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MEMOIRS:

Some Observations upon the Development of the Teeth of Physeter macrocephalus.

By

Frank E. Beddard, D.Sc., M.A., F.R.S.

With 13 Text-figures.

On the following pages I describe the nature of the tooth rudiments in the very young embryo of Physeter macrocephalus, whose general external characters I have already commented upon and figured (Beddard, **1**). Since the material upon which I have worked is, so far as I am aware, a unique specimen only $4\frac{1}{2}$ inches long, I am particularly grateful to the Curator of the Durban Museum (Mr. E. C. Chubb) for placing it in my hands and to the authorities of the Museum of the Royal College of Surgeons of England for allowing their highly skilled assistant, Mr. Steward, to prepare a series of sections for study.

General Characters of Teeth in Foetal Sperm Whale.

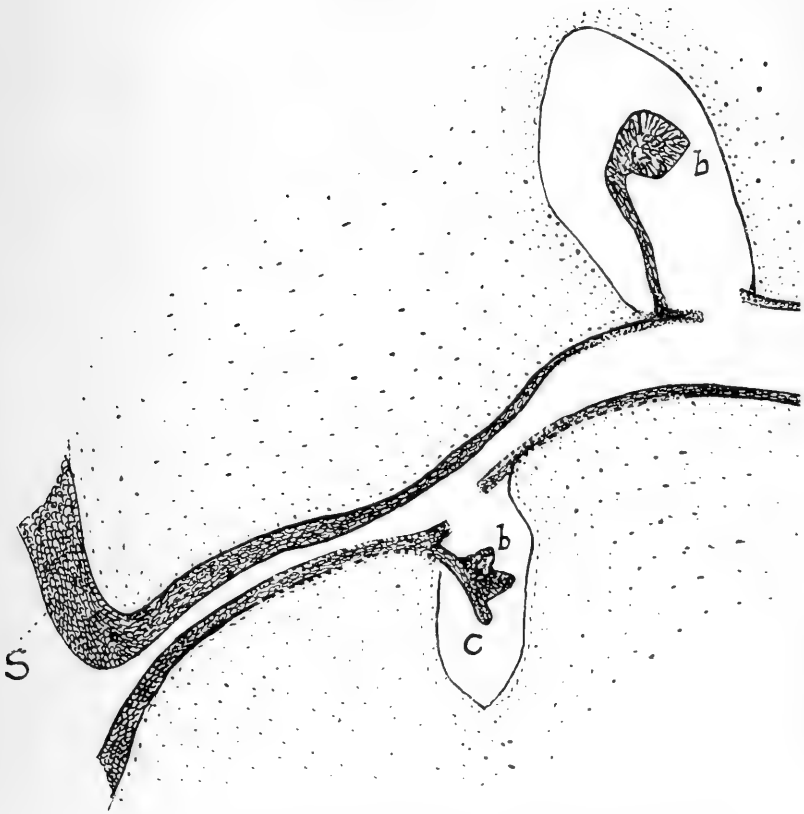
Although the teeth of the adult sperm whale, of both lower and upper jaws, are well known (Ritchie and Edwards, **5**), the development of the teeth in this cetacean is described in only one memoir (Pouchet and Beauregard, **4**), so far as I am aware. This memoir contains a description of the teeth in an embryo of 30 cm.; those of both jaws are described and figured. This embryo, it will be noted, is about twice the size of that dealt with in the present communication. There is not, however, as it would appear, a great deal of difference in the condition of development of the teeth; hence I have not very much to add to the description of Messrs. Pouchet and Beauregard. Apart, however, from such new facts as I am

able to set forth here, certain peculiarities in the growth of the teeth of Physeter macrocephalus are worth confirmation, though I doubt whether I am able to settle definitely the question of the homologies of the teeth of the adult, whether—that is to say—they belong to the milk or permanent dentition. The former view of the odontocyte dentition seems to be the one generally held ; but by others the question is considered to be still open. It should be added, however, that these views are not at all based upon a consideration of the facts described by Pouchet and Beauregard, whose memoir has been largely overlooked. This is a further reason for again directing the attention of zoologists to this subject.

I may commence by directing attention to a matter not illustrated in the figures of the teeth published by Messrs. Pouchet and Beauregard ; this is the position of the teeth of the upper and lower jaw with reference to each other. It will be seen by an inspection of Text-fig. 1 that the teeth of the upper jaw are divided from each other by a space that is less than the space dividing the two teeth rows of the lower jaw—that is to say, the upper teeth are distinctly within the lower teeth. Furthermore, the upper teeth are quite vertical in position, and at right angles with the longitudinal axis of the head, while the two rows of teeth in the lower jaw are at an angle to each other, and to the same axis of the head. Thus the teeth of the lower jaw look outwards as well as upwards, while those of the upper jaw are directed downwards only. This state of affairs is more marked in the anterior region of the lower jaw. It is due to the varying contour of the lower jaw, which anteriorly is more rounded while posteriorly the upper surface is straighter. Thus the teeth are, so to speak, carried on to what is largely the lateral surface.

This figure also shows a character which is to be seen more in detail in subsequent illustrations of the teeth of this foetus. The cavity in which the tooth rudiments lie is not at all invaded by the upgrowth from below forming the dental papilla, which is only represented in this stage by a thickening of the mesodermic tissue shown by a closer approximation of the nuclei

TEXT-FIG. 1.



In this and the ensuing figures the following general statement holds good of all, and need not be repeated. The figures are of sections cut deliberately thick, the diameter being 25 μ (i. e. $\frac{1}{40}$ mm.). The direction of all the sections is transverse to the long axis of the head. Where necessary for explanation, the sign * is on the lingual side. The following lettering is employed :

a, rudiment of milk tooth. *b*, rudiment of permanent tooth. *c*, residual lamina. *d.p.*, rudiment of dentine papilla. *E.*, Epithelium of mouth cavity which gives origin to dental lamina. *S*, side of head. *U.J.*, upper jaw. *L.J.*, lower jaw.

Fig. 1 represents a portion of the upper and lower jaw to show the position of the tooth germs of these two jaws in relation to each other. The tooth of the lower jaw is seen to lie outside of that of the upper jaw.

of this tissue. The peculiarly large extent of this cavity, which will be dealt with immediately, is possibly to be looked upon as a preparation for the subsequent growth of the dental papilla.

The ingrowths of the epidermis to form the enamel organ, instead of lying within—and firmly embedded therein—the mesoderm tissue underlying the epidermis, depend freely into a spacious cavity just referred to, which forms the tooth follicle. Pouchet and Beauregard figure extensive spaces surrounding these ingrowths, but not to so large an extent as I have found in the young embryos examined by myself. In the lower jaw of my foetus the space invariably commenced immediately below the epidermis ; but in the upper jaw there was frequently a layer of mesoderm immediately underlying the epidermis and perforated by the ingrowth. In other mammals spaces are apt to occur in the same way. The cavities are so large that they are only moulded in the roughest way to the enamel ingrowth ; it is to be remarked, however, that —in the upper jaw at any rate—the labial outgrowths (see Text-figs. 9–12) of the dental lamina, which will be dealt with later, lie in one or two diverticula of this cavity, and in the same way free within it ; they are not closely adpressed to its walls. I imagine that this state of affairs is not altogether natural, but is due to reagents and consequent shrinkage. I have no means, however, of ascertaining whether any part of this cavity is normal. In any case the practical result is that both in upper and in lower jaw a canal is formed which is quite continuous from one end to the other of both jaws. This cavity gradually narrows at the extreme end of the series of teeth and finally ceases to exist close beneath the epidermis. It is possible that it is associated with the groove which in this and other cetaceans lodges the teeth in the adult animal.

It is, furthermore, possible that something of the same kind led to the erroneous views upon the development of the teeth expressed by Goodsir, whose figures persisted until quite lately in text-books such as ' Quain's Anatomy '.[1] Here the

[1] 8th ed., vol. ii, 1876, p. 315, fig. 214, 3 and 4.

growing tooth is represented as the dental papilla only, growing upwards into a cavity.

A rough survey of the series of sections, one of which is shown in Text-fig. 1, shows also that there is apt to be considerable difference in size between some of the tooth cavities of the upper jaw and those of the lower. This is not always the case; but posteriorly (i.e. nearer to the condyle of the jaw) the follicles of the upper jaw teeth are deeper than those of the lower jaw, even twice as deep, in that and other regions. This is correlated with a greater length of the dental lamina, which will now be described.

It seems to be a general rule—so general that Sir Charles Tomes (**6**) makes his diagrams of the developing teeth conform to it—that the dental lamina is oblique in direction, running indeed at times almost parallel with the oesophageal epithelium, of which it is a downgrowth. In Physeter, as the figures of Pouchet and Beauregard indeed show, this lamina is absolutely at right angles to the oesophageal epithelium. This will be apparent from an examination of Text-figs. 3 and 4, &c., annexed hereto. The origin of the lamina shows no points of particular interest. It arises from the malpighian stratum, at both sides of an ingrowth of the superficial layer of cells which thus forms its core.

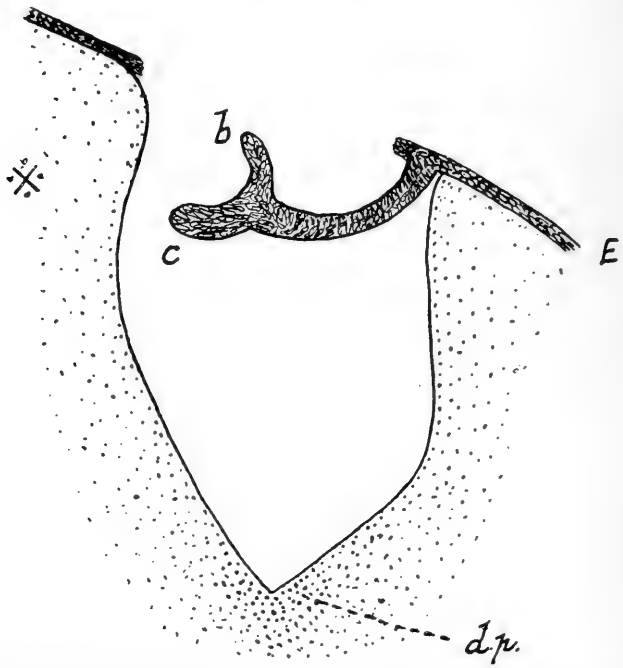
The histological condition of the material was good enough to show the difference between the central core of the lamina and the often cubical layer of epidermis which surrounds it externally. I do not, however, attempt any special description of the various cells, as they do not seem to present any features of disagreement with those of the developing teeth in other mammals.

Teeth of the Lower Jaw.

My examination of the teeth of the lower jaw was hindered by the condition of the sections of the anterior region of these jaw rami. The tooth follicles upon which I have already commented were often entirely empty of contents, the jaws being here obviously more exposed to external injury. I have, how-

ever, no other reason to doubt that the teeth rudiments were like[1] those situated more posteriorly, which presented very few such lacunae. Still, in attempting general statements concern-

TEXT-FIG. 2.



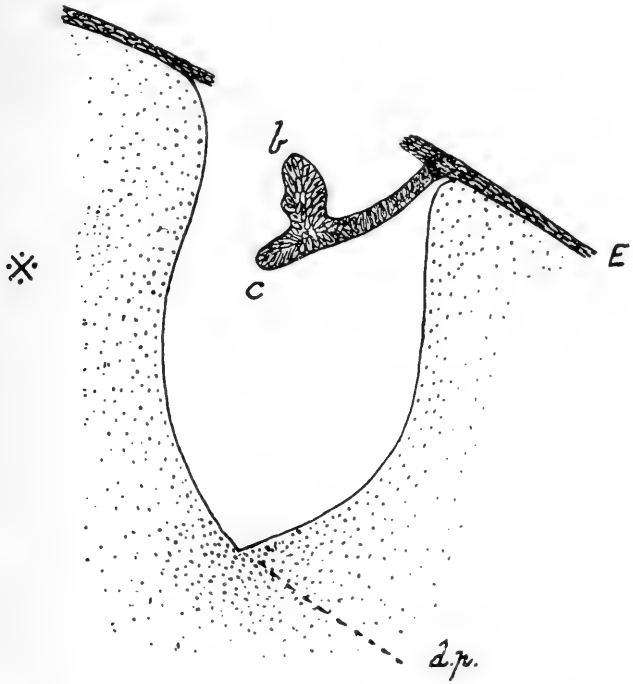
Tooth of lower jaw. This figure and the two following are a nearly continuous series, one section only lying between each section figured. The mouth epithelium is seen to be ruptured owing to the swelling of the tooth follicle, and the dental lamina with its tooth germs to be dislocated towards the lingual side. The dental papilla is no more than a closer agglomeration of the mesoderm cells at the base of the tooth follicle.

