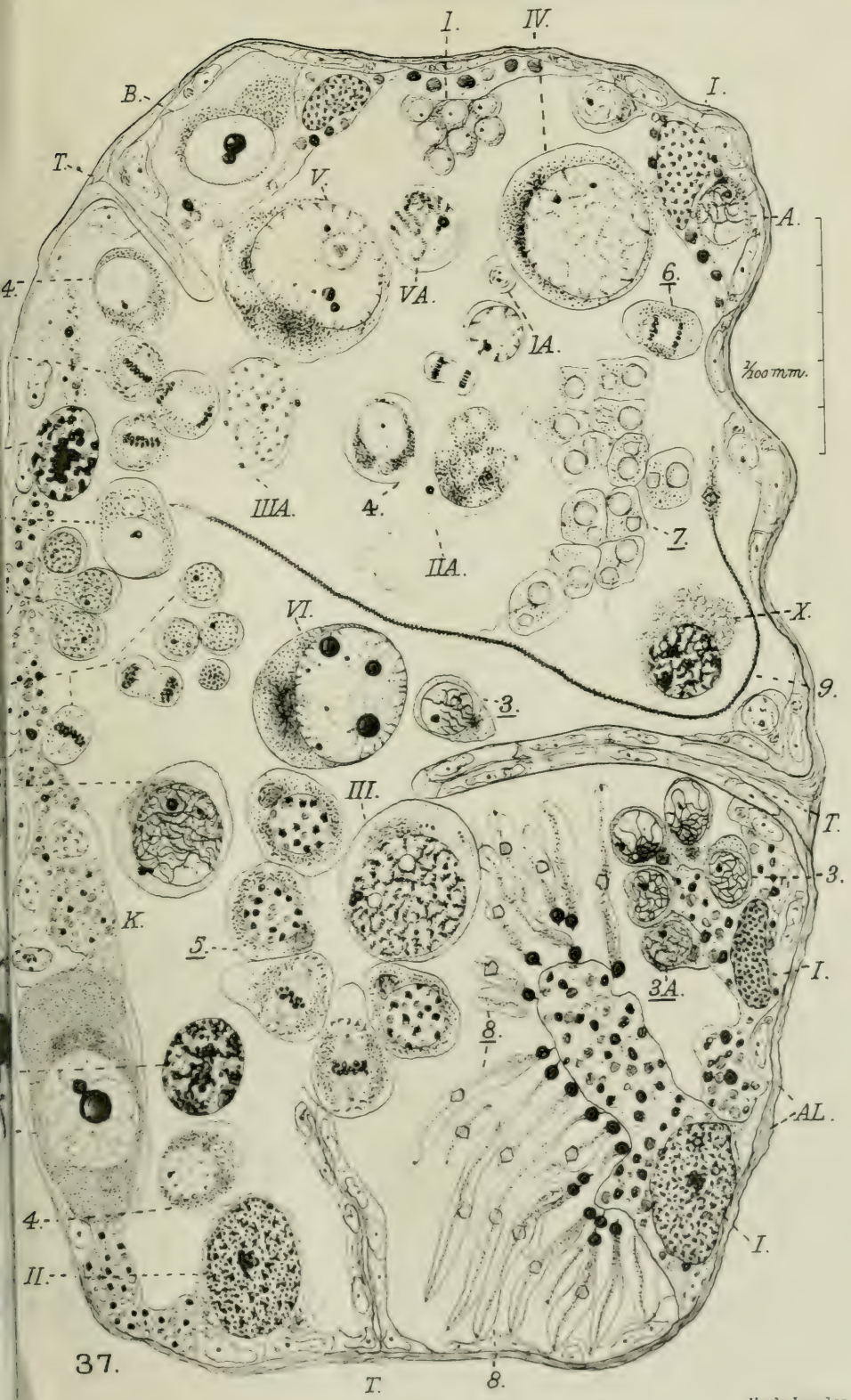

Huth, London

TACELLA.

The Cytoplasmic Inclusions of the Germ-Cells.

PART V. THE GAMETOGENESIS AND EARLY DEVELOPMENT OF LIMNÆA STAGNALIS (L.), WITH SPECIAL REFERENCE TO THE GOLGI APPARATUS AND THE MITOCHONDRIA.

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With Plates 27 and 28 and 6 Text-figs.

TABLE OF CONTENTS.

Introductory.

In the previous part (**20**) of this series of papers I gave a general account of the Golgi apparatus, and showed that the latter was present in the germ-cells and in at least all the more important somatic cells of the Metazoa. Reasons have been advanced which are considered adequate to demonstrate that the molluscan chondrioplasts (Nebenkern rod) are in reality the representative in the germ-cell of the nerve-cell Golgi apparatus (**61**). The molluscan Golgi apparatus has in spermatogenesis been the subject of several exhaustive researches (**19**), and in this section of my work I have described my attempts to trace out this apparatus in the oögenesis, during segmentation, and in the early germ-layer stages. In the same way the mitochondria are followed out.

The oögenesis and early development stages of the organism comprise periods about which no other section of embryology has raised so much discussion and theory, and it is a matter for satisfaction that modern methods should enable us to carry out new researches with a delicacy and certainty hitherto thought impossible. Reference to the table in another paper

(**61**) will suffice to show that the cytologist has been able to treat his subject from an analytical point of view, which enables him to trace out many bodies with practical certainty.

Previous Work.

No other work has been carried out on the gametogenesis and early development of Limnæa stagnalis, from the point of view of the cytoplasmic inclusions.[1] Several authors have described stages in the development of this Mollusc, or the closely allied Planorbis (**48**). The oögenesis stages described herein are quite new.

In development one finds the eight-cell stage to consist of four micromeres and four macromeres.

Conklin (**7**) describes the egg of Limnæa just after maturation as consisting of a clear "well" of protoplasm in the animal pole derived from the nucleoplasm of the burst germinal vesicle, of a finely yellow granular substance close around this clear "well," and beneath a mass of protoplasm with yolk. The clear "well" of karyoplasm afterwards spreads over the animal hemisphere; immediately beneath this clear cap lies the yellow substance, and the nucleus (or pronuclei) lies between the clear and the yellow substances. Conklin shows that the disposition of both the clear and yellow substances undergoes great changes during the time between the first maturation and first cleavage.

Centrifuging just after deposition and before maturation, the egg was found to show three layers—grey, clear and yellow—the clear protoplasm forming the middle layer. The yellow

[1] Since this was written a new paper by Jan Hirschler (**58**) has come into my hands. This observer has studied the fate of the Golgi apparatus also in Limnæa stagnalis, and has come to the conclusion independently arrived at by me, i. e. that the Golgi elements in the segmentation of the egg are equally distributed and pass through development without losing their identity. The oögenesis stages here given, and the work on the mitochondria are quite new, but I am glad to find that another worker has arrived at the same conclusions as myself with regard to the Golgi apparatus.

zone was the heavier pole of the egg. It contained "yolk"-spherules.

Conklin finds that the injurious effects of centrifuging increase rapidly from the time of the first maturation to that of the first cleavage. Eggs centrifuged during the maturation division usually develop normally; those centrifuged in the resting stage before the first cleavage rarely do. Conklin thinks that this increase in the injurious effects of centrifuging as the egg approaches the first cleavage stage is due to (i) increasing differentiation of the egg, and (ii) decreasing opportunity for readjustment of displaced substances. In all cases where the three substances, clear, grey and yellow, have been sharply separate, the clear protoplasm afterwards diffuses slowly into the grey and yellow zones. Differentiation of the oöplasm takes place mainly between maturation and cleavage. Finally Conklin says that his experiments show that the differently coloured substances of these eggs (Limnæa, Physa and Planorbis) are not "organ-forming" in the sense that each can give rise to only one organ or set of organs. In normal development the clear and grey substances are largely contained in the micromeres, or ectomeres, the yellow substance in the mesomeres or entomeres. But in the centrifuged egg the stratification of these substances may take place at any axis, and yet the form of development may be perfectly normal in every case. In cases the grey material may be cast out of the egg without interfering with normal development. Conklin makes the interesting statement that he got the impression that the grey and yellow substances are mere inclusions in the protoplasm, and that neither is essential to development. The clear substance, which increases rapidly, seems the real protoplasm of the egg, in which the heavier and lighter inclusions are contained. Conklin considers that the yellow substance decreases in quantity during development, being converted into clear and grey (protoplasmic) substances.

Morgan, in the same journal ('Journ. Exp. Zool.,' vol. ix), has studied the effect of the centrifuge on the eggs of

Cumingia (sea-urchin), Cerebratulus (Nemertine), Hydatina (Rotifer), and a fish. His results are hard to interpret from the cell-inclusion point of view, as are Conklin's. With regard to Cumingia he has some interesting remarks to make. He says that "the visible substances of the egg that can be centrifuged are not organ-forming." Morgan considers that abnormal development after centrifuging is not caused by the segregation of the visible substances of the egg. Such abnormal developments are due to mechanical difficulty of transport of nuclei into the mass of shifted yolk, or because of mechanical difficulties of such a mass in the gastrulating cells. Finally, despite such difficulties, Morgan considers that normal development may follow even when the visible centrifuged substances are unequally distributed, and are carried over into the blastomeres, redistribution being thereby prevented.

Technique and Material.

Egg-masses of Limnæa stagnalis were collected from water-weeds or from the sides of small aquaria. Eggs or embryos were either extracted singly from the jelly and fixed in a capsule, or large masses were treated as follows:

(1) They were left in Flemming-without-acetic for from three days to a week, or in Champy's fluid in the same way. They were then washed in running water for one night. After this the entire masses were thrown into the following mixture and left for two days: Chromic acid 1 per cent., 100 c.c.; bichromate of potash 2 per cent., 100 c.c.; nitric acid, 6 c.c. The masses were occasionally shaken up in this fluid (about 25 c.c. should be used), and this treatment dissolved away all the outer capsule and the inner gelatinous substance of the inner egg-capsule, leaving only the membrane, which did not interfere with the sectioning. In this way large quantities were easily done. The mass of eggs and membranes so procured was passed through up-graded alcohols from 50 per cent. alcohol, sectioned in wax, and generally stained in Heidenhain's iron-hæmatoxylin, with or without subsequent treatment in acid fuchsin or orange G.

(2) Masses of eggs were dried on blotting-paper to get rid of as much water as possible, and then fixed in 2 per cent. OsO_4 for a fortnight, according to Kopsch's method. After this they were washed overnight in running water, the outer membranes were separated with needles, and the inner capsules were passed through alcohols, embedded in celloidin and wax and cut into 6 μ sections, and either left unstained or stained in Altmann's acid fuchsin and picric acid.

(3) Egg-masses were first fixed in Carnoy and embedded in celloidin and wax.

After Kopsch's method (No. 2) the subsequent treatment in the chrome-solvent mixture could not be used, because the chromic salts interfere with the specific reduction action of the OsO_4, and the Golgi apparatus was difficult to see. The presence even of a trace of chrome salt tends to spoil the Kopsch reaction.

Warm Flemming-without-acetic or OsO_4 of 2 per cent. penetrated the masses in about a quarter of an hour, and even cold fluid penetrated quickly enough to provide maturation and fertilisation stages if the egg-mass had been just laid before treatment.

The Oögenesis of Limnæa stagnalis.

In Pl. 27, fig. 1, is a germinal epithelial cell of L. stagnalis; it is a flattened cell containing a nucleus with one karyosome and irregular lumps of chromatin; in the cytoplasm is found (Kopsch method) a Golgi apparatus formed of a few separate batonettes or chondrioplasts. No mitochondria could be demonstrated, but I am unwilling to claim that no mitochondria are present. Between the stages in Pl. 27, figs. 1 and 2, the nucleus breaks into filaments and the prophases of the heterotypic division are undergone. These have been given in part in my paper on another mollusc (**18**). By the synizesis stage of the prophases there is found around the Golgi-cum-archoplasmic apparatus a cloud, formed of matter which constitutes the mitochondria; the method of appearance

of this cloud is as already described for Helix aspersa (**18**). In Pl. 27, fig. 2, the mitochondria are clearly defined, while the Golgi rods are more numerous and the whole apparatus more conspicuous (*G.A.O.*). The nucleus has its chromatin arranged in the manner characteristic of the young oöcyte. Between the stages in Pl. 27, figs. 2 and 3, the archoplasm gradually grows larger, the number of batonettes also increases, and the former becomes constricted, first into larger parts (Pl. 27, fig. 3, *G.A.O.* 1 and *G.A.O.* 3), but soon into smaller parts. I think that it is the archoplasm which is responsible for this primary constriction. The fate of the centrosomes at this period I have been unable to ascertain; possibly, if it does not for the moment altogether degenerate, it detaches itself from the archoplasm and keeps near the nuclear membrane, but I think a centrosome appears later at the time of the formation of the polar bodies. I am inclined to believe that the centrosome becomes detached and lies near the nuclear membrane. Comparing the Golgi apparatus of Pl. 27, fig. 3, with that of Pl. 27, fig. 1, it will be seen that there has been a great increase, the small part marked *G.A.O.* 3 in Pl. 27, fig. 3, being as large as the whole apparatus (*G.A.E.*) in Pl. 27, fig. 1.

The individual batonettes or dictyosomes of the Golgi apparatus do not increase very perceptably in size; it is their number which becomes so large as to cause the apparatus to assume such importance. By the stage drawn in Pl. 27, fig. 4, the Golgi apparatus has spread out through the cytoplasm of one side of the oöcyte (*G.A.O.*), and in the larger oöcyte, in Pl. 27, fig. 5, it has spread on every side of the nucleus. Each Golgi group consists of several batonettes or collections of batonettes which are in process of dividing and then growing. In Pl. 27, fig. 14, the Golgi rod and its archoplasm is shown in process of growth and division.

Eventually in the full-grown oöcyte each Golgi element consists of a sphere of archoplasm, upon one side of which lies the batonette or dictyosome; the latter may be single or multiple; generally in Limnæa it consists of two rods, whose

ends touch at one point. Every part of the oöcyte cytoplasm is strewn with these Golgi elements in a fairly even manner, and every one of these elements has been derived by a process of growth and fission from the Golgi apparatus of the original germinal epithelial cell (Pl. 27, fig. 1, *G.A.E.*).

By the stage drawn in Pl. 27, fig. 2, the mitochondria have appeared as a rapidly-growing cloud of granules embracing the nucleus. If the ovotestis be fixed in Kopsch's method (OsO_4) and stained in Altmann's acid fuchsin and picric acid, the mitochondria stain reddish, while the Golgi apparatus goes quite black; any yolk-granules do not stain, but remain yellowish-brown (with a green tinge) from the osmic acid fixation. In Pl. 27, fig. 5, the yolk would, after Kopsch-Altmann, be greenish-brown to dark brown, mitochondria red, and Golgi apparatus black. There can be no mistake as to these elements, though it is impossible in unstained Kopsch sections to distinguish between egg-yolk and egg mitochondria, both of which are vaguely greenish-yellow to brown. The Golgi apparatus is, however, quite black.

In Pl. 27, figs. 3 and 4, the mitochondria are seen to be in process of growth and dispersal through the cytoplasm. Some of the granules grow faster than the others, and not all the mitochondria remain the same size.

As the oöcyte grows the mitochondria gradually pass evenly through the cytoplasm, and many of the individual grains attain a large size (Pl. 27, figs. 5 and 6). After the stage drawn in Pl. 27, fig. 3, the egg mitochondria grow denser as they become larger, and they no longer remain histochemically of the same nature as the mitochondria in the spermatocytes and spermatids. Nevertheless that these are the egg mitochondria there can be no doubt, as all manner of fixation and staining tests show. The alteration of the older oöcyte mitochondria during the growth period seems traceable to the fact that they become much denser, and therefore are able to respond differently from the more delicate spermatocyte mitochondria. This added denseness is in some way due to the metabolic conditions in the growing

oöcyte—conditions not found in the spermatocyte, which is not surfeited with formed nutritive materials, as is the full-grown oöcyte.

The mitochondria early become impregnated with a yellow pigment, which is destroyed by fixation in chrome-osmium and alcohol, and which gives the fresh egg of Limnæa its bright yellow colour. This pigment (a lipochrome?) is only present in the mitochondria, not in yolk or ground plasma. The full-grown mitochondrium of Limnæa is undoubtedly enlarged by the addition of some lecithin or other fatty matter, apart from its ordinary size. Such materials possibly serve to store energy used in subsequent development, and it is the using up of such material which causes the mitochondria to shrink in size during organogeny.

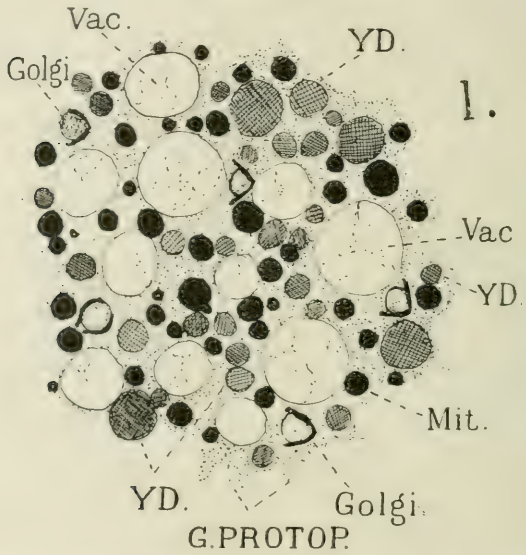
Deutoplasmagenesis.

Deutoplasmagenesis, or the formation of the yolk in the oöcyte, is a simple process. Yolk-discs are often found in the indifferent germinal epithelial cell, but the main formation of yolk-discs takes place after the stage of Pl. 27, fig. 4. While some of the yolk (i.e. the older discs) appears to stain black in iron-hæmatoxylin, fixation in Kopsch or in Flemming-without-acetic acid followed by Altmann's acid fuchsin and picric acid fails to stain the yolk, and leaves the latter greenish-brown, just as it has been coloured by the osmic acid. It is then a very easy matter to distinguish between yolk and the cytoplasmic inclusions. Pl. 27, fig. 5, *Y.C.*, is drawn from an iron-hæmatoxylin-stained preparation. In Pl. 27, fig. 10, is a cell drawn from an unstained Kopsch preparation; Golgi apparatus is black (*G.A.N.*), while yolk is yellowish to brown. In many cases the yolk-discs do not grow much larger than the largest mitochondria, but in other cases they grow about one and a half to three times the size of the largest mitochondrium. Examination of the centrifuged egg in Text-fig. 2 shows this plainly.

Structure of the Cytoplasm of the Ripe Ovarian Oöcyte.

In Text-fig. 1 I have drawn semi-diagrammatically a part of the ripe egg cytoplasm, after the latter is fully differentiated. The cytoplasm of the egg of Limnæa is frothy; this appearance is due to the presence of a large

TEXT-FIG. 1.



Diagrammatic high-power drawing of a part of the cytoplasm to show its structure. The cytoplasmic is vacuolated, and the vacuoles (*VAC.*) contain a coagulum. In the regions between the vacuoles is protoplasm (*G.PROTOP.*) containing yolk-granules (*Y.D.*), mitochondria (*MIT.*), and Golgi elements (*GOLGI*).

number of vacuoles, so numerous as almost to cause the entire egg to have a spongy structure. These vacuoles consist of a fluid substance which almost entirely becomes extracted in finished sections, but which leaves behind a slight coagulum. Whether the vacuoles are oily or watery I find difficult to say, but I think they are watery; the coagulum therefore might not be a fat, but a proteid

substance. These vacuoles appear fairly late in oögenesis, but are always present in the ripe ovarian egg. The "field" of the maturing egg, in which the polar spindles lie, contains no vacuoles, being quite smooth. In the stages following the gastrula vacuoles are hard to find, and are rapidly used up, either as food, or for some process connected with the physiology of development (Pl. 28, fig. 13, *VAC.*, in endoderm). Between the vacuoles of the egg lie the trabeculæ (or sponge-work) of ground cytoplasm, in which are embedded the three elements—yolk-spheres, mitochondria and Golgi grains. This is shown in Text-fig. 1, at *Y.D.*, *MIT.* and *GOLGI*; the ground cytoplasm is stippled (*G. PROTOP.*). In inferior preparations the granules may be carried into the vacuoles, but the best preserved sections show that yolk and other spheres are not normally a part of the vacuole system. This is in opposition to what I found in Helix aspersa (**18**), but I now believe that when yolk or other spheres lie in the vacuoles of eggs sectioned and stained, this is due to mechanical shocks during preparation. Possibly the edge of the knife draws some granules out of position.

Personal Work on Staining Egg and Sperm Mitochondria in Separate Colours.

It has been shown that the egg mitochondria become denser and more chromophil than those of the spermatid. I found it fairly easy, using this fact to guide me, to stain the egg mitochondria black and those of the spermatocyte and spermatid red. The ovotestis was stained in iron-hæmatoxylin as directed in my Helix aspersa paper (**18**), and then differentiated to a stage at which there was still too much hæmatoxylin left in the sections. These were then stained in Altmann's acid fuchsin and picric acid. The egg mitochondria, resisting the differentiation in iron-alum more successfully than the spermatocyte and spermatid ones, were left black before the application of the Altmann; the spermatocyte and spermatid ones were grey to bluish before

the addition of Altmann; the picric acid of the latter completed the washing out of the spermatocyte and spermatid mitochondria, and allowed these to stain in the red fuchsin, while the egg mitochondria managed to hold their hæmatin, and if any fuchsin entered them it only contrived to make them look still darker.

It will be seen that this process, which is capricious, depends on the correct degree of washing out of the ovotestis sections after staining in iron-hæmatoxylin. I found it rather difficult to get the spermatid mitochondria as brightly red as drawn by Held (**28**) for his Ascaris sperm mitochondria, but this may have been due to a difference in the fuchsin. It should be noted that the ripe sperm tail, like the egg mitochondria, stains black, not red.

The Mitochondria in the Early Development of Limnæa stagnalis.

At maturation, certain stages of which I have been able to examine, the germinal vesicle bursts and the contents of the latter flows upwards to form a zone clear of vacuoles, mitochondria and Golgi rods, wherein the maturation spindles arise.

At this period there is no flow of mitochondria to special regions, all the egg cytoplasm, with the exception of the cap of clear nucleoplasm at the animal pole, being evenly provided with granules, not only mitochondrial, but also yolk. In Pl. 28, fig. 11, is a two-cell stage, the mitochondria being drawn as circles. In Pl. 28, fig. 12, is an obliquely sagittal section of an eight-cell stage; the mitochondria are evenly distributed, even the clear cap of nucleoplasm having spread out flat over the surface of the animal pole.

In subsequent segmentation stages each cell gets a subequal amount of mitochondrial granules. Pl. 28, fig. 13, is a section through the gastrula showing the equal distribution of mitochondria to ectoderm (*ECT.*), endoderm (*END.*) and mesoderm (*MES.*) cells, the mitochondria being drawn as circles.

In subsequent organogeny the mitochondria are still subequally divided. Careful examination of the mitochondria in the unsegmented egg and in the advanced differentiating organ or germ-layer seems to establish the fact that the mitochondria shrink gradually in size pari passu with the differentiation of the tissue. They ultimately reach a minimum size, but do not disappear from the differentiating somatic or germ-cells during any stages I have examined.

The Golgi Apparatus in the Early Development of Limnæa stagnalis.

During maturation the even distribution of the Golgi elements throughout the egg is not altered. In Pl. 28, fig. 11, is a two-celled stage showing the Golgi rods black, archoplasm stippled. In Pl. 28, fig. 12, the Golgi elements, like the mitochondria, are evenly distributed to both micromeres and to macromeres. The rods, just like the mitochondria, lie passively in the cytoplasm, being attracted by neither centrosomes nor nuclei. In all the subsequent segmentation stages this rule is adhered to, and the Golgi elements eventually become distributed to the endoderm, mesoderm and ectoderm cells of the gastrula, as in Pl. 28, fig. 13; here the Golgi elements are black. With the exception of a cell here and there, in almost every case the Golgi rods with their archoplasm lie apparently inert in the cytoplasm of the cells. In the exceptions, such as the ectoderm cell above in Pl. 28, fig. 13, marked *ECT.* and *G.A.*, the Golgi apparatus may lie in a juxta-nuclear excentric position, possibly around a centrosome. In later stages, as the germ-layer cell elements become smaller, the Golgi rods gradually become attracted by either nucleus or centrosome (the latter probably), and become placed in a juxta-nuclear excentric position. In the ectoderm the Golgi rods lie towards the outside of the cell-layer. In division the Golgi rods keep within the zone of the asters. This change in the behaviour of the Golgi elements takes place gradually after the formation of the gastrula.

Centrifuging the Ovarian Egg.

The approach of winter and various duties prevented my complete study of the newly laid egg, but during the November of 1918 I carried out a number of experiments on the ovotestis. The latter in November and subsequent months contains many full-grown oöcytes and provided good material for this study. Ovotestes were removed from the Limnæas, and several were quickly transferred to some salt solution in a centrifuge tube. These were then centrifuged from five to ten minutes at 3500 revolutions a minute on an electric centrifuge.

Immediately afterwards the liquid was poured off and the ovotestes were jerked out into a capsule of fixing solution. Such material was then treated as I had previously done the normal ovotestis. My results were very successful, and have enabled me for the first time, I believe, to study correctly the nature of the layers of the centrifuged egg, already described in the fresh by Conklin. The latter, as has been shown, considered that of the three layers in the centrifuged egg, the bottom yellow and heaviest layer was formed in part at least of "yolk-spheres," then there came the middle "clear substance" and the uppermost "grey substance."

Examined by the best modern methods the egg layers are found to be composed as follows (see Text-fig. 2).

(1) Upper grey substance consists of yolk-discs and a very little protoplasm.

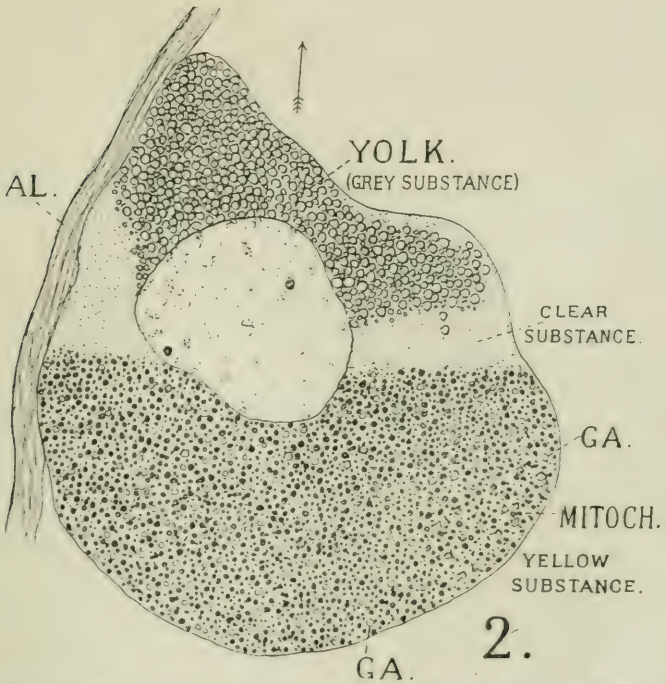
(2) The middle "clear substance" is pure protoplasm without any inclusions.

(3) The lower "yellow substance" consists of the bright yellow mitochondria, and of the Golgi apparatus suspended in protoplasm.

It was noticed that even in the smallest oöcytes containing yolk-discs, the latter came to the top of the cell. With regard to the diffusing of the clear substance into the yellow and grey zones which happens after leaving the centrifuged egg for a little time—a fact noticed by Conklin—I have found

that only those eggs on the outside of the ovotestis (i. e. best exposed to fixative) showed the three clear layers. This was due to the fact that during the time the fixative had taken to penetrate to the inner regions of the ovotestis, the grains dislodged from their normal position by the centrifuge began

TEXT-FIG. 2.



Camera lucida drawing of a centrifuged ovarian oöcyte. *G.A.* Golgi apparatus. *A.L.* Ancel's layer of ovotestis wall.

to regain their normal relations, the yolk sinking, the mitochondria floating up into the clear middle zone.

Conklin found the grey zone (which I consider yolk) could sometimes be completely disrupted from the egg without affecting subsequent development. The fact that this grey substance is yolk explains Conklin's experience. Conklin is wrong I believe in considering the yellow zone contains

"yolk." It consists entirely of yellow mitochondria and of the Golgi apparatus, together with protoplasm.

It was not a difficult matter to fix the centrifuged ovotestis overnight in the modification of Flemming-without-acetic acid, and subsequently stain in iron-hæmatoxylin and van Gieson. The layers were then as follows: Grey (yolk) zone, yellowish-green; clear zone, red; and yellow (mitochondria and Golgi grains) zone, black. With the same fixation and Altmann's stain the zones were as follows: Yolk, greenish; clear zone, yellowish; and mitochondrial zone, red.

Finally I may say that my centrifuge experiments completely uphold my interpretations of the bodies in oögenesis, and such experiments will undoubtedly help observers to clear up doubtful points in studying oögenesis.

Discussion.

Modern research has show that the architecture of the ovum is remarkably complicated, for within its small compass are the potentialities which unfold to form the differentiated embryo and thence the adult. We know that at least two categories of living cell organs, the mitochondria and the Golgi apparatus, are important in oögenesis, and the fate of these structures has been followed out during oögenesis, segmentation and early development; that is during the stages when we know that the potentialities of the ovum are being organised, and are subsequently unfolding themselves.

On the one hand we have a goodly number of excellent observations on the coloured or more or less opaque substances in the fresh egg before, during, and after fertilisation. I refer to such valuable work as that of Conklin and Morgan. On the other hand we have the latest modern work on the cytoplasmic inclusions of the gametes during their formation; there are now a few papers on the mitochondria in early development. It is interesting, therefore, to see how such lines of work compare with each other. I have endeavoured herein to undertake such a comparison, but because of the

fewness of the studies of the inclusions during early development the work has been difficult, and the result somewhat unsatisfactory. The near future will see the elucidation of these questions.

One result of modern work on the elements in the cytoplasm has been to show that the mitochondria and the Golgi rods or granules have the power of binary fission, and in the case of the Golgi rods possibly also the power of multiple fission; for the necessary proof of these facts see **1**, **13**, **18**, **23**, **28**, **29**, **41**, **50**, **52**, **53**, **54**, **56**, and **59** in the bibliography. This result is undoubtedly very important, because it demonstrates that the power of division is not limited to centrosome and chromosomes or nucleus, but is shared by other elements which seem to possess a high degree of morphological independence; both mitochondria and Golgi bodies are able to assimilate, grow and divide in the cytoplasm somewhat as a protist assimilates, grows and divides in its watery medium. I do not believe that either mitochondria or Golgi bodies are symbiotic organisms, as has been claimed for the yellow cells of Radiolaria, but it seems true that the cytoplasmic inclusions have a marked degree of independence. The movements of chondriokonts in the cells of plants and animals are often very elaborate and peculiar, as has been shown by the Lewis's and Guilliermond. Such movements, and even fission, might be directed by special stimuli emitted from the nucleus, but one seems forced to admit that the cell is much more a colony of semi-independent though perfectly regulated elements than was before held to be the case. The exact relationship between nucleus and cytoplasm and between these two parts of the cell and the Golgi elements and mitochondria are at present little known. The fact that both Golgi apparatus and mitochondria have a rôle in gland-cells, such as pancreas and salivary alveoli cells, causes one to favour the view that the cytoplasmic inclusions are not merely growing and dividing at the expense of the ground cytoplasmic and nuclear activity, but are contributing in some way towards the growth and formation of the differentiating cytoplasm; it is to be

remarked, however, that of positive evidence we have very little either way at the present moment.

The complete demonstration of both Golgi apparatus and mitochondria in all animal cells appropriately examined is a fact of cardinal importance to zoologists, and leads us to consider that by the aid of experimental methods a flood of light may in the near future be shed on the obscurities surrounding our knowledge of the modus operandi of the cell organs in various vital phenomena.

Definition of Organ-forming Substances.

In order not to cloud the following remarks it is necessary for me to state exactly what is meant by the words "organ-forming substances." Speaking generally, these words simply mean those materials in the egg-cell which, as in ascidians, become segregated into special regions and ultimately come to form organs or parts of organs—such would be yolk which in many animals comes to lie in the endoderm. But when one considers, it seems likely that in the latter case the yolk is not the only endoderm-forming substance which is situated in the embryonic endoderm; I cannot believe that the cytoplasm of the latter is not different from that of the ectoderm or mesoderm. The substances which confer on the endoderm-cell its quality of "endodermness" are presumably derived from the nucleus at some stage of oögenesis or development, but such substances are in reality the "organ-forming materials" in the truest sense of the word. Whether

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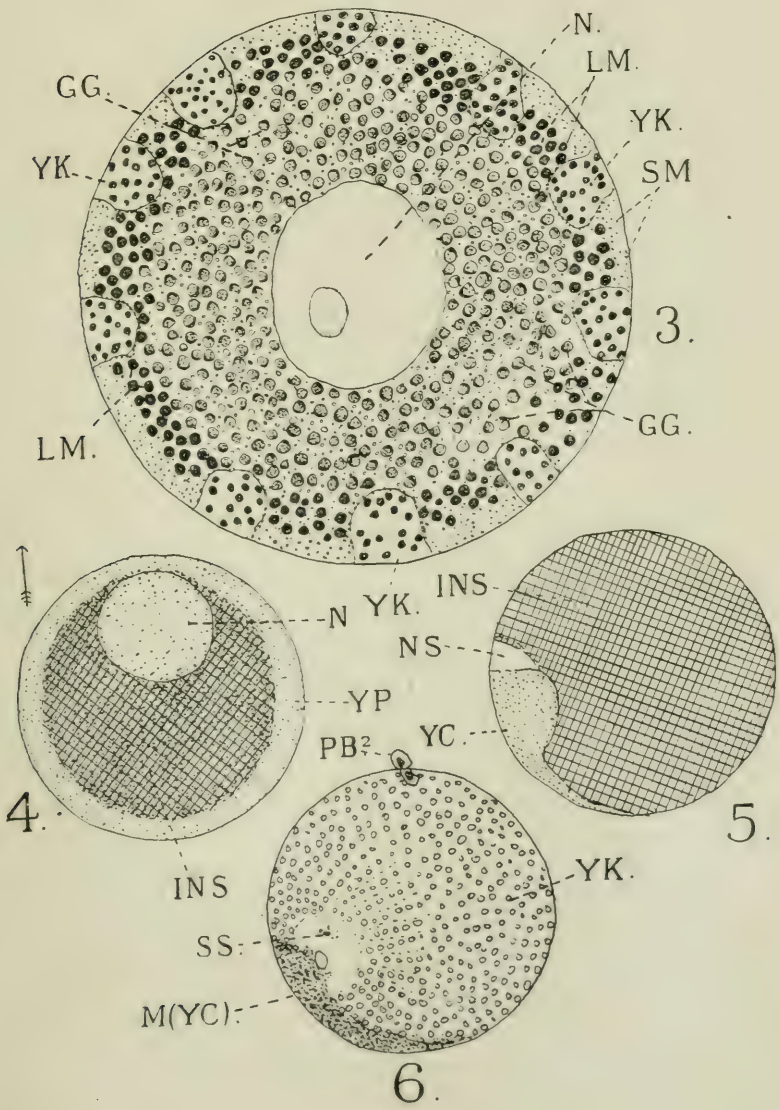
TEXT-FIGS. 3–6.