[1] I could only find one series of teeth in the anterior part of the jaw and these were quite early. The only difference from those which are described more in detail below is that the actual tooth germ is longer and more parallel with the residual lamina, and that the latter tends to disappear between successive tooth germs; there is thus an approximation in structure to the teeth of the upper jaw. But the dental lamina remains very short as in the posterior teeth of this jaw. These anterior teeth are evidently more advanced in development.

ing the mandibular teeth the defective condition here referred to must be borne in mind. I shall have, for example, to indicate actual structural differences between the teeth of the mandibles and those of the maxillae and premaxillae.

These teeth are readily comparable at first sight with the

TEXT-FIG. 3.

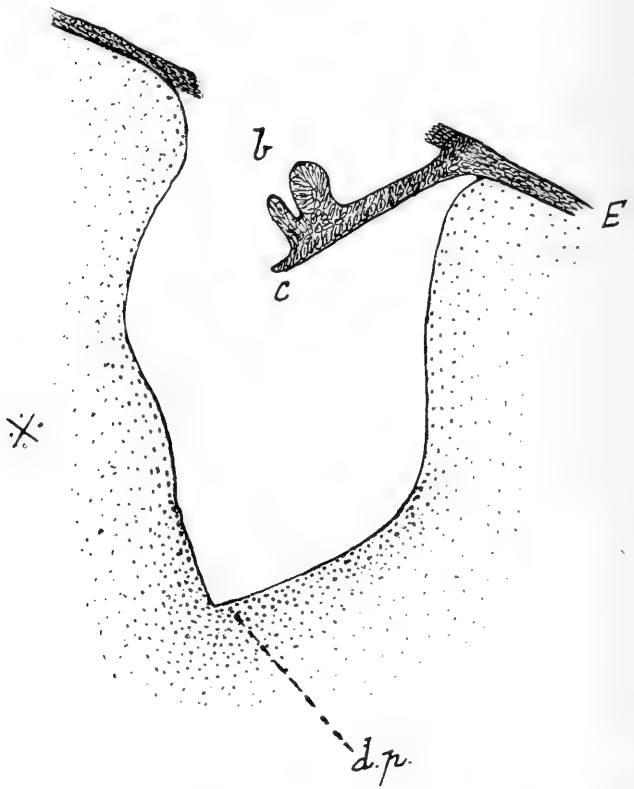


As Text-fig. 2, but the rudiment of permanent tooth is larger.

teeth of other mammals in a corresponding stage of development. The tooth rudiment shown (Text-fig. 4) as a bell-shaped swelling seems to be clear; and beyond this, i.e. distally from the place of origin of the dental lamina, is a prolongation which would seem to correspond to the residual lamina of other teeth. They all presented more or less the appearance shown in Text-figs. 2, 3, 4. The entire organ developed from the enamel germ often lay closely adpressed to one side of the

copious tooth follicle, but in other cases it lay more in the middle of that follicle removed from its walls. The growing tooth was small compared with those of the upper jaw, which will be dealt with immediately. Each was distinctly marked into three

Text-fig. 4.

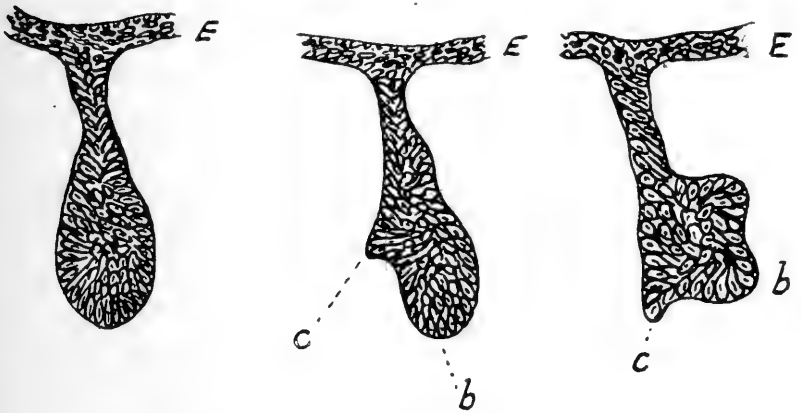


As in two previous sections; the rudiment of the permanent tooth has acquired its full size.

regions, which are very plain—as is shown in the figures already referred to. The tooth itself lies to the lingual side of the ingrowth, and at its middle or thereabouts is rather bell-shaped, or at least divided into two lobes; these look inwards and not downwards. The stalk of which this is an outgrowth, i.e. the

dental lamina is straight and quite at right angles to the oesophageal epithelium. Beyond the origin of the tooth rudiments, as will be seen in the figures, it is continued onwards in the same straight line as the dental lamina, and it is this region which I have termed above the residual lamina. In neighbouring sections to that represented in Text-fig. 4 (see Text-figs. 2, 3) the dental and residual laminae show no differences, but the actual tooth germ is more slender. But

TEXT-FIG. 5.



Three sections, near to each other, from the condylar end of the lower jaw, representing three stages in the growth of the tooth germ. The left-hand figure represents the initial stage, in which the entire tooth germ is a mere swelling of the dental lamina. Later (the middle figure) the residual lamina becomes differentiated; and the third section shows the complete differentiation of the rudiment of the permanent tooth.

it does not appear ever to vanish between successive teeth, but to be continued as a lamina, the actual tooth germ being local thickenings of this. The residual lamina undergoes no change in the intervals between the teeth, but it may at times terminate in a more club-shaped or at least slightly swollen extremity than in other places. At the very beginning of the series of tooth rudiments of the lower jaw—at the end nearest to the condyles—the rudiment consists (see Text-fig. 5) of a swollen extremity supported by a short stalk. This resembles

the first of the series of the upper jaw, which will now be described. It will be noted, therefore, that here, as in the upper jaw, the tooth series develops from behind forwards.

Teeth of the Upper Jaw.

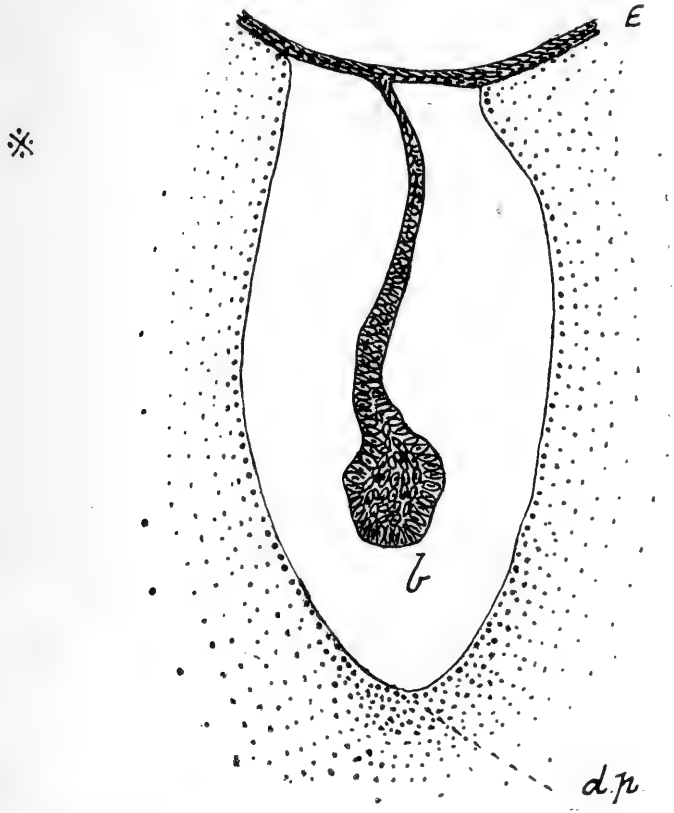
In the case of the upper jaw I was also able to trace the dental lamina to the very end, close to the condyle. It begins here as a much shorter fold than it becomes farther forward. The fold at the very first is more or less oval in transverse section, and later appears club-shaped. It becomes separated in fact into the oval free extremity and a much more slender stalk. The expanded free edge of the dental lamina may be traced forwards into the series of rudimentary teeth. The only change at first in this region of the dental lamina is the increase in size, at intervals and for the distance of a few sections, of the expanded free extremity. Nevertheless, it is particularly to be noted that where there are no signs of tooth formation the ending of the lamina is still swollen. It has been asserted and denied [1] that a swollen extremity of the dental lamina argues the actual presence of a rudimentary tooth germ. It would seem likely in the present case that the region of the jaw which we are now considering will ultimately be furnished with teeth. But I have no positive facts to fix the validity of this decision. And, moreover, in view of the apparent agreement in age of all the undoubted tooth germs in both jaws, it might be argued with equal force that the terminal region is not to be invaded by teeth. In this event the swelling of the edge of the dental lamina will be an argument in support of those who see in a terminal swelling no actual prophecy of teeth in the same situation, however rudimentary those teeth may be.

The accompanying figure (Text-fig. 6) shows an early tooth follicle with the dental lamina therein and that it ends in a slight swelling. It will be noticed that the terminal swelling is continued in the same straight line as the rest of the dental lamina. The next figure (Text-fig. 7) shows a section some way farther on towards the symphysis of the jaws; and in this a slight

[1] See, for a brief summary of these views, Tomes (**6**, p. 357).

alteration is to be noted. The terminal swelling—not particularly strongly marked and so far like the first of this group of sections which has just been described—is not in the same

TEXT-FIG. 6.

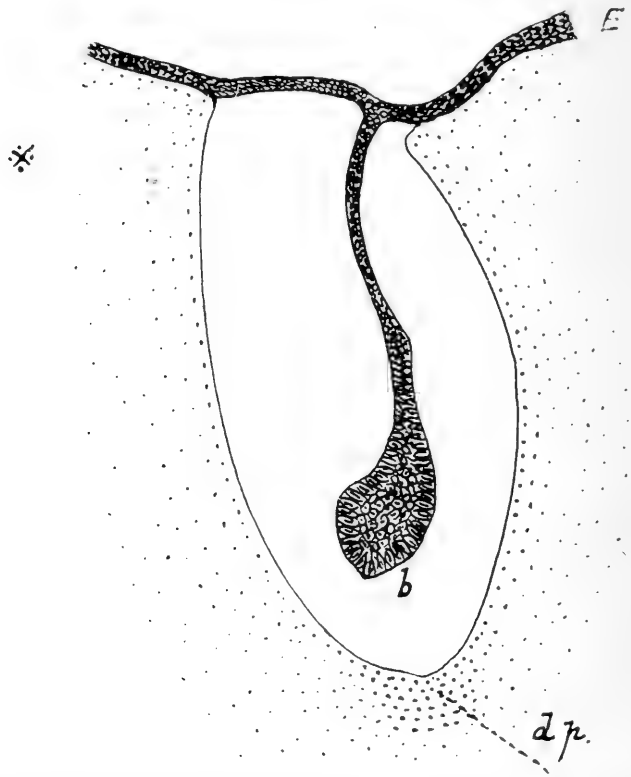


An early stage in the development of a tooth of the upper jaw. This, with Text-figs. 7, 8, is to be compared with Text-fig. 5, which represents three more or less equivalent stages in the development of the lower jaw teeth. The much greater length of the dental lamina will be noted.

straight line with the remainder of the dental lamina. It is distinctly turned lingually at almost, or in some sections quite at, right angles to the rest of the lamina. A further stage of

development of the tooth series of the upper jaw is to be seen in Text-fig. 8, which is the third of the present series. Here we have a dental lamina with terminal tooth swelling shaped—as in the last—much as a tobacco pipe, the 'bowl' being

Text-fig. 7.

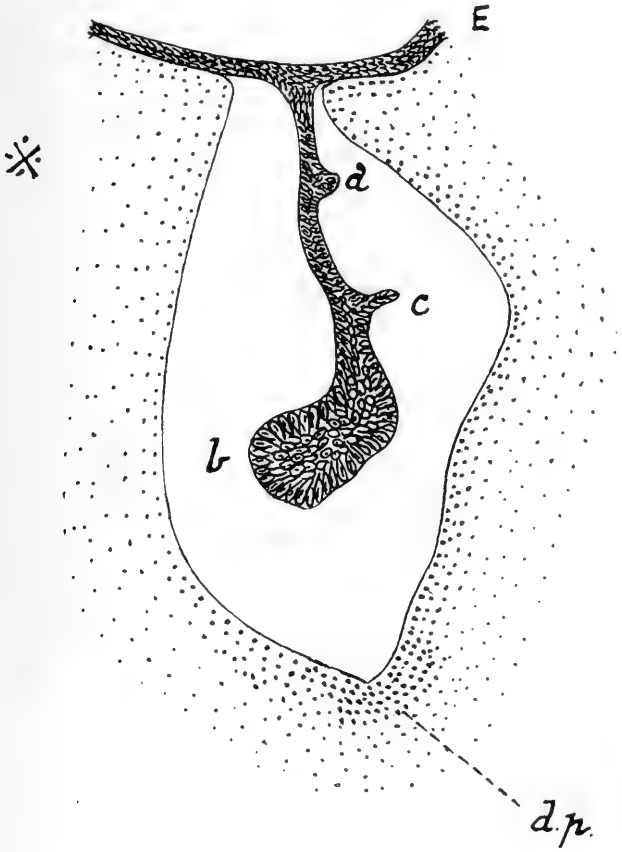


A stage farther than that represented in Text-fig. 6. The tooth rudiment, instead of being a straight continuation of the dental lamina, is slightly bent to lingual side.

turned inwards (lingually) at right angles to the 'stem'. But there is here a further addition. There are two slight processes upon the labial side which are very short but evidently correspond to the more highly developed processes whose

form and nature will be dealt with presently. We see in these tooth rudiments, early in the series, a commencing of the various characteristics of the fully developed embryonic teeth of this

TEXT-FIG. 8.



A more advanced tooth rudiment. In addition to the more marked bending of the rudiment of the permanent tooth, the commencement of the milk rudiment and the residual lamina is to be seen. In all these three figures the dental papilla is a mere thickening of the mesoderm tissue.

young foetus. It must not, however, be assumed that the labial bud-like outgrowths of the dental lamina are subsequent

in time of origin to the germ of the persisting tooth of the adult which arises from the end of the dental lamina. If this were proved to be the case the homologies of both would need another view than that put forward here. The actual time at which the end of the dental lamina becomes the rudiment of a tooth is hard to decide.

In the memoir of Messrs. Pouchet and Beauregard the authors figure [1] six sections of teeth, of which one only (Text-fig. 6) represents a tooth of the lower jaw. All of the figures are approximately alike, and an inspection of these drawings does not lead to any possibility of distinguishing between the teeth of the two jaws in this cetacean, or at any rate in an embryo of 30 cm. in length. I have already mentioned the fact that I was only able to discover anything that looked definitely like a residual lamina in the teeth of the lower jaw in my younger embryo. Nor do the figures of Pouchet and Beauregard (**4**) show anything similar beyond all doubt to the projection of enamel tissue which I have figured (see Text-fig. 4) to the labial side of the undoubted tooth rudiment. There is, however, in the former figures a thick process, forming really part of the terminal expansion of the dental lamina, to be seen ; but it will be noted that this is on the lingual side. The process does not seem to me to be distinctive enough to set it down as a residual lamina, even without going into the question of its position with regard to the main ingrowth of the dental lamina. And in any case those authors complain of the condition of their specimen, which would render it unwise to do more than call attention to such characters as are obviously not masked by the inferior histological state of this specimen.

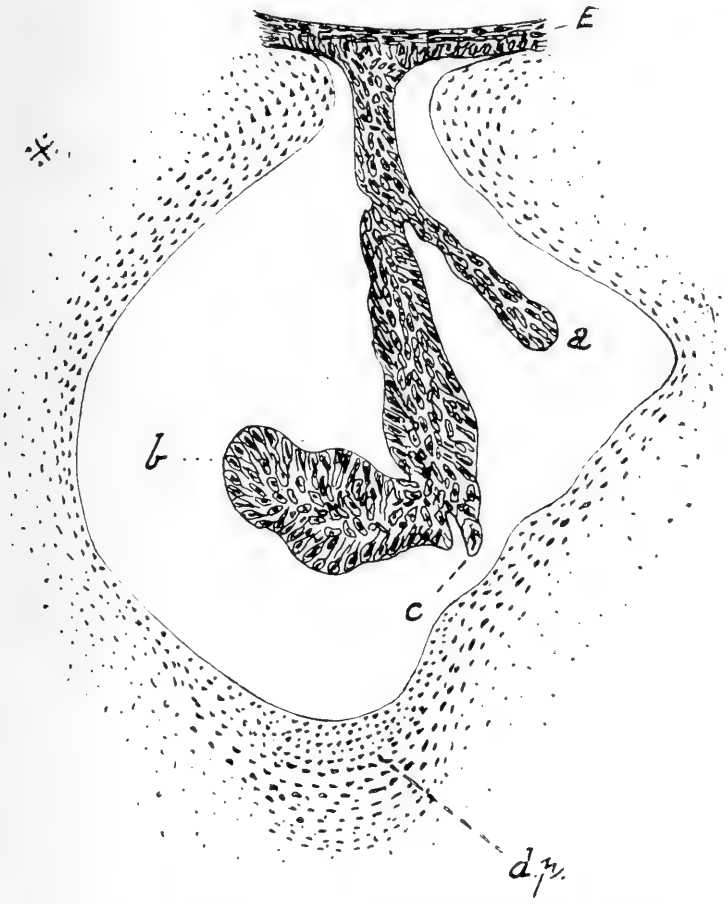
In my younger example there was nothing that could be compared to this residual lamina (if it be so) which is figured by Pouchet and Beauregard.

This, however, may easily be due to the fact that the state

[1] On Pl. vii of the memoir already quoted. I may call attention to the slight confusion in the description of Pls. vii and viii : the former is stated to be of an embryo of 1·30 m., the latter one of 0·30 m. The reverse is obviously the case.

of the tooth development was younger than that of the foetus of Pouchet and Beauregard, and that therefore a definite residual lamina could hardly be expected until a little later.

TEXT-FIG. 9.

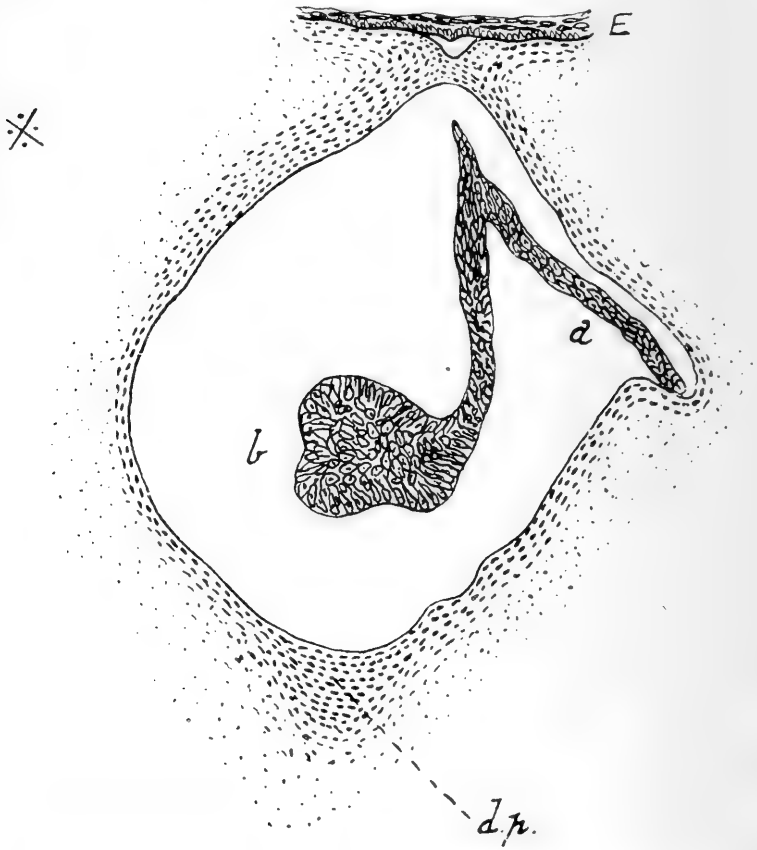


Tooth germ from upper jaw at a more advanced stage of development. The milk rudiment is long and conspicuous, expanded at the extremity.

The teeth of the upper jaw, however, though they show no prolongation of the dental lamina precisely comparable to that

to which attention has just been called, are provided with an outgrowth or outgrowths of which the nature is also difficult to decide. These are figured in Text-figs. 9, 10. They lie

TEXT-FIG. 10.



The next section but one to that figured in Text-fig. 9. The residual lamina is not to be seen.

in the sections which I have in my possession invariably on the labial side of the dental lamina. There are quite frequently, perhaps always, two of them—or even three, of which two may even arise by a common origin (Text-fig. 11) from the

dental lamina well behind its termination in a tooth germ. These outgrowths end in a swollen termination egg-shaped in

TEXT-FIG. 11.

Another tooth rudiment in the neighbourhood of those represented in Text-figs. 9, 10. It shows the peculiarity of a double milk outgrowth.

outline. The appearances which I have seen, and which are represented in the annexed figures (Text-figs. 9–11), are

obviously like those of the developing teeth of the older foetus described by Pouchet and Beauregard as regards these outgrowths. But there is one important difference which is particularly to be noted. In my foetus the outgrowths are always on the labial side ; but the figures of the two writers just referred to show that these outgrowths may be also on the lingual side, a fact which will have to be borne in mind in considering the nature of these parts of the tooth germs of the cachalot. They figure two sections in which the outgrowths are on the lingual and not on the labial side (**4,** Pl. vii, figs. 3 and 5). Furthermore, in the older foetus these outgrowths may become, or be accompanied by, a luxuriant crop of similar outgrowths (Pouchet and Beauregard, **4,** Pl. vii, fig. 1) on the same side as the single outgrowth or from the oesophageal epithelium (**4,** Pl. vii, fig. 5). I have seen nothing of the kind in my specimen. The nearest approach to it is the double outgrowth (Text-fig. 11) in my specimen. Finally, I call attention to the fact that in one section represented by Pouchet and Beauregard there are two outgrowths precisely as in several sections of the series from my younger foetus (**4,** Pl. vii, fig. 4).

I should mention also that in my specimens one of the two outgrowths only, or it may be two where there are two or three, certainly corresponds to the diverticulum or diverticula of the tooth follicle to which I have referred on a previous page. I am not sure whether this is always the first of the series where there are two or three ; for the great development of the cavity prevents an accurate fitting to the enamel organ. It should be noted also that the long ' stalk ' of these lateral outgrowths ending in a swollen extremity reproduces more exactly the form of the stalked tooth germ of which they are an outgrowth than in the case of the older foetus. This will be apparent from a comparison of Text-figs. 9–11 with those of the French authors. What are these outgrowths of the dental lamina ?

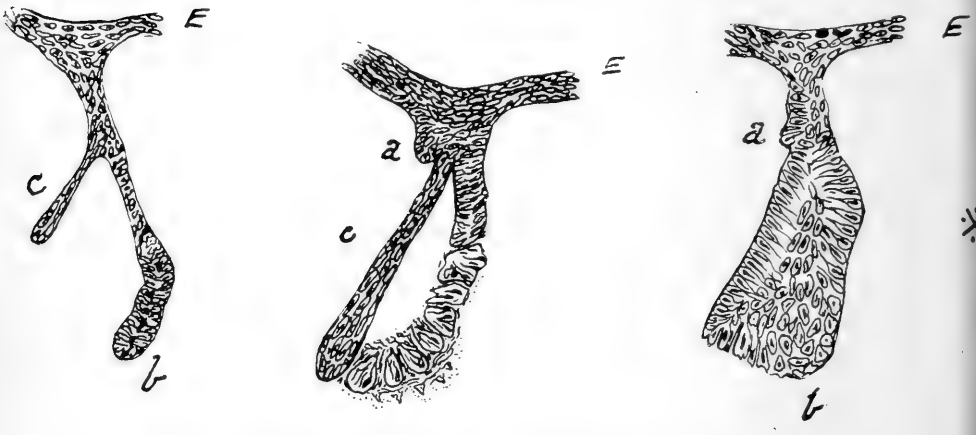
Messrs. Wilson and Hill (**7,** Pl. ii, figs. 52–4, woodcut figs. 2, 3, pp. 568, 569) figure and describe certain outgrowths from the dental lamina of marsupials (in Perameles) on the labial side, and call attention to the reference by the late Mr. Martin

Woodward (**9,** Pl. xxlvii, figs. 25 *a* and *b*) to similar outgrowths, which (in Petrogale) the latter regarded simply as tooth germs. Wilson and Hill, however, find that these outgrowths (in Perameles it must be remembered, not Petrogale) are really sheets arising from the dental lamina, and not to be confused with dental rudiments. They refer them to the 'labio-alveolar lamina' and trace them back in their origin to the oesophageal epithelium, finding sometimes no connexion at all with the dental lamina. I do not attempt to criticize, and content myself with the briefest account of this view of the labial outgrowths in question. I am convinced, however, that the structures which I describe here in Physeter macrocephalus really originate from the dental lamina, and are limited to narrow outgrowths like tooth germs. They are not to be seen throughout a long series of continuous sections; but only in three or four consecutive sections. I have dwelt upon their resemblance to the undoubted tooth germs in this cetacean; indeed, the only difficulty in the way of regarding them as tooth germs might be considered to be their origin from the labial instead of from the lingual side of the dental lamina. But in the first place these rudiments do occasionally originate from the lingual side, as I presume from the figures of Pouchet and Beauregard; and in the second place in a vertically developed dental lamina the actual side of origin seems less important than in an obliquely disposed dental lamina.