3. Full-grown ovarian oöcyte of Ciona (diagrammatic after Hirschler). *G.G.* Golgi apparatus. *L.M.* Large mitochondria. *S.M.* Small mitochondria. *Y.K.* Yolk pockets. 4. Schematic figure of Cynthia oöcyte, after Conklin. *N.* Nucleus. *Y.P.* Yellow-pigmented cytoplasm. *I.N.S.* Inner region. 5. Maturing egg during fertilisation. *N.S.* Clear substance (karyolymph). *Y.C.* Yellow crescent. 6. Ciona egg at same stage after Duesberg. *YK.* Duesberg's "yolk," which Hirschler calls Golgi elements. *S.S.* Clear space around male pronucleus. *M.* (*Y.C.*). Mitochondria in yellow-crescent region.

TEXT-FIGS. 3–6.



the mitochondria and Golgi elements are carriers of these suggested inner substances, or themselves constitute these substances, is a moot point which is further discussed below; but it is believed that some distinction should be made between such organ-forming substances as yolk and between the more subtle bodies which I have assumed to exist in the ground cytoplasm. Yolk, fat, pigment, and possibly also the mitochondria and Golgi apparatus might be looked upon as purely "nutrient" organ-forming substances, engaged in supplying materials for the work being carried out by the "definitive" organ-forming substances. These definitive or positive organ-forming substances might be what one well-known writer has assumed to be special enzymic bodies. In the following remarks the words "organ-forming substances" are taken to mean both the "nutrient" and "definitive" organ-forming substances unless otherwise stated.

Mitochondria, Organ-forming Substances, and Idioplasm of the Cytoplasm.

MacBride (**34**), in his recent valuable work "Text-book of "Embryology—Invertebrata," describes organ-forming (definitive?) substances as materials emitted from the nucleus during the ripening of the egg—materials which confer on the cytoplasm a definite character. Organ-forming substances are, according to this interesting writer, most plausibly to be regarded as of the nature of hormones or ferments.

By the majority of workers the hypothetical substance called "idioplasm," the physical basis of inheritance, is thought to be identical with the chromatin. We are driven then to ask, What relationship is there between the idioplasm and the organ-forming substances? and between both these and the cytoplasmic inclusions, Golgi apparatus and mitochondria?

Again, MacBride writes (**35**): "We have been gradually led to view the nucleus as a storehouse of all the characters of the species, and to look for the cause of the first differentia-

tions seen in development in the modification of the cytoplasm through the emission of substances from the nucleus." MacBride (**35**) quotes the work of Schaxel (**49**) and of myself (**16**) as support that such emissions can be seen in prepared specimens of developing oöcytes. Schaxel's work has been shown to be wrong in so far as this observer describes emanations of solid chromatin particles from the nucleus, through the nuclear membrane into the cytoplasm (see Beckwith **3a**, and **18**), while my own work, done some years ago by imperfect technical methods, cannot be interpreted as supporting Schaxel. In my work on the frog (**16**) I showed that a basophil cloud could be seen to appear around the nuclear membrane at a certain period. Further work shows that this cloud is formed of mitochondria which were present in the young cell, but which at a certain period of activity of the nucleus of the metamorphosing cell begin to grow and become denser. Nevertheless, it must be pointed out that in reality MacBride's interpretation of the fact is correct, because few would care to deny that the growth and changes of mitochondria at this period were not directly due to emanations from the nucleus. The point to be noticed is that the mitochondria are not chromatin, and do not usually appear in themselves directly to be due to emanations from the nucleus, but their primary stimulation and growth are probably initiated by fluid substances passed out from the nucleus. These substances are not chromatin apparently, nor can they be seen in the form claimed by Schaxel.

In a paper on the snail (**18**) I showed that, with the Flemming-without-acetic acid and iron-hæmatoxylin technique, the nucleus of the indifferent Helix germinal epithelial cell, when becoming "progerminative," could be seen to be capped on one side by a cloud of almost structureless stainable matter (see **18**, Pl. 31, figs. 11 and 19). I regard this for the time being as perfect evidence, in MacBride's sense, of emissions of substances from the nucleus into the cytoplasm, but I cannot say how further improvement in technique will lead us to interpret the peri-nuclear cloud.

The cloud drawn by me in the figures already mentioned is due to the coagulation by the fixative and subsequent staining by the hæmatoxylin of some substance which, intra vitam, must have occupied that region of the cell. Whether this substance existed intra vitam in the form of grains, of bulk colloid, or of a filamentous cloud, as drawn by me, is of no real importance to this part of the discussion. The really vital fact is that such a cloud was present in some form during the life of the cell, and that this cloud is developed in such a relation to the nucleus as to allow of no other interpretation but that it comes under the direct influence of the nucleus. But I agree with Miss Beckwith (**3a**) in this: "There is no evidence of formed material passing through the nuclear membrane into the cytoplasm either early (Schaxel) or late (Smallwood) in the growth period." By the term "formed material" I would mean chromatin or other visible "solid" particles or grains as drawn by Schaxel,[1] and this apparently is Miss Beckwith's view.

Duesberg considers that the mitochondria do not represent the organ-forming substances, but that the mitochondia of the egg represent that idioplasm (**10**) which is situated in the cytoplasm. Unlike Duesberg, at present I do not see my way clear to distinguish between idioplasm and organ-forming substances, but I am prepared to endeavour to distinguish between the *definitive* organ-forming substances and the mitochondria.

With regard to Duesberg's interesting views something may now be written. This observer, in his latest paper (**10**), no longer dogmatically insists that the tail of the sperm is that part of the male cytoplasm which takes part in fertilisation, and he shows every sign of preparing his ground for a

[1] In certain Insecta the nucleus of the oöcyte appears to extrude some of its chromatin in the form of grains, which constitute the "secondary nuclei." The latter are distinct from either mitochondria or Golgi apparatus; the secondary nuclei are treated in a forthcoming paper of this series. Dendy has described extrusion of chromatinic matter in the oöcyte of a sponge ('Quart. Journ. Micr. Sci.,' vol. 60 1914–15).

complete rejection of his former view. But, while Duesberg has doubts with regard to the idioplasmic function of the middle-piece of the sperm, he now adopts a new view: it is that the mitochondria of the egg represent that part of the idioplasm supposed to be situated in the cytoplasm; while to explain the difficulties with regard to the mitochondrial part of the sperm, he assumes that the gametes are really inequivalent, and that the idioplasmic function of the egg mitochondria is possibly not shared by that part of the spermatozoon.

Duesberg, however, fails to give any explanation as to the function of the sperm-mitochondria. The very constancy of their presence in all sperms shows that they must have some function. Duesberg quotes the views of Jenkinson, Schreiner and Broman on the supposed inequivalence of the gametes, and uses this as support for his view that the egg and sperm mitochondria have a different function.

It seems true that in some forms at least (Molluscs and Nematodes) the egg mitochondria are much denser than the mitochondria of the spermatid or spermatozoon, but this greater density of the egg mitochondria is possibly connected with the differing metabolic conditions in the egg, as contrasted with the sperm, where there is very little storage of food materials. Moreover, one would expect the sperm (not the egg) mitochondria to be denser, since denseness might be associated with concentration.

Duesberg considers that the idioplasm of the cytoplasm of an egg might be located in the mitochondria. More recent researches (Weigl, Hirschler, Gatenby) show that in some cases the bodies in the cytoplasm are not all mitochondria, many being derived from the Golgi apparatus—a distinct cytoplasmic organ, which, however, is somewhat related to the mitochondria in some of their chemical reactions. Duesberg should now explain what place the Golgi apparatus fulfils in his conception of the location of the idioplasm, and he should bring it into apposition with his views on the mitochondria, for both Golgi apparatus and mitochondria are alike definite cytoplasmic organs evidently sharing in the formation of the

ground-protoplasm of the egg. Further, it seems certain that some definite relationship exists between these two cytoplasmic organs; in Limnæa and other Mollusca (Helix, Arion) and in Hydractinia (Beckwith) the Golgi apparatus is small in bulk when compared with the mitochondria, while in Hirschler's Ciona the Golgi apparatus is very large in bulk and the mitochondria relatively smaller. (Miss Beckwith's "pseudo-chromatin granules" are, I believe, the Golgi apparatus, for her description of them corresponds very closely with that of the Golgi apparatus in mollusc eggs.) It therefore seems probable that the smallness in bulk of one sort of cytoplasmic inclusion may be made up by the largeness in extent of the development of the other—a fact which points to some relationship of function between the two.

If one fixes the ovary in Carnoy's fluid (absolute alcohol, acetic acid and chloroform), and subsequently treats in alcohol and xylol, one sweeps almost everything out of the cytoplasm, and leaves the latter smooth; the cell then is seen to consist of a nucleus and a ground-cytoplasm. To my mind the ground-cytoplasm left after this operation is the place of location of the cytoplasmic idioplasm, and definitive organ-forming substances. In a given germ-layer of an embryo I consider one might get in the cytoplasm of the cells several groups of materials. One might have an organ-forming substance distinct from the pure cytoplasm of the species, one would have in addition yolk, and the two categories of cytoplasmic inclusions. The relationship between, and common identity of, the organ-forming substance and the pure cytoplasm is a matter of doubt. When I use the term "pure cytoplasm," I conceive that cytoplasm which must be common to all cells of the body no matter what their function, and which is, after the nucleus, in itself the basis of the largest and smallest characteristics of that organism. In special organs this cytoplasm may be temporarily or permanently altered, or infused with special substances necessary for the organ to fulfil its functions. In this conception the mitochondria, instead of taking a major place as prime substances of

heredity, take the minor position as substances important in some parts of growth and general metabolism. But a great deal more work will need to be done properly to clear the various issues above mentioned. This I readily admit.

The mitochondria and the Golgi grains may also take part in the storage or elaboration of substances which may be drawn upon to provide energy during segmentation and organogeny; but these views are very different from that of Duesberg, who wishes to identity with the mitochondria functions directly connected with the hypothetical idioplasm. Moreover, that the mitochondria have some function either as the storers of energy-producing materials, or the providers of such, is, I consider, made more probable by Duesberg's own demonstration that the mitochondria take a great part in the formation of the myoplasm (muscles) in Ciona. One cannot but believe that the works of Guilliermond on plants (**22–25**), Dubreuil (**12**) on mitochondria and fat, and Arnold (**2a**) on the pancreatic zymogen granules and the mitochondria all argue strongly against the Meves-Duesberg view.

Under this section it may safely be concluded that present researches on the mitochondria in gametogenesis and development lead us strongly to believe that the mitochondria are unconnected with idioplasm or other hypothetical hereditary substances. Their function seems to be to elaborate certain materials which are utilised in the up-building of the gamete.

Mitochondria, Golgi Apparatus, and the Organ-forming Substances in Ascidia.

Jan Hirschler (**29**) describes the oögenesis of severa ascidians, and shows that the egg contains yolk, mitochondria and Golgi granules. In Text-fig. 3 is a diagrammatic drawing showing the final arrangement of the granules in the ascidian egg (Ciona). At the periphery of the egg lie a number of pockets containing yolk (*YK.*), and in the same region lie large mitochondrial grains (*L.M.*), about four or five deep. More inwardly and around the nucleus one finds a very large

number of Golgi granules (*G.G.*). Interspersed between the latter there are smaller granules which are identical in histochemistry with the larger Golgi grains, and mixed up with the peripheral mitochondrial grains are much smaller mitochondrial elements (*S.M.*), also identical in their histochemical reactions with the large mitochondria. Hirschler's main points are as follows:

(1) The mitochondria lie peripherally and are not mixed up with the Golgi elements.

(2) The Golgi elements form the bulk of grains in the egg, and lie internally to the peripheral mitochondrial elements.

(3) There are smaller Golgi and mitochondrial grains lying in the special regions occupied by their larger fellows.

(4) Yolk is scanty and lies in pockets on the periphery of the egg.

Hirschler gives satisfactory evidence of the difference in morphology and histochemistry between Golgi elements and mitochondria, both of which he has traced out from the earliest germ-cell. Personally I find nothing to doubt in his description of the oögenesis of the ascidian.

Conklin's work on the "organ-forming substances" in Ascidia is too well known to need lengthy description. MacBride has given a careful account of Conklin's work in his recent 'Text-Book on Embryology.' In Text-fig. 4 is a diagrammatic copy of one of Conklin's figures of the mature oöcyte. The periphery of the egg (*Y.P.*) is occupied by a rind of "yellow protoplasm" (compare with Text-fig. 3). The inner part of the egg contains much more opaque materials (*I.N.S.*); at *N.* is the nucleus. During maturation and fertilisation the nucleus bursts, and the karyolymph flows upwards and forms a cap on the animal pole; this material (or part of it) and the yellow rind of cytoplasm now flow downwards and around the sperm-aster, as in Text-fig. 5; *Y.C.* is the yellow cytoplasm (mitochondria) or myoplasm, and *N.S.* the clear cytoplasm (karyolymph). The inner more opaque substances (Golgi granules ?) are at *I.N.S.*

Duesberg finds the yellow-cresent substance (*Y.C.*) to be

mitochondria. In Text-fig. 6 is a diagrammatic copy of his interpretation of the unsegmented maturing egg. The substance at *X.*, corresponding to Hirschler's Golgi elements, Duesberg calls "yolk." Duesberg seems to be in agreement with Hirschler with regard to the grains (yellow pigment) in the outer rind of the egg; both observers find it to be mitochondrial. Since Duesberg does not pay any attention to the Golgi apparatus and has not used the correct technical methods, one can understand his calling Golgi granules "yolk."

Shortly, Hirschler's or Duesberg's work shows:

(1) The outer peripheral layer of the egg (yellow cytoplasm with pigment) contains the mitochondria.

(2) The rest of the egg is mainly occupied by Golgi elements (Duesberg's "yolk").

(3) The yellow crescent (myoplasm) is formed of protoplasm, in which lies the bulk of the mitochondria.

Unfortunately Hirschler does not discuss his work in the light shed by Conklin's studies on the fresh ovum of ascidians. The interpretations of the "plasms" of Conklin's ascidian egg are still much clouded, and the matter is far from being settled by either Hirschler or Duesberg. Both observers contribute valuable evidence. Finally it may be stated that Hirschler and Duesberg have brought forward evidence which shows that certain of the "organ-forming" regions in the fresh ascidian egg owe their differentiation to a definite segregation in those special regions of granules which are probably the mitochondria and the Golgi apparatus.

Golgi Apparatus.

There can be no doubt now that the Golgi apparatus and mitochondria are distinct and separate from each other. The attitude of Duesberg in his comprehensive 1912 review can only be upheld in view of the work of Hirschler, Weigl, Perroncito, Nussbaum, Golgi, Cajal and myself by disregarding the facts. In his paper on Ciona, Duesberg 10 uses no Golgi apparatus method such as that of Kopsch or

Cajal, and further adherence to his standpoint is impossible. When Duesberg uses the proper methods I have no doubt that he will find the Golgi apparatus to be distinct from the mitochondria.

The reader who is not specially acquainted with the latest literature on the Golgi apparatus and mitochondria may be assured that while there is distinctly good evidence of a relationship of chemical constitution between Golgi apparatus and mitochondria, there is now quite sufficient evidence which has been independently produced by several reliable workers that the Golgi apparatus is a distinct entity in all active cells of the metazoan; in germ-cells especially is the Golgi apparatus distinct and evidently separate from any mitochondrial apparatus. In the case of Monocystis ascidiæ, Hirschler ('Anat. Anz.,' vol. xlvii) found that there was a typical Golgi apparatus distinct from the mitochondria. I have little doubt that other Protozoa will also be found to possess these two distinct categories of cell-organs.

The study of the Golgi apparatus in "zoological" material has been somewhat neglected. Curiously enough, some zoologists seem to consider that this cell-organ is rightly neglected; such a valuable paper as that of Hans Held (**28**) is incomplete, because he neglected to study the Golgi apparatus, previously shown to be present in both egg and ripe sperm of Ascaris by the excellent Polish observer Jan Hirschler (**59**). Held's remarks on Ascaris may therefore be interpreted with Hirschler's paper. In all probability the few Golgi batonettes present in the Ascaris sperm are, like the mitochondria, carried over during fertilisation, and continue afterwards to grow and divide; whether, however, they spread throughout the egg cytoplasm like the sperm mitochondria it is impossible to say. The Golgi apparatus, this present paper shows, is during oögenesis equally scattered throughout the cytoplasm of the ovum, and in segmentation the blastomeres, before the process of gastrulation, are each provided with a portion of the original apparatus of the germinal epithelial cell. The out-spreading of both Golgi apparatus and mitochondria of Limnæa we now

know to be, among other things, a preparation for the subsequent disposal of parts of these elements to every cell of the morula, and thence of the embryo and adult organism.

In Mollusca of the Pulmonate type, such as Limnæa, Helix or Arion, and in Paludina, the Golgi apparatus seems to be sloughed off the sperm during spermateleosis, and can take no part in fertilisation and thence in heredity. The Golgi apparatus of Molluscs seems to be passed on from generation to generation through the ovum. Subsequent work might reveal a Golgi apparatus in the ripe sperm, but so far my own researches have failed in this respect, and I do not believe that Golgi elements are present in the ripe mollusc spermatozoon. Weigl (**53**) gives a figure showing the presence of the Golgi apparatus in the sperm of Cavia. Weigl's work is illustrated by convincing microphotographs, and may be recommended to those who desire to deny that the Golgi apparatus and mitochondria are not separate entities.

In Lepidoptera (**17**) I described the presence of certain bodies specially in spermatogenesis, which I traced ultimately to vesicles in the spermatid; I described these vesicles as running together to secrete a granule which was the acrosome of the sperm. I have now little doubt that these sickle-shaped rods —called by me acroblasts—are really the Golgi apparatus of Lepidoptera. I have re-examined all my material and have made more preparations, and can come to no conclusion other than that they form the acrosome and do not slough off. Several friends who have examined my sections have come to the same conclusion. The matter is still being examined by me. Weigl (**53**) has described similar bodies in a Sphingid, but does not follow out their fate. Casteel's (**5**) bodies in Argas are likewise possibly Golgi apparatus, and they also behave very like my bodies in Lepidoptera. Further work is needed to clear up these questions with reference to Arthropoda, and especially Insecta.

Our knowledge that the Golgi apparatus (and the mitochondria) spreads out throughout the egg cytoplasm during oögenesis might be interpreted in two ways: it may be con-

sidered that this spreading out is to enable the activities of the Golgi rods to be felt in every corner of the cytoplasm, or that the spreading out of the apparatus is only in preparation for subsequent segmentation of the egg. I think both interpretations are true, and there seems little doubt that the two categories of cytoplasmic inclusions whilst spreading out are taking some part in the building up of the oöcyte.

It was previously pointed out that the spermatid mitochondria are immensely smaller in bulk than the egg mitochondria; exactly the same unequal relationship exists between egg Golgi apparatus and spermatid Golgi apparatus. The explanation of this may be that the cytoplasmic inclusions, being concerned in metabolism, are naturally proportionately small in the small spermatid cell and proportionately large in the oöcyte, which contains so much more nutrient and formed matter. While it can be shown that the chromatin matter in egg and sperm is equal in bulk (e. g. the pronuclei at fertilisation), this relationship has only been shown to apply to the mitochondria at the same period in one case—Ascaris megalocephala (**28**).

The great importance of the Golgi apparatus may be gauged when one remembers that every sort of metazoan cell carefully examined has been found to possess the typical apparatus. Every mitosis or karyokinesis is also, as well, a dictyokinesis, or a nearly equal sorting out of the Golgi rods between the daughter-cells (**20**). Moreover, it seems probable that when the possibly universal occurrence of Golgi apparatus in Metazoan, protozoan and plant cells is recognised widely, and the attention of more workers is brought to bear upon these problems, some relationship between the amphiaster and the Golgi rods or granules may be discovered, and the real function of the cytoplasmic inclusions definitely ascertained.

The main fact which it is desirable to emphasise in this section is that a Golgi apparatus has been described in every animal order, and is throughout of the same general type. In my previous article I gave some description of the morphology of the apparatus. It nearly always consists of a sphere of

archoplasm, such as is found around a centriole (centrosome), upon which lie several more or less deeply curved batonettes or little rods. In mitosis the rods are sorted out whole—a process called by Perroncito dittokinesis or dictyokinesis; each daughter-cell gets about half of the original number of rods; in some cases the rods are branched and may fuse to form a reticulum.

Centrolecithality, Telolecithality and Alecithality with regard to Yolk-discs and Mitochondria.

The yolk in Limnæa is not massed on one side, nor does it lie in the middle: it is evenly scattered through the cytoplasm; this has been called a homolecithal egg. In Limnæa there is some evidence that the vacuoles in the protoplasm, already described, are more numerous in the vegetative hemisphere; that they ultimately mainly come to lie in the large endoderm cells seems certain. There is every likelihood that the large mitochondria, which are possibly partly laden with food material of a lipin nature, and which are less easily destroyed by certain techniques than the delicate yolk-spheres, might be mistaken for the latter. It must be noticed by embryologists that such yolk as one finds in molluscs is very delicate, and does not stain black in iron-alum hæmatoxylin. The bodies found in many invertebrate eggs, which stain black in hæmatoxylin, are nearly always mitochondria. In another paper I have carefully entered into the histo-chemistry of yolk and mitochondria (**61**).

What it is necessary to point out here is that in the eggs of molluscs (Helix, Paludina, etc.), insects (Apanteles, Sphinx, etc.), amphibians (Rana, Triton), mammals (Mus, Lepus), the mitochondria extend throughout the egg and are not especially segregated into regions; undoubtedly this applies to the majority of animals, examples of which have been left out in the above list.

In some forms, such as Ciona or Cynthia (**29**), it is equally certain that the mitochondria (and Golgi apparatus) are arranged in a special manner. For these rare examples the

paper of Hirschler on Ascidian oögenesis may be consulted (29). It is quite certain that in the majority of forms studied, the mitochondria and Golgi apparatus do not take part in producing any definite polarity of egg substances such as yolk does. Even in the markedly telolecithal ovum of Rana the mitochondria are spread through the entire cytoplasm, and it would not be possible to say that the upper hemisphere had a smaller quantity than the lower, or vice versâ.

Embryologists not specially acquainted with recent advances in cytology are in the habit of calling any round, stainable granules of the egg cytoplasm yolk. This is wrong, and what such workers call yolk may often be really Golgi granules and mitochondria; the latter are quite different, being formed of a protoplasmic basis impregnated with some phosphatide. Yolk is a dead storage substance and not a true cell organ as is the mitochondrium or Golgi granule or rod. Neglect to distinguish between yolk, mitochondria and Golgi elements only introduces confusion; in another paper this matter is treated more fully from the practical view-point.

The Mitochondria in Metazoan Spermateleosis.

In spermateleosis, or the metamorphosis of the spermatid into the spermatozoon, the mitochondria vary remarkably in their behaviour. After a perusal of the latest work on the subject, together with the results I have myself ascertained, I find that five main classes exist:

(A) In the amœboid spermatozoon of Ascaris and in the Decapod sperm the mitochondria remain morphologically unchanged, though grouped loosely in a special region of the spermatozoon.

(B) In the Mammalian type also the mitochondria generally maintain their individuality, but become grouped around the upper part of the axial filament often to form a spiral, and generally in a specialised region between the head and second centrosome (so-called middle-piece).

(C) In many Insecta the mitochondria fuse to form a more

or less elaborate coil, or macromitosome, losing their individuality.

(D) In Pulmonate Mollusca the mitochondria do not bodily form part of the sperm-tail, but seem to be drawn upon as reserve material for the secretion around the axial filament of a new layer of mitochondrial nature. This is probably what occurs in Peripatus (**44**).

(E) In many other Mollusca the spermatid mitochondria are few in number, and bodily fuse to form a solid structure around the axial filament. This also occurs in some scorpions (Wilson, **54**).

It is noteworthy that while this variation in behaviour of the mitochondria take place, the nuclear phenomena are throughout fairly regular.

From the above descriptions it will be evident that during spermateleosis in some animals the mitochondria are, as far as we know, morphologically as well as chemically unaltered, while in others it is almost equally certain that not only does a morphological change take place, but, what is more important, a chemical one. In Ascaris the sperm mitochondria, being unaltered, and being carried into the egg, may pass on from generation to generation. In the mollusc of the pulmonate type the spermateleosis stages are different; the mitochondria lose their individuality, and do not bodily form a part of the tail. In the case of insects it is equally clear that a complete change comes over the mitochondria at spermateleosis (**17**).

I believe that the above will be found to explain the varying behaviour of the mitochondria during fertilisation in various groups of animals. Further reference to this important question is made in the following pages.

The Behaviour of the Sperm Mitochondria after Introduction into Ovum.

In most animals properly studied it has been established that the entire sperm penetrates into the egg at fertilisation.

The only possible exception seems to be the Nereidiformes, where Lillie (**33**) and Just (**30**) both conclude that the middle-piece, i.e. the mitochondrial part of the sperm, is left outside and never penetrates the egg-membranes. In the majority of animals studied by skilled cytologists the sperm after entry is seen to break up into three pieces (at the least)—the nucleus, the centrosome, and the mitochondrial part behind the head centrosome. The fate of the nucleus and the centrosome need not detain us here. The fate of the mitochondrial matter so introduced by the male element is our immediate concern. In Phallusia (**41**) and in Ascaris (**40**) Meves describes the sperm mitochondria as being attracted into the fertilisation area (in the case of the ascidian, around the centrosome), and taking direct part in fertilisation, in the case of Ascaris at least, by fusing with the egg mitochondria, as the sperm nucleus fuses with the egg nucleus. In Phallusia Meves did not show that the ♂ and ♀ mitochondria fused, but he establishes that the ♂ mitochondria do grow and continue actively dividing in the egg cytoplasm after the disintegration of the sperm. In the case of Echinus, Wilson (**55**) and then Meves showed that the middle-piece does not become active after entry of the sperm. Meves (**42**) carried the matter further in two papers, and demonstrated that the middle-piece of the sea-urchin sperm may eventually become haphazardly segregated entire into a cell of either the animal or vegetative pole; the fate of the middle-piece in the sea-urchin is quite different from that of the ascidian. In the Lamellibranch Mytilus edulus, Meves (**43**) showed that the mitochondria enter the egg, but he was unable to demonstrate any subsequent activity as in Phallusia. In Ciona, Duesberg (**10**) failed to show any activity of the ♂ mitochondria; in the mammals no activity of the middle-piece has been shown, and as far as known it may be segregated whole to one or the other blastomere of the two-cell stage. The suggestion that the blastomere of the two-cell stage which gets the middle-piece becomes the formative (embryonic) part, while the other becomes the trophic

part, is interesting, but does not accord with the hypothesis which accounts so well for the origin of identical twins; for, if the middle-piece made a difference in the blastomeres, one would not expect the twins to be identical. Finally, the case of Ascaris may be mentioned: Held (**28**) recently shows that by staining in molybdate hæmatoxylin, and then in Altmann's acid fuchsin, the ♂ mitochondria become red, the ♀ keep black, and the two sorts can be followed out during fertilisation and even to segmentation. The "specificity" in coloration merely depends on the washing out of the first stain from the less dense ♂ mitochondria. Held shows that the ♂ and ♀ mitochondria of Ascaris do not fuse as claimed by Meves, and that after entry of the sperm the ♂ mitochondria grow, divide and multiply very rapidly, till ♀ and ♂ granules are of a like quantity.

Tentative Explanation of the Behaviour of the Mitochondria during Fertilisation.

We have seen that in Ascaris and possibly also in such an ascidian as Phallusia the sperm mitochondria begin to grow and divide after transference to the egg, while in such a form as Parechinus the mitochondrial part of the sperm does not fragment and grow in the same way. Assuming that subsequent researches will prove that in certain groups this growth phenomenon of the mitochondria of the sperm holds good, it may be well to attempt to give some explanation other than that the process is connected with the transmission of cytoplasmic factors. The explanation which occurs to me is as follows: Believing that the mitochondrium is a living plastid-like body of semi-independent automatic functions of purely metabolic nature, and that its main function in the sperm is that of storing (or taking from the surrounding medium) enough energy-producing materials to enable the sperm to live and move till it reaches the egg, I consider that after the sperm enters the egg the mitochondrium merely begins to undergo the vital metabolic phenomena in the new

cytoplasm to which it has been carried. It is as if a bacterium was transferred from an old culture medium to a fresh one.

The reason why the Ascaris sperm mitochondria begin to grow when transferred to the egg is that they are provided with a new field of activity and at once begin to utilise it. It may, then, be asked why the mitochondria do not go on dividing while in the middle-piece, since their vital functions are assumed to be semi-automatic in the sense that they are not directly due to another body. The answer to this would be that the functions of the mitochondria can be controlled and directed by stimuli emitted from the nucleus—a supposition which few will care to doubt—and that the sperm mitochondria are temporarily under the influence of some substance which keeps them from dividing, but not from functioning as energy-providers. If it be objected that such a view would not apply to the sea-urchin fertilisation where the middle-piece remains inert, it may be explained that in all probability the spermateleosis stages leave the Echinus mitochondrial matter in a modified state, which inhibits a recovery of growth after entry of the sperm.

In Ascaris the mitochondria are not altered, but probably in the sea-urchin the ripe middle-piece is not really formed of normal mitochondrial matter, but of modified material which dies on entering the egg, or which is unable to recover its former qualities. In this connection the evidence collected on p. 476 may be consulted, when it will be seen that my contention that the mitochondrial matter in the ripe sperms of different animals is rarely formed in the same way, or exists in the same quality, will be seen, I believe, to have been proven. Hence the varying behaviour of this mitochondrial matter after transference to the egg cytoplasm. Studying pulmonate molluscan spermateleosis stages in Kopsch's method (OsO_4, fourteen days), I found that at the time the sperm-tail was being formed the mitochondria went much darker than at any other stage, showing that a chemical change had overtaken them. (See also **19**, p. 248, for further evidence.)

Possible Objections to the above Tentative Explanation.

I have assumed that the mitochondrium consists of two sorts of substance, one the protoplasmic living basis, the other the lipin or phosphatide. That the mitochondrial granule is not metaplasm, but a living entity, I conclude from these facts: It had long been believed, and in several cases has now been actually demonstrated, that the mitochondrium has the power of dividing by binary fission. As remarkable evidence of this I may mention Held's recent work (**28**), where it is shown that the mitochondria of the male element divide and grow rapidly inside the cytoplasm of the egg (refer also to Wilke, **56**, Wilson, **54**, and Fauré-Fremiet, **13**).

Secondly, the behaviour of the mitochondria in the spermateleosis stages of spermatogenesis can only be explained by the assumption that these bodies have the power of spontaneous movement (**56**, **17**). If it be objected that such evidence does not necessarily allow one to conclude that the mitochondrium is living substance, it can be pointed out that it is exactly similar evidence that enables us to conclude that the chromosomes are living. Further evidence that the mitochondrium consists of these two parts is got by fixation experiments, where it is found that mild fat-solvents remove some part of the mitochondrium and leave a residue.

In addition, it is now firmly believed that the mitochondrium is formed mainly of a phosphatide; this is merely the name of a dead substance extracted from the cell, and it is clear that such a substance must, *intra vitam*, have been accompanied by some other material which would enable the above-mentioned reactions, such as that of binary fission, to take place.

We may now inquire into the position in which these assumptions lead us. In the first place, believing that the mitochondria, unlike fat, yolk, or pigment masses, are able to undergo movements which we can only interpret as vital, and that such movement is due to the fact that the basic substance

of the mitochondrium is living protoplasm, it is clear that in such a case as the fertilisation in Phallusia or Ascaris, sperm protoplasm must be introduced into the egg through the instrumentality of the sperm mitochondria. This being so, some may take the view that the Benda-Meves theory is proven—for Ascaris at least. I believe that such is far from being the case. There are many who take the Darwinian view that "inheritance must be looked upon as merely a form of growth" (8a), but it is to be noticed that the supposed function of the mitochondria as elaborators of certain "fatty" substances which partly enable the cell cytoplasm to grow does not necessitate our believing that these are in themselves the substances of heredity, or that the mitochondria can in any way influence the substances of heredity. The conclusion arrived at above (p. 468) with regard to the mitochondria in development has already been stated: it is that those materials which must be present in the cell and which bear in themselves the hereditary factors when not actually located in the nucleus are found in the ground-cytoplasm of the cell. That such is the case I consider indicated by the evidence already produced with regard to the erratic behaviour of the mitochondria in the germ-cell cycle, and by the centrifuge experiments on eggs.

Finally it may be explained that while I believe that the plant or animal plastid or mitochondrium has as its basis living protoplasm, it is conceived that this protoplasm might be specialised for metabolic or trophic functions, as apposed to reproductory ones. A further advance in these views will only be made possible by further research, and I am prepared to admit that there are many points which are most obscure, and which occasionally may even be found to give more or less specious support to the mitochondria-idioplasm hypothesis. My present views are due to my dissatisfaction with the evidence offered by such observers as Meves and Duesberg, but were the latter to produce further and more convincing facts I would still be prepared to abandon my present attitude. But that such evidence will be produced is doubtful, and at all

events it is quite certain that the function of the mitochondria is of secondary importance in the life of the cell as compared with the nucleus. This conclusion is further supported by observations on the behaviour of the mitochondria during mitosis, gametogenesis and organogeny, treated elsewhere (**61**).

Present General Conclusions with Regard to the Cytoplasm in Fertilisation.

It is now known that the following cell organs possess the power of fission and are self-propagating units : chromosomes, centrosome, mitochondria and Golgi apparatus. I overlook the cases in which a centrosome seems to appear out of the nucleus. Mitochondria and Golgi rods or granules have a very high degree of morphological independence in most if not all parts of the developmental cycle of the organism. The exact relationship between the nucleus and the Golgi elements and mitochondria and degree of independence of the latter are not known, but it seems unlikely that the nucleus would not have a great measure of control over all the cytoplasmic inclusions. There is now strong probability that the ripe sperm in some forms contains, in addition to nucleus, not only mitochondria but Golgi apparatus, and it seems likely that in cases both categories of inclusions are carried over to the egg. The latter has been shown to contain the same elements as the spermatid, only in much larger quantity.

In a few cases the sperm mitochondria, after introduction into the egg, are found to grow and divide, somewhat like bacteria in a nutrient medium. Though it is possible that the Golgi apparatus of the Cavia sperm and of the Ascaris sperm is introduced into the egg, so far no evidence has been brought forward as to their subsequent activity.

Finally, it is probable that the behaviour of the sperm mitochondria in various animals is so variable, that it is unsafe to look upon them as bearers of hereditary factors of any kind. The same will probably apply to the elements of the Golgi apparatus, which are more rarely carried over to the egg.

Summary.

Oögenesis.

(1) In the germinal epithelial cell of Limnæa stagnalis a Golgi apparatus is present. It is excentric and lies around the archoplasm, consisting of a number of rods (chondrioplasts or dictyosomes, dittosomi).

(2) In the progerminative oöcyte mitochondria appear at a very early stage, but it is not known whether they exist in the indifferent germinal epithelial cell. The mitochondria lie at first in the zone of the Golgi apparatus.

(3) The rods of the Golgi apparatus divide by binary fission and keep growing in number. The archoplasm upon which they repose gradually becomes divided into regions; these regions again subdivide till each Golgi rod is discrete and provided with a small part of the archoplasm, which it partly embraces. As each Golgi rod divides transversely the archoplasm does not divide. The latter only divides by binary fission after it has become studded with a number of rodlets.

(4) The Golgi apparatus gradually, from its excentric position, spreads completely throughout the egg cytoplasm, and in the full-grown oöcyte is evenly distributed here and there in all parts of the egg cytoplasm. No segregation into special regions was noticed.

(5) The mitochondria, from their excentric position near the Golgi apparatus, grow, divide, and spread evenly throughout the cytoplasm. The mitochondria are not all the same size; this is apparently due to the fact that some granules grow larger and more quickly than others.

(6) While the egg mitochondria grow much larger than the spermatid mitochondria, it has been shown that the individual Golgi batonette or rodlet never grows beyond a certain size. The difference between the Golgi apparatus of a young oöcyte and a full-grown ovum lies, not in the fact that the Golgi rods of the latter are individually very much larger (if at all) than those of the former, but mainly in the fact that the rods have increased enormously in number by binary

fission. The individual Golgi rodlet of spermatid, young and old oöcyte are approximately subequal in size.

(7) Deutoplasmagenesis, or the formation of yolk, does not begin very early; the first yolk-discs make their appearance after the Golgi elements and mitochondria have progressed far in the process of spreading throughout the growing oöcyte. The yolk-discs do not appear in any special region of the cytoplasm, but eventually become evenly spread out. The discs at first are very small, and later grow some two or three times larger than the largest mitochondria. In Flemming-without-acetic (overnight) + iron-alum hæmatoxylin, yolk goes dark brownish-green, mitochondria black.

(8) Towards the end of oögenesis the cytoplasm gradually becomes filled with vacuoles of a fluid nature. These leave a coagulum on fixation, but most of the vacuole is empty. The granules in the cytoplasm only abnormally lie in these vacuoles; yolk, Golgi elements and mitochondria lie in the trabeculæ between the vacuoles.

Spermatogenesis.

(1) The spermatogenesis of Limnæa stagnalis agrees in the main with that of other Pulmonata which have previously been studied (**18, 19**).

(2) No micromitochondria were discovered. In spermateleosis there is a mitochondrial residue sloughed off. The mitochondria do not bodily form the tail of the sperm; the tail of the sperm appears as a new formation of mitochondrial matter around the axial filament. The spermatid mitochondria are drawn upon to provide material for this process; as a result they alter chemically.

(3) With Kopsch's method (OsO_4 of 2 per cent., fourteen days) at the time of the formation of the mitochondrial tail, the mitochondria go dark brown, as apposed to the much lighter colour (i.e. power of reduction of OsO_4) of the mitochondria in the spermatocyte and early spermatid. They also become more resistant to injurious fixation.

(4) The Golgi apparatus is sloughed off during spermateleosis.

(5) In the fully formed spermatozoon, the nuclear head of the sperm is very small as compared with the immense length of the mitochondrial tail.

Centrifuge Experiment.

The egg centrifugalised before maturation has three layers: the upper or grey substance is yolk; the middle or clear substance is protoplasm; the lower and largest layer (yellow substance) is protoplasm, in which are suspended yellow mitochondria and Golgi elements.

Segmentation.

(1) In segmentation of the egg the mitochondria are equally divided, and keep so in organogeny stages examined.

(2) The same applies to the Golgi apparatus.

(3) In organogeny neither mitochondria nor Golgi apparatus disappear.

Bibliography.

1. Alexeieff, A.—"Sur le cycle évolutif et les affinités de Blastocystis enterocœla," 'Arch de Zool. expér. Notes et Rev.,' 1915–17.

2. Altmann, R.—'Die Elementarorganismen und ihre Beziehungen zu d. Zellen,' Leipzig, 1894.

2a. Arnold, G.—"The Rôle of the Chondriosomes in the Cells of the Guinea-pig's Pancreas," 'Arch. f. Zellf.,' Bd. viii.

3. Bayliss, W.—'Principles of General Physiology,' Longman.

3a. Beckwith, C.—"The Genesis of the Plasma-structure in the Egg of Hydractinia echinata," 'Journ. Morph.,' vol. xxv, 1914.

4. Best, F.—"Über Karminfärbung des Glykogens und der Kerne," 'Zeit. wiss. Mikr.,' Bd. xxiii, p. 319.

5. Casteel, D. B.—"Germ-cells of Argas," 'Journ. Morph.,' vol. xxviii.

6. Cowdry, N. H.—"Comparison of the Mitochondria in Plant and Animal Cells," 'Biol. Bull.,' vol. xxxiii, p. 17.

7. Conklin, E. G.—"The Effects of Centrifugal Force upon the Eggs of Fresh-water Pulmonates," 'Journ. Exp. Zool.,' vol. ix, 1910.

8. Dangeard, P. A.—"Sur la nature du chondriome et son rôle dans la cellule," 'Compt. rend.,' t. clxvi.

8a. Darwin, Ch.—'Variation of Plants and Animals.'

9. Duesberg, J.—"Plastosomen, 'Apparato reticolare interno,' und Chromidialapparat," 'Ergeb. der Anat. u. Entwickl.,' Bd. xx.

10. ——— "Recherches cytologiques sur la fécondation des Ascidiens et sur leur développement," 'Contributions to Embryology,' vol. iii, Nos. 7, 8, 9, 1915.

11. ——— "Nouvelles recherches sur l'appareil mitochondrial des cellules séminales," 'Arch. f. Zellf.,' Bd. vi, 1911.

12. Dubreuil, G.—"Transformation directe des Mitochondries et des Chondriocontes en graisse dans les cellules adipeuses," 'C.R. Soc. Biol.,' t. lxx.

13. Fauré-Fremiet, E.—"Etude sur les mitochondries des Protozaires et des cellules sexuelles," 'Arch. d'Anat. micr.,' t. xi.

14. ——— "Le Cycle germinatif chez l'Ascaris megalocephala," ibid., t. xv.

15. ——— Mayer et Schaffer.—"Sur la constitution et le rôle des mitochondries," 'C.R. Soc. Biol.,' t. lxvi.

16. Gatenby, J. Bronté.—"The Transition of Peritoneal Epithelial Cells into Germ-cells," 'Quart. Journ. Micr. Sci.,' vol. 61.

17. ——— "The Cytoplasmic Inclusions of the Germ-cells: Part I, Lepidoptera," ibid., vol. 62.

18. ——— "The Cytoplasmic Inclusions of the Germ-cells: Part II. Helix aspersa," ibid., vol. 62.

19. ——— "The Cytoplasmic Inclusions of the Germ-cells: Part III, Other Pulmonates," ibid., vol. 63.

20. ——— "The Cytoplasmic Inclusions of the Germ-cells: Part IV, Notes on the Golgi Apparatus and the Dimorphic Spermatozoa of Paludina vivipara," ibid., vol. 63.

21. ——— "The Segregation of the Germ-cells in Trichogramma evanescens," ibid., vol. 63.

21a. ——— "Polyembryony in the Parasitic Hymenoptera: A Review,' ibid., vol. 63.

22. Guilliermond, M.—"Recherches sur le chondriome chez les champignons et les algues," 'Rev. génér. de Botan.,' t. xxvii.

23. ——— "Nouvelles remarques sur la signification des plastes de W. Schimper par rapport aux mitochondries actuelles," 'C.R. Soc. Biol.,' t. lxxv.

24. ——— "Quelques remarques nouvelles sur la formation des pigments anthocyaniques au sein des mitochondries," ibid., t. lxxv.

25. Guilliermond, M.—"Nouvelles recherches sur les caractères vitaux et les altérations du chondriome dans les cellules épidermiques des fleurs," ibid., 1917.

26. Handwerk, C.—"Beiträge z. Kenntniss v. Verhalten d. Fettkörper zu Osmimmsäure u. zu Sudan," 'Zeit. f. wiss. Mikr.,' Bd. xv, 1898.

27. Hegner, R.—'The Germ-cell Cycle,' New York, 1914.

28. Held, H.—"Untersuchungen über den Vorgang der Befruchtung," 'Arch. f. mikr. Anat.,' Bd. lxxxix.

29. Hirschler, J.—"Über die Plasmakomponenten der weiblichen Geschlechtszellen," ibid., Bd. lxxxix.

30. Just, E. E.—"Fertilisation in Platynereis megalops," 'Journ. Morph.,' vol. xxvi.

31. Koltzoff, N.—"Untersuchungen über die Spermien der Decapoden, als Einleitung in das Problem der Zellengestalt," 'Arch. f. mikr. Anat.,' Bd. lxvii.

32. Lankester, E. Ray.—'Notes on Embryology and Classification,' London, 1877. (This Journal, vol. 17.)

32a. Lams et Doorme.—"Nouvelles recherches sur la maturation et fécondation de l'œuf des Mammiféres," 'Arch. de Biol.,' t. xxiii.

33. Lillie, F. R.—"Studies in the Fertilisation of Nereis, IV," 'Journ. Exp. Zool.,' vol. xii.

34. MacBride, E. W.—'Text-Book of Embryology,' vol. i. MacMillan.

35. ——— 'Section D, Brit. Assoc.,' 1916.

36. Maclean, H.—'Lecithin and Allied Substances: The Lipins,' Longmans, 1918.

37. Mann, G.—'Methods and Theory of Physiological Histology,' Oxford, 1902.

38. Meves, F.—"Über Umwandlung von Plastosomen in Sekretkügelchen nach Beobachtungen an Pflanzenzellen," 'Arch. f. mikr. Anat.,' Bd. xc, p. 18.

39. ——— "Verfolgung des Mittelstückes des Echinenspermiums durch die ersten Zellgenerationen des befruchteten Eies," ibid., Bd. lxxxv.

40. ——— "Über die Beteiligung der Plastochondrien an der Befruchtung des Eies von Ascaris," ibid., Bd. lxxvi.

41. ——— "Über das Verhalten des plastomatischen Bestandteiles des Spermiums bei der Befruchtung des Eies von Phallu mamillata," ibid., Bd. lxxxii.

42. Meves, F.—"Verfolgung des sogenannten Mittelstückes des Echinidenspermiums im befruchteten Ei bis zum Ende der ersten Furchungsteilung," ibid., Bd. lxxx.

43. ——— "Über den Befruchtungsvorgang bei der Miesmuschel (Mytilus edulis)." ibid., Bd. lxxxvii.

44. Montgomery, T. H.—"Complete Discharge of the Mitochondria from the Spermatozoon of Peripatus," 'Biol. Bull.,' vol. xxii.

45. Rabl, C.—"Ueber die Entwicklung der Tellerschnecke," 'Morph. Jahrb.,' Bd. v, 1879.

46. Regaud, Cl.—"Sur la Signification physiologique du Chondriome des cellules sexuelles mûres, et notamment des Spermatozoïdes," 'C.R. Soc. Biol.,' 1909.

47. ——— "Attribution aux 'formations mitochondriales' de la fonction générale d'extraction et de fixation électives," ibid., 1909.

48. ——— "Etude sur la structure des tubes séminifères et sur la spermatogenèse chez les Mammifères," 'Arch. d'Anat. micr.,' t. xi.

49. Schaxel, J.—"Das Zusammenwirken der Zellbestandteile bei Eireifung, Furchung, und ersten Organbildung der Echinodermen," 'Arch. f. mikr. Anat.,' Bd. lxxvi.

50. Sjoväll, E.—"Ein Versuch das Binnennetz von Golgi-Kopsch be der Spermato- und Ovo-genese zu homologisieren," 'Anat. Anz.,' Bd. xxviii.

51. Starke, J.—"Über Fettgranula u. eine neue Eigenschaft d. Osmiumtetroxydes," 'Arch. f. Physiol.,' Bd. lxx, 1895.

52. Strasburger, E.—'Text-Book of Botany,' MacMillan, 1912.

53. Weigl, R.—"Vergleichend-zytologische Untersuchungen über den Golgi-Kopschen Apparat, etc.," 'Bull. de l'Acad. Scien., Cracovie,' 1912.

54. Wilson, E. B.—"The Distribution of the Chondriosomes to the Spermatozoa in Scorpions," 'Nat. Acad. Sci. Phil.,' vol. ii, 1916.

55. ——— 'The Cell in Development and Inheritance,' New York, 1900.

56. Wilke, G.—"Chromatinreifung und Mitochondrienkörper in der Spermatogenese von Hydrometra paludum," 'Arch. f. Zellf.,' Bd. x, 1913.

57. Zoja, L. und R.—"Über die fuchsinophilen Plastidulen,' 'Arch. Anat. u. Entwickl.,' 1891.

58. Hirschler, J.—"Über den Golgischen Apparat embryonaler Zellen," 'Arch. f. mikr. Anat.,' Bd. xci, 1918.

59. Hirschler, J.—"Über die Plasmastrukturen in den Geschlechtszellen der Ascariden," 'Arch. f. Zellf.,' Bd. ix, 1913.

60. Weismann, A.—'The Evolution Theory,' vol. i, Arnold, 1904.

61. Gatenby, J. Bronté.—"Identification of Intracellular Elements," 'Journ. Roy. Micr. Soc.,' 1919. (In press.)

EXPLANATION OF PLATES 27 and 28.

Illustrating Mr. J. Bronté Gatenby's paper on "The Gametogenesis and Early Development of Limnæa stagnalis."

Lettering.

A.L. Ancel's layer of ovotestis wall. *AR.* Archoplasm. *C.C.* Clear cytoplasm. *ECT.* Ectoderm cell. *END.* Endoderm cell. *G.A.* Golgi apparatus element. *G.A.E.* Golgi apparatus of epithelial cell. *G.A.O.* Golgi apparatus of oöcyte. *G.A.N.* Golgi apparatus of nurse- (yolk-) cell. *G.A.S.* Golgi apparatus of spermatocyte or spermatid. *G.E.* Germinal epithelium. *G.R.* Golgi rod. *J.B.* Juxta-nuclear body. *M.* Mitochondrium. *MA.* Macromere. *MES.* Mesoderm cell. *MI.* Micromere. *N.* Nucleus. *P.B.* Polar bodies. *T.* Sperm-tails (mitochondria). *VAC.* Vacuole. *Y.* Yolk-disclet. *Y.C.* Yolk- or nurse-cell. *X.* Line of division of archoplasm.

PLATE 27

Fig. 1.—Indifferent germinal epithelial cell, showing Golgi apparatus. Kopsch.

Fig. 2.—Later progerminative cell after "appearance" of mitochondria. Kopsch-Altmann.

Fig. 3.—Young oöcyte showing growth of Golgi apparatus. F.w.a.; iron-hæmatoxylin.

Fig. 4.—Little older oöcyte showing outspreading of Golgi apparatus and mitochondria. Ditto.

Fig. 5.—Half-grown oöcyte, Golgi apparatus and mitochondria; yolk drawn as circles. Ditto.

Fig. 6.—Nearly full-grown oöcyte, showing juxta-nuclear body (*J.B.*), clear cytoplasm (*C.C.*), Golgi elements (*G.A.O.*), and mitochondria (*M.*). Yolk drawn as circles throughout the cytoplasm. Ditto.

Figs. 7 and 8.—Spermatocyte and spermatid respectively showing Golgi apparatus and mitochondria. Ditto.

Fig. 9.—Ripe sperm, drawn to same scale as oöcyte in Fig. 6. Smear.

Fig. 10.—Nurse-cell, showing Golgi apparatus, yolk, and heads of spermatozoa adhering to cell. Kopsch unstained.

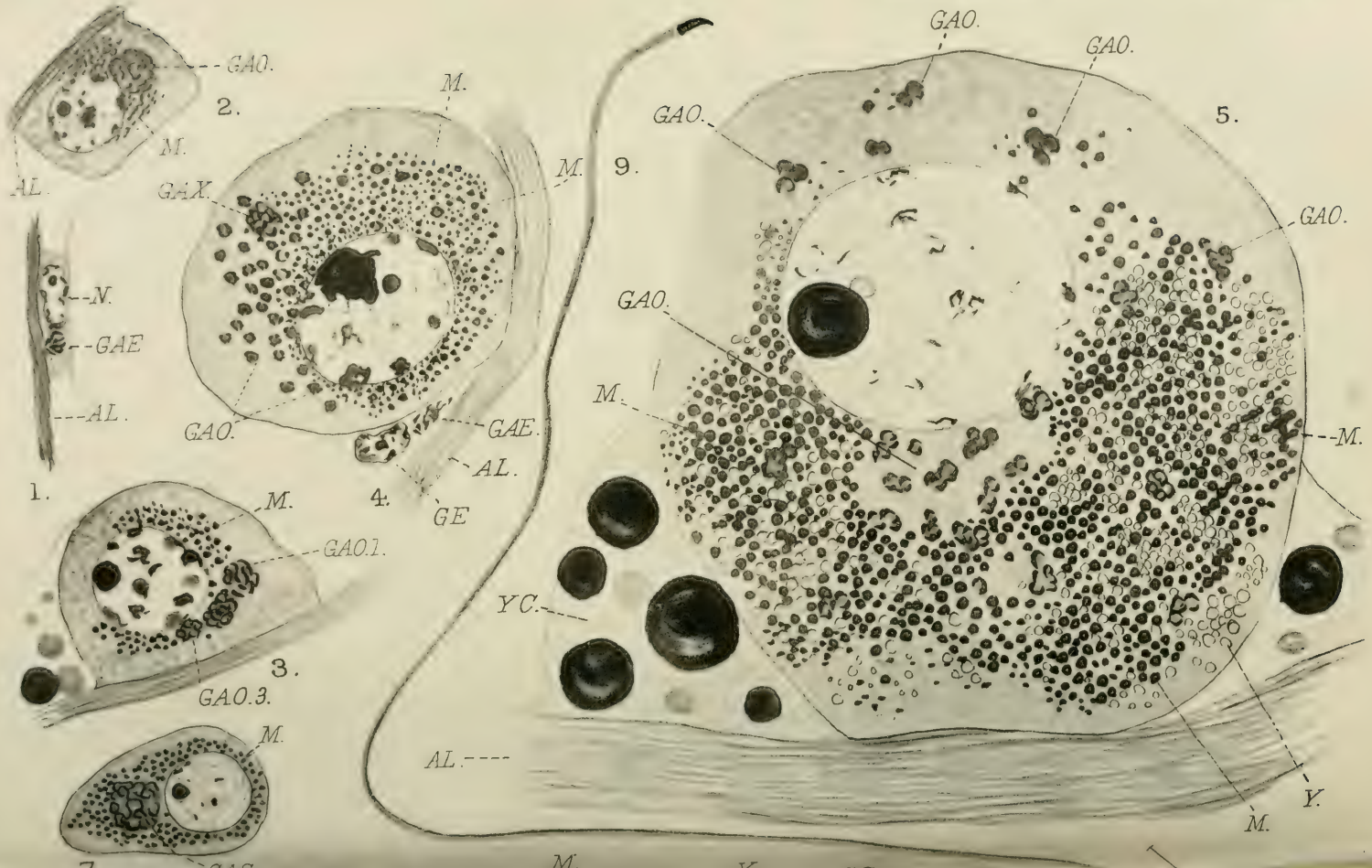
PLATE 28.

Fig. 11.—Two-cell stage, showing equal distribution of mitochondria and Golgi apparatus elements. Kopsch.

Fig. 12.—Obliquely sagittal section through micromeres and macromeres of eight-cell stage showing equal distribution of same elements. Kopsch-Altmann.

Fig. 13.—Median section through gastrula, to show equal distribution of all cell elements except oily (?) vacuoles (of endoderm). Mitochondria drawn as circles, Golgi apparatus elements black. Cells of all three germ-layers shown. Kopsch-Altmann. (6 com. × $\frac{1}{15}$th semi-ap., reduced $\frac{1}{3}$.)

Fig. 14.—Semi-diagrammatic scheme of method of division and multiplication of Golgi elements.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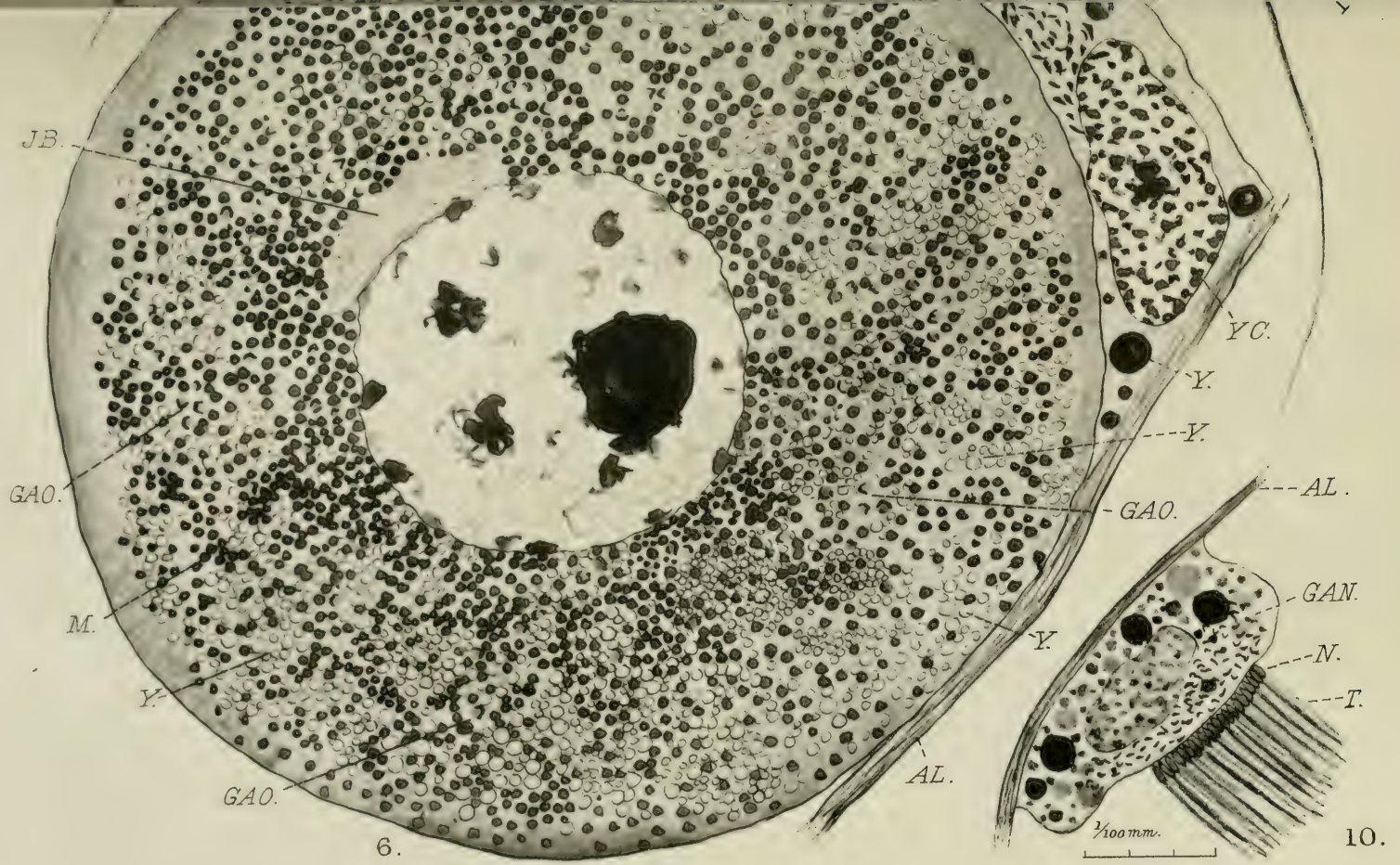

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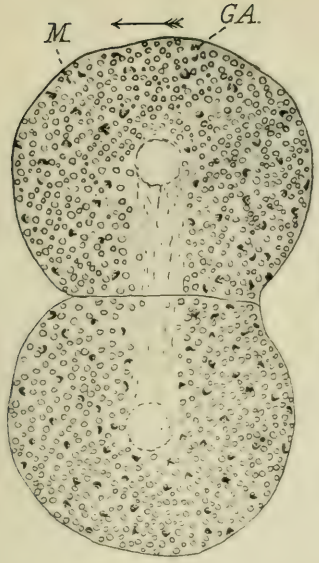

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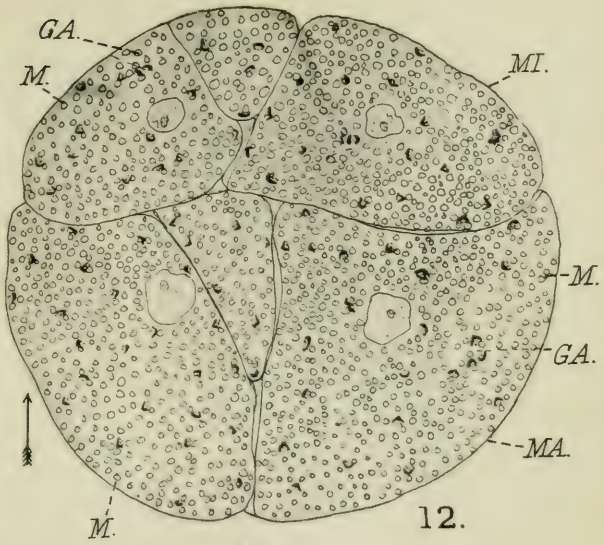

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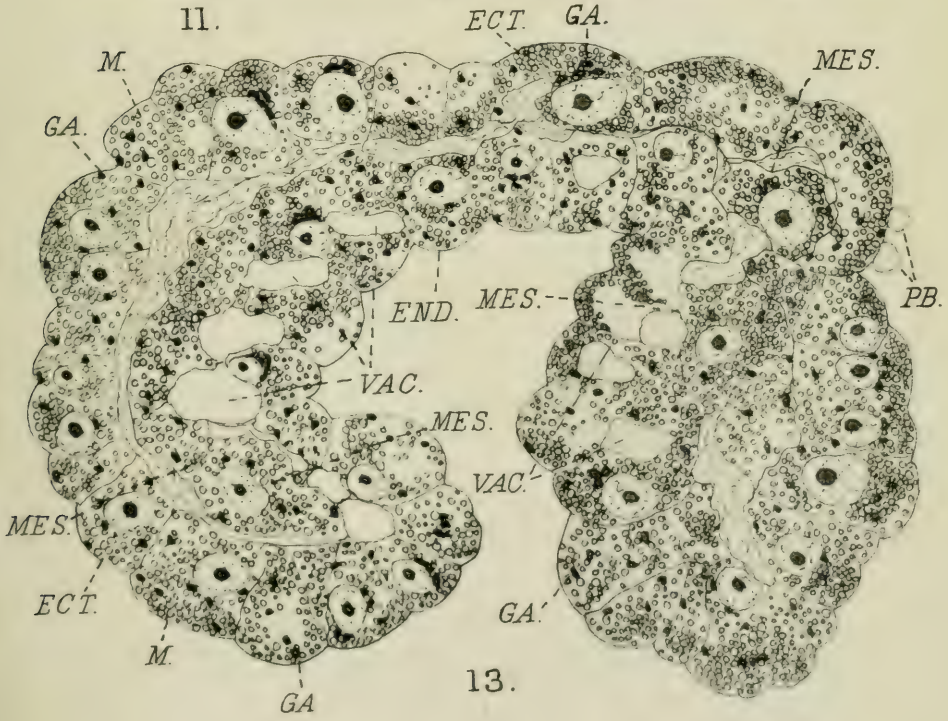

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Reproduction by Transverse Fission in Phoronopsis.

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With Plate 29.

The occurrence of asexual reproduction in the Phoronidea, though apparently an easy matter to determine, has not proved to be so. Species of Phoronis occur in groups of closely associated individuals or colonies, and it has often been observed that they have the power of casting off and rapidly regenerating the whole of the lophophoral or distal end of the body. Several observers have noted the occurrence, and the process of regeneration has been described in detail by Schultz, who also found that regeneration occurred in both parts of a Phoronis cut in two. Though the naturally detached part can be readily kept under observation, it was never found to develop into a new individual, and this may be the reason why no suggestions were made of the possible reproduction of the animal by simple division of the body. In 1907 Selys-Longchamps (**7**), in repeating the experiments of Schultz, came to the conclusion that natural division of the body probably does occur, and that the animal reproduces in this way. It was not, however, until 1917 that any definite proof of this suggestion was brought forward, when Harmer (**3**), in his rediscovery of Phoronis ovalis, found definite evidence, which seems to place it beyond doubt that such an occurrence does take place.

A species of Phoronis (B. capensis) is very common in

South Africa, but no direct evidence of asexual reproduction has been observed. The colony from which the species was originally described in 1907 was lodged in a piece of limestone. This was suspended in a tank by a piece of copper wire, and, up to the present date, still shows the animals in a flourishing condition; it was suspected therefore that some mode of asexual reproduction does take place, but no proof of this was obtained. Phoronopsis albomaculata was found about the same time (**2**). It has since proved to be not so rare as was then thought, and indeed another species, since recorded from Vancouver Island by Miss Pixell (**5**), seems to occur in abundance. The Cape species is not readily procurable, but has again been found on pieces of limestone in the sea from a depth of 10 to 15 fathoms, one being found at extreme low tide. These stones invariably contained numerous individuals of Phoronis capensis, but only one or two Phoronopsis. All were placed in a well-aërated tank for observation, with a view more especially to obtain some information as to their development, and the occurrence of a possibly interesting larval form, different from that of Phoronis. No further information, however, was obtained on this point, beyond the originally observed occurrence of eggs within the tube, except that the eggs may also be deposited on the rock at some distance from the tube. This was effected by the protrusion of the body to a surprising distance from the tube. The body, during this protrusion, including the lophophoral end, was closely applied to the substratum, and, on two occasions, the eggs were seen to be deposited, and subsequently covered over and attached securely to the rock by a mucous secretion from a large glandular lophophoral organ. The eggs, however, did not develop further, apparently not being fertilised. With a view to overcoming this difficulty, the parts of the large stones containing the animal were cut out and placed together in a suitable vessel, so arranged that the animals could readily be observed by lens or microscope. These were kept under observation throughout a year, but without any further

light being thrown on their development. Another result, however, though not the one looked for, was accidentally obtained. It had been repeatedly observed that, as in other Phoronidea, the lophophoral end of the body was thrown off, and once or twice the actual process, which occupies about half an hour, was observed. For some time after the loss of this region, the rest of the body, which is withdrawn into the tube, is invisible until a new lophophore arises. It was not surprising, therefore, that individuals appeared on stones where they had not been seen before. It was indeed suspected that the cast-off part might develop into a new individual, but no indications of this were detected, though the cast-off portion of the body was carefully watched. On one occasion, however, an undoubted case of reproduction by division of the body was observed, and the details of the process present some interesting features.