Woodward also found no difficulty in referring such outgrowths to a milk dentition, while in the case of the incisors he referred a lingual outgrowth to the permanent series. This position as to the nature of a particular rudimentary tooth is accepted and asserted by Tomes, who writes (**6,** p. 356): 'we are justified in saying that any additional specialization of the dental lamina which is situated on the lingual side of a formed germ belongs to a later generation of teeth, and conversely that any similar outgrowth of the lamina which lies on the labial side of a formed tooth germ belongs to an antecedent generation.' But it would, as I think, be pushing this

generalization too far to regard the (? same) outgrowths of this embryo of Physeter as a rudiment of a milk dentition when they appear on the labial side and of a postpermanent generation when they are processes of the lingual surface of the dental lamina. There are, however, as it appears to me from the facts represented in my sections, and from the literature briefly referred to above, considerable grounds for believing these outgrowths of the dental lamina in Physeter to represent

TEXT-FIG. 12.



Three sections at an interval from each other of one section only, nearer to the anterior end of the upper jaw than those sections represented in preceding figures, and therefore at the most complete stage of development shown in the foetus examined. They show certain differences from the more posteriorly situated sections of the upper jaw series. This chiefly affects the relative positions of the milk rudiment (*a*) and the residual lamina (*c*) to each other and to the germ of the permanent tooth (*b*) (assuming that these several outgrowths are correctly identified).

vestiges of a milk dentition which never comes to maturity, and that the permanent teeth of this cetacean are therefore to be looked upon as the equivalent of the permanent dentition of other mammals. This conclusion is not that of Kükenthal (**2**), who, however, did not (probably was not able to) refer to the memoir of Bouchet and Beauregard owing to nearly simultaneous publication.

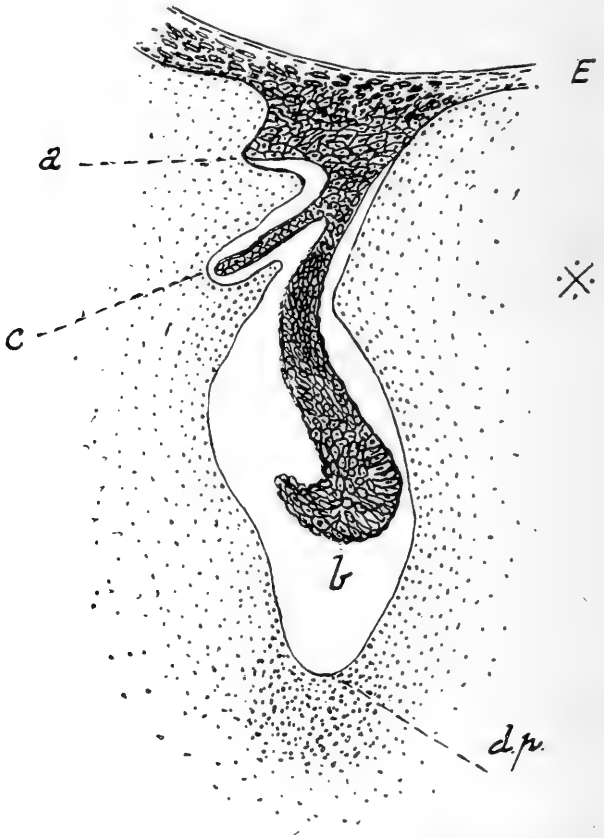
I have in a few cases (Text-fig. 12) found an apparent absence of a second outgrowth of the dental lamina. There is here a specially long single outgrowth which is less like a residual lamina than in other teeth, as may be noted by a comparison with Text-fig. 9. Nevertheless, there are some reasons for regarding this outgrowth as the representative of the residual lamina though the usually present additional outgrowth seems to be absent. I believe, however, that this structure is not absent, but present in the form of a process of epithelium of a somewhat different appearance and origin. Close to the stalk of the dental lamina, and close to its origin from the oesophageal epithelium, is a pyramidal heap of cells which is continuous with the stalk but also arises separately from the oesophageal epithelium. This pyramid has its apex directed upwards, its wider base being continuous with the oesophageal epithelium. It is not large, as I could only find it in one to three continuous sections. I think that this outgrowth may be compared with that which I have described above as existing in most of the teeth of the upper jaw. If this supposition is wrong, I cannot at the moment compare it with anything else, except perhaps as a dwarfed equivalent of the mass of independent outgrowths which Pouchet and Beauregard have figured as growing from the oesophageal epithelium in the immediate neighbourhood of a tooth germ.

It should be noted (as is shown in Text-fig. 13) that this pyramidal outgrowth is received into an excavation of the mesoderm tissue surrounding the tooth follicle, as are other parts of the developing tooth series. It is not, therefore, to be looked upon as merely an outgrowth of the oesophageal epithelium having no relation to a particular tooth germ.

It is possibly the case that the absence of the basal pyramidal outgrowth of the oesophageal epithelium to which reference has just been made is not always a reality, for in the three consecutive sections in one case (Text-fig. 12) I could find this outgrowth in only one or two sections, and there but small and rather of a rounded than conical form. It may be, therefore, that this structure has been missed or—if present—not

regarded as I regard it (i.e. as a part of a definite tooth outgrowth), in sections such as that which Kükenthal represents of the developing tooth of the Beluga. This latter figure

TEXT-FIG. 13.



Tooth germ belonging, like Text-fig. 12, to those of the anterior part of the upper jaw, but situated farther back than that represented in Text-fig. 12.

(2, p. 391, fig. 60) consists of a tooth rudiment which is older than those figured in the present paper, inasmuch as one germ is already bell-shaped, while the accompanying germ is racket-shaped like those which I figure on the preceding pages. On

the other hand the small anterior outgrowth, characteristic of so many of the teeth of the upper jaw in Physeter, may perhaps have been missed owing to its non-occurrence in the sections actually figured by Kükenthal. Or the pyramidal outgrowth may have been missed for similar reasons, as suggested above on general grounds. If this be so, then the teeth of Physeter will come more into line with those of other Cetacea, and be less abnormal than the facts described and illustrated here would imply. But again it is as likely—perhaps more likely—that the figure of Kükenthal is to be compared rather with the lower jaw teeth of Physeter.

Finally, the fact is to be emphasized that these more basally situated outgrowths of a more or less pyramidal form are only to be found among the teeth which are in the anterior part of the upper jaw; and it is only farther back that the long filiform outgrowth is to be seen. There is thus a differentiation of the upper jaw teeth into an anterior and a posterior series, which is remarkable.

Comparison of Teeth of Lower and Upper Jaw.

Having now dealt with the structure of the teeth of the upper jaw, we are in a position to compare them more accurately with those of the lower jaw. There is, I think, on the whole, reason for believing that there are differences between these two series. It is remarkable, however, that Pouchet and Beauregard figure no differences between teeth of the upper and lower jaw in their memoir. Such differences as I shall point out, or recall from the above references, from the teeth of the lower jaw, may be therefore merely a matter of age. Whether this be the case or not, the younger foetus shows the following apparent differences between the two series of teeth—those of the upper and those of the lower jaw. Apart from size, and shortness of the dental lamina in the teeth of the lower jaw, the chief—and indeed perhaps the only—difference between the two series lies in the fact that whereas the dental lamina has only one outgrowth in addition to that which forms the persistent tooth in the case of the teeth of the lower jaw, there are at

least and generally two such outgrowths in the teeth of the upper jaw. The most anterior of these (i. e. that closest to the persisting tooth rudiment) is to be regarded as the residual lamina which is alone (?) met with in the teeth of the lower jaw. Whether I am right or not in regarding the second outgrowth as a milk rudiment, it is at least a point of difference between these teeth and those of the lower jaw, where it appears to be non-existent—at any rate in the embryo which I have examined.

This again may be a matter of age. As to the residual lamina its exact likeness to that of the lower teeth is not absolute. There is this important difference. While in the case of the lower teeth the lamina is a lamina continuous from section to section, it is not so with the upper teeth ; here in fact the small process (see Text-fig. 10) which may be its equivalent disappears and reappears every two or three sections, thus indicating a series of processes and not a continuous lamina (cf. Text-figs. 9, 10). There is next to be seen a difference—perhaps more apparent than real—between the mode of origin of what I regard as the permanent tooth in the upper and lower jaws.

As has been already mentioned, the tooth germ in both arises as a thickening of the end of the dental lamina, which is continuous as a thickened edge to that lamina. In examining the whole series of teeth rudiments in the lower jaw, from their commencement at the condylar end of the jaw, the following stages may be detected. The oval thickening, shown in Text-fig. 5, persists in section after section, but gradually alters its shape to a more triangular outline, and at the pointed end away from the origin of the dental lamina the residual lamina gets gradually to be free, the rest of the thickening remaining behind, so to speak, as the actual tooth germ.

In the upper jaw the series of events is rather different. The same thickened edge appears at first, but its stalk grows longer until a racket-like structure is produced, as shown in Text-fig. 6. Instead of remaining as it is—as is the case with the lower jaw teeth—it is bent over lingually, and the residual

lamina appears as a new structure. There is, so to speak, no freeing of the residual lamina from the compound mass. Strictly speaking, therefore, there is not an exact homology between the concave surfaces of the future cup-shaped enamel cap in the two series of teeth. It is terminal in one and lateral in the other.

But it must be remembered that, as I have pointed out above, the more mature teeth, i.e. those at the symphysis end of the jaw, apparently approach the teeth of the upper jaw in this last-mentioned characteristic. The tooth germ, that is to say, is more elongated and oval. But what we are dealing with here is not the form of the growing tooth germ but its mode of origin. This is undoubtedly different in the teeth of the two jaws, as has been emphasized. But this latter consideration may be regarded perhaps as suggesting comparisons between the teeth of the two jaws which have not yet been closely examined. On the views just advanced the one outgrowth of the dental lamina beside the outgrowth which results in the tooth of the adult is a residual lamina. Its form, moreover, is highly suggestive of such an interpretation as is to be seen in Text-figs. 1 and 4; and I have put forward other facts. On the other hand, in the more mature teeth of the lower jaw the shape of the whole tooth germ is not unlike that of the Beluga as figured by Kükenthal, as pointed out on another page, and is of course also like that of the upper jaw of the present species and specimen represented in Text-fig. 10 of the present paper. Are there, in fact, after all, reasons for regarding the process which I have lettered '*c*' in the teeth of the lower jaw (Text-fig. 4) as really the equivalent of the process lettered '*a*' in the teeth of the upper jaw (e.g. Text-figs. 9, 10)?

If this is so, it is clear that a different view may have to be taken of the homologies of the two teeth rudiments than that advanced in the present paper.

For if the labial outgrowth immediately following the lingual outgrowth is a tooth germ, and not a residual lamina, it would appear to follow that it is this which is the rudiment of the tooth of the permanent dentition; and therefore that the tooth

which actually arrives at maturity is in reality of the milk dentition—a view which is held of the cetacean teeth. But to support this view requires some ' manipulation ' of the facts set forth in the above pages, and in the memoir of Messrs. Pouchet and Beauregard. It is true that the labial process in the lower jaw teeth at the symphysis extremity of the jaws is very like the tooth rudiments of Beluga, and in fact many mammals, a likeness which is increased by the fact that this outgrowth does not form a continuous lamina as does its supposed homologue in earlier sections (i. e. at the condylar end of the jaw), but decreases in successive sections and seems to disappear. This, however, need only remind us of the residual lamina (as I regard it) in the upper jaw (see Text-figs. 9, 10), which is not a continuous lamina but a series of outgrowths.