In one of the animals kept under observation a constriction appeared round the body about 7 mm. from its free lophophoral end. This became more marked, until a part was ultimately cut off altogether. It moved away from the rest of the body, with periods of rest, and so slowly that its progress could be actually observed only under the microscope. In a few hours it had moved about half an inch. This movement was probably effected by the cilia of the tentacles and body, but may have been assisted by an occasional well-marked bending of the lophophoral end from side to side. There was apparently no adherence to the stone by any mucous secretion, which is often abundant in the disturbed animal. When looked for the following morning the free portion had disappeared, but was ultimately found on the bottom of the vessel under another stone about four inches distant. On the fourth day after division the free portion again divided, below the base of the lophophore, and only a small piece of the body, about 3 mm. in length, remained. Both parts were apparently alive, as the cilia of the tentacles were still active. The lophophoral fragment, which had moved away from the other, began to show indications of a change, as the

tentacles, hitherto straight, commenced to curl up. On the following day no trace of this part could be found anywhere, and it had presumably become disintegrated. The other part, however, was in an apparently healthy condition. Formerly it had been more or less truncate at both ends, but one end now was markedly more pointed than the other, and at times slowly swayed from side to side. It was difficult to keep trace of the animal without running the risk of interfering with its normal course of develpment, and, for this reason also, it was not removed from amongst the stones and sandy mud in the vessel or isolated for closer observation. It continued wandering about for some time, and, when a suitable opportunity was afforded, it was measured, and found to have apparently increased in length somewhat. It was also noted that the circulation, or at least movement of the blood, had again started, as it could be seen through the semi-transparent body, appearing and disappearing at intervals. There was no trace of the lophophore or of the tentacles, but there appeared a small, rounded projection at the smaller end, which seemed to be the epistome. That this projection was ciliated was apparent from the fact that a current of water with contained particles was observed to flow towards it.

On the following day, the tenth after the first division, an opportunity was afforded of observing the other end of the animal, as it had penetrated into a small heap of muddy débris, leaving its posterior part exposed. This presented a somewhat swollen appearance, which, however, varied fairly rapidly, being sometimes almost globular in outline. This movement was well marked. The transverse corrugations, characteristic of the general surface of the body, were continued on to the swollen part, except at its centre posteriorly. Here a small, circular area was devoid of corrugations, and somewhat whitish in colour, but not raised above the general rounded surface of the body. The animal gradually penetrated into the débris, and became nearly hidden in a small crevice of the stone.

This was supposed to be the beginning of the tube-formation and the sedentary life, but on the following day the animal was found to have moved off again, being found under a stone a few inches away. The movements were now much more definite, the anterior end turning readily to any loose material and penetrating it; occasionally also the body in progression became much elongated. Its rate of progression was noted, and it was found on occasions to pass over a distance equal to the length of its body in about two minutes. The rate of movement, however, was not regular, and it frequently ceased completely, the anterior end meanwhile moving about from side to side. The most marked feature, however, of this stage was the appearance of a knob-like projection at the posterior end, at the place occupied by the whitish spot already mentioned. It was of a clear but not homogeneous appearance, as, internally, it appeared to have convoluted strands or corrugations.

The animal could not be found on the following day without the risk of undue disturbance and probable injury, but it was again seen next morning, and two new features were observed, namely, the appearance of a low but distinct ridge round the anterior projection. As subsequent development showed, this was the beginning of the lophophore. A second and more striking change was the growth of the posterior prominence, which had now become about half a millimetre in length (Pl. 29, fig. 1).

On the following day the animal was found with difficulty and only after all the stones had been removed from the tank. Its presence was detected by a slight protrusion of the regenerating lophophoral region from a small heap of mud and sand. This was carefully cleared away, when it was found that the animal was no longer free, but was securely fixed to the bottom of the tank by the pedunculate posterior projection. The whole of this part, but more especially its terminal portion, was covered with a very adhesive mucus, which adhered to the surrounding particles of sand, etc., as well as to the substratum. Apparently the animal was

at a critical stage in its development, and it was therefore detached from its point of adhesion and disentangled from the surrounding material. It was then examined microscopically in a watch-glass full of water, and the following particulars observed. The total length of the body, including posterior appendage, was 2·76 mm., of which the body was 1·9 mm., the appendage 0·86 mm. (Pl. 29, fig. 2). The length of the body, however, varied with the movement of the animal, but was decidedly shorter though thicker than in the previously observed stages. This may have been due to the irritation of the animal on removal to new conditions. The lophophore (Pl. 29, fig. 2, *l.*) was more defined, the tentacles being marked out as lobular protrusions of the lophophoral ridge. The lophophore-opening was well marked, and through it could be seen a distinct prominence, apparently the epistome (Pl. 29, fig. 2, *epist.*) The body became somewhat narrower below this region, and widened out greatly towards its aboral end. An indistinct white line could be traced along the left side—apparently the nerve-cord. At its aboral extremity the body was very rounded, and a slight indentation appeared where it joined the appendage. This tail-like appendage, which for convenience may be referred to as the peduncle, is of more particular interest. In the living animal it was observed to be about one-third of the total length. It did not merge gradually into the body, its point of attachment being situated in the centre of a depression, so that in a lateral view the point of junction was not observed. The other or free end appeared to be irregular in shape, but was partly hidden by the very adhesive mucus and entangled particles which surrounded it. The peduncle was of a slightly yellow colour, like that of the animal generally, and was irregular in outline, exhibiting three or four protrusions on each side. Its structure, as seen by transmitted light under the microscope, was not homogeneous. The protrusions were very transparent and homogeneous, but the main part appeared to have a few thick strands running lengthwise. This appearance was not due to strands of internal tissue,

but to epidermal foldings, as was shown in sections (Pl. 29, figs. 8–13). The secretion of the mucus appeared to be confined chiefly to the distal or free end of the peduncle. There was little or no contraction of this part, though it was well marked in the body of the animal.

As the animal at this stage appeared to be about to assume the normal tubicolous habits, and as there was some risk of losing sight of it altogether, it was thought best to preserve it for minute examination by sections after removing such particles as might interfere with cutting. It was accordingly fixed in sublimate with 5 per cent. acetic acid, and stained in alum carmine.

The various stages of the transformation may be briefly recapitulated:

March 21st	1st day	Division from parent.
,, 22nd	2nd ,,	Crawling movement.
,, 23rd	3rd ,,	,, ,,
,, 24th	4th ,,	Second division; lophophoral region cast off.
,, 25th	5th ,,	Anterior end became narrower.
,, 26th	6th ,,	Not seen.
,, 27th	7th ,,	,,
,, 28th	8th ,,	,,
,, 29th	9th ,,	Indication of epistome; circulation re-started.
,, 30th	10th ,,	Posterior end rounded, with white patch.
,, 31st	11th ,,	Projection at posterior end.
April 1st	12th ,,	Not seen.
,, 2nd	13th ,,	Appearance of lophophore and elongation of posterior projection to ·5 mm.
,, 3rd	14th ,,	Further elongation of posterior projection and fixation of animal by a mucous secretion at its free end.

The process of regeneration occurring meanwhile in the parent animal may be noted here. This was at the normal rate observed in other cases, and much more rapid than in the detached portion. On the first day it remained completely withdrawn into its tube, but was visible as a truncate projection on the second day, and on the ninth day the tentacles of the lophophore could be made out, being at the stage only reached by the detached part on the fourteenth day, on which latter date the tentacles of the parent had grown out to a length of about 1 mm.

No other case of natural division and growth of a detached part was observed, and as it was desirable to obtain other stages for further examination, the experiment was made of cutting off the protruding parts of normally growing individuals and observing their subsequent changes. About a dozen in all were so treated, a part of the body being cut off about equal in length to that observed in the spontaneous division. Various results followed which need not be detailed, as in all except two cases the part cut off became disintegrated. In one case the piece again divided, but subsequently disappeared; in another, however, after the second division development was seen to proceed, as in the case of natural proliferation. In this case a larger piece of about 22 mm. was cut off. This was on April 8th. On the 9th it was found not far off, but reversed in position. On the 10th a constriction appeared about a millimetre from the base of the lophophore, and on the same day a slight protuberance was seen at the aboral end, measuring about ·24 mm. in breadth and ·15 mm. in length. Special note was made of this part. Internally it was of a whitish colour, the outer parts being of a yellowish colour, similar to that of the body generally. It was not seen again until the 12th, when it had considerably advanced in development. A collar-like ridge, representing the beginning of the lophophore, was seen, and projecting from its centre was a conspicuous prominence, which apparently represented the early appearance of an epistome, as in the previous case. The posterior projection

was more pronounced. It will be observed here that the times and order of the various changes were different from those in the first case. Thus the second division was on the third day in place of the fourth, and the posterior prominence appeared much earlier. The fragment was also much less active, and did not move about freely. As it did not look very lively, and no movement of blood was observed, it was preserved for longitudinal sections with a view specially of ascertaining the mode of origin of the posterior projection.

The following is the record of the changes with their dates:

April	8th	First (artificial) division.
,,	9th	Unaltered, but removed a short distance.
,,	10th	Second (spontaneous) division, and appearance of posterior projection.
,,	12th	Epistome and rudiment of lophophore.

These observations are given in some detail as they serve to show that transverse division may be a normal mode of reproduction in Phoronopsis and yet not be readily observed even under favourable conditions, and in spite of the fact that this animal lives in isolated tubes which do not penetrate the substratum. The difficulties of observation in the case of species of Phoronis forming colonies would be greater, but a somewhat similar process to that above described may occur in this genus, and it would be well worth while to examine by sectioning the structure of the fragments of the animal said to be found in such colonies, more especially their aboral extremities.

Minute Structure of Free Part.

For convenience the piece set free by natural division of the animal may be referred to as A, of which transverse sections were made; the second, obtained by artificial division, may be designated B, of which longitudinal sections were made.

Some features are worthy of note in the process of

regeneration of the oral region of the body, but as we are chiefly concerned here with the changes which take place in the aboral region, the origin and structure of the peduncle may first be considered. In specimen B (Pl. 29, fig. 3) the beginning of the change is clearly seen. The ruptured aboral ends of the ascending and descending parts of the alimentary tract have closed up and become somewhat pointed at their extremities, which are close to each other, but there is, as yet, no connection between their cavities. Just above their extremities and almost completely encircled by them is a large space occupied by blood (Pl. 29, fig. 3, *bl.*), and from this both afferent and efferent parts of the vascular system can be traced, so that free circulation is already possible. Lying below this, and immediately over the aboral projection, is a lenticular and compact mass of cells of a well-defined nature (Pl. 29, fig. 3, *cœl. ep.*) ; the nucleus of each cell is comparatively large, and the body is drawn out into two long, tapering ends. The mass of cells is not at this point directly connected with the blood-vessels, but other sections further off show that there are fine blood-vessels, often of the diameter of one blood-corpuscle, connecting it with the main vascular system. As to the origin of these cells, there seems to be little doubt from their general appearance, and from the fact that they can be traced on each side to the layer of cells lining the wall of the body-cavity, that they constitute a mass of proliferating cells of the cœlomic epithelium. Below this mass and completely in the aboral projection these same cells are found, not in close contact with each other, but forming a sort of loose network. There are as yet no other kinds of cells in the cavity of the projection.

The outer walls of the projection are a continuation of the single-celled layer of epidermal cells of the body generally, and of the well-developed basement-tissue (Pl. 29, fig. 3, *b.t.*). These regenerating cells, however, are smaller than those of the normal epithelial cells, and not so well defined, though a few glandular cells may be seen among them.

In the median section figured there were no traces of the foldings of the epithelium so characteristic of a later stage, but in more lateral sections the commencements of these were indicated.

The muscular layers of the body-wall cease abruptly near the edge of the protuberance, and are not continued into its cavity; the same is true also of the nerve-cord.

The further development of the projection was seen in the fragment A, in which it attained a length of ·86 mm. in four days. The ends of the alimentary tract are not yet joined, though that of the descending limb has become enlarged and sends out diverticula (Pl. 29, fig. 6), between which blood-vessels may be seen in some sections. The joined ends of the vascular system are as before, but there is an increase of small ramifying branches. Below this is again to be found a mass of the characteristic elongate cells, which were found to originate from the cœlomic epithelium. These extend into the lumen of the peduncle, but in a somewhat modified condition; they are less definite, and scattered throughout them are small globular particles (Pl. 29, fig. 9, *ft.*), apparently of nourishing material, for they closely resemble the fatty globules seen in the vaso-peritoneal epithelium of the body-cavity. Cori (**1**) has described these, and Ikeda (**4**) has shown that they arise in connection with a proliferation of the cœlomic epithelium covering the blood-vessels, and serve as nourishment for the developing gonads; they probably serve here as nourishment for the growing peduncle. Miss Pixell (**5**) describes and figures them in the genus Phoronopsis.

A conspicuous feature of the inner tissue of the peduncle is also muscular tissue, varying from thick strands like the longitudinal muscles of the body to thin elements similar to the radial muscles. A few elongate cells lying immediately beneath the epithelium in some sections may represent the beginnings of the circular muscles. The epidermal elements were similar to those already noted in the fragment B, but were smaller, and there was no trace of the thick basement-tissue. There were amongst the cells numerous clear

glandular cells, and the peripheral parts, more especially of the cells at the free end, contained fine yellow granular material.

A conspicuous feature of the epidermis was the manner in which it was folded in some places, more particularly at the beginning and the middle (Pl. 29, figs. 7–12). This folding was carried to such an extent in places that there was no lumen in the peduncle (Pl. 29, fig. 12). That these foldings were not due to shrinking in the preservative is evident from the fact that they were seen in the living condition as thick strand-like structures in the semi-transparent tissue of the peduncle.

Some features in the origin of the epistome are worthy of note. At an early stage in the regeneration of the oral end of the body there appeared between the ruptured ends of the ascending and descending limbs of the alimentary tract a conspicuous conical prominence (Pl. 29, figs. 1 and 4, *epist.*). It first appears shortly before the ridge of tissue which will form the lophophore, but as development proceeds it is not so well marked. It still appears in section as a prominent organ, but has apparently shrunk in the preservative. In life it may contain a space, but in the sections it is seen that the whole of its interior is solid. At the base of the organ is a mass of tissue resting on a large cavity filled with blood. This tissue (Pl. 29, fig. 4, *cœl. ep.*) is in close contact with the blood and also with the regenerating mouth and anus, which have not as yet appeared. It appears to be a mass of proliferating cells of the cœlomic epithelium, similar to that observed at the aboral end of the body.

It may be suggested that the comparatively large structure here referred to, though arising between the mouth and anus, is not the homologue of the epistome. This may possibly prove to be so when the further stages are known.

Significance of the Mode of Development of Peduncle.

The peduncle, after its first appearance, is formed by a rapid multiplication of the cells of the single-layered

epidermis, which is thus thrown into a number of folds or involutions, and its apparently rapid growth and elongation in subsequent stages (·36 mm. during the last day) is probably due to the unfolding or eversion of these. This is a process which at once recalls a somewhat similar phenomenon in the larval development, namely, the rapid proliferation of the epidermal cells at a point in the body and the consequent involution of the epidermis, which, later on, becomes somewhat suddenly everted, and into which the alimentary tract penetrates. The later stages of the peduncle are not known, but it seems to be probable that here also the lumen of its folded walls will be dilated to receive the growing visceral elements of the body. In other words, the process of regeneration is here a repetition, or at least a recapitulation, of the process of ordinary development from the ovum.

Summary.

(1) Phoronopsis has been observed to reproduce asexually by transverse division of the body.

(2) The division occurs in the muscular region of the body.

(3) The detached part is capable of locomotion, and divides a second time below the lophophore, which is thrown off and disintegrates.

(4) The remaining part, after moving about freely, develops an anterior projection (epistome ?), a lophophoral ridge, and later an aboral projection.

(5) The epidermis of the aboral projection is thrown into a number of folds or involutions, by the unfolding of which it somewhat suddenly increases in length at later stages and assumes the form of a peduncle.

(6) The animal then becomes fixed by a mucous secretion at the free end of this peduncle.

(7) The whole process, from first division to pedunculate fixed form, occupied fourteen days.

(8) The peduncle consists externally of a proliferation of

the epidermis of the body and internally of modified cells of the cœlomic epithelium, fatty particles and muscular elements.

References.

1. Cori, C. J.—"Untersuchungen über die Anatomie und Histologie der Gattung Phoronis," 'Zeit. f. wiss. Zool.,' Bd. li, 1890, p. 480.
2. Gilchrist, J. D. F.—"New Forms of the Hemichordata from S. Africa," 'Trans. S. Afr. Phil. Soc.,' vol. xvii, 1907, p. 151.
3. Harmer, S. F.—"On Phoronis ovalis, Strethill Wright," 'Quart. Journ. Micr. Sci.,' vol. 62, 1917, p. 115.
4. Ikeda, I.—"On the Development of the Sexual Organs and their Products in Phoronis," 'Annot. Zool. Jap.,' vol. iv, 1903, p. 141.
5. Pixell, H. L. M.—"Two New Species of the Phoronidea from Vancouver Island," 'Quart. Journ. Micr. Sci.,' vol. 58, 1912, p. 257.
6. Selys-Longchamps, M. de.—"Ueber Phoronis und Actinotrocha bei Helgoland,' 'Wiss. Meeresunt. Kiel u. Helgoland,' N.F., Bd. vi, Abt. Helgoland, Heft 1, 1903.
7. ——— "Phoronis," 'Fauna u. Flora G. v. Neapel,' 30 Monogr., 1907.

EXPLANATION OF PLATE 29.

Illustrating Dr. J. D. F. Gilchrist's paper on "Reproduction by Transverse Fission in Phoronopsis."

The magnification of figs. 1 and 2 is indicated by the scale accompanying fig. 1, that of the other figures by the scale accompanying fig. 3. All the figures refer to Phoronopsis albomaculata, Gilchrist.

List of Abbreviations.

al. c. Alimentary canal, descending part. *c.m.* Circular muscles. *al. c'.* Ascending part. *bl.* Blood-corpuscles. *b. t.* Basement-tissue. *cœl. ep.* Cœlomic epithelium. *d.* Débris. *ep.* Epidermis. *epist.* Epistome. *f. t.* Fatty tissue. *l.* Lophophore. *l. m.* Longitudinal muscles. *n.* Nerve. *ped.* Peduncle.

Fig. 1.—Free crawling form of Phoronopsis, produced by two transverse divisions of the body of the adult, thirteen days after first division.

Fig. 2.—Pedunculate fixed form, fourteen days after first division.

Fig. 3.—Sagittal section of specimen B (free form), showing commencement of posterior projection.

Fig. 4.—Sagittal section of oral extremity of specimen B.

Fig. 5.—Transverse section of body of specimen A (pedunculate attached form) near the extremities of the digestive tract, at the point of junction of the afferent and efferent blood-vessels.

Fig. 6.—Transverse (somewhat oblique) section of aboral end of body, showing two diverticula of the extremity of the descending part of the alimentary canal, and the position of the cœlomic epithelium which marks the origin of the peduncle.

Fig. 7.—Transverse section of attached form, showing mode of origin of the peduncle from the body.

Fig. 8.—Transverse section of proximal end of peduncle, free from body.

Figs. 9–11.—Transverse sections of peduncle, showing nature of its contained tissue.

Fig. 12.—Transverse section of peduncle, showing excessive foldings of epidermis and obliteration of central space.

[Sections 7–12 are at about regular intervals in the first half of the peduncle.]

Fig. 13.—Section of distal and more rounded part of peduncle, showing particles entangled in mucus secreted by this part. The central tissue is omitted.


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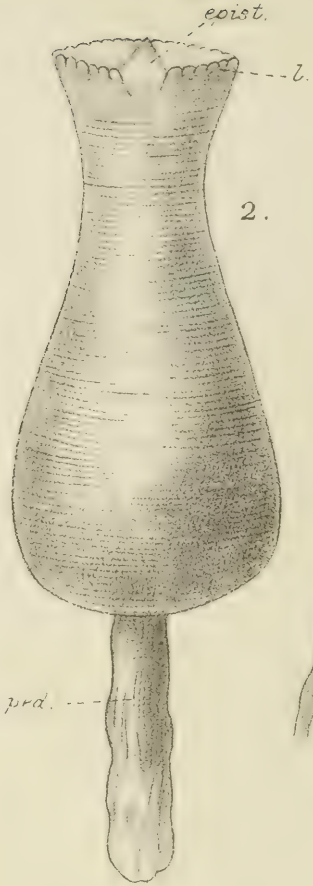
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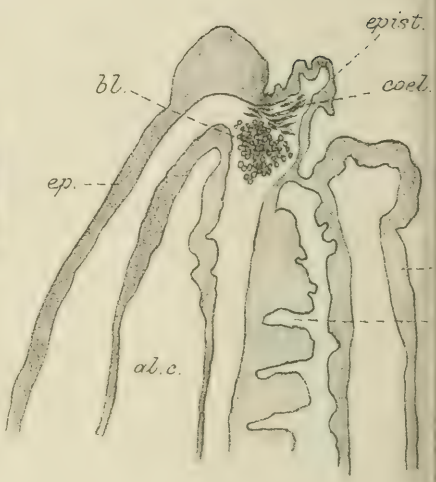
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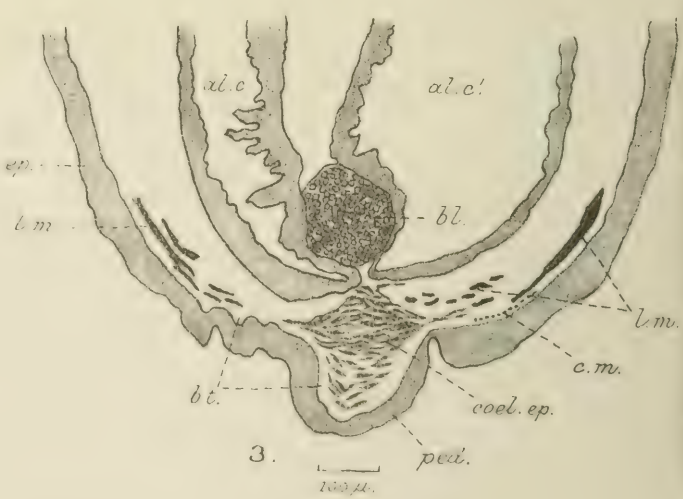
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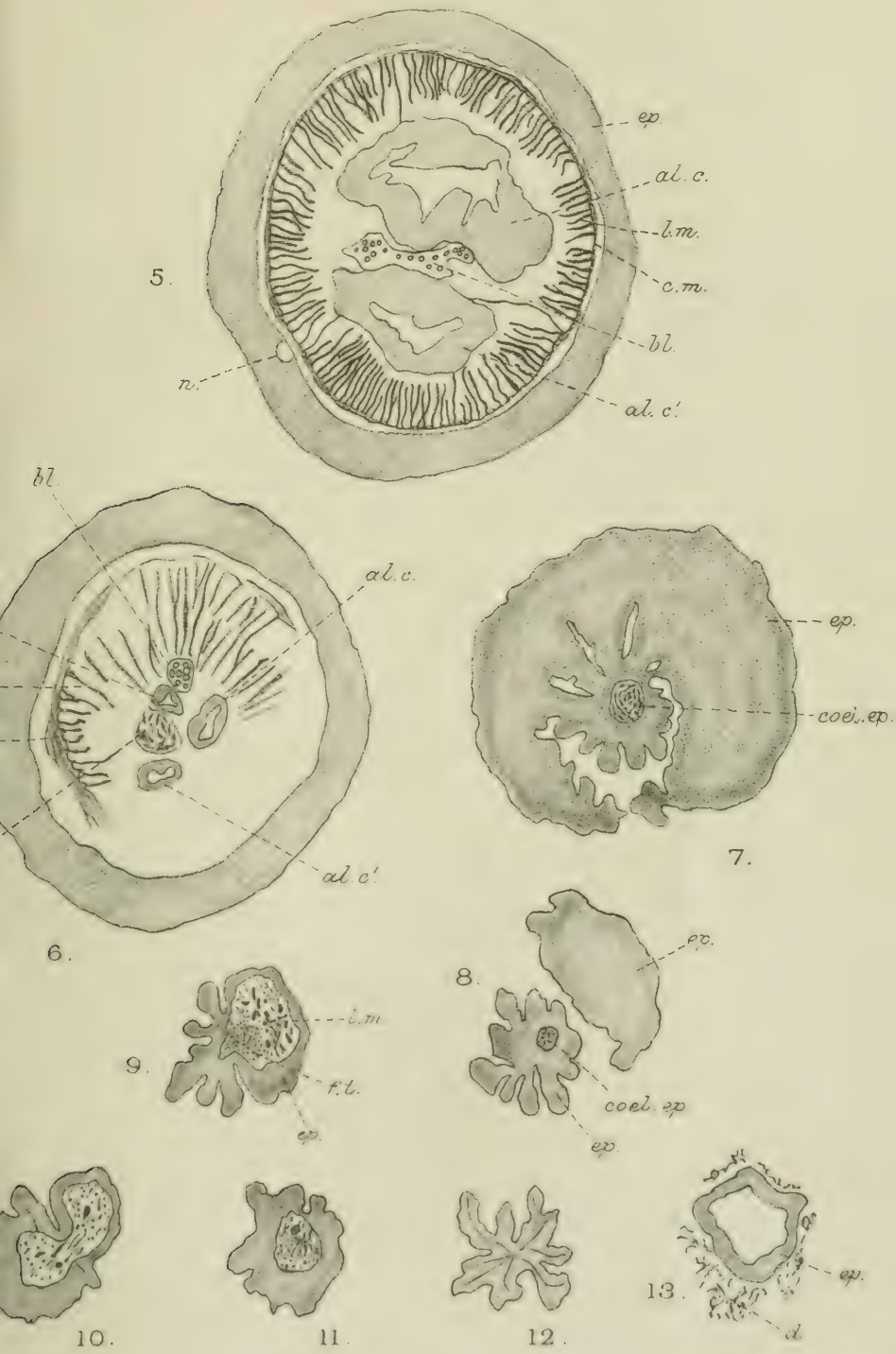

Huth, Lith. London.

On a Species of the Crawling Medusa, Eleutheria, from the Cape of Good Hope (Cnidonema capensis, g. et sp. n.) and the Southern Eleutheriæ.

By

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With Plate 30.

I. Medusa.

The crawling or creeping Medusa Eleutheria is one of the most interesting of the Cœlenterates. While the tentacles are modified into ambulatory organs and suggest a possible mode of transition between the fixed polyp and the free-swimming medusa, the sub-umbrellar cavity is more complex than in the ordinary medusa, being, it is said, modified into a large brood-cavity, which in one species, at least, extends over the stomach. Unfortunately, the animal is rather rare, so that of the two species of the Northern Hemisphere only one has been traced to its hydroid form (Hinks, 1861), and there is still some doubt as to the differences between the species. In more recent years three new species have been described by Browne from the Southern Hemisphere, from the Falkland Islands, Wandle Island and McMurdo Bay, and Vanhöffen procured a species from Kerguelen, from an examination of which he concludes that all the species from the Southern Hemisphere are identical.

It is of special interest, therefore, to find that a species occurs at the Cape of Good Hope, which at certain times and

places can not only be procured in fair abundance, but can be readily kept in confinement, thus affording an opportunity of observing its habits, which are little known.

Occurrence.

The animal was first observed in a tank of the Government Marine Laboratory, near Cape Town, shortly after a number of crawfish had been put in, and was thought to have been brought in with them. On a later occasion it appeared in a smaller tank, and had apparently been carried in with the supply water. A search was then made on the sea-shore, where at first only one was found, but on another occasion about twenty were found at the bottom of a large basin in which sea-weed from low water (spring tide) had been left for some hours. Curiously enough, weed procured from the same spot the following day produced no specimens. In spite of their apparently delicate organisation they were found in localities most exposed to the breaking waves.

Habits.

The most striking feature in the behaviour of the animal is, of course, its method of locomotion. It may remain stationary for several days, but is usually very active. When it was lightly touched on one side the crawling, or rather walking, movement could be readily observed; the tentacles on the side opposite the source of irritation were released and applied at a point further from the body, which was then moved in this direction. Progression was assisted by a reverse movement of the tentacles on the other side. When viewed from the side during this process, it was seen that the whole body was raised from the substratum, so that the movement was more that of walking than creeping or crawling. If the irritation was applied to the other side of the body the direction of movement was reversed, so that the animal could be made to move in any direction. There was always a tendency, however, to move off to the under side of the weed

or other object on which it rested. This active movement was more marked in the early stages; the older and mature individuals remained, as a rule, stationary.

The adhesive power of the tentacles is a relatively powerful one, for the jet of water, playing on the animal in the tank, was frequently strong. In fact the animals seem to prefer being in such a strong current, as on one or two occasions they moved off from quiet, though apparently sufficiently aërated water, to the strongest part of the current. The animal could not be picked up by a pipette without first releasing the tentacles one by one. Often it remained adherent by one tentacle only. When placed on its back the animal had great difficulty in recovering its right position.

The tentacles exhibited another movement which was almost constant and very characteristic. This consisted of a sudden jerking upwards, so that the upper nematocyst-bearing branch was thrown over the body, the lower or sucker-bearing branch meanwhile loosening its hold on the substratum and sharing in the upward movement. This movement was kept up when the animal was stationary, and differed from the slow and more deliberate movement in walking. It may have some protective function, as by it the clusters of nematocysts on the upper side of the tentacles were thrown over the upper surface of the body.

The feeding action of the animal was observed. This consists of a slow movement of the mouth over the surface of the substratum under the body of the animal, apparently for the purpose of securing small particles of a vegetable or animal nature. A much more active method of procuring food was, however, observed on one or two occasions. Thus, in one, whose tentacles were in a state of great activity, it was observed, on closer examination, that a small animal, apparently a copepod, had been captured, and was held by the tentacles clustered round it. The manubrium was extended beyond the edge of the umbrella and the mouth was applied to this object. In another case a similar activity was observed, and here it was a small larval chætopod that had

been captured. Such a chætopod is usually a very active animal and it made frequent attempts to escape, but on each occasion, when the head was protruded, the nematocyst-clusters were brought sharply to bear on it, driving it back. On another occasion, however, an Eleutheria, which had been observed to be perfectly healthy, was observed, a few hours later, surrounded by about half a dozen larval chætopods, which were devouring the remains of its disintegrated body.

European Eleutheriæ are not known to swim, but Vallentin (1910) states that the Falkland species is able to do so. The Cape species was never observed to swim.

External Characters of the Body.

The breadth of the largest male was 3·3 mm., the largest female 2·24 mm. These large specimens, however, were rare, and most were less than 1 mm. down to ·27 mm.—the diameter of the newly-detached bud. In life the body is usually flattened, the height being about one-third of the breadth. In preserved material there is much variation, some being almost spherical in shape. None were so flattened as is E. hodgsoni.

In colour the body is mostly a dark reddish-brown by transmitted light, being of a somewhat bright red by reflected light. This is due to pigment-granules lodged in the stomach and circular canal, but absent in the radial canals. Thus in the younger specimens there appeared a circular brown patch in the centre of the disc; in the older this assumed a hexagonal shape with six radiations, which in still older specimens became enlarged into saccular structures extending almost to the circular canal (Pl. 30, figs. 1, 2, 5).

The exumbrellar surface also had a distinctive colour, which lies above and partly conceals the brown pigment mentioned. It, however, varied considerably in pattern. This was a pure white, which usually disappeared in the preservative. It was best seen by reflected light, and consisted in the well-developed condition of a hexagonal ring above the stomach produced

into six radiating lines to the circular canal (Pl. 30, figs. 1 and 2). In other cases the hexagonal ring was absent and only the radiations were present, and these were sometimes reduced to patches halfway between the apex and the margin of the body. Some cases were observed in which the white colour covered almost the entire upper surface, and others in which it assumed a ring-like form.

Another and entirely different pigment pattern was found in some large individuals, which proved on being sectioned to be mature. In these the gonads, which are of a clear whitish colour, extend round and above the brown stomach, concealing it all except a small circular patch in the centre, from which there are six thin, radiating lines—the only part of the stomach visible.

The ocelli were of a dark brown colour, usually surrounded by a pure white circle of pigment. In sections the ocelli showed scattered pigment-spots, but no lens.

External Characters of the Tentacles.

The number of the tentacles varies very considerably—from six in the youngest to about forty in the largest. Though they are apparently irregularly arranged with no reference to the radial canals in the larger specimens, they are quite regular in younger forms. In the newly-detached bud there are six (sometimes eight) long tentacles, arising from the semi-circular canal, opposite the ends of the six radial canals. Between them there are sometimes smaller tentacles. The subsequent origin and growth of additional tentacles, however, does not appear to be regular, as they are frequently seen in numbers which are not multiples of six or eight.

Each of the tentacles is divided into two branches, one long, upper branch and a shorter lower branch, about the length of the main stem of the tentacle (Pl. 30, fig. 1). The upper branch is about three times the length of the lower branch in the living and fully-expanded condition. It,

however, contracts much more in preservative, and is then only about the length of the lower branch. In some preserved specimens the main stem is much longer than either of the branches, being about two and a-half times their length. This fact may be of importance, as the chief difference between the two northern species, according to Hartlaub and Mayer, is the relative length of the branches and main stem of the tentacle. None of the living or preserved specimens, however, showed such a wide difference in this respect as is indicated in the figures of those species.