A stronger argument in favour of the view advanced here is that on the hypothesis now being considered we should have to pay no attention at all to the conspicuous outgrowths of the upper jaw, which are difficult to explain away as of no importance and without meaning. But even then, it will be noted, we are left with an undoubted difference between the teeth of the two jaws, lower and upper, which is evident in other characteristics of these organs, and which is set forth in the present section of this paper.

Peculiarities of Teeth of Physeter and comparison with those of other Mammals.

It is possible to deduce from the foregoing pages such a comparison, which does not, however, shed a great deal of light upon the zoological relationships of the Cetacea, except perhaps in one of the points raised.

It is clear, at any rate, that Physeter agrees with other mammals in having the usual two dentitions and—as in many cases—a residual lamina containing the promise or possibility of a third dentition, sometimes abnormally developed farther (e. g. in man). Furthermore, I have shown reasons for believing that the permanent dentition, in this cetacean at least, is

preceded by a milk dentition, thus conforming to the generally accepted view that (as far at any rate as the facts contained in the present paper allow of a statement) the Cetacea are the offspring of a stock already provided with the typical Eutherian dentition.

Messrs. Pouchet and Beauregard, as has been duly pointed out in the above pages, register an apparent peculiarity of the developing teeth of Physeter in the form of tufts of outgrowths from either the dental lamina or as a direct series of buds arising—not from, but beside, the dental lamina. These I have not been able to discover in the younger foetus described by myself. But in any case they are not, as I believe, to be regarded as a peculiarity of Physeter or of the Cetacea. For others have dealt with structures which are, I think, essentially similar.

Thus Leche (**3,** Pl. ix, fig. 70 ; Pl. xi, figs. 64, 90 ; Pl. xvi, figs. 140–2) figures and refers to a number of small 'tags' attached to the dental lamina in Phoca groenlandica, in the bat Phyllostoma, and in the marsupial Phascolarctus. The latter figures are copied by Wilson and Hill (**7,** Pl. xxxi, figs. 76, 77). How far such outgrowths have anything to do with tooth formation—phylogenetically for instance—the facts at my disposal do not allow of a guess. They suggest themselves as a mere state of perhaps abnormal activity.

There is a final matter, however, in which a possibly important difference from that generally observed in mammals is to be seen in the developing teeth of Physeter macrocephalus. This concerns the continuation along the jaw not only of the dental lamina but of the actual tooth germs of the permanent series only. The more usual state of affairs in mammals must be referred to first. Thus in the earliest stage (Stage II) of the embryos of Perameles studied by them Messrs. Wilson and Hill (**7,** Pl. xxv, figs. 1, 2, and woodcut fig. 1 on p. 475) represent the origin of a third deciduous incisor which grows out of the dental lamina. In the first of these sections the dental lamina is shown alone without a trace of the tooth which

appears suddenly in the next sections as an outgrowth of the dental lamina. There is no trace of any direct connexion—additional to the dental lamina—between the germ of this tooth and preceding teeth; its enamel organ is a separate outgrowth of the dental lamina. In the same way these authors represent the growth of a premolar in the same animal in a later stage (Stage III).

There are seven sections figured (A–G), each three sections apart. It is not quite clear what is the exact connexion between the second premolar (represented in figs. A and B) and the dental lamina; but in any case the latter is shown as such (i.e. without any tooth outgrowths) in figs. C–F. Then in G suddenly appears—as an outgrowth of the dental lamina—the rudiment of deciduous premolar three; the whole tooth germ —that is, the actual tooth, the dental lamina, and the residual dental lamina, extending beyond the tooth—possessing a close resemblance to one of the teeth of the lower jaw in Physeter, such as is represented in Text-fig. 1 of the present paper. There is no trace here either of any continuous lamina connecting the individual tooth germs. A final instance is shown by Woodward in a reconstruction of the teeth, deciduous as well as permanent, of Sorex (**10,** Pl. xxv, fig. 19). In this figure the teeth are seen to depend from the dental lamina only, and to be completely separate from each succeeding and preceding tooth. Quite different is the state of affairs shown in my sections of Physeter. The processes of the dental lamina which I have identified above with the milk dentition are in fact a series of processes only arising at intervals from the dental lamina. But the permanent teeth are produced at the free end of the dental lamina (in the case of the upper jaw) or from its lingual surface, leaving a continuous residual lamina (in the case of the lower jaw). In the upper jaw the position of the future teeth is shown by a bending inwards of the entire dental lamina (see Text-figs. 6–8), and a thickening of the same at intervals; but there is no projection of the rudiments of teeth beyond the edge of the dental lamina, which is continuous between the successive teeth and is only different in

the interdental regions by its less swollen character. Precisely the same is to be seen in the lower jaw, where (Text-figs. 2–4) the dental rudiments are definite outgrowths of the dental lamina, which is, however, a continuous outgrowth, being merely thinner in the interdental intervals (Text-figs. 2, 3). This will, I think, be made plain by an inspection of the figures referred to. There is to be seen, as I interpret the facts ascertained and figured by Messrs. Wilson and Hill (**8**, p. 141, Text-fig. 1), a perhaps comparable state of affairs in the developing teeth of Ornithorhynchus.

In the younger of two foetuses examined by those two anatomists the entire dental lamina of both upper and lower jaw (of one side) is figured and described. From those figures and the descriptions it is to be inferred that the enamel organs of two teeth are differentiated in the substance of the lamina of each half-jaw as a thickening of it, and not as an outgrowth therefrom—the connecting part of the dental lamina remaining unaltered between those rudiments. This is, as I think, to be compared exactly with any two succeeding teeth of the upper jaw of Physeter, where the tooth thickening is merely the lamina itself, and the unaltered lamina remains in the same way between successive tooth germs. This—as it will not be forgotten—is different from the lower jaw teeth of Physeter; for these are outgrowths of the dental lamina in the form of a continued laminal outgrowth thickened at intervals to form the actual teeth rudiments, which remain connected by the unaltered laminal outgrowth.

The fusion between successive teeth in this the youngest stage of Physeter as yet known may have some bearing upon the theory of tooth origin, i. e. as to whether separate teeth, like those of Physeter, are primitive, or show signs of the breaking up of a complex tooth series. Are the unions between the individual teeth a promise of a later concrescence, or the remains of a separation of the cusps of a complex tooth? I have not, however, been able to ascertain any further facts which bear upon this most interesting topic. I can, for example, see no gaps which might mark the boundaries of pre-existent

multicuspidate teeth, or, on the other hand, show by this arrangement the specialization of sets of separate cusps—a promise of separate multicuspidate teeth in the future. I may remark, however, that these connexions between successive teeth may possibly be related to the fact that in the adult the individual teeth are connected by a tough gum which comes away with them when they are forcibly removed from the bony trough in which they lie.

Résumé.

As to the facts contained in the above pages they are really summed up in the illustrations which accompany the letterpress.

There are tooth germs from end to end of both upper and lower jaws, except at the posterior end of the series, where the dental lamina is not specialized in the upper jaw for some little distance. The teeth, in fact, are developed from behind forwards.

The dental lamina extends into the subjacent mesoderm at absolutely right angles to the horizontal plane of the head. The dentine papilla is represented only by a condensation of nuclei in the mesoderm ; it does not yet extend into the cavity surrounding the enamel organ.

The tooth rudiments of the lower jaw are borne upon a shorter dental lamina than those of the upper jaw. They consist of the dental lamina which is prolonged beyond the tooth germ as a ' residual lamina ' and of the tooth germ arising from the lingual surface of the lamina.

The tooth rudiments of the upper jaw are borne upon a longer dental lamina than those of the lower jaw. They consist of the dental lamina which is prolonged beyond the tooth germ as a ' residual lamina ' ; but this consists, not of a continuous lamina as in the lower jaw, but of a series of processes one to each of the successive tooth germs. In addition to these there is always a second outgrowth of the dental lamina on the labial side (sometimes doubled) lying nearer to the origin of the dental lamina, which do not form a continuous lamina but are separate outgrowths corresponding

each (or each two) to a tooth germ. In the anterior half of the jaw the tooth rudiments also possess two labial outgrowths, of which the first, i.e. that nearest to the tooth germ, is longer than in the posterior series of tooth germs, while the second, i.e. that nearest to the origin of the dental lamina, arises partly from the dental lamina and partly from the oesophageal epithelium, and forms a short pyramidal process. I have not seen intermediate conditions.

There is also a differentiation into two series of the teeth of the lower jaw, but the anterior teeth seem to differ merely through greater age.

In both the teeth of the upper and of the lower jaws the permanent tooth rudiments (i.e. those outgrowths from, and on the lingual side of, the dental lamina) are not isolated outgrowths of the dental lamina but are connected successively by a continuous outgrowth of the dental lamina, as follows :

In both lower and upper teeth the individual tooth germs arise from a marginal thickening of the dental lamina, but the subsequent course of the development differs in the two series. In the teeth of the lower jaw the thickening is shifted to the lingual side of the dental lamina by the freeing from it of a residual lamina on the labial side. In the upper jaw the corresponding thickening at the distal edge of the dental lamina is bent over to the lingual side, while a later formed residual lamina continues at intervals the straight line of the dental lamina ; thus the tooth germ has grown to lie laterally instead of being formed in situ.

As to the homologies of the various regions of the embryonic teeth, it has been attempted to show that there are reasons for believing that the teeth of the adult correspond to the permanent dentition of other mammals, that there are also rudiments of precedent milk dentition, and that a residual lamina succeeds the rudiments of the permanent dentition.

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The 'Segmentation Cavity' of the Egg of the Frog.

By

Alexander Meek.

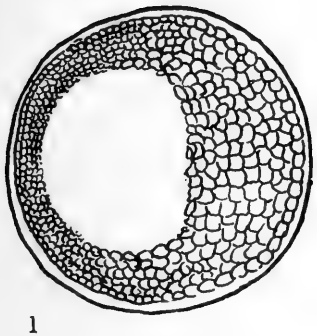
With Plate 1 and 6 Text-figures.

The egg of the frog, Rana temporaria, starts its career of segmentation as does that of Amphioxus, but after the one-celled blastula stage a delamination of cells takes place, with the result that the wall becomes multicellular. The cavity is excentrally situated, the anterior wall being thin and the posterior wall thick. The latter consists of endoderm, and while internally it is impossible to point to a distinct demarcation between ectoderm and endoderm, externally an equatorial zone is defined which marks the limits of ectoderm and endoderm, and this is emphasized when the zone becomes a ring of proliferation indicating the beginning of the activities of the blastopore. The proliferation is followed by the appearance of a dorsal groove, the dorsal lip of the blastopore. An invagination takes place, confined, however, to the dorsal aspect of the embryo, and a horizontal slit-like cavity is produced which penetrates anteriorly above the segmentation cavity and is extended posteriorly as the dorsal lip of the blastopore advances over the large cells of the yolk-plug. This cavity is the enteron, and while it is forming the original cavity, which is still called the segmentation cavity, acquires a cup shape, the anterior wall being definitely resolved into ectoderm, and the endodermal margin of the cup gradually narrows and ultimately fuses, so that the cavity becomes enclosed in endoderm. It is almost universally believed that the segmentation cavity is compressed by the expanding enteron and that it finally disappears antero-ventrally in the endoderm. But

A. M. Marshall, O. Schulze, and O. Hertwig have all drawn attention to cases of fusion between the two cavities, cases which indicate that sometimes the segmentation cavity may become converted into the forward part of the enteron. Such have been regarded as abnormal, and upholders of the orthodox view of the procedure which takes place within the egg of the frog explain them as being due to faulty preservation or preparation. On the other hand, the accepted view is not supported by a convincing series of figures. A stage is shown which presents the segmentation cavity and the enteron. The next stage is one which displays the enteron occupying the place of the segmentation cavity and the latter as non-existent or as a small cavity of the endoderm ventral to the enteron. Even if such a change actually takes place it is plain that the subsequent excavation and thinning of the ventral and lateral walls of the endoderm restores the cavity to the enteron, and the process could be explained as a variation of that produced by an earlier fusion of the two cavities. With this in mind it may be said, to begin with, that, if the segmentation cavity sometimes produces the anterior part of the enteron and usually disappears as a cavity enclosed by endoderm, the nomenclature of the cavity is wrong and misleading.