An important point in the specific determination of the southern Eleutheria is the position of the clusters of nematocysts on the tentacles, and this was therefore specially noted both in the living and preserved condition. In all, the position, but not the number of nematocyst-clusters, was constant. There was always a knob-like terminal cluster, and immediately behind it a cluster on the upper or aboral side of the tentacle. This latter, however, in large specimens extended down on each side of the tentacle. In the younger specimens it was entirely dorsal in position. Towards the base of the tentacle another and smaller cluster occurred, but entirely on the ventral or oral side of the arm. A fourth occurred at some greater distance, again entirely on the dorsal or aboral side. These were all on the upper branch of the tentacle. A fifth and much smaller cluster appeared in most specimens on the dorsal or aboral side of the main stem. In some large individuals, however, this last was entirely absent. None were ever observed on the lower branch.

The development of these nematocyst-clusters is of interest. The first to appear (in the bud) is the terminal cluster, and this was sometimes the condition found in a free bud, so that this species passes through a stage similar to that of the adults of the Northern Hemisphere, which have a terminal cluster of nematocysts on the upper branch. Soon after the appearance of the terminal cluster a second arises close behind it, and the others at later stages.

Macroscopically the tentacles do not appear to be pigmented,

but grains of yellowish pigment are seen in the endodermal cells under the microscope, and these are grouped together in little heaps under the bases of the nematocyst-clusters, where also patches of white were seen.

Nematocyst Ring.

This is a very prominent feature in Eleutheria, and consists of a thick cushion of nematocysts in the form of a ring, under the circular canal, apparently in all species except E. hodgsoni. It is, in all the sections of the Cape, and apparently of the Kerguelen species, distinctly marked off from the base of the tentacles, with which it has no connection (Pl. 30, figs. 3 and 4, *n.r*).

Velum.

Eleutheria has been described by some early observers as devoid of a velum. This is due to the fact that the velum often fits closely round the manubrium, and lies appressed to the body. It may always be readily made out, however, in sections, sometimes being closely applied to the manubrium, at others drawn out into a tubular or funnel-shaped structure, extending well beyond the mouth (Pl. 30, fig. 3, *vel.*). In one case it appeared to be partly fused to the manubrium. In Eleutheria it has apparently become transformed from an accessory locomotory organ into an organ whose chief function is the closing up and protecting of the large cavity of the gonads.

Alimentary Tract.

The stomach is a wide sac, occupying at its widest the greater part of the body. It is produced into six wide pouches, the sides of which when fully developed are more or less rectangular in sections (Pl. 30, fig. 5). Above and below this part the pouches become smaller, and appear in the form of slight diverticula of an angular shape. The radial canals join the stomach at the wider parts (Pl. 30, fig. 5, *r.c.* 3). The cells of the walls of the stomach are laden with granular

material, and consist of glandular cells and nematocysts, but no ova, which are said to have been found in similar gastric pouches in Cladonema.

In younger and smaller animals the stomach is also wide, but the diverticula are small and acute, giving the whole a star-like appearance in sections. In still earlier stages the stomach is also wide, but with thinner walls, and sometimes with no diverticula.

The lower or manubrial part of the alimentary tract showed a marked development of muscular tissue. As has been noted it is in a very motile part, and may be protruded to a very considerable extent. If the animal is placed on its back the main stem of the manubrium, as it moves about from side to side, may be seen to have six thick strands, more pronounced in its middle and distal portions, but fading away towards its upper parts. Transverse sections of the manubrium, near the mouth, show that these strands are of a muscular nature and arranged here in the shape of a star with six rays. Between the rays are nematocyst cells. Towards the upper part of the manubrium a slit appears in the centre of these rays, giving rise to a star-shaped space—the lumen of the manubrium. These rays become wider, and ultimately expand into the six diverticula of the stomach. Near the stomach they become much reduced, and spread out on its inner surface. Meanwhile the other cells of this part of the body, largely composed of nematocyst cells, become much more numerous.

Radial Canals.

The radial canals were six in number in all the sections made, and they appeared to be so in all other specimens, though they could not be seen clearly in the living or preserved whole specimens, as they are unpigmented or slightly so. As already noted, there is in the mature individual an appearance of pigmented radial canals, but this is due to the gonads spreading over the stomach, which can then be seen only as brown radial lines between them.

Circular Canal.

The circular canal is wide, and contains pigment-granules on its inner side only. The inner margin of this ring of pigment is well defined; the outer is irregular, with projections, which, however, do not extend into the tentacles, as figured in Vanhöffen's species, except in the young forms, in which the endodermal part of the tentacles is of a reddish-brown colour, similar to that of the circular canal. The projections mentioned do not occur in the adult opposite the tentacles, but opposite the spaces between them.

Gonads.

The gonads in the mature condition are very well developed, in contrast to the condition in Eleutheria dichotoma, and when fully developed they occupy almost the whole of the large sub-umbrellar cavity, extending from the velum upwards alongside of the stomach and to a considerable extent above it (Pl. 30, figs. 3 and 4), as indicated by sections.

They are separated from each other by partitions formed of a double layer of ectoderm, so that they may be described as occupying six pouches or vertical diverticula of the sub-umbrella (Pl. 30, figs 5, 6, 7). These pouches extend from the circular canal towards the apex of the body, as may be seen in the living animal and in sections. They do not, however, fuse together at their apex to form a brood pouch, and there is a central area above the stomach, about equal to half the diameter of the animal, quite devoid of gonads.

Whether or not such partitions between the gonads exist in other southern Eleutheria is not known, except in the case of the male of Vanhöffens's species. The females of this species do not appear to have them, though Vanhöffen suspects they may be present in the young females.

As to the nature and origin of the pouches in the Cape species, they are obviously associated with the comparatively

short radial canals, which enter the stomach at a low level. The enlargement of the sub-umbrellar space has therefore been upwards between the radial canals. This is illustrated in Pl. 30, fig. 5, which is a transverse section of a large male. The section, being somewhat oblique, shows a radial canal (*r.c.* 1) at its point of origin from the circular canal (*c.c.*). The radial canal to the right (*r.c.* 2) is free from the circular canal, while that above (*r.c.* 3) is at the point where it enters the stomach. At the upper part of the figure the section passes above the radial canal, which therefore does not appear here, but in its place there is the double fold of the ectoderm, lining the sub-umbrellar space. These have come in contact over the radial canals (Pl. 30, figs. 5, 6 and 7, *s.e.p.*).

This does not agree with the condition found in the Kerguelen species, for here, according to Vanhöffen, the radial canals are continued up on the outside of the septa, which therefore cannot have arisen by the ectoderm of the sub-umbrellar cavity meeting over the radial canals (1911, p. 203, Pl. 30, fig. 5, *c.*).

Asexual Reproduction.

Budding is a very frequent occurrence, but only in the earlier and smaller stages of the medusa. In them half-a-dozen buds at various stages may be seen arising from the circular canal, between the tentacles and ring of nematocysts. The process is fairly rapid, buds being separated off from an individual observed, at the rate of one in every two or three days. Budding may begin early, as in one individual ·42 mm. in diameter a bud ·17 mm. in diameter was given off.

Relation of the Cape Eleutheria to other species.

The northern species of Eleutheria are readily distinguishable from the southern by the fact that the former have a single terminal cluster of nematocysts in the upper branch of the tentacles. In all the southern species there are

additional clusters in the course of the branch between its distal end and the point where it joins the main stem. There seems to be still some doubt as to the two European forms being specifically distinct from each other. Hartlaub (1889), and Mayer (1910), following him, state that the chief distinction between E. dichotoma and E. claparedii is that in the latter the branches of the tentacles are much shorter (cf. variation in this respect noted in the Cape species), while Browne (1910) states that E. claparedii differs from E. dichotoma in having "both branches of the tentacles terminating with clusters of nematocysts." He adds that "it is quite probable that it is only an abnormal form of E. dichotoma with some nematocysts in the adhesive disc" (cf. the variation in nematocyst-clusters noted in the adults of the Cape species). Hæckel (1879, p. 106) gets over this difficulty by supposing that the alleged presence of nematocyst-clusters on the lower branch, noted by Quatrefages (1842), was founded on a mistaken observation.

Similar difficulties have been encountered in distinguishing the southern Eleutheria. The first representative of these was described by Browne (1902) from a single specimen, found by Vallentin in Stanley Harbour, Falkland Islands. He named the species E. vallentini, and amongst other characters mentioned that the gonads occupy the whole of the upper part of the umbrella above the stomach, and the nematocyst of the tentacles are in "two or three clusters on the upper (aboral) side, and occasionally on the under side."

He also (1910) recognised that the animal described by Bedot (1908) as Wandelia charcoti, taken off Wandel Island, was a species of Eleutheria which he called E. charcoti, characterised by the fact that the radial canals have slender lateral branches, the clusters of the nematocysts being, not oral and aboral in position, but lateral.

Browne (1910) recognised another species obtained in the National Antarctic Expedition, naming it E. hodgsoni characterised by ten to twelve clusters of nematocysts

arranged as in E. charcoti, but distinguished from this and the other species by having an incomplete ring of nematocysts under the edge of the bell.

Finally, in 1911, another Eleutheria was found by the German Deep Sea Expedition at Kerguelen, and described by Vanhöffen (1911). He considers that all the three species described by Browne, together with his own, are identical, the supposed differences being due to mistaken observations.

Thus he thinks that as Browne examined only a single specimen of E. vallentini, a mistake could easily have arisen as to the position of the clusters of nematocysts. The fact, however, that in the Cape species the clusters of nematocysts are, in all cases, in the position described by Browne, seems to indicate that his statement cannot be set aside merely on the ground that a mistake could easily have been made.

With regard to E. charcoti, Vanhöffen doubts that its distinctive feature, the branching of the radial canals, is a fact. He has seen and sketched in the living animal an appearance which, he thinks, might have given rise to the supposition that the radial canals are branched, but this was not confirmed by sections. It is probable that this pigmentation is of the same nature as the white pigment described in the living Cape species; it usually disappears in preservative and was not seen in sections. Browne does not state exactly on what evidence he makes his statement, but it will be erring on the safe side to accept it until disproved by sections.

E. hodgsoni is distinguished from all other species by its interrupted band of nematocysts, these being isolated patches on the basal portions of the tentacles according to Browne. This Vanhöffen doubts, as the tentacles are very crowded together, so that there is scarcely any space between them. In the Cape species, and apparently also in the Kerguelen species, this band is well separated from the bases of the tentacles.

Vanhöffen states, as a further argument for the identity

of the species of Eleutheria in the Southern Hemisphere, that it would be very strange if the Falkland Island species should differ from that from Kerguelen, both having been found on the kelp (Macrocystis) which is carried in the Antarctic current round the south polar continent. This is not very convincing evidence, but may also be taken for what it is worth as evidence that the Cape species, which was not found on this weed, is distinct.

On the whole, in absence of definite evidence to the contrary it may be advisable to retain Browne's species provisionally, and, if so, we must regard Vanhöffen's species as a fourth, which may be called E. kerguelenensis. The Cape species, which may be designated E. capensis, agrees with E. vallentini, and, like it, differs from all other species in having the clusters of nematocysts oral or aboral in position; it differs from it, however, markedly in that the gonads do not occupy the whole of the upper part of the umbrella above the stomach, as they do, according to Browne, in E. vallentini.

With regard to the placing of these species under one genus, it may be noted that an apparently important character of the genus Eleutheria, which seems to be of more fundamental significance than the character of the tentacles, is the presence of a brood-pouch above the stomach along with the reduction of the gonads, as described by Hartlaub (1889), who regarded it as one of the characteristics of the genus, and of such importance that its absence, if proved, in the only other Eleutheria then known (E. claparedii) would necessitate the establishment of a new genus. This suggestion has not been accepted by later authors, who have definitely described forms in which it is absent. The reconsideration of this, however, seemed to be desirable in view of the fact that in the mature female of the Cape species there is probably no brood-cavity at all, and certainly none above the stomach, and it appeared to be necessary to establish a new genus for the reception of such forms. The subsequent discovery, however, of the hydroid threw a new light

on the above question, and at the same time disclosed further reasons for separating the two groups generically.

II. The Hydroid Stage.

The preceding description of the medusoid form of the animal was completed before the hydroid from which it arises was found, and it may be as well to leave it in its present form, with a few necessary alterations, in order to indicate the position with regard to our knowledge of the southern "Eleutheria," and how this has been altered by the characters which the polyp proves to possess.

The determination of the hydroid, which seemed at first a difficult matter, proved ultimately to be very simple. A small hydroid-like Hydranthea was very abundant in the tank, in which the medusa was mostly found, and this was suspected to be the parent stock, but no definite evidence was procured. A smaller vessel, kept for another purpose, and in which the medusæ had appeared in two successive summers, was then carefully examined, and a beautiful, but inconspicuous and small Cladonema-like hydroid was found, with buds in all stages, one just set free, and two crawling about slightly larger. As the medusoid form of Cladonema is in some respects closely related to Eleutheria, as has been pointed out by Haeckel, it was obvious that this would prove of importance in clearing up some difficulties mentioned in the inquiry.

This hydroid (Pl. 30, fig. 8) may first be briefly described. The hydranth is of varying length, the longest being about a millimetre and a half; at its broadest part, just below the upper tentacles, it is about ·2 mm., narrowing down to ·12 mm. at its proximal end. Coloration is not conspicuous, except in the endodermal parts, which are of a slightly reddish colour. The medusoid buds were of the same colour, but much more conspicuous. The rounded heads of the distal tentacles were of a transparent white colour. The animal could only be clearly recognised under the microscope on account of its

small size and the fact that it is usually concealed by adhering débris.

There are usually three capitate tentacles below the prominent conical or rounded hypostome. These tentacles are short and stout, about ·25 mm. in length in the individual measured when fully expanded. In one only of the specimens (about twenty) examined were these tentacles four in number. About a millimetre from the distal end of the polyp is a circle of non-capitate tentacles, thinner and usually longer than the other tentacles. They are usually six in number, but four were also observed. Pl. 30, fig. 8, is drawn from a living individual, with a large medusoid bud; its dimensions are somewhat different from those stated, and the tentacles are somewhat contracted.

The hydrocaulus was in the specimen measured about 2 mm. in length. The length may vary, however, considerably, and it may be straight or bent in various ways. The perisarc is thin and transparent towards its distal end.

The hydrorhiza is sometimes closely applied to and penetrates the substratum, or it may be free for a considerable portion of its length. It is of about the same diameter as the hydrocaulus; the perisarc is of a yellowish-brown colour, is fairly tough, and coated with débris of various sorts.

The buds arise at or slightly above the level of the lower tentacles. One, two or three may be seen at one time in this position in all stages of growth. They are well advanced before they are set free, and the tentacles, which are then in active motion, show a well-developed nematocyst-cluster at the end of the upper branch of the tentacle, with the rudiment of a second beginning behind it on the aboral side in some.

Except for the reduced number of tentacles the hydroid closely resembles the genus Cladonema, and we may now consider the significance of this in relation to some of the characters of the species of Eleutheria.

Though the southern differ markedly from the northern species, as, for instance, in the absence of a brood-pouch

above the stomach and the character of the tentacles, which, moreover, closely resemble those of the young medusa of Cladonema, Browne (1902, 1910) and Chun (1900) seemed to have no hesitation in referring them to the genus Eleutheria. Vanhöffen (1911), however, had some suspicion of the affinities of these forms with Cladonema, as he at first (1911) named the unknown polyp, to which the southern Eleutheria probably belonged, Cladonema vallentini, as, according to the form and arrangement of its tentacles, it belonged to this genus and not to Clavatella (the hydroid of the northern Eleutheria). His further examination of the medusa, however, led him to abandon this suspected affinity to Cladonema, and he returned to the genus Eleutheria on the following grounds of resemblance: simple mouth without stinging tentacles; simple division of tentacles into two branches; ring-shaped mass of nematocysts under the margin of the bell and the utilisation of the sub-umbrellar space as a brood-cavity. Two objections to this are that there is no special brood-chamber above the stomach as in Eleutheria, as described by Hartlaub (1886), and that there is no conclusive evidence that the sub-umbrellar space functions as a brood-cavity in the southern form—in fact, there is evidence to the contrary in the Cape species.

His first suspicions therefore prove to have been justified, but there is some difficulty as to placing this Cape medusa and hydroid (probably along with other southern "Eleutheria") in the genus Cladonema. The reduced number of capitate tentacles in the hydroid and the increased number of non-capitate tentacles may not be of great significance, and are not constant, but the oral tentacles, terminating in nematocyst knobs, found in the medusa of Cladonema and not in the southern Eleutheria, presents a more serious difficulty. The presence or absence of the oral tentacles has, indeed, been used (Mayer, 1910) to separate the family of the Cladonemidæ into two sub-families, and though this is avoided in Günther's classification, this character is still used to separate genera of the sub-families into groups.

In view of the present generic classification of the Cladonemidæ it seems, therefore, necessary to distinguish this representative of the family both from Eleutheria and Cladonema, and, to mark a distinctive feature, namely, the existence of several clusters of nematocysts on the upper branch of the tentacle, it may be called Cnidonema. The following list of outstanding features in the three genera will indicate their differences and similarities.

Genus Eleutheria, Quatrefages.

Medusa:
- Adapted for crawling or walking.
- Brood-pouch above stomach.
- Gonads reduced, lodged in brood-pouch.
- Hermaphrodite.
- Radial canals simple, four to six in number.
- Tentacles of the same number as radial canals, dichotomous; upper branch with one terminal nematocyst-cluster.
- No oral tentacles.
- Thick nematocyst ring under margin of bell.

Hydroid:
- With one verticil of capitate tentacles only.

Genus Cnidonema, g. n.

Medusa:
- Adapted for crawling or walking.
- No brood-pouch above stomach.
- Gonads well developed, in ectodermal inter-radial pockets around stomach.
- Sexes separate.
- Radial canals usually six.
- Tentacles numerous, increasing with age, and not corresponding to number of radial canals, dichotomous;

the upper branch with several clusters of nematocysts in addition to a terminal cluster.

No oral tentacles.

Thick nematocyst ring under margin of bell.

Hydroid:

With one verticil of three capitate tentacles, and a second of six non-capitate tentacles.

Genus Cladonema, Dujardin.

Medusa:

Not adapted for crawling or walking.

No brood-pouch above stomach.

Gonads around stomach continuous.

Sexes separate, occasionally hermaphrodite.

Radial canals simple, or more or less fused together, eight to ten in number.

Tentacles of the same number as radial canals, branched, or with simple or branched appendages.

Four or five oral tentacles terminating in spherical masses of nematocysts.

No thick nematocyst ring under margin of bell.

Hydroid:

With one verticil of four capitate tentacles, and a second of four non-capitate tentacles.

With regard to the inclusion of all the described species of the southern Eleutheria under the genus Cnidonema as here defined there are certain difficulties, which, however, are not serious, and may disappear with a fuller knowledge of the species. Thus the existence of ectodermal pockets in which the gonads are partly lodged has not been described in Browne's species, and only in the male in Vanhöffen's, and the hydroid form is only known in the case of the Cape species. The following key to the various species indicates the chief differences between them as they have been described.

I. Nematocyst clusters oral and aboral in position:

A. Gonads entirely above stomach

1. C. vallentini (Browne).

B. Gonads not entirely above stomach
2. C. capensis, n. sp.

II. Nematocyst clusters lateral in position:
A. Radial canals branched . 3. C. charcoti (Browne).
B. Radial canals not branched.
A′. Complete nematocyst ring
4. C. kerguelensis, n. sp.
B′. Incomplete nematocyst ring
5. C. hodgsoni (Browne).

Further information is, however, required with regard to these specific differences.

There are also some interesting points which are worthy of attention, such as the origin of the gonads, said to be ectodermal in Eleutheria, and endodermal at least in one species of Cladonema. Whether the sub-umbrellar space serves as a brood-cavity in which the young are developed is still questionable. There is evidence also that the tentacles appear in a definite order in development. The development of the Cape species, which can be readily procured, will probably throw some light on these points.

Summary.

(1) A species of "Eleutheria" is found in fair abundance at certain times and places at the Cape of Good Hope.

(2) It can readily be kept in confinement, and some of its habits are noted.

(3) The chief characteristics of the species are described and compared with those of other southern species.

(4) Its hydroid form has been found, and proves to be very similar to that of Cladonema (Stauridia), not of Eleutheria (Clavatella).

(5) It differs, however, from Cladonema chiefly in the absence of oral tentacles, and a new genus (Cnidonema) is proposed for the reception of this and probably all the other southern Eleutheria, the hydroid forms of which, however, are not yet known.

References.

1842. Quatrefages, A. de.—"Memoire sur l'Eleutherie dichotome (Eleutheria dichotoma, Nob.) nouveau genre de Rayonnes, voisin des Hydres," 'Ann. des Sc. Nat.,' ser. ii, t. 18, p. 270.

1861. Hinks, Th.—"On Clavatella, a new genus of Corynoid Polypes, and its Reproduction," 'Ann. Mag. Nat. Hist.,' vol. vii, ser. 3, p. 73.

1861. Krohn, A.—"Beobachtungen über den Bau und die Fortpflanzung der Eleutheria, Quatref.," 'Wieg. Arch. fur Naturgesch.,' 27 Jahrg., Bd. i, p. 157.

1863. Claparede, E.—'Beobachtungen über wirbellose Thiere.'

1866. de Filippi, F.—"Spora due Idrozoi del Mediterranes," 'Memorie della R. Accad. delle Sc. di Torino,' ser. ii, t. xxii, p. 357.

1872. Allman, G. J.—'A Monography of the Gymnoblastic or Tubularian Hydroids,' Ray Society.

1879. Haeckel, E.—'Das System der Medusen,' Jena.

1886. Hartlaub, C.—"Über den bau der Eleutheria, Quatref.," 'Zool. Anz.,' ix, p. 706.

1887. ——— "Zur Kenntnis der Cladonemiden," 'Zool. Anz.,' x, pp. 651–658.

1889. ——— "Über die Claparedesche Eleutheria," 'Zool. Anz.,' xii, p. 665.

1900. Chun, C.—'Reiserbericht über die Tiefsee-Expedition.'

1902. Browne, E. T.—"A Preliminary Report on Hydromedusæ from the Falkland Islands," 'Ann. Mag. Nat. Hist.,' vol. ix, pp. 272–284.

1908. Bedot, M.—"Sur un Animal pélagique de la Région antarctique" (Wandelia charcoti), 'Expéd. Antarct. Française (1903–5) Expédition Charcot.'

1910. Mayer, A. G.—'Medusæ of the World,' vol. i.

1910. Browne, E. T.—"Cœlenterata: V, Medusæ," 'National Antarctic Expedition, 1901–1904,' vol. v, pp. 1–62.

1911. Vanhöffen, E.—"Die Anthomedusen und Leptomedusen der deutschen Tiefsee-Expedition," 'Wissen. Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia."' Bd. xix, pp. 193–233.

EXPLANATION OF PLATE 30,

Illustrating Dr. J. D. F. Gilchrist's paper "On a Species of the Crawling Medusa, Eleutheria, from the Cape of Good Hope (Cnidonema capensis, g. et sp. n.) and the Southern Eleutheriæ."

[Sections (figs. 3–7) are magnified to the scale shown with fig. 3. The magnification of other figures is indicated by the scale accompanying them.]

Reference Letters.

c.c. Circular canal. *m.* Mouth. *n.r.* Ring of nematocysts. *ov.* Ovary. *r.c.* Radial canal. *sep.* Septum, separating gonads. *s.um.c.* Sub-umbrellar cavity. *t.* Testis. *vel.* Velum.

Fig. 1.—Immature and actively crawling medusa.

Fig. 2.—Mature and more stationary medusa viewed from above, with tentacles fully expanded.

Fig. 3.—Female medusa: vertical section passing through the radial canals.

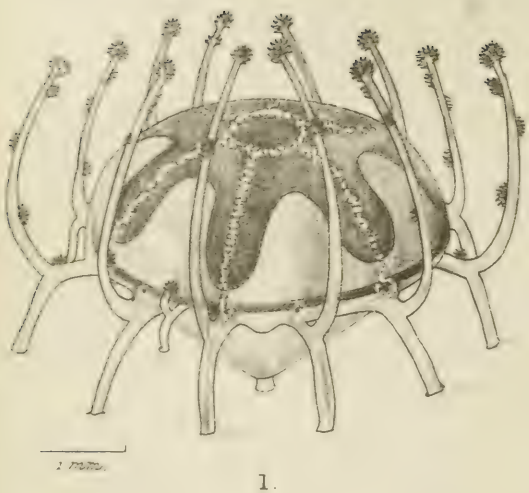
Fig. 4.—Female medusa: vertical section passing through inter-radial region.

Fig. 5.—Male medusa; oblique section passing through the circular canal, the radial canals and septa. *r.c.* 1. A radial canal where it joins the circular canal. *r.c.* 2. A radial canal at a higher level. *r.c.* 3. A radial canal entering the stomach. *sep.* Septa separating the gonads at a level above the radial canals.

Fig. 6.—Male medusa; transverse section above stomach.

Fig. 7.—Female medusa; transverse section above stomach.

Fig. 8.—Hydroid stage with a fully-formed medusoid bud.



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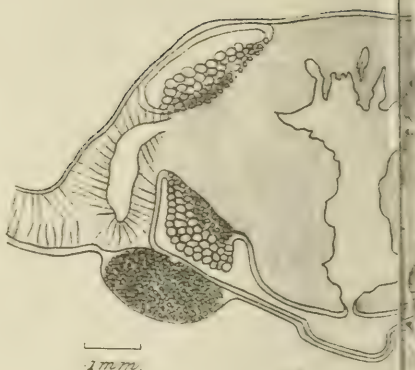

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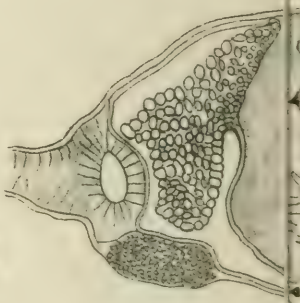

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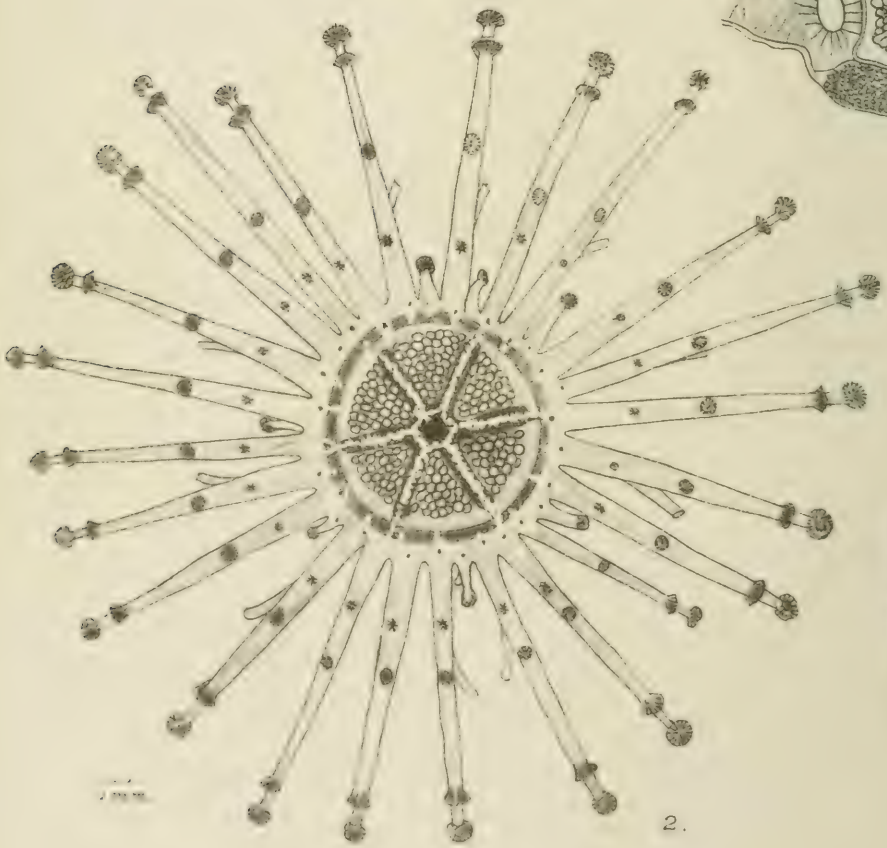

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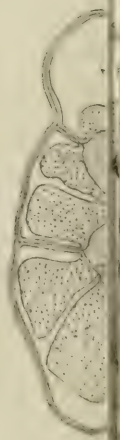

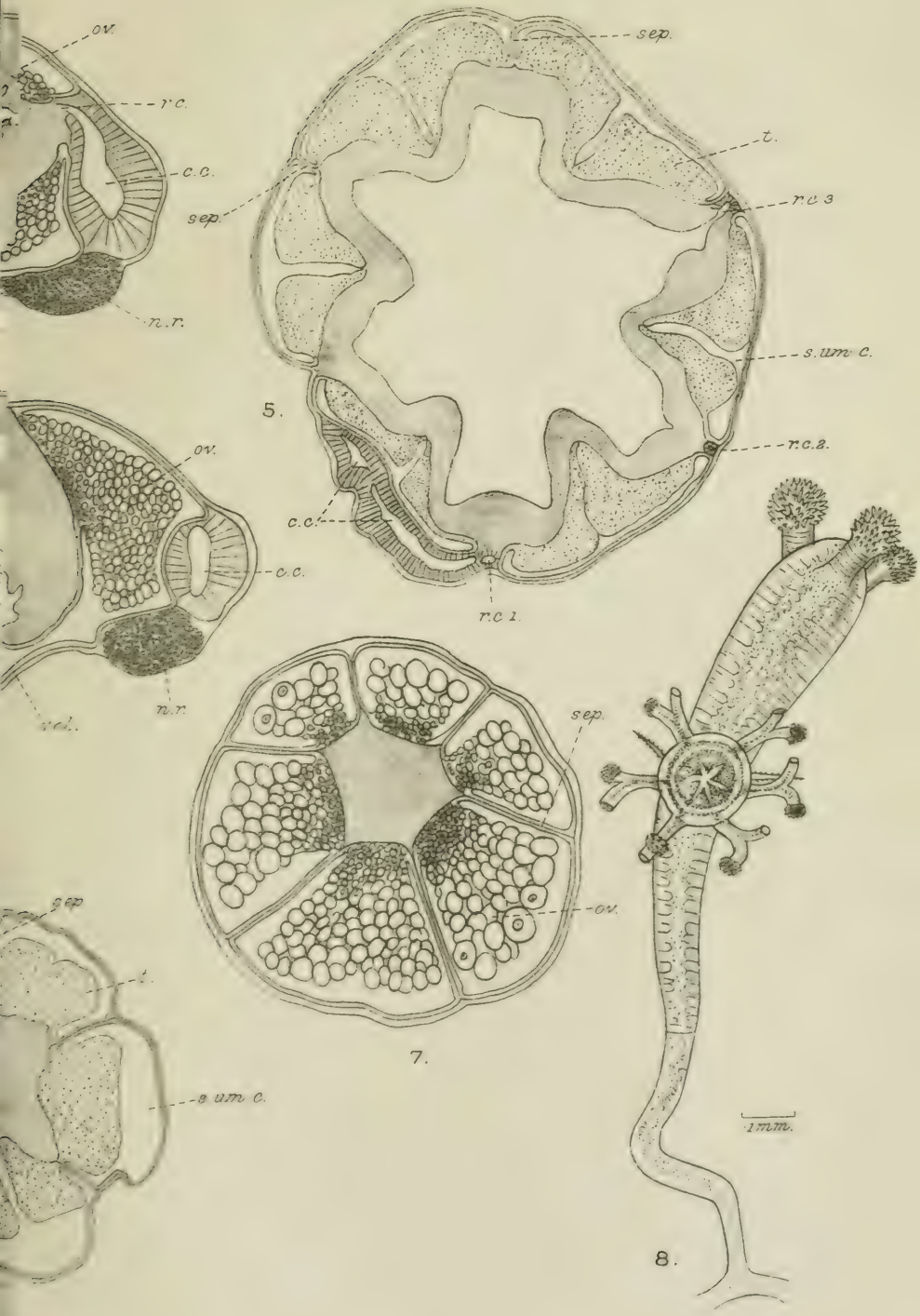

Huth, Lith^r London

The Terminology of Parthenogenesis.

By

Sir Ray Lankester, K.C.B., F.R.S.

The word "parthenogenesis" has become established in biological science to signify the production of offspring by a virgin mother. The term does not embrace reproduction by buds or by fission, but refers to parentage by a mother who produces egg-cells identical in character with those which, in the vast majority of instances, fuse with male sperm-cells before proceeding to develop. In those instances which are distinguished as instances of "parthenogenesis," the egg-cells proceed to develop *without* fusion with the male reproductive element or sperm-cell.

Parthenogenesis may accordingly be defined as an exceptional and historically super-induced modification of the normal process of sexual reproduction or gamogenesis in which the female gamete or egg-cell does not unite with a male gamete or sperm-cell to form a "zygote," but proceeds to develop independently.

The term should not be applied to reproduction by unfertilised unicellar "spores" common in the lower plants and Protozoa, nor to any cases except those in which the "parthenogenetic" reproductive cell is either (1) a normal egg-cell capable of sexual zygosis, or (2) demonstrably a comparatively recent modification of such an egg-cell. The latter is an important special group, and at one time these modified egg-cells—incapable of fertilisation—were incorrectly described as "pseud-ova" (Huxley). The egg-cell

thus independently developing may be described as "autoblastic" and the process as "autoblastesis." And again the autoblastic egg-cell may be described as "lipospermic" and the embryonic history as one characterised by "lipospermy" or "lipospermia."