With a view to obtaining evidence of what actually occurs, I requested the laboratory steward, D. C. Geddes, to prepare serial sections of a large number of embryos. The eggs were gathered in the neighbourhood of Newcastle. They were in the process of segmentation, and those preserved six to seven days after collection furnished all stages from the end of the period of delamination to that in which the yolk-plug is reduced to a small spot at the posterior end of the egg. These I have now examined.

I was quite prepared to find that the first-formed enteron, the so-called segmentation cavity, was actually replaced by the secondary or neurenteric enteron, but no such example presented itself. In all cases I found that the segmentation cavity is converted by delamination into a primitive enteron distended by dissolved yolk-products, and that it is put by fusion into communication with the enteron formed underneath the



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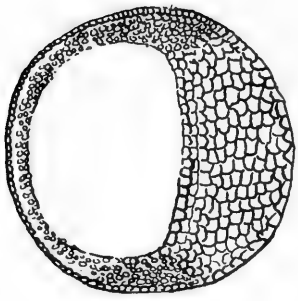

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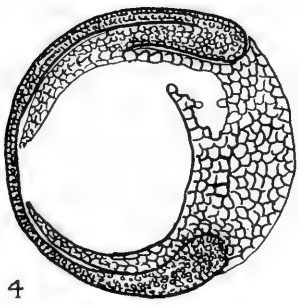

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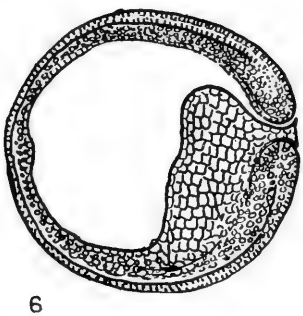

6

TEXT-FIGS. 1–6.

Diagrams of sagittal sections to illustrate the stages of development concerned.

dorsal lip of the blastopore. The changes which take place are illustrated by photographs of sections.

Passing over the stage of completed delamination, figs. 1 and 2 (Pl. 1) are taken from slides 4 and 12 of a series of horizontal sections of an embryo with a blastopore and invaginated dorsal lip (Text-fig. 2). The primitive enteron is, as will be observed, abstracting food material from the yolk-cells, and the yolk-cells are being changed and broken down in the process. As a result, the anterior wall is thinner than in the previous stage. The blastopore is ushered in as an area of proliferation uniting ectoderm and endoderm. It is later that the groove appears dorsally, and still later that it extends ventrally to form a circular lip. During the earlier period the activities of the blastopore are mainly confined to the dorsal lip, and in that region the blastopore is concerned not only in producing the secondary enteron but in yielding mesoderm which presses outwards on each side separating ectoderm from endoderm.

Fig. 3 (Pl. 1) is a typical sagittal section of a somewhat later stage (Text-fig. 3), but it is scarcely necessary to point out that in this series the anterior end has unfortunately become flattened. The anterior wall may now be said to consist of ectoderm. In association with the invagination of the dorsal lip of the blastopore a margin of endoderm projects anteriorly, providing for the forward extension of the enteron. The margin, however, also advances all round the primitive enteron, thus producing a cup-shaped cavity of the endoderm. The condition defines more clearly the layers and may be described as a reversion to a segmentation cavity under the influence of the blastopore. But, as has already been stated, this condition of the primitive enteron is a temporary one.

Figs. 4 to 8 (Pl. 1) are from successive slides of a series of sagittal sections bearing the numbers 4 to 7 and 9 (Text-fig. 4). These show that the invagination which produces the secondary enteron is confined to the dorsal aspect of the embryo, and that the margins of the cup of endoderm are rapidly approaching and fusing at the anterior pole of the egg. But, more importantly, they indicate that the invagination of the secondary endoderm is accompanied by a breaking down

of the endoderm intervening between the primitive enteron and the secondary enteron. The intervening cells are thereby reduced to a single layer. I have also a series of transverse sections of this stage. This process creates an extension of the primitive enteron and provides for the expansion of the secondary enteron. The expansion takes place by the thin floor being depressed into the primitive enteron, and during the process the intervening cells are absorbed.

Figs. 9 to 14 (Pl. 1) are from a series of transverse sections of the stage (Text-fig. 6) after the fusion of the two cavities has taken place, and are photographs of sections from slides 3, 4, 5, 6, 8, 9. It will be seen that they bear no evidence of the loss of the primitive enteron; the cavity is practically as large as ever. Incidentally they show that the notochord is continuous with the rest of the mesoderm, and that it is formed by splitting off from the lateral wings of mesoderm. These latter completely encircle the wide anterior region of the enteron and already begin to exhibit the splanchnocoel.

But the process of fusion is better illustrated by the transverse sections of the younger stage, figs. 15 to 18 (Pl. 1), from slides 11, 12, 13, 15—a stage intervening between Text-figs. 5 and 6. In the first of the sections photographed, fig. 15 (Pl. 1), the secondary enteron is seen above, not far from the dorsal lip, and the primitive enteron below. The succeeding figures demonstrate that the two cavities are converted by fusion into one cavity. It will be observed that even at this stage the mesoderm has made rapid progress in enveloping the endoderm, and not by a process of delamination.

I have preparations besides which show in sagittal section the fusion depicted especially by Schulze and Hertwig (Text-fig. 5). These, with the transverse sections submitted, prove that the thin wall of cells which intervenes between the two cavities is depressed and absorbed during the process. Some degree of variation is possible as to the completion of a process which I am led to regard as the normal one. If it is not the normal process then a demonstration to the contrary is called for.

The morphological aspects of the results I do not intend to discuss. But I may be allowed to point out that my observations

demonstrate that the frog and its allies come into line with the meroblastic Amphibia and with the rest of the terrestrial Vertebrata. It is evident that the segmentation cavity of Petromyzon is not, for the reasons given above, a true segmentation cavity, and evidence is required as to its fate; and this raises the question of the interpretation of the segmentation cavity in other aquatic vertebrates. Even amongst the Urochordates there are examples of the formation of the enteron from an excavation of endoderm.

Summary.

The examination of series of sections of frog embryos has shown that in all cases the 'segmentation cavity' is converted, by fusion with the secondary or neurenteric enteron, into the forward part of the enteron.

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EXPLANATION OF PLATE 1.

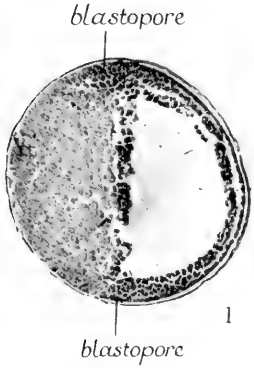
Figs. 1 and 2.—Horizontal sections of an early stage (Text-fig. 2).

Fig. 3.—Sagittal section of typical stage indicating an early phase of the invagination of the dorsal lip (Text-fig. 3).

Figs. 4–8.—Sagittal sections of a later stage, wherein the excavation of the cells intervening between the primary and secondary cavities has reached an advanced stage (Text-fig. 4).

Figs. 9–14.—Transverse sections of a stage after fusion of the cavities (Text-fig. 6).

Figs. 15–18.—Transverse sections of a younger stage than the preceding to illustrate the process of fusion (Text-fig. 5).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blastopore

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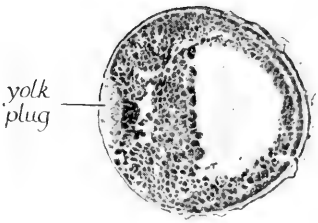
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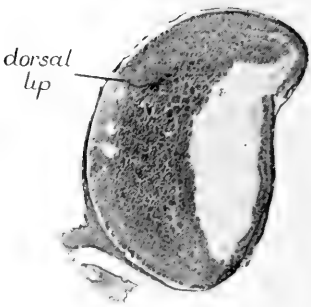
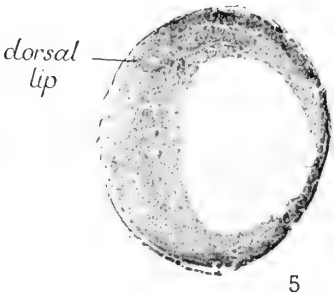

dorsal lip

3


enteron

4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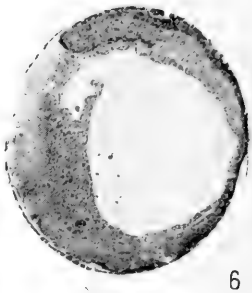

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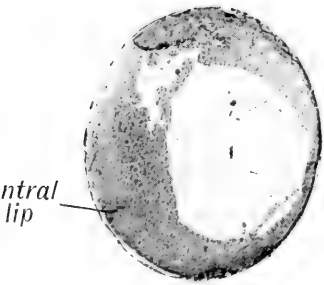
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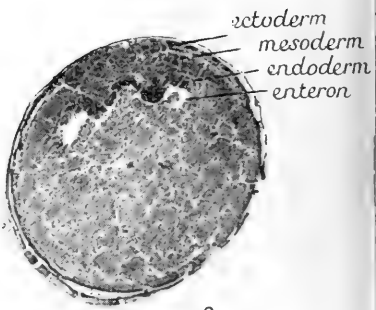
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mesoderm
endoderm
enteron

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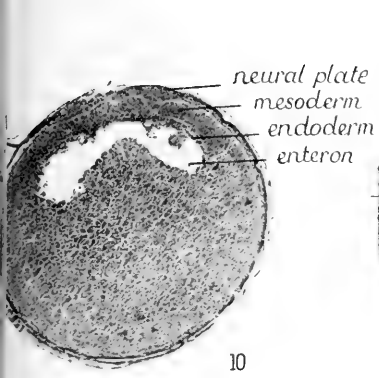

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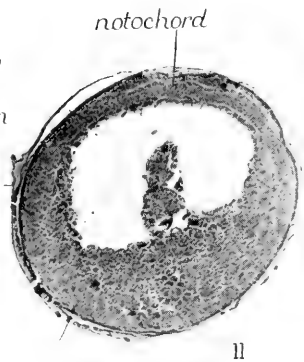

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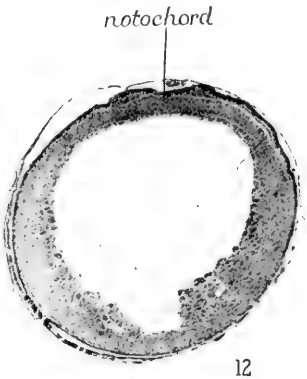

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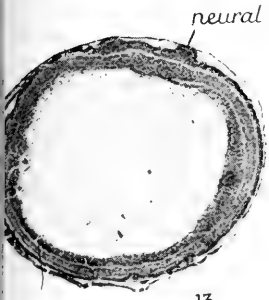

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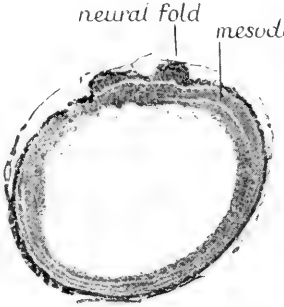

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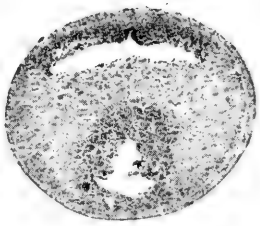

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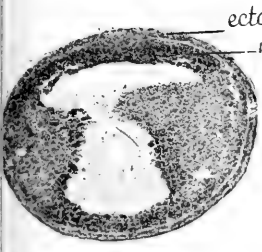

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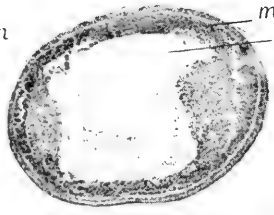

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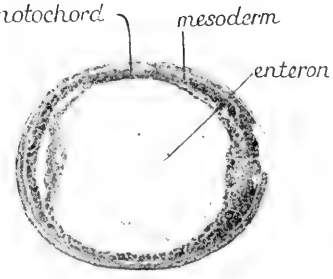

18

Nuclear Divisions in Amoeba proteus.

By

Monica Taylor, S.N.D.

With Plate 2.

No definite answer, so far as I have been able to ascertain, has yet been given to the question that is inevitably provoked by the spectacular rapidity with which amoebae increase in number in laboratory cultures, viz. ' How does the nucleus divide ? ' In attempting to answer this question it is important to remember, a fact which five years' experience in cultivating amoebae on a large scale has taught, that the most luxuriant cultures are subject to periods of depression. Unless the necessary precautions be taken (**5**) they die down and remain dormant for a varying period of time, then gradually recover.