A difficulty of nomenclature has lately arisen in describing and discussing the offspring so produced—for instance, when the eggs of the frog have been experimentally induced by the mechanical method of Bataillon (scratching with a needle) to develop so as to give rise to tadpoles, and even adult frogs, without fertilisation by sperm-cells. By oversight the tadpoles so produced have been referred to as "parthenogenetic," and by a similar error the broods of green-fly produced without the intervention of a male parent have been called "parthenogenetic young." Clearly the word "parthenogenetic" has been, and must be, used to describe the virgin mother, and therefore cannot at the same time be applied to her offspring without causing confusion. It seems to me that the word "impaternate," or "fatherless," should be used for the offspring. I have failed to excogitate any other term which will so well meet the case.

If we call individuals so produced "autoblastic"—a term applicable to the egg-cells which give rise to them—we leave it doubtful as to whether we may not be referring to their future reproductive capacity rather than to their origin; and if we call them "lipospermic" we may possibly intend by this word to indicate that they are devoid of male reproductive gonads, and not merely that no sperm-cells were concerned in their genesis. The term "impaternate" is readily intelligible and admits of no such ambiguity.

A further difficulty in regard to the nomenclature of virgin reproduction or parthenogenesis is that the word "virgin" and its Greek equivalent refer to the condition of the mother, and not to the history of the egg-cells which she produces and passes from her body. The "virgo intacta" is an adult female who has not been "covered" or "impregnated" by a male, or, to use another term, has not been

"mated." In most species of frogs and fishes, and in many other aquatic animals, the female parent is always a "virgo intacta." Such females are always "parthenogenetic" in the strict sense of the word. The fact that the eggs are not "autoblastic," but are fertilised after they leave the mother's body, does not alter her physiological condition or "status" in any way as compared with that of a mother whose eggs on being deposited by her are capable of "lipospermic" embryogenesis. She is never "mated" or "impregnated." The difference between her and the more familiar impregnated or fecundated mother arises from the persistence in the one case of the original and primitive method of free discharge of both the female and the male reproductive cells into the water in which the parents live, and, by contrast, the secondary development in the other case (comprising a vast variety and number) of arrangements for the fertilisation of the egg-cells while still actually within the protective body of the mother or in close contact with it. These secondary developments are determined by the fact (1) that they favour both economy and certainty in the operation of the male gametes or spermatozoa, and (2) by their provision of advantageous maternal protection to the minute egg-cells and the early stages of their growth when fertilised. It is obvious that in non-aquatic animals intra-maternal fertilisation of the egg-cells is obligatory.

The egg-cells which are freely discharged and fertilised by free-swimming sperm-cells "in the open" may be called "planktogamic" (plankton = freely swimming), whilst egg-cells which are subjected to the secondary protective arrangements may be called either "hysterogamic" (hysteron = uterus), if fertilised within the oviductal chamber of the mother, or "propylogamic" (propylon = a gateway), if fertilised on the surface of the mother's body or in immediate relation thereto (as in the case of many Crustacea and of some Amphibia).

There is no word in use to indicate the physiological status of an adult female which is no longer a "virgin," but has been

"mated" or "covered," and has received into her oviduct sperm-cells from a male. We might designate such a female as a "mate" in contrast to a "virgin," but "mate" is in ordinary use for any kind of comrade. Though the words "wife" and "spouse" have too definite a reference to human legal and social status, yet the Latin word "conjux," implying as it does a "conjugium" (the significance of which is given in Virgil's account of wind-fertilised mares, "sine ullis conjugiis vento gravidæ"), may well be used as the antithesis of "virgo." Any female bearing hysterogamic egg-cells is accordingly a "conjux," whilst one discharging "planktogamic," or it may be "propylogamic," egg-cells is a "virgin."

The existence of "hysterogamesis" leads on to that phenomenon which was by Aristotle regarded as a highly important "differentia" in the clasification of animals, and is loosely described as "viviparity." Animals which pass a large part of their embryonic growth within the mother's body and are born naked and with much of the shape and locomotive capacity of the adult are called "viviparous." But really all animals are viviparous, for the birth product is a living thing whether it is a naked egg-cell or more or less advanced in development. The enclosure of the birth-product in a shell or case, which has given rise to the term "oviparous," is not of any value as indicating the real degree of development of the young at birth, for in some cases unfertilised egg-cells, in others mere discs of developing embryonic cells (as in birds, etc.), and in yet other cases well-shaped young ranging from the early larva up to the completely formed miniature of the adult, as in some of the shell-bearing snails, may be enclosed within an egg-shell when "laid" by the mother. There is accordingly no great general importance to be attached to the distinction between "viviparous" and "oviparous" animals. The egg-shell has, of course, its protective value, but the exact phase and nature of the living thing within it must be considered in any comparison of the reproductive processes of different animals.

I may now show how far the considerations and the des-

criptive terms here suggested apply to certain typical cases of what is usually called parthenogenesis, but is better designated "autoblastesis" or "lipospermia."

(1) The greenflies, or Aphides, are, as are all insects, characteristically hysterogamic. They are propagated by males and mating females (conjuges) in autumn. But the spring and summer broods are females only. They are virgins, and produce true egg-cells which are autoblastic and develop into impaternate females (lipospermia or parthenogenesis). These impaternate females in their turn produce impaternate females, and this process may continue for several generations. The egg-cells of these virgin mothers are modified and enclosed so as to be incapable[1] of zygosis, whilst the maternal structures connected with hysterogamesis (intra-maternal fecundation) are aborted, although the intra-uterine gestation is retained and the young are born naked in a fully formed condition, and are accordingly said to be "viviparous."

(2) The phyllopod Crustacean Apus normally gives birth to egg-cells encased each in a delicate egg-shell. These are autoblastic, and produce with very rare exceptions only impaternate females. At rare intervals, owing to conditions not ascertained, a few impaternate males are hatched from some of the eggs, and "propylogamic" fertilisation of the eggs of some of the virgin mothers of the same generation then takes place.

(3) The breeding queen bee (Apis) and the breeding queens of some other hymenopterous insects are at the same time both parthenogenetic and gamogenetic! They are definitely "conjuges," or mated females, but some of their eggs are hysterogamic and give rise to females only, whilst others are agamic (lipospermic) and give rise by autoblastesis to impaternate males (drones) only. This remarkable double character of the "queen" is due to the fact that the sperm-cells of the drone received and stored by her in her spermatheca can be withheld from contact with the egg-cells about to be laid or

[1] Possibly a skilful experimentalist might succeed in artificially fertilising these eggs with the appropriate spermatozoa.

admitted to them according to circumstances. Fertilisation of the egg-cell is (to use a French term) "facultative."

(4) Silkworm moths and some other female Lepidoptera sometimes lay eggs without having mated or come into contact with a male. Not infrequently these eggs, which in normal conditions should be hysterogamic, proceed to develop by autoblastesis, and produce impaternate males and females. This lipospermic reproduction is stated to have been experimentally carried out through three successive generations. The autoblastesis can be favoured, if not determined, by brushing the shell of the egg with a camel's-hair pencil.

(5) The female of the common frog is in all circumstances a "virgin." Her eggs are planktogamic. The eggs of some other Amphibia may be propylogamic or even hysterogamic. When received into carefully purified water, the unfertilised eggs of the common frog, which are naturally enveloped, each in a jelly-like coat, can be caused to enter upon the curriculum of cell-division and embryonic growth by scratching the surface of the dark-brown egg-cell with a needle. The impaternate offspring thus produced have been reared to late stages of the tadpole phase, and more rarely to the adult form. The impaternate or fatherless young thus reared have, so far as present records stand, always proved on examination to be males.

(6) Female bony fishes with a few exceptions, such as the viviparous blenny, are, like the female frog, virgins. Their eggs are planktogamic—that is to say, are fertilised when floating in the water.

Other cases of lipospermia or autoblastesis, such as those revealed by the experiments of Loeb, Deslages, and others, could, I think, be with advantage summarised by the use of some such nomenclature as that here suggested. Autoblastesis is contrasted with gamoblastesis, but its occurrence is not "spontaneous." It depends upon either mechanical or chemical conditions which could be enumerated and classified.

[The present article is a revision of one which was printed in 'Nature,' August, 1917.—E.R.L.]

Rhythmic Pulsation in the Madreporic Vesicle of Young Ophiuroids.

By

James F. Gemmill, M.A., D.Sc.

With 1 Text-figure.

In view of the interest attaching to the nature and function of the madreporic vesicle, I made a careful examination of a number of young living Ophiuroids from tow-nettings at the Millport Marine Station in June, 1918. In particular specimens (probably of Ophioglypha albida), at the stage when the young star is a flattened disc with five blunt arms, each with five tentacles, the madreporic interradius is slightly wider than the rest, and is marked by the projection in its neighbourhood of a still persisting larval arm-rudiment. Careful focussing through the tissues of this interradius, in recently taken specimens examined in salt water, never failed to reveal the presence of a rhythmically pulsating thin-walled cavity entirely comparable with the pulsating madreporic vesicle of an Asterias larva. The pulsations are extremely regular, occurring once in every eleven or twelve seconds, and they continued for over an hour in several of the specimens examined. They could be made out both from the oral and from the aboral side, and nothing similar was revealed by search in the other interradii. One gets the impression that the essential part of the pulsation is the emptying and filling of spongy tissue to one side of the vesicle, but this appearance is not nearly so definite as in Asterias (**2**, p. 248) and Porania (**3**, p. 40) larvæ. Bury (**9**, p. 76, 1896) noted in Echinus micro-

tuberculatus that during contraction the floor of the vesicle projects far up into its cavity, and that the pulsation is certainly continued in the earliest post-larval stages, though whether it occurs in the adult he was unable to say. Recently MacBride has figured and called attention to a similar projection in sections of late larvæ of Echinocardium cordatum (**5**, p. 263, Pl. 19, fig. 10A).

In a bilaterally symmetrical double-hydrocœle Porania

TEXT-FIG. 1.

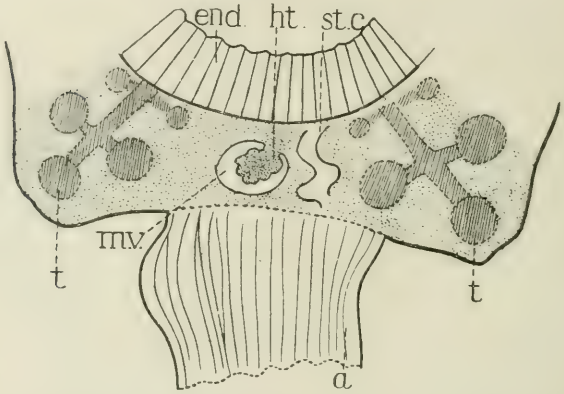

Diagram (optical section from aboral side) to illustrate position of pulsating cavity in madreporic interradius of young Ophiuroid near end of metamorphosis. *a.* A larval arm rudiment. *ht.* Spongy tissue invaginating the madreporic vesicle, and emptied and filled rhythmically. *end.* Lining of gut. *mv.* Madreporic vesicle. *st. c.* Stone canal. *t.* Terminal tentacle of arms I (on left) and II (on right).

bipinnaria (**2**, p. 249; **4**, p. 72) the filling and emptying progressed from behind forwards, indicating that the contained "blood" flows in the same direction as that within the dorsal vessel and heart sinus of Balanoglossus. In starfish at metamorphosis (**2**, p. 249) the spongy tissue becomes permanently invaginated into the madreporic vesicle to form the so-called head process of the axial organ, which remains connected at its neck with the main part of the axial organ and with the rest of the adult hæmal system, and still ex-

hibits rhythmic pulsation (Asterias, Porania, Solaster, Echinus, Echinocardium) (**2**, p. 272). Accordingly, following out the view of Bury (**1**, p. 129) and Masterman (**6**, p. 398) that the madreporic vesicle = pericardium of Balanoglossus, I homologised the head process of the axial organ and its antecedent spongy tissue with the heart sinus of Balanoglossus (**2**, pp. 249, 278; **4**, p. 63)—a conclusion taken as probably right by MacBride (**5**, p. 263), at any rate with reference to the larval spongy tissue. The homologies of the heart complex in Balanoglossus, and in Asteroids, Ophiuroids and Echinoids may thus be taken as on a moderately sound basis, and it seems legitimate to extend the comparison between the hæmal systems of Balanoglossus and Echinoderms to other details (cf. **2**, p. 278[1]), e.g. to the axial organ, which can represent the left pharnygeal or collar vessel of Balanoglossus with the addition of the left half of the glomerulus. The vessel in question is a spongy channel, comparable in structure to the axial organ (Spengel, **7**, p. 753), and forming a fold or ridge within the left collar cœlom, like the axial organ within the axial sinus. In double-hydrocœle Asterias larvæ at metamorphosis there is a right as well as a left axial organ, and these meet aborally in a single head-process ("heart") invaginating the single madreporic vesicle ("pericardium," cf. **4**, p. 63).

To consider the axial organ as primarily a genital stolon (see MacBride, 'Text-Book of Embryology,' i, pp. 480, 500, 516) does not appear to me to meet the case, in view of the following considerations: (1) There exists in Enteropneusts a spongy channel (the left collar vessel continued from the left part of the glomerulus), efferent from the heart sinus, comparable in structure to the axial organ and with similar relations, but unconnected with gonal rudiments. (2) The rudiment of the axial organ, including both vessels and lymphoid elements is formed prior to the downgrowth into it of the cells identified as primitive germ-cells by MacBride.

[1] Note † on this page should read . . . fold of the collar cœlomic wall, instead of . . . fold of the pharyngeal cœlom, etc.

(3) The axial organ persists in the adult, retaining its vessels and parenchyma but without recognisable germ-cells, and it cannot well be the seat of permanent production of germ-cells too embryonic to be recognisable, since the germ-tissue of each gonad is seen to be shut off by a membrane, as it is in the adult from the cellular contents of the aboral sinus (Gemmill, Solaster). On the histological side the resemblances between the hæmal systems of Enteropneusts and Echinoderms are very striking and have long received recognition.

References.

1. Bury, H.—"The Metamorphosis of Echinoderms," 'Quart. Journ. Micr. Sci.,' vol. 38, pp. 45–131, 1896.
2. Gemmill, J. F.—"The Development of the Starfish Asterias rubens, L.," 'Trans. Roy. Soc., Lond.,' B, cccxix, 1914, pp. 213–294.
3. ——— "The Larva of the Starfish Porania pulvillus O.F.M.," 'Quart. Journ. Micr. Sci.,' vol. 61, 1915, pp. 27–50.
4. ——— "Double Hydrocœle in the Development and Metamorphosis of Asterias rubens L.," ibid., pp. 51–80.
5. MacBride, E. W.—"The Development of Echinocardium cordatum. Part II: The Development of the Internal Organs," ibid., vol. 63, 1918, pp. 259–282.
6. Masterman, A. T.—"The Early Development of Cribrella oculata (Forbes), with Remarks on Echinoderm Development," 'Trans. Roy. Soc., Edin.,' vol xl, pp. 373–417 (1902).
7. Spengel, I. W.—"Die Enteropneusten," 'Fauna u. Flora des Golfes von Neapel,' vol. xviii (1893).

An Alcoholic Eosin and Methylene-Blue Staining Method.

By

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(From the Department of Physiology, Edinburgh University.)

Aqueous or alcoholic solutions of eosin and aqueous solutions of methylene-blue have long been individually and successively employed for the double staining of sections. (It is not intended here to deal with the mixed or combined staining method of Romanowsky (8), Ehrlich (1), and others.) The various methods now in use (Schafer (9), Sims Woodhead (10), Miller (5), etc.) may be generally described as follows:

Sections are stained with a water-soluble eosin solution for periods of 5–20 minutes or longer. They are then washed with water and brought into contact with a methylene-blue solution for a usually shorter time. After being again washed, they are differentiated and dehydrated in absolute alcohol, and subsequently cleared and mounted. Mallory and Wright (4) employ Unna's methylene-blue, which contains 1 per cent. of potassium carbonate. Richard Muir (6) uses a saturated solution of alcoholic eosin in rectified spirit, but drives off the alcohol by heat during the process of staining, leaving the eosin in watery solution. He then rinses in water and places in saturated potash alum for 3 minutes, subsequently decolorising with alcohol containing a trace of ammonia, and, after washing with water, stains with methylene-blue.

For blood-films the general method given above for sections

has been advantageously used after adequate fixation, e. g. with methyl-alcohol or with formol-alcohol (Gulland (2)), with the exception that the film is dried and mounted after the methylene-blue has been washed off. Türk stains with 0·5 per cent. eosin in 60–70 per cent. alcohol; he both dries and heats the film before applying methylene-blue solution.

Films of pus or of other exudates are also stained in a somewhat similar way (Muir and Ritchie (7)).

The results obtained by the above methods mainly depend upon the experience of the histologist. A successful preparation demonstrates well the nucleus, cytoplasm, and cell-granules, the latter especially if Richard Muir's method is used. The failure to obtain constant results is due to the difficulty of obtaining good differentiation. This difficulty is largely overcome with formalin-fixed tissues by the use of the following solutions, viz. 1 per cent. solution of alcohol-soluble eosin in rectified spirit, and 1 per cent. solution of methylene-blue in distilled water. In employing these for sections the latter are treated as follows:

1. Remove paraffin with xylol or benzol, then wash with absolute alcohol.

2. Pour on alcoholic eosin solution and leave for one minute.

3. Wash with water (distilled or tap).

4. Pour on methylene-blue solution and leave for one minute.

5. Wash with water; the sections should appear purplish.

6. Wipe slide dry with a fine cloth, leaving only the section moist.

7. Pour on absolute alcohol liberally to differentiate the staining, and immediately carry out the next step.

8. Pour on xylol or benzol to stop the differentiation and to clear the section for mounting.

During the manipulations the slide should be held obliquely in order that the reagents may run off.

The section may now be examined, and if found to be insufficiently differentiated, steps 7 and 8 may be rapidly

repeated; as a rule this is not necessary. When correctly differentiated and cleared, mount in dammar.

It is sometimes more convenient to have the methylene-blue and xylol in vessels large enough to accommodate a slide. In this case the slide should be agitated within the xylol until the section is cleared, when it may be mounted in dammar.

For blood or exudate films the same technique, if carried out implicitly, will give good results. The film is allowed to dry as slowly as possible in a cool place—the slower the better. When quite dry, staining may be commenced. Fixation is accomplished by the alcohol of the eosin solution, although for rapid work the film may be inundated with absolute alcohol for from 1 to 3 minutes prior to staining.

The above-described method has been used successfully upon sections for some time in this laboratory. It is rapid, simple, and certain, and is well suited as a routine procedure for most tissues. The preparations do not readily fade; some made in 1916 are as yet quite unchanged. It is particularly useful for the central nervous system and for the peripheral ganglia. It stains axis cylinders a deep red, Nissl's granules blue. Connective tissue is also stained an intense blue. It is valuable for glands, especially the pituitary, pancreas, and suprarenal. The oxyphil and basiphil granules of the anterior lobe of the pituitary are clearly differentiated; while in the pars nervosa (in man), free, coarse, greenish (basiphil?) granular masses, seemingly not identical with those described by Herring (**3**), are shown.

The results with blood-films are equal to, but are not claimed to be better than, a good Leishman (**8**) stained film. Nuclei of white blood-corpuscles are stained blue, granules according to their affinities, while the red blood-corpuscles come out bright red. In films of pus, etc., the bacteria are also stained blue.

In connection with this part of the subject, I wish to thank Dr. A. K. Towers for providing me with clinical material.

Literature.

1. Ehrlich and Lazarus.—'Die Anämie,' 1898, Wien.
2. Gulland.—'The Blood: a Guide to its Examination and to the Diagnosis and Treatment of its Diseases,' 1914, p. 30.
3. Herring.—'Quart. Journ. Exp. Physiol.,' 1908, vol. i, p. 149.
4. Mallory and Wright.—'Pathological Technique,' 1915, pp. 327–328.
5. Miller.—'Practical Pathology,' 1914, pp. 341–342.
6. Muir, Richard.—'Journ. Path.,' 1905–06, vol. xi, p. 373.
7. Muir and Ritchie.—'Manual of Bacteriology,' 1913, pp. 103–104.
8. Romanowsky.—'St. Petersburg med. Wochenschr.,' 1891. See also 'Enzykl. d. mikr. Tech.,' 1910, Bd. i, s. 121–122.
9. Schafer.—'Essentials of Histology,' 1916, p. 547.
10. Sims Woodhead.—'Practical Pathology,' 1912, p. 97.
11. Türk.—'Enzykl. d. mikr. Tech.,' 1910, Bd. i, s. 121.

On the Orientation of Minute Objects for the Microtome.

By

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With 7 Text-figures.

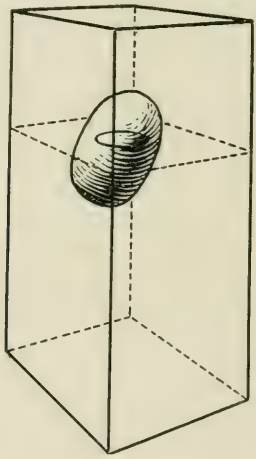
For some time past the work I have been engaged upon has necessitated the sectioning of large numbers of minute larvæ, whose small size and lack of obvious symmetry or external "landmarks" made them extraordinarily difficult things to prepare for the microtome, and in no degree amenable to the ordinary methods of embedding. None of the various devices that are current will give, in my experience, anything approaching exact orientation—upon which often depends the very possibility of interpreting the sections when they are cut; and this is because in none of them are the essential operations conducted at leisure, at the ordinary temperature, and with the object plainly seen under the microscope. A method which combines the satisfaction of these requirements with the advantages of double-embedding in collodion and wax is given below. By its use I have been able to obtain precise orientation of such small objects as the early larval stages of Amphioxus, Ciona and Cucumaria, and of the cleavage stages of Echinus and Ascidiella.

I am persuaded that the usefulness of such a technique to workers whose researches are not (as mine are) interrupted by the war will excuse its present publication unfortified by

a context of material results. Modifications will doubtless suggest themselves to the reader, and indeed the ensuing description is designedly only an outline.

The principle of the method is to enclose the specimen to be cut in a mass of collodion large enough to be seen and handled in paraffin wax and with a definite geometrical form, the long axis of which is at right angles to the plane of intended section (Text-fig. 1). This primary object may be

TEXT-FIG. 1.



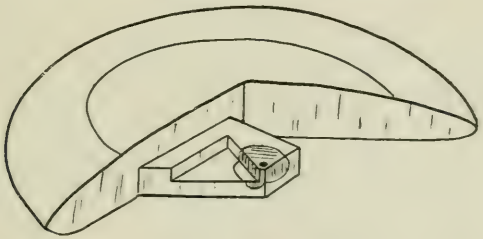
attained in one or other of the following two ways, according as a greater or a less degree of accuracy is necessary in the orientation. The first furnishes a convenient means of handling small objects in all cases where the greatest nicety is not required.

(i) The fixed and hardened specimens, lightly stained, are transferred from "absolute" alcohol to oil of cloves, and thence to a thin syrup made by mixing a thick alcohol-ether solution of collodion[1] with an equal volume of oil of cloves. Such a syrup has a high index of refraction (i.e. is a clearing reagent), and can be thickened to any convenient consistency

[1] Grübler's or Schering's "celloidin" was used.

by allowing the volatile constituents to evaporate. After remaining in it for twenty-four hours, in a covered capsule, the specimens are picked up singly in a pipette, and allowed to fall, each surrounded by a drop of syrup, into chloroform, in which they are left till they are quite clear and have sunk to the bottom of the receptacle. Each glassy globule of hardened collodion is then embedded for from twenty to thirty minutes in paraffin wax (melting-point 52°–56° C.) in the thermostat, transferred to molten wax in a glycerine-coated watch-glass, and cooled quickly in the ordinary way. The cast so obtained is now pared away on one side till the globule stands

TEXT-FIG. 2.



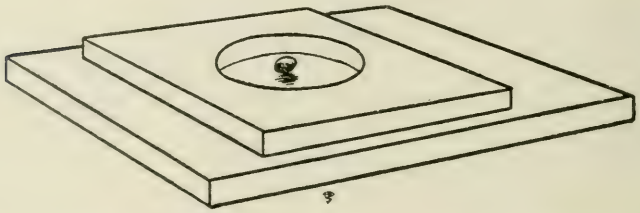
in a salient solid angle of wax, care being taken to retain a sufficient mass of wax to serve as a handle during the next operation, which involves cutting the collodion itself. In doing this it is important to shave away only a little at a time, so that the passage of the knife shall deform the small piece removed and cause no compression of the parent block —a matter of importance when the embedded object is a delicate one. With a thin, sharp knife (a safety-razor blade is excellent for the purpose), and preferably under a binocular dissecting microscope, the collodion is cut away until the object comes plainly into view (Text-fig. 2) and is made to occupy the end of a slender rod, the axis of the specimen either coinciding with that of the rod, or being at right angles to it, according as the sections are to be transverse or longitudinal. The little rod is then separated from its parent mass, embedded for about a minute in hard wax, and

prepared for the microtome in the usual way, the collodion rod being set vertical upon the carrier.

It is possible to obtain in this way, with a considerable degree of precision, transverse or longitudinal sections of objects of which only one main axis can be determined in the external view, or in which no discrimination between longitudinal planes is desired; but where the plane of section is required to be in relation to the planes of internal organs, visible only in the cleared object, under the microscope, a greater refinement of means is necessary.

(ii) A microscope slide, cut down to two-thirds of its original length, is coated on one side with a thin, continuous

TEXT-FIG. 3.



film of paraffin wax by drawing a drop of molten wax over its heated surface with the edge of another slide, as in making a blood-film. A square of glass with a circular hole in it is coated thinly with wax and cemented on the prepared slide by means of heat; or if such squares are not available, four slips of glass, cut from a thin slide, may be used to the same end. In this way a shallow cell is obtained, lined throughout with wax, and to this the specimen is transferred with sufficient collodion syrup to fill the cell, the amount being adjusted by means of a fine pipette so that the surface of the liquid is a plane (Text-fig. 3). The depth of the cell must be suited to the diameter of the object, but it should not be less than a millimetre, or the resulting rod of collodion will lack rigidity, on which the success of the method depends.

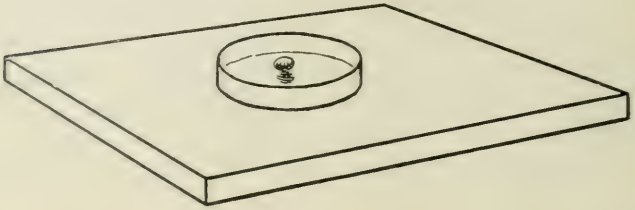
The slide is now placed in a shallow Petri dish upon the stage of a vertically set microscope under the lowest power

objective that will serve to show the structures to be orientated, and the specimen is manipulated with the point of a fine needle till the plane of intended section is vertical. Generally there is no great difficulty in achieving this, because it matters not at all what aspect of the object is upward so long as one line in the chosen plane is vertical, and in the case of the most minute objects this initial orientation is obtained by tilting the slide or moving the medium with the point of a needle. But in refractory cases it may be necessary to prop the object with a fragment of collodion-impregnated tissue, or, better, with a flake of egg-albumen that has been fixed, stained, and embedded in the same way as the specimen itself.

When the specimen has been so arranged enough xylene is poured into the Petri dish to cover the surface of the preparation, which is then left untouched on the stage of the microscope for fifteen to twenty minutes, after which the collodion is sufficiently "set" to prevent rotation of the specimen. Xylene is used here in preference to chloroform or cedar oil as being a lighter liquid and less liable to disturb the surface of the collodion. As soon as this is set the preparation is transferred to a second dish and covered with cedar oil, in which it is left overnight. This completes the clearing and hardening of the collodion, and, by dissolving or softening the paraffin wax, makes it possible to remove the glass square. The object is now contained in a thin plate of collodion, with its plane of section normal to the surfaces of the plate, which in practice always remains attached to the microscope slide (Text-fig. 4). It remains to fix one other spatial direction. The excess of cedar oil having been drained off the slide, and the surface, except in the neighbourhood of the collodion wafer, cleaned with alcohol and dried, the preparation is placed upon the mechanical stage of a microscope and the specimen brought into the centre of the field, the slide being rotated until the plane of intended section is at right angles to the lateral movement of the mechanical stage. The position of the specimen in the field of a low-power objective

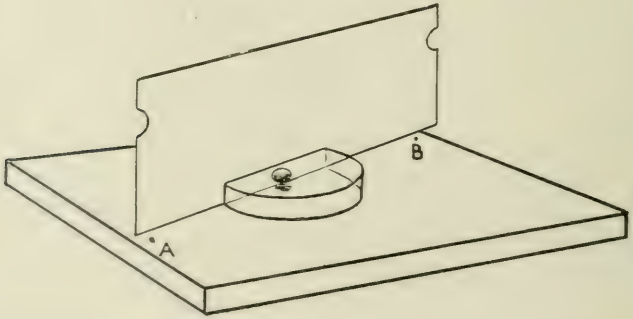
is now noted (by means of an ocular micrometer or the like) and the stage shifted laterally till an area of cleaned glass slide fills the field. A mark is made with a fine brush and Indian ink on that part of the slide which occupies the

TEXT-FIG. 4.



position in the field already noted, and the stage is moved in the reverse direction and the operation repeated on the other side of the collodion wafer. Two points (Text-figs. 5 and 6 A and B) are thus obtained which accurately fix the axis of

TEXT-FIG. 5.

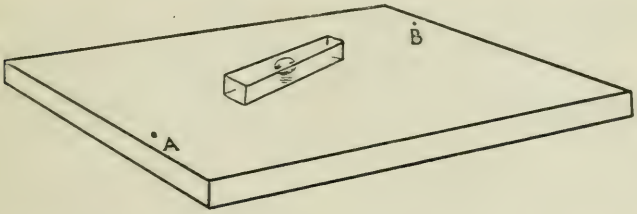


the desired rod of collodion, and using these as guides this rod can now be cut with a straight-edged knife. It is detached from the slide and prepared for the microtome as before.

It may be asked: Why not pour the collodion into a waxed rectangular gutter and orientate the object once and for all

with the plane of intended section at right angles to the length of the gutter—i. e. to the axis of the resulting rod of collodion? The answer is that the operation of placing a body in a viscous liquid so that one of its planes shall be at right angles to a given straight line (the axis of the gutter) is many times more difficult than that of placing it with the given plane at right angles to a plane (the upper surface of the collodion). Added to this is the difficulty of working with needles in a narrow gutter. A method, brought to my notice by the late C. H. Martin, in which a streak of collodion syrup containing the object is drawn across a waxed slide by means of a pipette, orientation being then effected with

TEXT-FIG. 6.



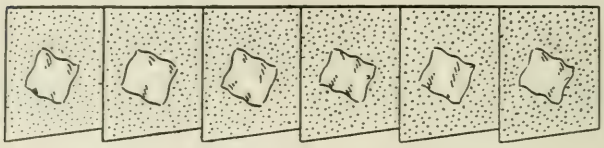
reference to the direction of the streak, is open to these same and other objections. Manipulation is made difficult by surface-tension effects; the curvature of the surface of the highly refractile liquid distorts the image of the object, and the resulting rod of collodion is not a symmetrical one.

In conclusion, a few general considerations concerning the cutting of double-embedded objects may be added.

The greatest bugbear of the method is undoubtedly the difficulty, which arises from time to time, of flattening the sections upon the slide. Folding of the collodion under the shear of the microtome knife will always occur to some extent, but so long as this folding is approximately equivalent to that in the surrounding wax it will disappear automatically in the ordinary process of flattening. It is differential folding, or puckering, of the collodion that must be reckoned

with. This is due to the difference of elasticity between the two media: the wax is compressed and telescoped under the shear of the knife, while the collodion retains its area undiminished, and is thrown into puckers in order to accommodate its perimeter to the reduced total area of the section. Such puckers (Text-fig. 7) are unaffected by flattening the surrounding wax—unless, indeed, it is actually melted—and prevention should be relied upon rather than cure. The microtome knife should be sharp, and inclined as little as possible to the plane of section, its cutting movement slow. A machine, such as Jung's, in which the knife is directly actuated by the hand, is better than one in which a system of

TEXT-FIG. 7.



levers, or the like, intervenes. Paraffin of high melting-point (about 56° C.) gives the best results, and the thickness of the collodion syrup should be adjusted to the hardness of the wax by means of two or three blank experiments. The collodion rod must be made as narrow as is consistent with the safety of the contained object.

If, with these precautions taken, there are signs of puckering in the early stages of cutting a block, a satisfactory series can often be obtained by increasing the thickness of the sections by a micron. Where, however, this cannot be done, or is ineffective, and the collodion is still puckered after the wax has been flattened, the following method should be tried. The excess of water used in flattening the sections is run off the slide, and the lengths of ribbon moved into the position they are to occupy. A cigarette-paper, wet with absolute alcohol, is now cautiously brought down on them, and over this is laid a piece of stout filter-paper. The whole

system is held firmly down on the bench by placing the thumb of the left hand over the part of the slide where the label will be put, and the pad of the right thumb or forefinger is then drawn several times with firm, even pressure over the covered slide, from left to right. On removing the filter-paper, the cigarette-paper quickly dries and separates from the surface of the slide, leaving the sections firmly adhering. This seemingly heroic method is perfectly safe when applied to sections in a tough, elastic medium like collodion. It is quite inapplicable to ordinary wax sections.

Observations on the Protozoa Parasitic in the Hind Gut of Archotermopsis wroughtoni Desn.

Part I.—Ditrichomonas (Trichomonas) termitis, Imms.

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With Plates 31, 32, and 33, and 3 Text-figures.

CONTENTS.

1. Introduction.

In a recent paper Dr. Imms (**12**)[1] has described some of the species of Protozoa parasitic in the hind gut of an Indian termite—Archotermopsis wroughtoni Desn. As many of these insects have been living in Dr. Imms' laboratory at Manchester, I had the opportunity, a few years ago, of examining their Protozoa, which showed a number of features of great interest and importance. Early in 1918 I decided to make a thorough investigation of these parasites, and Dr. Imms kindly placed at my disposal the remainder of his material. I therefore express to him my sincere thanks for thus rendering possible my research.

The object of the present paper is to record as fully as possible the life-history of one of the Protozoa. I hope to publish from time to time results of my observations upon the other species already described by Imms.

The flagellate forming the subject of this paper is one of the commonest species of Protozoa found in the termites; it occurs in all casts in great numbers, and offers an excellent opportunity for work on its method of division.

The Trichomonads have been objects of research by many naturalists, but up to the present no species has been recorded from termites[2]; also, as will be seen later, the species I have investigated is in many respects totally different from any hitherto described.

Imms has already pointed out that the animal differs from other Trichomonads in the possession of only two anterior flagella, and this fact makes it desirable, though not essential, to place it in a new genus. As the genus Tetratrichomonas has been established for those forms possessing four

[1] Although the above paper is still in the press, Dr. Imms has kindly given me permission to publish the results of my work.

[2] While this paper was in the hands of the printer I was able to read Grassi's latest publication on termite protozoa ('Mem. R. Accad. Lincei,' ser. 5, vol. xii, 1917). In this paper he describes a Trichomonad, which, however, differs considerably from D. termitis.

anterior flagella, but otherwise like Trichomonas, and the genus Pentatrichomonas for those possessing five anterior flagella, I have decided to create the new genus Ditrichomonas for the animal under discussion.

2. Methods.

The animals were examined alive in 0·75 per cent. NaCl, but even when the utmost precautions were employed in the preparation of the slides, it was found impossible to keep the flagellates alive for more than about two hours. On occasion albumen in salt solution was used as a medium, but, though possessing the advantage of slowing the animal's movements, it caused rapid death, preceded by degeneration changes, thus rendering the use of this medium unsatisfactory.

From time to time I have endeavoured to obtain a pure culture of these parasites, but with no success. The ordinary bacteriological media were tried, as was also the culture media used by me in my experiments on Entamœba histolytica (**5**). Finally, I used an extract obtained from the wood in which the termites were living, and also a medium prepared by teasing up in salt solution the abdomen of a termite, but in all cases the results were completely negative.

Stained preparations were made by squeezing the contents of the hind gut of a termite out on to a clean slide; thin films were then prepared and the slides immediately placed in the fixing fluid. It is important that this should be done as rapidly as possible, as shrinkage effects are very soon produced after the Protozoa have left their host.

As fixing fluids I have used Bouin's picro-formol-acetic solution and Schaudinn's sublimate-alcohol mixture. Both these mixtures gave excellent results, especially when used at a temperature of about 56° C. The former, however, is disadvantageous in that the picric acid takes a long time to wash out before staining can be commenced. Equally good results were obtained by the use of Schaudinn's mixture as modified by Dobell and Jepps (**8**) as a result of their work on

Entamœba histolytica cysts. These bodies are very susceptible to shrinkage, and a fluid which obviates this is obviously one of great service to protozoologists. Such a fluid is prepared as follows: Saturated corrosive sublimate in water, two parts; absolute alcohol, one part; glacial acetic acid, 4 to 5 per cent.

For staining, Heidenhain's iron-hæmatoxylin is probably the best for the details of nuclear division, though Dobell's iron-hæmatein, described by him in 1914 (**7**), also gives fine results; especially is it useful for work on the flagella and axostyles, which are better coloured by this method than by any other I have tried.

From time to time I have made use of alcoholic safranin, thionin, Grenacher's carmine, and Mayer's hæmalum, but have not obtained as good effects with any of these as with those mentioned above. Methyl green and Schneider's aceto-carmine have proved useful in those cases where permanent preparations were not essential.

Most of my slides have been made by one of the methods described above, but for the demonstration of the parabasal body I employed Flemming's strong fixing solution, omitting the acetic acid, as described by Gatenby (**11**).

3. General Considerations as to the Living Animal.

(a) Morphology and Movement.

Ditrichomonas termitis is a large flagellate measuring on the average $50\,\mu \times 22\,\mu$. There is, however, much variation in size, as occurs in most species of Protozoa; indeed, Wenyon (**27**) states that in Trichomonas intestinalis he observed forms ranging from $3\,\mu$–$20\,\mu$.

Imms gives the size of Ditrichomonas termitis as $30\,\mu$–$88\,\mu$ in length by $13\,\mu$–$57\,\mu$ broad, with an average of $64\,\mu \times 38\,\mu$. These figures are undoubtedly too high, and this is due to the fact that Imms has included in his measurements both dividing and non-dividing animals, the former of which are of course much larger than the latter.

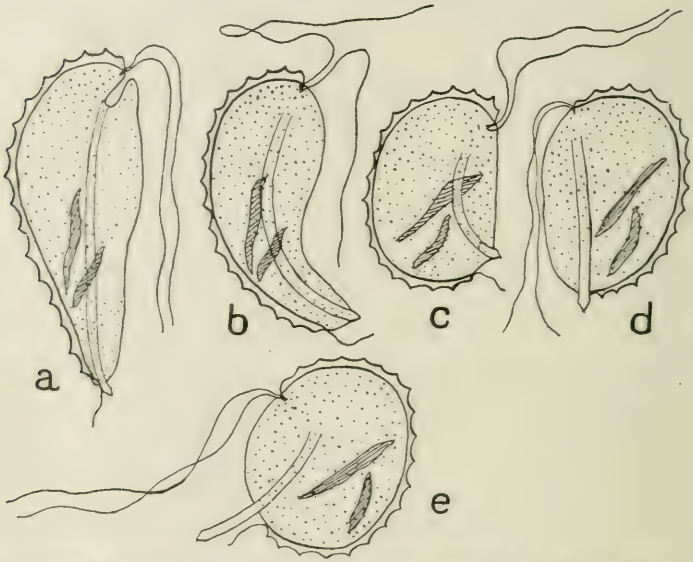
At the anterior end of the animal there is found a large cytostome, situated a little to one side of the middle line. Close to this structure there is the point of origin of the flagella, which are three in number. Two of these are directed anteriorly and are of equal length, while the third runs down one side of the body to form the edge of the undulating membrane, which is supported by a firm, rod-like structure characteristic of Trichomonas. The flagellum, however, becomes free at the posterior extremity of the body.

The body is not sharply differentiated into ectoplasm and endoplasm, but appears homogeneous throughout; the protoplasm is usually granular, though small vacuoles are occasionally seen, but in no case is there evidence of a contractile vacuole. In the interior of the body there are numerous food-particles, chiefly consisting of what appear to be fragments of wood, many of which are so large it is difficult to realise how the animal could engulf them. I have, however, been unable to detect any method of feeding other than by the cytostome.

As Imms has observed, the shape of the animal is not constant, but when progressing forwards it has a characteristic appearance, the anterior end broad and rounded, with this width maintained for about two-thirds of the body-length, the posterior third is, however, much more slender and in some cases almost tapering. Further, this slender region is capable of independent movement so that it is possible to speak of it as a "tail" (Text-fig. 1, a). The undulating membrane runs from the anterior end of the animal down to the end of this "tail" and the axostyle is seen projecting from it (Text-fig. 1, a, b). This shape is, however, not the only one found, for when the animal is at rest—or at any rate not moving forward—an entirely different appearance is seen. The "tail" portion of the body disappears and the animal assumes an almost round shape. The posterior end of the undulating membrane is now no longer separated from the anterior by the whole length of the animal, but is more closely applied to it (Text-fig. 1, c and e, and Pl. 31, fig. 1).

An intermediate condition between the two extremes is seen in Text-fig. 1, d. These changes in shape are brought about by contraction, and it is often possible, when examining these animals alive, to see this cycle of changes taking place with great rapidity in the same animal. Text-fig. 1 was drawn freehand from a living preparation; it represents the

TEXT-FIG. 1.



Freehand drawing of the form changes observed in a living Ditrichomonas termitis during a period of five minutes.

successive movements of one of these flagellates after it had come to rest, the whole of the changes occurring in a period of five minutes.

I have spoken above of the animals being at rest; this, however, is only a relative term, for though they may not be undergoing translation, yet the free flagella and undulating membrane are quite active; indeed, the membrane appears to undergo its rhythmical changes throughout the whole of the animal's life. Minchin's suggestion (**21**, p. 57) that when the animal is perfectly still, "the function of the membrane is

probably to cause currents in the fluid surrounding the body, and to change and renew the liquid bathing the body surface" appears to me well founded.

Kuczynski (**19**, p. 163), has described the formation of pseudopodia in Trichomonas augusta observed alive in a bouillon culture. He says: "9h54′ Vorn rechts entsteht (ventral) ein Pseudopodium, um rasch nach hinten zugleiten, 3–4mal hinterein ander, dadurch wird der Eindruck der undulierenden Bewegungen hervorgerufen. Dabei macht die Membran 90 Schläge in der Minute bei geringer Windungszahl. Sie ist klar erkennbar. Nach allen Seiten werden spitze Pseudopodien ausgestreckt und wieder eingezogen.

"Blitzschnell mehrere zugleich. Die Pseudopodien wandern den Körper entlang. Die Geisseln arbeiten kaum. Das Tier bewegt sich ganz langsam ein wenig von der Stelle (kriecht). Es ist freigekommen, nimmt seine alte Gestalt wieder annähernd auf, schwimmt fort, zunachst anregelmassig bewegt. Der Achsenstab steht weiter aus dem Körper als vorher. (Dauer des Geschilderten drei Minuten.)

"9h57′: Es zeigt wieder am Hinterende den Haken.

"10h7′: Das Tier hat eine ganz normale Form; es schwimmt, wobei sich 90 Umdrehungen in der Minute feststellen lassen. Etwa ebenso oft schlagen die Greisseln."

Appearances such as the above I have sometimes seen in Ditrichomonas, when examined in media containing albumen. In 0·75 per cent. NaCl, however, the animals show no trace of pseudopodial formation. I do not doubt, therefore, but that this is an abnormal feature of the life-history, and that it is due to the effect of artificial media; this conclusion finds support in that the forms which developed pseudopodia were in a feeble condition, and if watched for a short time became moribund. Buscalione and Comes record the same fact (**4 a**).

(B) Axostyle.

The axostyle, which is such a constant organ of the Trichomonas body, arises from the anterior end in front of

the nucleus, and probably has its origin in the blepharoplast, described on p. 563. It appears as a hyaline structure running throughout the body length (Pl. 31, fig. 9). At the posterior end it reaches the outer surface, which it pierces, and is continued for a short distance, finally terminating in a sharp point (Pl. 31, figs. 2, 3, 6, and Text-fig. 2, p. 564).

Kuczynski (**19**, **19a**) considers that it is composed of two threads running parallel to each other; this I think to be a mistake, but apart from this error his description could apply very aptly to the axostyle of D. termitis.

On p. 143 of his paper (**19**) he says: "Er besteht (i. e. the axostyle) bei sämtlichen bisher untersuchten Trichomonaden aus zwei Fribrillen, welche vom Basalkorper ventral vorn Kern und diesem dicht angeschmiegt, aber dorsal vom Cytostom, wenn dieses gut augebildet ist, die eine über der anderen entlang ziehen und nach geraden oder mehr oder minder gekrummtem Verlauf die Körper—peripherie erreichen."

Arranged in a linear series down the middle of the axostyle there are small, deeply-staining granules (Pl. 31, figs. 4, 6, 10) I have been unable to determine the significance of these, but they are of constant occurrence, and have been described in other species by previous workers. It is possible that they are the result of metabolic activity.

When preparations are made by fixing with the fluids mentioned on p. 580, it is found that clustered round the anterior portion of the axostyle there are another set of cytoplasmic bodies. These are small, deeply-staining, rod-like structures (Pl. 31, fig. 8). From their general appearance and from the fact that they are well seen only after treatment by the methods recently described by Gatenby (**11**) I believe that they probably represent mitochondria. It may be mentioned at once that similar bodies are sometimes found scattered through the cytoplasm in an irregular manner (Pl. 31, fig. 5), but in the majority of cases these mitochondria are aggregated round the axostyle in the way described. I am able to offer no suggestion as to the reason for this, but possibly they corre-

spond to the "Zona chromidiale" described by Grassi (**11a**) as encircling the anterior end of the axostyle (Mestolo) in Jœnia.

On observing a large number of living animals it is noticed that the amount of axostyle projecting from the body is very variable. Sometimes only the extreme point is seen (Pl. 31, fig. 7), while at others a relatively large part protrudes (Pl. 31, fig. 3). This also is observed when a single animal is studied through the form changes previously described. It appears as though, when the body is contracted into the rounded condition, the axostyle is too long to be accommodated, and is therefore pushed out. It is not, however, absolutely rigid, for when living animals are examined in those forms which are long and tapering in shape, the axostyle is seen running through the body in a straight line, but in the rounded forms it is flexed at some point in its course, as is seen by reference to Text-fig. 1.

I have not observed the axostyle used as an organ of attachment as Wenyon (**27**) described in T. intestinalis, but disputed by Dobell (**6**) from his observations on T. batrachorium. This structure, however, exhibits in D. termitis a very peculiar movement when the animal is at rest. The portion projecting from the body undergoes a slow, lateral jerking movement, which is confined to the region outside the body, as may be readily seen by the flexion occurring at the place where the axostyle becomes free.

Kofoid and Swezy (**18**) state that the axostyle exhibits "a vigorous lashing from side to side, sometimes constant, sometimes intermittent." Such a vigorous motion I have not observed, but it is evident, I think, that the authors have seen a phenomenon similar to the one recorded by myself.

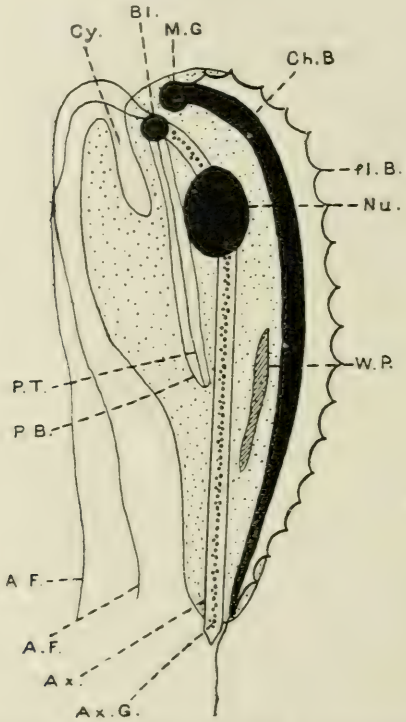
4. Description of Stained Preparations.

(a) Anterior Granules and Nucleus.

In stained preparations there is seen at the anterior end of the body two distinct granules. From one—the blepharoplast—there arise the two free, anteriorly directed flagella of length

about equal to that of the body. From the second granule, which Imms does not seem to have noticed, the chromatic base of the undulating membrane and the flagellar border

TEXT-FIG. 2.



Semi-diagrammatic freehand drawing of a non-dividing Ditrichomonas termitis. *Bl.* Blepharoplast. *A.F.* Anterior free flagella. *Cy.* Cytostome. *M.G.* Membrane-granule. *fl.B.* Flagellar border of undulating membrane. *Ch.B.* Chromatic base of membrane. *Ax.* Axostyle. *Ax.G.* Axostyle granule. *P.B.* Parabasal body. *P.T.* Parabasal thread. *Nu.* Nucleus. *W.P.* Ingested food-particle of wood.

of the same take origin. This granule I propose to term the "membrane granule," as it takes no part in nuclear division (Pl. 31, figs. 4, 5, 6, and Text-fig. 2).

A little posterior to the granules there is found the nucleus,

oval in shape and surrounded by a distinct membrane, the chromatin completely filling it as an homogeneous mass. The size is 6–8 μ.

There is no connection with the blepharoplast by a rhizoplast as described by Kofoid and Swezy in T. augusta. Imms' statement that such a structure is to be found in D. termitis is incorrect, and the probable explanation of the mistake will be found on p. 567.

(B) Parabasal Body.

Arising from the blepharoplast there is a long, deeply-staining body, which is undoubtedly that named as parabasal by other authors (Text-fig. 2). This is a constant constituent of the animal's body, and when preparations are treated in a suitable manner (see p. 580) it stands out most prominently. The parabasal extends for about two-thirds of the distance down the body, and has an almost straight course, though occasionally it shows a few undulations (Pl 31, fig. 5). There is, however, no coiling round the axostyle as occurs in Devescovina striata described by Foa (**10**).

Apparently the parabasal is composed of an homogeneous plasma not enclosed in a definite membrane. When the stain, however, is greatly extracted from it there is seen running down the middle a thread, which arises at the anterior end, and is attached to the blepharoplast (Pl. 31, fig. 6, and Text-fig. 2). This is evidently the parabasal thread described by Janicki (**13**) in other flagellates.

In all the preparations fixed by the method described on p. 580 the parabasal body is constant in size and position, exhibiting none of the fluctuations recorded by other workers, and especially by Kofoid in his recent paper (**18a**), in which he puts forward the view that this structure is a reservoir of kinetic energy, which supposition he largely supports on the variations seen in the parabasal bodies of the same species of animal.

I should mention that in "Schaudinn"-fixed material such variations are frequently encountered in D. termitis.

5. Division.

(A) Anterior Granules and Nucleus.

The first indication of division is that the anterior flagella become four in number (Pl. 31, fig. 7). I have examined very many preparations in the hope that it would be possible to determine with certainty whether this doubling was brought about by division of the pre-existing flagella or by the growth of two new ones from the blepharoplast. I think that the latter is the correct view, for it is possible to find forms in which the flagella are of unequal length—two equal long ones and two equal short ones, which I conclude are the new ones in process of growth. The basal granule or blepharoplast from which they arise is not seen as a double structure until the four flagella have attained an equal length. When this occurs, however, it divides, and two equal granules are observed, from each of which two flagella spring (Pl. 31, fig. 8).

Simultaneously with the division of the blepharoplast—occasionally a little later—the membrane granule increases in size, and ultimately divides (Pl. 31, figs. 8, 9, 10). From the second granule so formed the new chromatic base grows out until it has attained a length equal to that of the old structure. There can be no doubt that this is the mode of origin, as all growth stages have been repeatedly seen, and are shown in Pl. 31, figs. 11, 12, 14, and Pl. 32, fig. 13.

During the growth of the chromatic base, the new undulating membrane is produced by the growth from the granule of a flagellar border, until a complete structure, similar in every way to the old one, is formed (Pl. 31, figs. 14, 15). I can find no evidence in D. termitis that the daughter-membrane is formed by division from the existing one as described by Dobell (6) and by Kofoid and Swezy (17); rather my results substantiate Wenyon's assumption (27) as regards the origin of the structure in T. intestinalis.

I would point out also that both the flagellar border and the chromatic base are produced from the same granule, and

not, as Wenyon states is the case, where the base arises from one granule, and the border from the same granule as do the anterior flagella.

When the animal has reached this condition its size is greatly increased, ranging from 60–80 μ. The nucleus has also grown, so that it is about 8 μ in diameter. At this stage the chromatin has contracted away from the nuclear membrane into a small mass, and lying between this and the membrane there are numerous small granules (Pl. 31, fig. 15, and Pl. 32, fig. 16), representing, I believe, the intra-nuclear cloud described by Kofoid and Swezy.

During these changes one of the blepharoplasts—probably the original one—divides once again, and the resulting body, the centriole, migrates towards the nuclear membrane, retaining its connection with the granule by means of a short thread—the rhizoplast (Pl. 31, fig. 11 A). Probably it was this stage in the life-history which led Imms to the conclusion referred to on p. 565—that the nucleus was attached to the blepharoplast by a rhizoplast.

The centriole now in its turn divides, and the two so produced are joined together by a solid strand of deeply-staining material—the paradesmose (Pl. 31, figs. 11A, 12, 14).[1]

At a later stage the second centriole acquires a connection with the other basal granule by the development of a new rhizoplast, which is of secondary growth.

As a result of these divisions a trapezoid figure is produced, very characteristic of the nuclear division (Pl. 32, figs. 13, 17, and Text-fig. 3, p. 568).

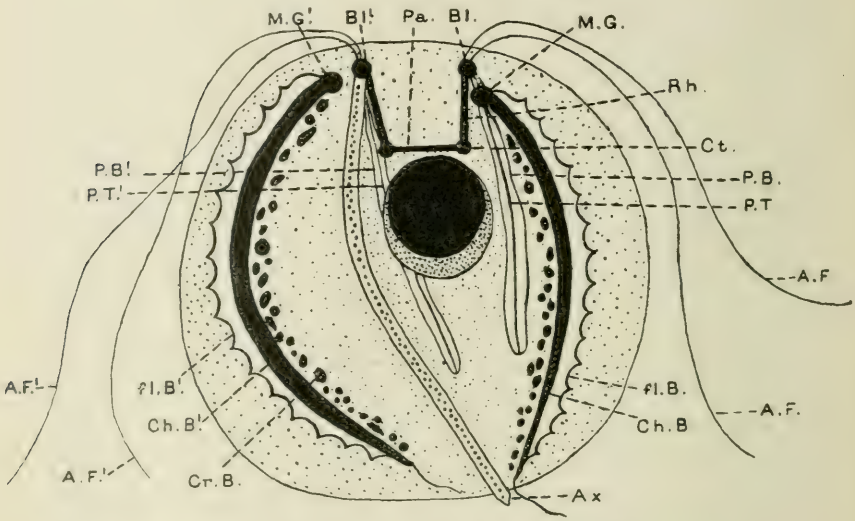
Finally the paradesmose assumes a position outside the nuclear membrane. This extra-nuclear position is retained throughout all stages; there is no evidence for its ever becoming intra-nuclear.

In the meantime the nucleus undergoes changes. Its

[1] I prefer to follow the nomenclature used by Kofoid and Swezy (17), for the reasons given by these authors. The paradesmose refers therefore to the similar structure designated as "centrodesmose" by other authors.

original homogeneous character is lost and it becomes resolved into a number of small granules (Pls. 31, 32, figs. 8, 14, 17); occasionally there is a well-marked karyosome (Pl. 33, fig. 32), but this a variable constituent of the nucleus and disappears before division takes place. Kuczynski (**19**) has described a similar body in the species of Trichomonas he has investigated.

TEXT-FIG. 3.



Semi-diagrammatic freehand drawing of a dividing Ditrichomonas termitis. A double set of organella has been formed, together with the paradesmose and centrioles. *Bl.* Blepharoplast. *Bl'.* Daughter-blepharoplast. *A. F.* Anterior flagella. *A. F'.* New anterior flagella. *M. G.* Membrane-granule. *M. G'.* New membrane-granule. *fl. B.* Flagellar border of membrane. *fl. B'.* New flagellar border of membrane. *Ch. B.* Chromatic base of membrane. *Ch. B'.* Chromatic base of daughter-membrane. *Cr. B.* Chromatinic blocks (probably metabolic granules). *Ax.* Axostyle *P. B.* and *P. T.* Parabasal body and thread. *P. B'.* and *P. T'.* Daughter-parabasal body and thread. *Rh.* Rhizoplast. *Ct.* Centriole. *Pa.* Paradesmose.

From the nucleus there is now extruded a part of its chromatin as a cloud of granules—the extra-nuclear cloud of Kofoid and Swezy (Pl. 32, fig. 13). I have not been able

to determine this point with certainty, but I think it highly probable that the intra-nuclear cloud furnishes this extruded chromatin. It is certain that after the development of the extra-nuclear cloud the intra-nuclear one disappears.

Numerous observers have described in the cytoplasm of the body small refractile bodies which stain intensely by chromatin stains, and have been termed chromidial blocks, cytoplasmic bodies, etc. These seem to be a very constant feature of most Trichomonads, and I have found them in D. termitis at certain stages of the life-history. In this animal they vary in size from 1–2·2 μ and are usually round in shape. In suitable preparations there is an indication that they are not homogeneous in character, but are formed of a central vesicle surrounded by a lightly-staining zone (Pl. 32, fig. 18).

Whereas, however, in many species of Trichomonas, such as T. gallinarum, described by Martin and Robertson (**20**), these bodies are a constant constituent of the animal, in D. termitis they are rarely seen in the non-dividing stage of the life-history. Division forms with a double set of organella and with the nucleus in the granular condition invariably contain them. In some cases they are few in number, but in others they are numerous and almost fill the body (Pl. 32, fig. 18); usually they are arranged in a series along the chromatic base of the membrane (Pls. 32, 33, figs. 23, 24, 25, 27, 30). When found in the vegetative forms they are present only in those which are the products of a recent division (Pl. 31, fig. 9); apparently as these young animals grow the granules are gradually absorbed so that the mature forms entirely lack them. Rarely have I seen them in a non-dividing form such as is shown in Pl. 31, fig. 5.

There is no evidence that these bodies undergo division during the reproductive stages of the flagellates.

It seems probable that they represent products of metabolic activity—a view which would to a certain extent account for their abundance in dividing animals.

To return to the account of the nuclear changes which occur during division. After the formation of the extra-

nuclear cloud the granules inside the nuclear membrane, which are indefinite in number, aggregate to form spherical masses—the so-called chromosomes—whose number does not seem to be definite; in most cases there appear to be six of equal size (Pl. 32, fig. 13). I have, however, found animals in which the number is between four and seven, and in these cases there is usually an inequality of size (Pl. 32, figs. 18, 19, 20).

Previous investigators, with one or two exceptions, record a constant number. Thus Wenyon states that there are six chromosomes in T. intestinalis; Kuczynski (**19**) finds eight in T. augusta; while Kofoid and Swezy state that in T. augusta, T. muris, Tetratrichomonas prowazeki and Eutrichomonas serpentis there are invariably five chromosomes. The discrepancy in the results of these authors, in some cases working on the same species, suggests that in many trichomonads the chromosome number is not constant. A discussion, however, of this point is to be found in Kuczynski's recent paper (**19a**), which unfortunately only came into my hands at the moment when the present paper was going to the press.

Soon after their formation the "chromosomes" elongate, forming short rods (Pl. 32, fig. 21) which split longitudinally (Pl. 32, fig. 22). The two halves of each body now draw apart (Pl. 32, fig. 23), so that two masses, each of approximately the same amount of chromatin, result (Pl. 32, figs. 24, 25).

It has been recorded by Kuczynski, Martin and Robertson that the chromosomes first pair and then split longitudinally before passing to each pole of the nucleus. I can find no evidence in D. termitis for this preliminary pairing, nor for any form of reducing division.

The whole of these processes occur within the nuclear membrane and there are no spindle-fibres produced. Hand in hand, however, with the nuclear changes the paradesmose elongates so that the centrioles occupy positions at each end of the nucleus, and during the whole period of separation the

chromosomes remain aggregated in loose bunches, with their apices very close to the separating centrioles (Pl. 32, fig. 23). The impression is forcibly given that the centrioles act as dividing centres for the chromosomes. It must, however, be clearly understood that the paradesmose and centrioles are during the entire cycle outside the nuclear membrane, and that there is no trace of any connection, in the shape of fibres, between the two structures. In the next and last stage of division the paradesmose and nucleus elongate still more until finally a constriction appears in the membrane of the latter (Pl. 32, fig. 26), so that ultimately two daughter-nuclei are produced (Pl. 33, fig. 27).

Further elongation of the paradesmose results in the separation of the nuclei so that a condition is reached like that seen in Pl. 33, fig. 28. Nuclear reconstruction occurs by a reversal of the process described above.

As the centrioles are connected by rhizoplasts with the basal granules, these, together with the flagella and membrane-granules, migrate from one another.

When the daughter-nuclei are separated by practically the whole width of the body the paradesmose disappears (Pl. 33, figs. 29, 30). There is no evidence that the centrioles remain in connection with the nucleus—indeed, I do not believe that this is the case—but that they share the same fate as the paradesmose.

6. Division of the Axostyle.

There is much discrepancy between the accounts given of the formation of the axostyles in Flagellates, and a short discussion of this will be found on p. 574.

Unfortunately I have not been able to work out as completely as is desirable the method of origin of the new structures in D. termitis. The axostyle does not stain at all well, and it is only occasionally that it can be traced throughout its entire length. In a few of my preparations, however, treated, as far as I am aware, in exactly the same way as others, the axostyles appear fairly plainly. These

preparations demonstrate that the old structure does not disappear during nuclear division, for it can be seen running throughout the whole body (Pl. 32, figs. 23, 24). In a few cases also there are indications that at the close of the telophase the old axostyle divides longitudinally, beginning at the anterior end (Plate 33, fig. 31). I believe that the process is similar to that described by Kofoid and Swezy in T. augusta, but unfortunately I cannot furnish such conclusive evidence as they adduce for this conclusion.

One thing, however, is certain—that the new axostyles are not derived from the paradesmose, for on one or two occasions there was seen the paradesmose, connecting the separated, nuclei together with the daughter-axostyles running through the body. Such a condition is seen in Pl. 33, fig. 28, which is, I think, sufficient evidence for concluding that there is no connection between the paradesmose and the axostyles.

7. Division of the Parabasal Body.

The parabasal body becomes duplicated directly after the blepharoplast and membrane-granule have divided, and before the formation of the paradesmose. Unfortunately I have been unable to follow the stages in this process, but I think that the new body is developed by the division of the old one. In all the many animals I have examined the two parabasals are seen lying side by side, each attached to its basal granule by the parabasal thread (Pls. 31, 33, figs. 15, 32 and Text-fig. 3). If, now, the daughter-structure arises by growth, one would expect to find the various intermediate stages as in Stephanonympha, and as one sees in the development of the undulating membrane. This I have never observed, however; in all the animals possessing two parabasals they are always of approximately the same size and appearance.

At the close of nuclear division the parabasals move in company with the basal granules away from each other, so that appearances such as are seen in Pl. 33, figs. 29, 30, are produced. In this way each daughter-animal possesses a

parabasal body and thread connecting it with the blepharoplast. The sequence of stages in the division of the animal is commonly that just given, but small variations in the course may occur. Thus the membrane-granule may divide before the blepharoplast (Pl 31, figs. 9, 10), or the nucleus may become resolved into chromatin granules before the development of the paradesmose (Pl. 31, fig. 8); these variations, however, are of little importance, and in no way affect the general course of events.

8. Sexual Process and Cyst-Formation.

In agreement with the majority of workers I have been unable to find any evidence for the presence of a sexual process.

Also there appears to be no cyst-formation—which is contrary to the general experience. However, after a careful search through many slides, involving the examination of the contents of numerous termites, I am forced to the conclusion that such a stage does not occur in the life-history of D. termitis. I may add that Imms, during the course of his research on these flagellates, was unable to find any cysts. Of course it is possible that during certain seasons of the year the animals may pass into the cystic stage; but these conditions evidently do not obtain in the laboratory, as the termites have now been examined, at short intervals, throughout the whole year. Again, it is possible that the temperature conditions under which the animals live in India are such as to induce cyst-formation, and that these conditions are not realised in this country. Owing, however, to the habit which termites have of eating the fæces of their companions, often doing so directly it has left the anus, infection from animal to animal can easily occur without the aid of cysts; this involves the assumption that the parasites are capable of withstanding the digestive juices of their hosts. That this is possible is indicated by the fact that I have often found the mid-gut of the termites heavily infected by all the species of Protozoa resident in the hind-gut.

This absence of cyst-formation is the experience of other workers on termite parasites, and Grassi (**11a**), in order to account for this in Jœnia and Mesojœnia, has put forward the same suggestion as the one outlined above. On p. 739 he says: "Probabilmente la soppressione dell' incistamento e for'anche quella della fecondazione sono rapportabili al costume or ricordato dei Termiti di mangiar la feccia dei propre compagni all'atto della emissione."

9. Multiple Fission.

Kofoid and Swezy (**18**), in their most recent communication to the American Academy, of which I have only been able to read a summary (**16**), state that in T. augusta, T. muris, Tetratrichomonas prowazeki and Eutrichomonas serpentes multiple fission occurs. According to these authors eight nuclei are produced with the accompanying flagella apparatus before the plasma divides. Eventually, however, eight daughter-animals are formed, which do not separate immediately.

Since this phenomenon was described in four closely related species, it seemed to me possible that it would also be found in D. termitis. After a careful search, however, I have failed to find any trace of such an occurrence.

A review and criticism of the above work is given by Kuczynski (**19a**).

10. General Considerations.

(a) Axostyle.

The axostyle has been the subject of discussion as regards its origin and connection with the nuclear division, and also as to its relationship in the various orders of Flagellates. From the various descriptions given of this organ it appears that there are, broadly speaking, three modes of origin. In the first the daughter-axostyles are formed from the para-desmose (centrodesmose) which persists after the close of nuclear division, as described by Prowazek (**23**), Dobell (**6**), and Janicki (**14**). In the second method the new axostyles

grow out from basal granules and have no connection with the paradesmose, which disappears at the end of division of the nucleus. This method is decribed by Kuczynski (**19, 19a**). The supporters of these two methods are in agreement that the old axostyle disappears and plays no part in the formation of the new ones. Although Kuczynski is, I think, incorrect in his statement that the new axostyles arise by the growth of threads from basal granules, yet he has made the interesting and important observation that they are developed before the disappearance of the paradesmose. As will be remembered, I was able to show the same thing occurring during the reproduction of D. termitis. The third type of origin of the axostyles was first described by Wenyon (**27**) in T. intestinalis, and has recently been re-described by Kofoid and Swezy (**17**). Here the old structures divide longitudinally at the close of the telophase, thus giving origin to the new axostyles. This is the method which I believe to have found in D. termitis.