The amoebae which first appear in such ' recovering ' cultures are minute. When they have attained full size they multiply at a great rate, luxuriance being eventually again established in the culture. Divisions of the nucleus by fission must therefore be distinguished from nuclear divisions connected with the ' sporulation ' process.

It will be useful to recall at the outset the ' resting ' nucleus and its behaviour in a living amoeba. In the following account the descriptions given by Doflein (**3**), Schaeffer (**4**), Carter (**1, 2**), having been verified, are embodied for the sake of viewing the subject as a whole.

The nucleus is a large discoid body which is rolled over passively by the streaming endoplasm. Its membrane is strongly marked. Immersed in the nuclear sap is (i) a centrally placed, plate-like, conspicuous karyosome which lies in a highly vacuolated achromatic substance, and (ii) the chromatin. The chromatin, according to Doflein (**3**) and Schaeffer (**4**), is

confined to the blocks situated normally just under the nuclear membrane (Pl. 2, fig. 1). The karyosome being plate-shaped may appear circular (Pl. 2, fig. 1) or band-shaped (Pl. 2, figs. 2, 3). For convenience of reference these two views of the karyosome will be referred to briefly as the karyosome in 'plan' and in 'elevation' respectively. It consists of two substances: (1) a ground substance which does not stain so deeply as (2) another substance in the form of small blocks or rods which stains like chromatin, but which, according to Doflein, takes no part in the formation of the chromosomes which appear on the spindle of the nucleus when it divides by karyokinesis. These karyosome blocks have a more or less round outline when viewed in the plan of the karyosome. They appear more rod-like in the elevation view of that structure (Pl. 2, fig. 2).

As the nucleus is rolled about by the endoplasm the karyosome presents not only alternate views of its plan and elevation positions, but a series of positions intermediate between these two extremes. Another complication now to be described gives the nucleus a successive variety of appearances when it is examined in the living condition. The rolling over brings about a redistribution of the nuclear sap. Now the more solid nuclear contents, i.e. the karyosome, the chromatin, and the achromatic network, are very flexible. They easily become temporarily folded. Most frequently two such folds are formed from the poles towards the equator, one fold being nearest the proximal surface, the other nearest the distal surface, of the nucleus under examination. This has the effect of making the nuclear contents appear lenticular or dumb-bell-shaped (Pl. 2, fig. 4). This appearance, which is exceedingly common, is succeeded by the normal (Pl. 2, fig. 1) as the nucleus gradually steadies itself, and becomes stationary, only however to begin a new series of convolutions as it is played upon by the streaming endoplasm. In a well stretched, fairly young and active amoeba which has got a good grip of the substratum these successive changes in appearance (cf. Pl. 2, figs. 6 and 7) of the nuclear contents can easily be observed. In order to

study the phenomenon in 'fixed' specimens a fairly large number of young amoebae should be placed on a slide in a small drop of water and left in a damp chamber until the animals begin to creep about on the glass. The fixative should then be dropped quickly on to them, when, a cover-slip having been provided, they can be examined microscopically (aceto-carmine is very useful for this purpose) and the whole range of positions studied. (The behaviour of a nucleus in a living amoeba is highly reminiscent of the tossing of a pancake on Shrove Tuesday !)

For the purpose of this investigation, in addition to an unlimited supply of living amoebae for examination and of specimens treated with aceto-carmine, whole cultures were fixed in corrosive alcohol, in warmed corrosive acetic, and stained in Delafield's haematoxylin, carmine,[1] light green, etc., and the individual nuclei studied whole or sectioned. I am greatly indebted to Sister Carmela Hayes for much assistance in making preparations.

In stained preparations the 'resting' nucleus is a much more striking object when seen with the karyosome in elevation than it is when the latter is viewed in plan. A newly formed daughter nucleus can easily be picked out from a number of older nuclei by the more brilliant colouring exhibited by the karyosome, especially in aceto-carmine preparations.

Doflein states that the division of the nucleus is effected when the amoebae have temporarily drawn in their pseudopodia and have assumed a spherical shape, having freed themselves from the substratum. They look extremely opaque in this condition. It is a common sight to see several of these opaque-looking spheres in any good culture of amoeba. In one instance, Sister Carmela, who was tending the cultures during my absence, reported to me that practically every amoeba in a particular culture (and there must have been many thousands) had rolled itself into a ball.[2]

[1] A modified formula of Picro-magnesia-Carmine suggested to me by Dr. J. S. Dunkerly was also applied.

[2] The subsequent history of this culture, which shortly afterwards

I have repeatedly verified the observation that such ball-shaped amoebae are multinucleate. The commonest number of nuclei is four, but binucleate, six- or eight-nucleate conditions occur. The extreme rarity of mitotic figures in the many preparations he examined caused Doflein (**3**) to leave it an open question as to whether or no there was another method of division. The results of my investigation lead me to conclude that the mitotic figures given by Lucy A. Carter (**2**) for A. proteus (var. X, Carter; A. dubia, Schaeffer), and by Doflein (**3**) for A. proteus (var. Y, Carter) are connected with sporulation, and 'encystment'. The ordinary vegetative multiplication, i.e. fission divisions, are effected by a sort of 'budding' of the nucleus. A search through innumerable specimens at all times of the year, and at all hours of the day and night, in many cultures of amoeba, some of them so luxuriant that the bottom of the glass trough (8 in. in diameter) appeared whitish because of the enormous numbers of amoebae lying on it, has failed to give one single example of a mitotic figure. It is the common experience of cytologists that wherever an organism is developing rapidly mitotic figures are sure to be forthcoming if such tissues be examined by a suitable technique. Hence it would seem that the published figures of mitosis in A. proteus belong to the sporulation cycle of the life-history.

In most of the spherical amoebae I have examined the separate nuclei have been close together. If, however, the spherical specimens be examined as soon as they have assumed this form (and this is quite a simple matter where large numbers of cultures are accessible) appearances similar to those drawn in figs. 8, 9, and 14 (Pl. 2) are to be found. Here the daughter nuclei are clearly not completely formed, and the mother nucleus is clearly not dividing by ordinary mitosis. In short, a series of preparations may be made showing the gradual conversion of a 'lobed' large nucleus into four daughter products. The

underwent a period of depression and is now (July) full of small amoebae, would seem to indicate that many of the amoebae must have been preparing to encyst.

spherical opaque amoebae vary greatly in size, the mother nucleus giving rise to only two daughter nuclei in the smaller spheres. To facilitate description of this non-mitotic method of division, I have used these smaller amoebae, and I have drawn a series of stages with the karyosome (1) in elevation (Pl. 2, figs. 15–19) and (2) in plan (Pl. 2, figs. 25–31 and 34), the product of the division being two daughter nuclei in each case.

The process is not a simple halving of the nucleus into two hemispheres which then roll themselves up into discoid figures, but seems to be as follows : a patch of chromatin blocks divide, each block into two (Pl. 2, figs. 10–13). This process gradually extends to the whole of the chromatin. Simultaneously the karyosome also divides into two, each daughter karyosome assigning itself to one daughter set of chromatin blocks. The outer set of daughter products sloughs off gradually from the inner by the increase of nuclear sap and by the formation of the necessary additional areas of nuclear membrane and two daughter nuclei thus result. The distance between these gradually increases. Very frequently the daughter nuclei in one preparation show different views of the karyosome (Pl. 2, figs. 32, 33). Very complicated figures necessarily result during such a process of division, a binocular eye-piece being essential for the complete elucidation of the preparations in some cases ; especially is this true when the mother nucleus is undergoing rapid successive divisions into four or more nuclear products (Pl. 2, fig. 14).

If a spherical individual in which the daughter nuclei have not been separated be placed on a slide and made to assume the expanded position, the nucleus is seen to resemble the ' folded ' nucleus described by Schaeffer (**4**) as being characteristic of old or large specimens of A. proteus. In the light of the above interpretations of fission divisions these folded ' lobed ' nuclei are due to the fact that some external physical condition has interfered with the amoeba when division was in process, the so-called ' lobed ' nuclei being incipient multinucleate structures. Now the amoebae are easily disturbed. They are

extremely sensitive to change of position, temperature, or light. When, therefore, a spherical amoeba is removed from its culture for purpose of examination it quickly accommodates itself to its new surroundings, and crawls away without completing the division of its nucleus. Very complicated appearances are due to such nuclei rolling about in an active amoeba. With care to preserve their cultural conditions, however, spherical amoebae can be removed and isolated, when they are seen to give rise to daughter products.

These 'lobed' nuclei, however, are formed before the amoebae become spherical. They are very early stages in the division process, for although the actual separation into daughter nuclei takes place when the amoebae are spherical, the division is initiated at a much earlier stage. Most of the large individuals in a watch-glass culture will be found to have a 'lobed' nucleus. If they become spherical this is converted into a varying number of daughter nuclei, unless, as described above, external conditions disturb the process.

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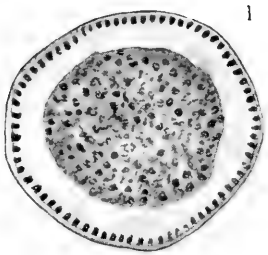
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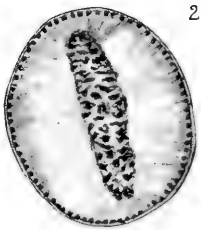
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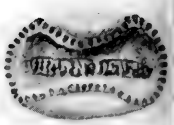

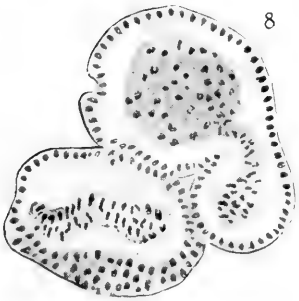
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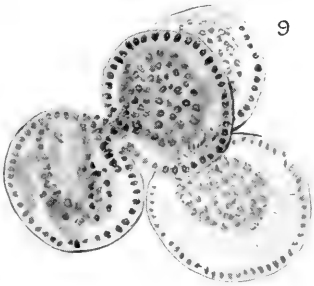
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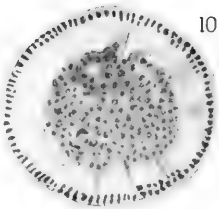
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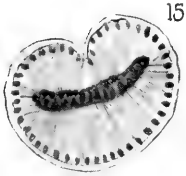
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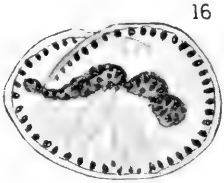
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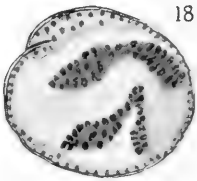
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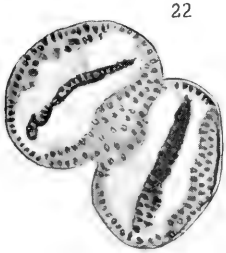
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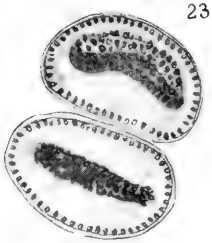
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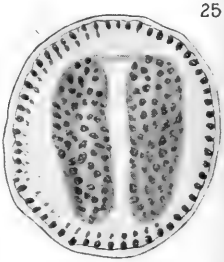
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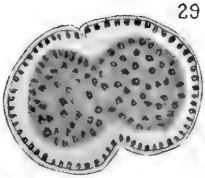
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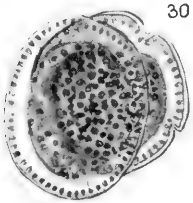
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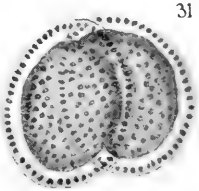
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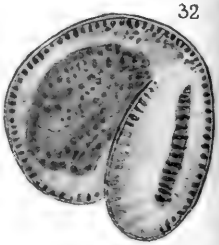
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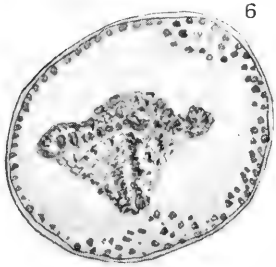
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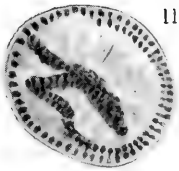
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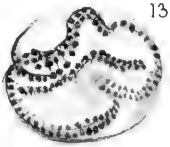
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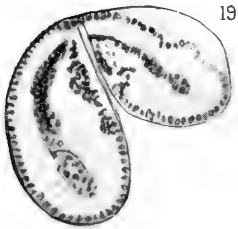
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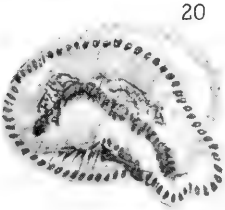
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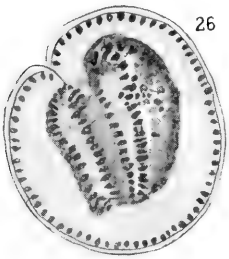
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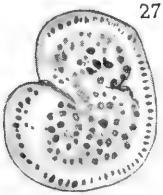
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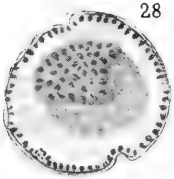
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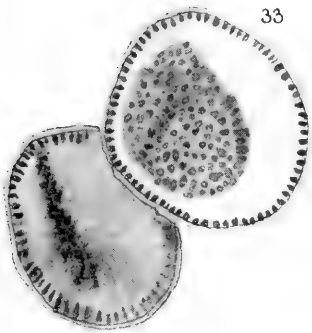
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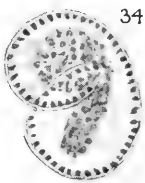
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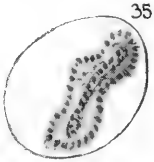
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EXPLANATION OF PLATE 2.