There seems to me, however, to be little doubt that some axostyles do arise from the paradesmose as Dobell stated. In Lophomonas this is most certainly the case, as may be verified by anyone who will study the various division-stages in these animals; further, the account given by Dobell for T. ranarum and by other students of different species of flagellates leaves little room for doubt that this method obtains.

On the other hand, the results obtained by Kofoid and Swezy completely negative this view, as does Kuczynski's statement of finding the paradesmose and axostyles present at the same time. Such a condition as I found in D. termitis and figure in Pl. 33, fig. 28, of this paper is to me conclusive.

It is, therefore, evident that the axostyles of various flagellates have different origins and are not homologous with one another, but rather analogous. Surely no one would assert that the bundle of axial fibres, running down the middle of the body of Calonympha and Stephanonympha, as described by Janicki (**15**), and formed by the union of an axial fibre from each nuclear complex, is homologous with the

Trichomonas axostyles, yet they are probably similar in function with one another.

As Jollos (16) truly remarks in his abstract of the work of Kofoid and Swezy, it is necessary to obtain a clear idea of the relationship of the axostyles in the various groups of Polymastiginidæ, and until this is done it is hopeless to homologise them with other structures as so many people have attempted to do, some regarding them as cytoplasmic flagella, and others likening them to the thread running down the middle of the spermatozoon tail.

My observations, and those of others mentioned above, make it impossible to support certain statements made by Janicki (14) in 1912. On p. 99 of his paper he says: "Was ich als allgemeines Resultat der vorliegenden Zusammenstellung besonders hervorheben möchte, ist der Umstand, dass das Auftreten der extra-nucleären Spindel wahrend der Kernteilung sich bei den Gattungen Konstatieren lässt, welche mit einem Achsenstab resp. dessen Homologa versehen sind."

And again on p. 100: "Das der Achsenstab, sei es in seiner Grundlage (Jœnia, Trichomonaden, Devescovina), sei es überhaupt in seiner Gesamtheit (Calonympha, Stephanonympha) auf die persistierende extra nucleäre Spindel (Zentralspindel) zuruckzufuhren ist, kann heute als gesicherte Erkenntniss gelten, die für Flagellaten, wie schon gesagt, zum erstenmal durch Grassi unter Mitwirkung von A. Foa an Jœnia begründet wurde."

Finally I may add as additional proof of the opinions which I have stated above, that in other parasitic flagellates of the Termites, an account of which I hope to publish later, the axostyle cannot arise from an extra-nuclear spindle, because such a structure does not occur in these forms.

(B) Undulating Membrane, Blepharoplast, and Nuclear Division.

So much has been written in the past regarding the first two of these structures that it is unnecessary for me to do

more than indicate the most important points which have arisen from my research.

A striking feature of the life-history of D. termitis is the perfect independence of the blepharoplast and the membrane-granule, though in Devescovina and Parajœnia the trailing flagellum has an origin distinct from that of the anteriorly directed flagella.

In many species of Trichomonas, however, the free flagella and the undulating membrane arise from a single body—the blepharoplast—such as in T. batrachorum and T. gallinarum, and there is no trace of differentiation into separate bodies. Prowazek (**24**) concluded that this terminal basal granule was in reality tripartite, and composed of three granules closely associated. Parisi (**22**) finds in T. prowazeki and T. orthopterorum a double blepharoplast, but he does not state whether the undulating membrane springs from one of these or not. Some unpublished observations of my own on this latter species, however, lead me to conclude that the arrangement is similar to that of D. termitis.

Wenyon (**26**) found in T. intestinalis two distinct granules lying close together, from one of which the anterior flagella and the flagellate border of the membrane arose, and from the other the chromatic base took origin. Benson (**4**), however, describes in T. vaginalis the same arrangement as I have given in this paper.

It appears as though we have before us in the species of Trichomonas a gradual elaboration of the blepharoplast. Starting from a primitive condition where each flagellum has its own basal granule, the next stage is found in those forms in which two or three granules have fused to form a complex such as we have in D. termitis, or as in T. vaginalis, if three granules fuse, leaving the membrane-granule free. In the final condition this also is absorbed into the complex, so that a single multipartite body is produced from which all the locomotor apparatus arises, and this may play a part in nuclear division, thus forming a true blepharoplast.

A further point of interest which has arisen out of the work

is the demonstration that in division the daughter-membrane is produced by independent growth and not by division of the existing membrane. This method has been described by most of the earlier workers, but Dobell (**6**) asserted that in T. batrachorum the flagellar border was formed by splitting of the old one, and this view has been revived recently by Kofoid and Swezy (**17**). As I have already said, there is no evidence whatsoever to be found in D. termitis for such an opinion.

Turning now to consider nuclear division, one is struck by its independence of the bodies governing the flagella. In many flagellates the blepharoplast acts as division centre, so that it has been suggested that this granule and the metazoan centrosome are homologous structures. Dobell (**7a**), however, in a recent communication on Oxnerella maritima, a Heliozoan, contests this view. On p. 535 of his paper he says: "The centroplast of the Heliozoa is thus closely comparable with the blepharoplast of the Mastigophora—an organ permanently subserving a skeletal function to the organs of locomotion (the flagella), and in some forms assuming the office of centrosome at division, in others playing no part in this process (as in some trichomonads and in Copromonas respectively, as I have shown in two earlier papers [1909 and 1908]). To say that either the centroplast or the blepharoplast is the homologue of the metazoan centrosome and to apply the same term to all these structures appears to me, therefore, inadvisable. . . . In the language of the older morphology, I would say that the centroplast and the centrosome may be analogous, but are not homologous, organs." With this view I am in complete accord.

In many Trichomonads there appear to be no division-centres developed. Recently, however, Kofoid and Swezy (**17**) state that in the forms they investigated the blepharoplasts migrate to the poles of the nuclear membrane and there divide to form centrosomes. On p. 318 of their paper they state that "the two blepharoplasts have migrated to the two poles of the pointed ellipsoidal nucleus, and each has divided

into a centrosome at the apex of the spindle and the adjacent basal granule to which the flagella are attached. In some instances the division of the blepharoplasts is not apparent. . . . Connecting the two blepharoplasts as they migrate to the polar position is a heavy chromatic thread, which lies outside the nuclear membrane. This we name the paradesmose, though in origin it may seem to be homologous with the central spindle of the metazoan mitotic figure." This statement is a little vague, and leaves one in doubt as to whether the paradesmose connects the centrosomes or the basal granules. It serves to show, however, that there is little demarcation between the two bodies. Now in D. termitis they are quite distinct and act independently, the basal granules retaining their position at the anterior end of the body, while the centrioles derived from them migrate to the nuclear poles. Thus it appears that in this animal the complex blepharoplast of other forms is here easily resolved during reproduction into its constituent parts.

Finally I would emphasise the fact that the paradesmose is a transitory structure. That it has no connection with the axostyles I have already shown, but I wish it to be understood also that the centrioles disappear at the close of nuclear division, and new ones are produced at the next division from the blepharoplasts. I have no wish to enter into a discussion regarding the centriole of the Protozoan nucleus, for this controversy has already become somewhat wearisome, but I might point out that my observations supply a further proof—if such is necessary—of the untenability of the now famous phrase of Hartmann and Chagas that "Das allgemeine Vorhandsein von Zentralorganem im Caryosom aller Protozoen kann jetzt als eine wohlbegrundete wissenschaftliche Tatsache gelten."

(c) The Parabasal Body.

On p. 565 I have said that in every specimen of D. termitis there is found a well-developed parabasal body. In the previous literature on Trichomonas this body has

been mentioned, but, with the possible exception of T. batrachorum, as recorded by Alexeieff (1) and Janicki (15), it has only been found sporadically. Because of this, Kuczynski, in his earlier paper (19), considered that it was of transitory appearance. In his recent communication (19a), however, he states that such a body is a constant character of the newly-described T. mirabilis. He says, however, that during division the structure passes over entire to one of the daughter-animals, and is formed "de novo" in the other. This is, of course, in direct opposition to the conclusion at which I have arrived.

I think that the non-appearance of the parabasal in so many cases is due to the method of preparing the slides. In all my preparations fixed with sublimate or Bouin's fluid the parabasal was only occasionally seen, and then very indistinctly—so much so that for a long time I was in doubt as to what the faintly-stained, badly-defined body was which I recorded in my notes. When, however, I fixed material with Fleming's solution, as modified by Gatenby (11)—that is, by omitting the acetic acid—and then stained with iron-hæmatoxylin, the body was most distinctly seen, and I at once recognised that I was dealing with the parabasal body described by other workers.

Subsequent work has shown that the same result is obtained after fixation with osmic acid, 5 per cent. formol, or any good fixative so long as sublimate and acetic acid are not constituents.

I may mention here that I have found Fleming's solution, modified as described above, an excellent fixative for Protozoa, and would recommend it to protozoologists. In the past workers have tended to use only one fixative, and that Schaudinn's fluid, which, though excellent in many ways, may lead to erroneous conclusions, as in D. termitis. I by no means advocate that Gatenby's mixture should be substituted for Schaudinn's, but that in an investigation these and other good fixatives should be more extensively employed, and so obviate as far as possible the errors which

arise through the different actions that fixing fluids exert upon the protoplasm of the cell. That these are often very great may be seen by reference to the papers recently published by Gatenby (**11**), where he demonstrates the existence of many "cell-inclusions" overlooked by previous observers who had been too conservative regarding their use of fixatives

For staining the parabasal any reliable Protozoan stain is suitable, including Delafield's hæmatoxylin, though this is contrary to Janicki's experience.

The structure of the parabasal in D. termitis is not remarkable in any way, though the presence of the parabasal thread is of interest, as it has not previously been described in Trichomonas. Such a connection with the blepharoplast, however, is recorded by Swezy (**25**) in Polymastix bufonis, and in other flagellates, such as Devescovina, Parajœnia and Stephanonympha. In division the parabasal seems to behave in three different ways among the various orders of Flagellates. Thus it may—

(A) Be destroyed in the mother animal and formed anew in each daughter-individual—Lophomonas.

(B) Divide and pass to the daughter-animals in connection with the division centres—Devescovina, Parajœnia.

(C) Remain undivided at one pole of the nuclear spindle and a new one grow from the other pole—Stephanonympha, T. mirabilis.

For the reasons given on p. 572 I consider that the body in D. termitis belongs to the second category.

It is of interest, however, that, owing to the independence of the centrioles and the basal granules, the parabasals remain attached to the latter, and have no connection with the division centres at the poles of the paradesmose, as occurs in the animals mentioned above.

Various suggestions have been made as to the significance of the parabasal—that it is of the nature of waste products, or that it is allied to the mitochondria (Janicki). For this latter view there is the support that the mitochondria and parabasal are affected by the various fixatives in the same

manner, but our knowledge of the parabasal is at present too small to warrant any definite statement. A great deal more detailed work is required before such discussions would be profitable.

It is necessary, however, to refer briefly to the latest suggestion of Kofoid and Swezy, who homologise the parabasal of other Flagellates with the chromatic base of the undulating membrane. This view is, I think, quite untenable. It has been shown that parabasals do occur in some species of Trichomonas, and though Kofoid and Swezy do not describe them in T. augusta, yet they were recorded in this species by Alexeieff in 1909 and by Kuczynski in 1914. It appears probable also that when research is made upon suitably prepared material the presence of these bodies will be found in most, if not all, the species of Trichomonas.

Such being the case, it is unlikely that the chromatic base represents the homologue of the parabasal of the Trichonymphidæ, and that the body described in trichomonads as parabasal is an entirely new structure. Of course such a condition is possible, but until very definite proof is adduced for such an hypothesis it is legitimate to view it with the utmost suspicion. A further criticism of this view is given by Jollos (**16**). In the 'University of California Publications,' vol. xvi, 1916, Swezy has elaborated her views as to the nature of the parabasal, and Janicki in his last paper gives a full discussion of the subject. Also Kuczynski (**19b**) adduced reasons for believing that the parabasal and the kinetonucleus are homologous, which view is also held by Kofoid (**18a**). To these papers I would refer the reader who is desirous of obtaining full discussions of this subject.

11. Diagnosis of Ditrichomonas termitis.

I will conclude this paper by giving a brief diagnosis of Ditrichomonas termitis, which may be useful to future observers, and will at the same time render unnecessary a summary of the foregoing facts.

Ditrichomonas termitis.—Tetramitidean flagellate of

large size (average 55 μ × 22 μ). At the anterior end there are two free flagella springing from a blepharoplast, and a posteriorly directed one forming the border of the undulating membrane and arising from a special granule. Cytostome present. From the anterior extremity of the body there arises an axostyle which runs through the whole body-length, emerging at the posterior end. This structure shows a peculiar lateral movement of its free portion in the living animal. An elongate parabasal body with a centrally situated thread springs from the blepharoplast. The cytoplasm is granular, and in some animals filled with small, deeply-staining granules resembling mitochondria. The nucleus is anteriorly placed, the chromatin completely filling the space bounded by a well-developed membrane.

The only method of reproduction observed is by simple division into two. The blepharoplast divides, as does the membrane-granule from which the new undulating membrane grows. By division of one blepharoplast a centriole is produced, which divides to form two. These migrate apart, and between them a solid band of fibres—the paradesmose—is formed. This becomes situated just outside the nuclear membrane and remains there throughout the division stages. Nuclear division is by a simple mitosis without fibres.

The daughter-parabasal is probably produced by the longitudinal splitting of the existing one. The old axostyle is not absorbed during the reproduction stages, but probably divides to form the daughter ones. It is certain that the paradesmose plays no part in their origin.

Habitat.—Hind-gut of an Indian termite—Archotermopsis wroughtoni Desn.

Food.—Particles of wood and cellulose.

I wish to express my thanks to Miss M. Dixon for redrawing for publication the figures which I made to illustrate Plates 31 and 32.

References.

1. Alexeieff, A. (1909).—"Les flagellatés parasites de l'intestin des batriciens indigènes," 'C. R. Soc. Biol.,' t. lxvii.

2. ——— (1909).—"Un nouveau Trichomonas à quatre flagellés antérieurs," ibid.

3. Alexeieff, A. (1911).—"Notes sur les Flagellés," 'Arch. Zool. exp. et gen.,' 5e ser., t. vi.

4. Benson, W. (1909).—"Untersuchungen über Trichomonas intestinalis und vaginalis des Menschen," 'Arch. Protistenk.,' Bd. xviii.

4a. Buscalione, L., and Comes, S. (1910).—"La digestione della vegetali per opera dei Flagellati contenuti nell intestino dei Termitidi e il problema della simbiosi," 'Atti. Accad. Gioenai Catania,' xxiii.

5. Cutler, D. W. (1918).—"A Method for the Cultivation of Entamœba histolytica," 'Journ. Path. and Bact.,' vol. xxii.

6. Dobell, C. C. (1908).—"Researches on the Intestinal Protozoa of Frogs and Toads," 'Quart. Journ. Micr. Sci.,' vol. 53.

7. ——— (1914).—"Cytological Studies on three species of Amœba," 'Arch. Protistenk.,' Bd. xxxiv.

7a. ——— (1917).—"On Oxnerella maritima, nov. gen., nov. spec., a new Heliozoon and its Method of Division; with Some Remarks on the Centroplast of the Heliozoa," 'Quart. Journ. Micr. Sci.,' vol. 62.

8. ——— and Jepps, M. (1918).—"A Study of Diverse Races of Entamœba histolytica, distinguishable from one another by the Dimensions of their Cysts," 'Parasit.,' vol. x.

9. Doflein, F. (1911).—'Lehrbuch der Protozoenkunde,' Jena.

10. Foà, A. (1905).—"Due nuovi flagellati parassiti," 'R. R. Acad. dei Lincei,' xiv (2).

11. Gatenby, J. B. (1917 and 1918).—"Cytoplasmic Inclusions of the Germ-cells," 'Quart. Journ. Micr. Sci.,' vols. 62, 63.

11a. Grassi, B. (1911).—"Intorno ai Protozoi dei Termitidi," 'Rend. Accad. Lincei,' ser. 5, xx (1).

12. Imms, A. D. (1919).—"On the Structure and Biology of Archotermopsis, together with Descriptions of New Species of Intestinal Protozoa and General Observations upon the Isoptera," 'Phil. Trans.,' B, in the press.

13. Janicki, C. (1911).—"Zur Kenntnis des Parabasalapparatus bei parasitischen Flagellaten," 'Biol. Centralb.,' Bd. xxxi.

14. ——— (1912).—"Bemerkungen zum Kernteilungsvorgang bei Flagellaten, namentlich bei parasitischen Formen," 'Verh. Naturf. Gesell. Basel,' Bd. xxiii.

15. ——— (1915–15).—"Untersuchungen an parasitischen Flagellaten," 'Zeit. f. wiss. Zool.,' Bd. cxii.

16. Jollos, V. (1916–17).—"Summary of Kofoid and Swezy's paper—'Mitosis and Multiple Fission in Trichomonad Flagellates,'" 'Arch. Protistenk.,' Bd. xxxvii.

17. Kofoid, C. A., and Swezy, O. (1915).—"Mitosis in Trichomonas," 'Proc. Nat. Acad. Sci.,' U.S.A., vol. i.

18. ——— (1915).—"Mitosis and Multiple Fission in Trichomonad Flagellates," 'Proc. Am. Acad. Boston,' vol. li.

18a. ——— (1916).—"Biological and Medical Significance of the Intestinal Flagellates": paper presented before the Second Pan-American Scientific Congress, Washington, U.S.A., December 27th, 1915, to January 8th, 1916.

19. Kuczynski, M. H. (1914).—"Untersuchungen an Trichomonaden," 'Arch. Protistenk.,' Bd. xxxiii.

19a. ——— (1918).—"Über die Teilungsvorgänge verschiedener Trichomonaden und ihre Organisation im allgemeinen," ibid., Bd. xxxix.

19b. ——— (1917).—"Über die Teilung der Trypanosomenzellen," ibid., Bd. xxxviii.

20. Martin, C. A., and Robertson, M. (1911).—"Further Observations on the Cæcal Parasites of Fowls," 'Quart. Journ. Micr. Sci.,' vol. 57.

21. Minchin, E. A. (1912).—'An Introduction to the Study of the Protozoa,' London.

22. Parisi, B. (1910).—"Su alcuni flagellati endoparassiti," 'Arch. Protistenk.,' Bd. xix.

23. Prowazek, S. (1903).—"Flagellatenstudien," ibid., Bd. ii.

24. ——— (1904).—"Untersuchungen über einige parasitische Flagellaten," 'Arb. Gesundhsamt.,' Berlin, Bd. xxi.

25. Swezy, O. (1916).—"The Genera Monocercomonas and Polymastix," 'Univ. Calif. Publ.,' vol. xvi, No. 11.

26. ——— (1916).—"Kinetonucleus of Flagellates and the Binuclearity Theory of Hartmann," ibid., vol. xvi, No. 15.

27. Wenyon, C. M. (1907).—"Observations on Protozoa in the Intestine of Mice," 'Arch. Protistenk.,' Suppl., Bd. i.

DESCRIPTION OF PLATES 31 TO 33.

Illustrating Mr. D. Ward Cutler's paper, "Observations on the Protozoa Parasitic in the Hind-gut of Archotermopsis wroughtoni Desn.: Part I.—Ditrichomonas termitis, nov. gen., nov. spec."

[With the exception of figs. 1, 2, 3, all the figures have been drawn from permanent preparations with the aid of a camera lucida and the following optical apparatus: Zeiss apochromatic oil-immersion objective 2 mm. (N.A. 1·3) and compensating ocular 6. Critical illumination was always employed. The magnification of all figures except figs. 1, 2, 3 is approximately 940 diameters; figs. 1, 2, 3 were drawn freehand from living preparations. Figs. 5, 6, 8, 10, 15, 25, 29, 30, 31, 32, 33 are from preparations fixed with Fleming's fluid, without acetic acid, as described by Gatenby. The remaining figures are from preparations fixed with either Schaudinn's fluid or the modification of it recommended by Dobell and Jepps. Figs. 6, 7, 9, 15, 23, 29, 31 are from preparations stained by Dobell's hæmatein stain, the remaining figures from preparations stained by Heidenhain's iron-hæmatoxylin. See Text-figs. 2 and 3 for reference to organella, etc.]

PLATES 31, 32 AND 33.

Figs. 1, 2, 3.—Living animals, examined in 0·75 per cent. NaCl, showing the general body characters. Note the large amount of axostyle projecting in fig. 3.

Fig. 4.—Normal non-dividing form. Parabasal body not visible. Axostyle with centrally-arranged granules.

Fig. 5.—Non-dividing animal with deeply-stained parabasal and scattered mitochondria. The distinction between blepharoplast and membrane-granule is well seen.

Fig. 6.—Same as above, but less deeply-stained so as to show the parabasal thread. Axostyle with the series of granules.

Fig. 7.—Animal in the first stage of division with four free flagella; blepharoplast still undivided.

Fig. 8.—Blepharoplast divided and also the membrane-granule. Parabasal with thread. Mitochondria clustered round anterior end of the axostyle.

Fig. 9.—Small form in which membrane-granule has divided before the blepharoplast.

Fig. 10.—Same as above: axostyle arising from blepharoplast.

Fig. 11.—Animal with two membrane-granules, from one of which the new chromatic base is beginning to grow out.

Fig. 11A.—Centrioles have been formed from one of the basal granules.

Fig. 12.—Centrioles joined together by the paradesmose. Further growth of the chromatic base of the new undulating membrane. Nucleus breaking into granules.

Fig. 13.—Trapezoid figure produced from the centrioles, basal granules and rhizoplasts. Paradesmose outside the nuclear membrane. Beginning of growth of the flagellar border of undulating membrane. Nucleus with extra-nuclear cloud and the chromatin aggregated into six "chromosomes."

Fig. 14.—Formation of the paradesmose and second undulating membrane; nucleus with intra-nuclear cloud.

Fig. 15.—Animal in which the organella are duplicated. Two parabasals seen. Nucleus with intra-nuclear cloud, and the beginning of extra-nuclear one.

Fig. 16.—New undulating membrane almost completed. Intra-nuclear cloud in nucleus.

Fig. 17.—Stage previous to fig. 13, with nucleus breaking into granules.

Figs. 18, 19, 20.—Forms in which the chromosomes are of various numbers. In fig. 18 the metabolic granules almost fill the body.

Fig. 21.—Chromosomes assuming the rod-like form.

Fig. 22.—Longitudinal splitting of the chromosomes.

Fig. 23.—Elongation of the paradesmose; chromosomes separating into two bunches. Axostyle still present throughout the body.

Fig. 24.—Chromosomes completely separated into two masses inside the nuclear membrane. Metabolic granules along the chromatic bases. Complete axostyle still present.

Fig. 25.—Similar to above, but parabasal bodies seen.

Fig. 26.—Animal in which nuclear membrane is beginning to constrict.

Fig. 27.—Nucleus completely separated into two, which are in the granular condition.

Fig. 28.—Daughter-nuclei widely separated, but still connected by paradesmose. Note the two daughter-axostyles.

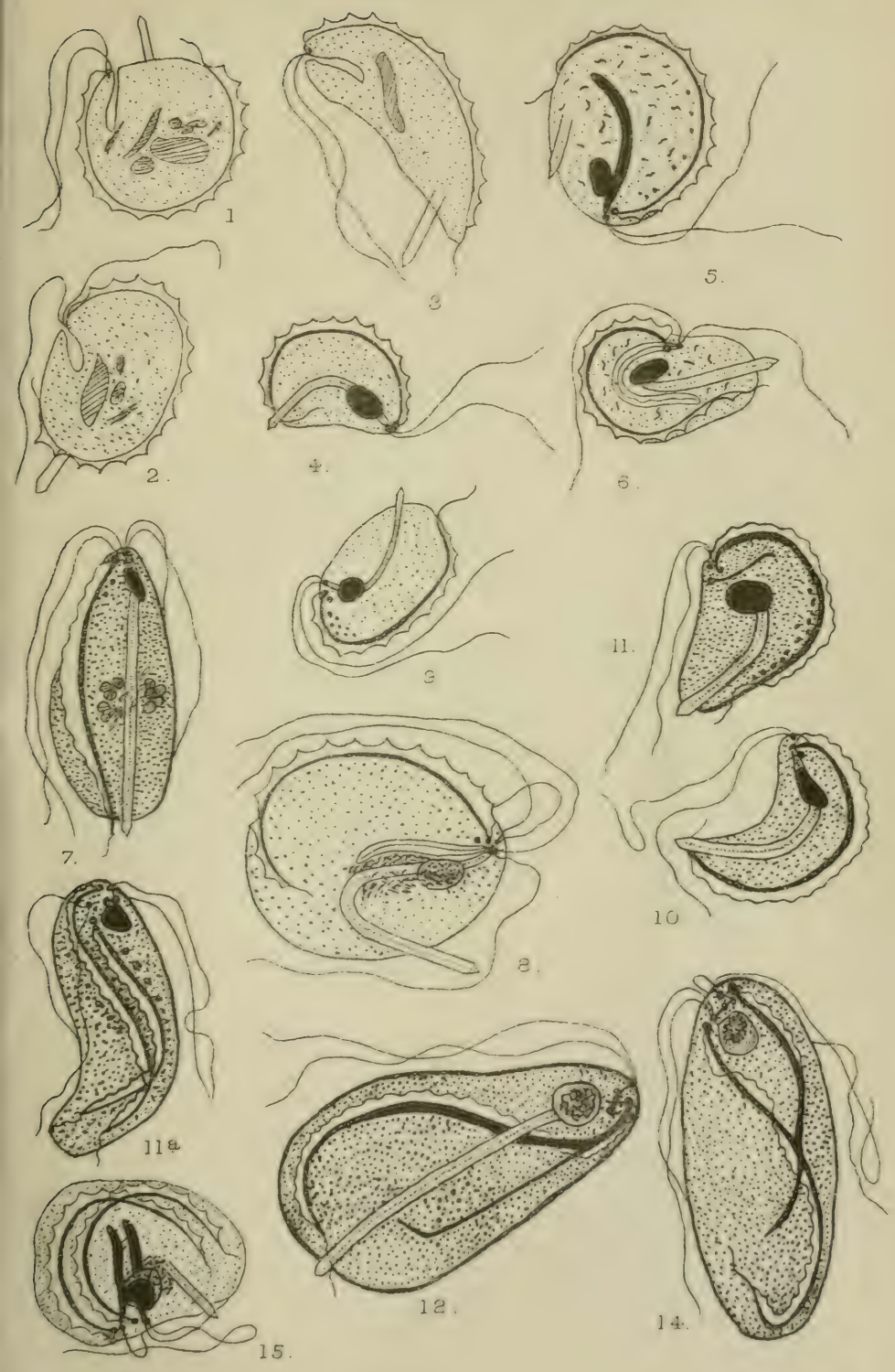
Fig. 29.—Paradesmose has disappeared. Two parabasals in connection with the blepharoplasts. Two axostyles present.

Fig. 30.—Similar to above, but without the axostyles visible. The nucleus is losing its granular condition.

Fig. 31.—Animal in which axostyle appears to be dividing.

Fig. 32.—Animal with stain extracted so as to show the parabasal threads. Mitochondria are visible; and the nucleus contains a karyosome.

Fig. 33.—Animal at the end of division: the two daughter-forms each possess an axostyle and parabasal body.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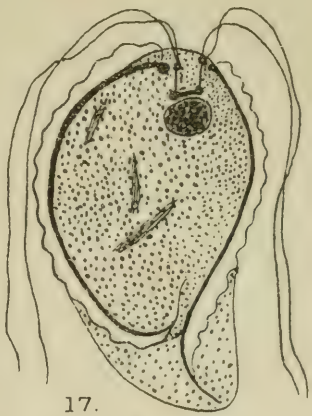

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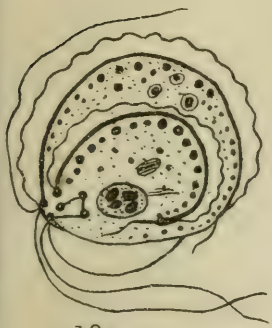

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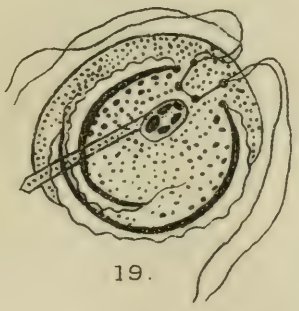

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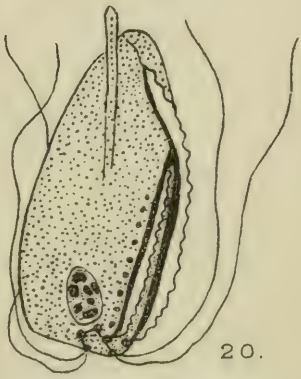

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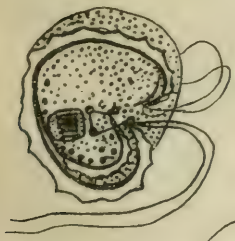

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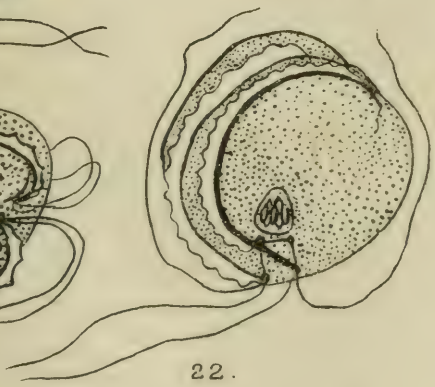

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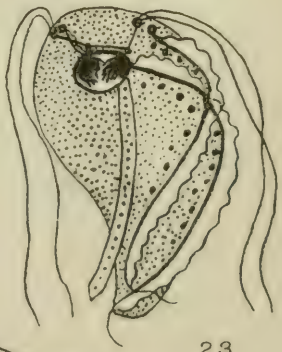

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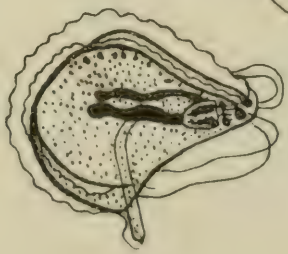

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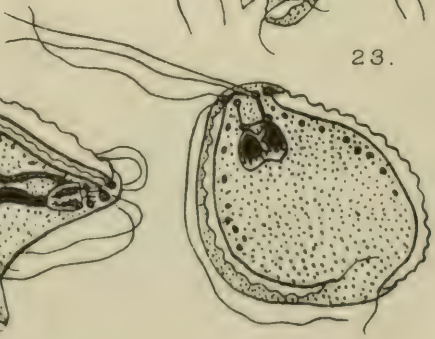

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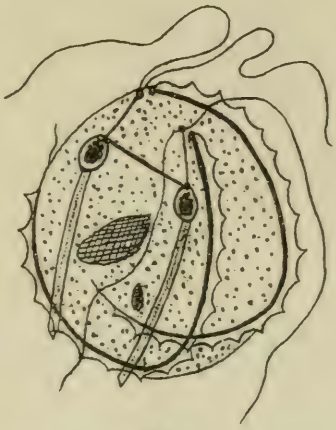

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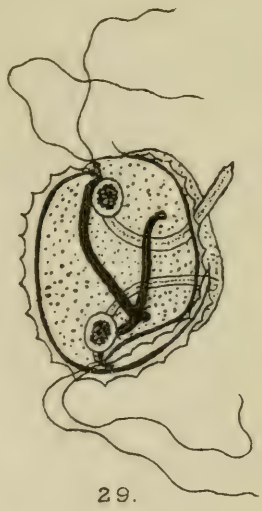

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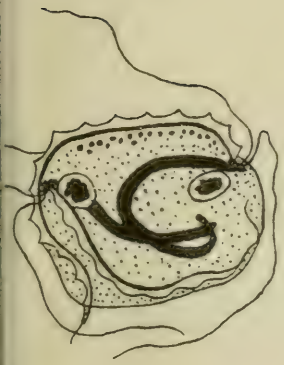

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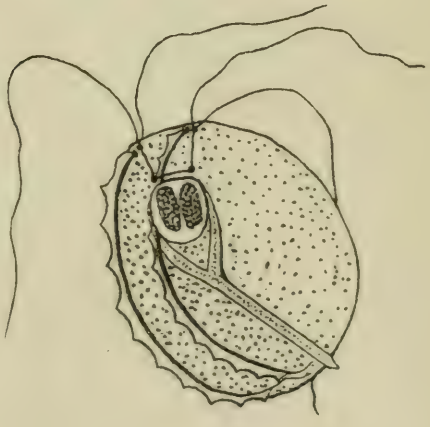

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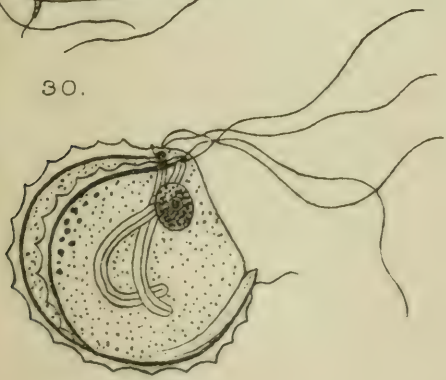

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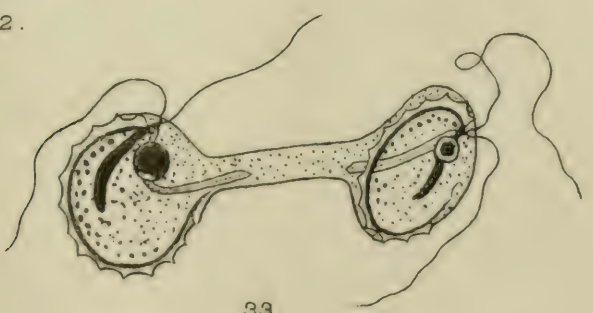

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