Illustrating Dr. Monica Taylor's paper on ' Nuclear Divisions in Amoeba proteus '.

All figures were drawn with the Abbé camera.

With the exception of figs. 4 and 8 the material for the drawings was fixed in warm corrosive acetic, and stained in Delafield's haematoxylin.

Fig. 1.—Resting nucleus. Karyosome in plan.

Fig. 2.—Resting nucleus. Karyosome in elevation.

Fig. 3.—Resting nucleus. Karyosome in elevation (daughter product of newly divided nucleus).

Fig. 4.—Resting nucleus. ' Biconcave ' form of contents (aceto-carmine).

Fig. 5.—Resting nucleus. Karyosome contracted.

Fig. 6.—Resting nucleus. Karyosome rolling over.

Fig. 7.—Resting nucleus. Nuclear contents assuming ' biconcave ' form.

Fig. 8.—Aceto-carmine preparation of nucleus from a spherical amoeba.

Fig. 9.—Permanent preparation of nucleus from a spherical amoeba. Two pairs of daughter nuclei not completely separated.

Fig. 10.—Early stage in division of nucleus. Increase in number of chromatin blocks.

Figs. 11 and 12.—Early stage in division of nucleus. Increase in number of chromatin blocks. Division of karyosome.

Fig. 13.—' Lobed ' nucleus—assuming ' biconcave ' condition. Chromatin and karyosome dividing. Nuclear membrane beginning to divide.

Fig. 14.—Complicated nucleus consisting of four incipient daughter nuclei.

Fig. 15.—Later stages in division. One daughter nucleus with its component chromatin blocks and karyosome is freeing itself from the other. Karyosome in elevation.

Figs. 16–19.—Later stages in division. Karyosome in elevation.

Figs. 20 and 21.—Later stage in division. Karyosome in elevation.

Fig. 22.—Daughter nuclei almost separate.

Fig. 23.—Daughter nuclei completely separate, but lying close together in the amoeba.

Fig. 24.—Daughter nuclei rolling away from each other. Karyosome partly in ' plan ', partly in ' elevation '.

Figs. 25 and 26.—Stages in division of karyosome (plan).

Figs. 27 and 28.—Later stages in division of nucleus. 'Plan' view corresponding to 'elevation' view of karyosome shown in fig. 17.

Fig. 29.—Stage in 'plan' corresponding to fig. 19. 'Elevation' view of karyosome.

Figs. 30 and 31.—Daughter nuclei separating. Plan view of karyosomes.

Fig. 32.—Daughter nuclei showing plan and elevation view of karyosome.

Fig. 33.—Ditto, but slightly older.

Fig. 34.—Slightly later stage than in fig. 29.

Fig. 35.—Nuclear contents greatly contracted—probably due to fixation.

On Amphilina paragonopora, sp. n., and a hitherto undescribed Phase in the Life-History of the Genus.

By

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With Plates 3–5.

Contents.

[1] A large portion of the work involved in this inquiry was done in India, during my tenure of the Chair of Zoology at the Muir Central College, Allahabad, U.P. (Indian Educational Service).

Part I.

The Structure and Bionomics of Amphilina paragonopora, sp. n.

The new species of Amphilina which forms the subject-matter of this paper constitutes the fifth[1] known to exist and the third to be described in detail. I have supplied a detailed description of the anatomy of this new species because it appears to me that the descriptions of Amphilina supplied by previous authors leave much to be desired in certain respects, because the present species differs in several particulars from A. foliacea and A. liguloidea, and because it is necessary to elucidate the exact nature and mode of function of the proboscis apparatus—the proboscis, the enormous attached proboscis muscle (or bundle of gland-ducts according to one interpretation), and the conspicuous giant cells which Salensky termed ' problematische Zellen '—an apparatus which has been much misdescribed and misunderstood. I am also able to describe an important phase in the life-history of this form, which has not been mentioned in connexion with any other species of Amphilina or, indeed, Cestodaria, so far as I am aware.

I may add that A. paragonopora is the second species of Amphilina discovered in or near India. The first (A. magna) was found by Southwell (**8**) in the coelom of a marine fish (Diagramma crassispinum) from the coast of Ceylon, and in general shape and structure is apparently similar to A. paragonopora, and it is to be regretted that Southwell's sole description of its genitalia is so vague. A. paragono-

[1] The other four are A. foliacea, Rudolphi, 1819 (vide Salensky, **7**; Cohn, **1**; Hein, **2**; Pintner, **6**; Wagener, **9**) and A. neritina, Salensky, 1874 (**7**), from Europe; A. liguloidea, Diesing, 1850 (vide Monticelli, **5**; Janicki, **3**), from Brazil, and A. magna, Southwell, 1915 (**8**), from Ceylon. The anatomy of A. neritina (assuming it to be a distinct species) is apparently very similar on the whole to that of A. foliacea, only differing in small details, and Southwell has only roughly indicated the general disposition of the genitalia in A. magna.

pora, as will be stated, is found in a fresh-water fish in the Ganges and Jumna.

(*a*) Habitat and External Characters.

Amphilina paragonopora is parasitic in the body-cavities of the two closely related species of Siluroid, Macrones aor and M. seenghala[1]—the common 'Tingra'—found in the Ganges and the Jumna at Allahabad and elsewhere in the United Provinces and in the Panjab, and supplied to the bazaars as food for the lower caste natives. In size the parasite varies greatly according to its stage of growth and the degree of contraction of the body musculature. The largest specimens (two) I have found measured when living (and uncontracted) 280 mm. in length (when preserved the specimens contracted to 170 mm.) and 5 mm. in maximum breadth; on the other hand, the smallest specimens only measured 10 mm. or 11 mm. in length and 1 mm. in breadth. Specimens measuring 60–70 mm. in length are fairly common; occasionally specimens are found which are slightly broader in proportion to their length than the above measurements indicate. The parasites are thus distinctly ribbon- or strap-shaped (Pl. 3, fig. 1), the thickness being one-fifth to one-tenth of the breadth. There is no scolex, but the 'anterior'[2] end tapers slightly, is either rounded or pointed (according to the state of contraction), and carries a short muscular globular or ovoid evaginable organ (Pl. 3, fig. 1, *k*)—the 'proboscis'—which is not a sucker (acetabulum) and is never used for

[1] The chief distinction between Macrones aor and M. seenghala is that in the latter the maxillary barbels are much shorter than in the former. These fishes are said to grow to a length of six feet, but my largest specimens did not exceed three feet and the majority were between one foot and two feet in length. I may add that the food of these fishes consisted of insects (chiefly Orthoptera) and small fishes, and an examination of the tissues of this food fauna may reveal later the intermediate host or hosts of Amphilina paragonopora.

[2] I apply the term 'anterior' to the mobile end of the body possessing the proboscis in a descriptive and not in a comparative morphological sense.

attachment. The ' posterior ' extremity of the body is similar in form to the anterior, save that at the extreme end a well-marked bay or semicircular inlet is situated, in the centre of which is a contractile papilla which bears the three separate openings of the ductus ejaculatorius, terminal excretory duct, and vagina (Pl. 3, figs. 1 *l*, 11, 12). In colour the parasites vary from a creamy-white to orange-yellow (the usual colour being a distinct yellow), and are almost always identical in tint with the masses of fat attached to the mesentery of the fish they infest.

The above statements concerning the dimensions and shape of the parasites are based on the appearance of the parasites when the body-cavity of the fish is first opened. So long as the parasites are not disturbed they exhibit no active movements, save perhaps a to-and-fro motion of the protruded ' proboscis ' which is only visible under the microscope (Pl. 4, fig. 15), but if a parasite be removed from the body-cavity and placed on a glass slide the body almost immediately shrinks to about one-half its former length and also becomes pinched by two, three, four, or more deep transverse constrictions [1] which usually travel down the body antero-posteriorly, new constrictions appearing anteriorly as the old disappear posteriorly (Pl. 3, fig. 1, *f*, *g*, *h*) ; the ' proboscis ' also either becomes extruded (Pl. 4, fig. 15, *a*) and active in penetration movements, or (more usually, especially when the animal is placed in water) becomes tightly retracted and immobile (Pl. 4, fig. 15, *b*).

Amphilina paragonopora is, as I have already stated, usually found in the body-cavity of the fish. I have most frequently found it on the mesentery, or on the peritoneum, or on the surface of the liver. On some occasions I have found specimens lying underneath the peritoneum, and once a specimen, 40 mm. in length, was discovered lying immediately dorsal to the gas bladder, in the dense connective

[1] Hein has described similar contractions as occurring in A. foliacea, but states that they are feeble (the worm is very broad) and move postero-anteriorly, i. e. in the reverse direction to that described in the text (!).

tissue attaching the bladder to the dorsal muscles and vertebral column. Another Amphilina, 200 mm. long, was found to have bored through the wall of the gas bladder into its cavity, half the worm being in the cavity and half still outside, and another large Amphilina was found to be buried in the muscles of the body-wall anteriorly. A. paragonopora is also occasionally to be found on the external surface of the fish, and my attention was first drawn to the parasite by discovering part (56 mm. long, and a portion anterior to this, including the proboscis, of unknown length, had become detached) of a large specimen emerging from a perforation at the base of the left pectoral fin and lying on the outside of the skin (Pl. 3, fig. 3). On cutting open the perforation I found that a further portion of the worm (34 mm. long) lay in a cavity in the muscles of the body-wall, and that the remainder of the worm (the hind portion, 40 mm. long) lay inside the body-cavity. This Amphilina, therefore, had evidently bored its way through the body-wall in the neighbourhood of the base of the left pectoral fin (where the body-wall is very thin), and, had it not been caught in the act, would doubtless have altogether escaped from the fish. The uterus of this Amphilina (130 mm. in length plus an unknown length of anterior portion detached) was full of nearly or fully mature larvae (Pl. 4, fig. 35). In another fish also I found a small worm (only 11 mm. long) lying on (not attached to) the surface of the external skin just behind the anus. I cannot say whether or not this worm had escaped from the body-cavity of this fish, but it is possible (although the worm was of course immature sexually) since in this fish a large perforation (some 3 mm. in diameter) was found at the base of the right pectoral fin, leading into a large inflamed cavity in the body-wall and thence into the body cavity. Out of fifty-one[1] Macrones aor and M. seenghala, most but not all of which I specially examined for perforations under the pectoral fins, seven were seen to possess these perforations (situated either under the left

[1] In all I examined during this inquiry about one hundred specimens of Macrones sp., in twenty-two of which I found active Amphilina.

or the right pectoral fin, or, as on one occasion, under both). From sixteen only of these fifty-one fishes did I obtain active A. paragonopora.

I may also mention that no relationship exists between the size of the fish and the degree of infestation by or size of the active parasite, since some of my largest Amphilina have been obtained from very small fish: thus e.g. in one Macrones aor 450 mm. long I found in the body-cavity my two largest specimens of Amphilina (each 280 mm. long), also one about 40 mm. long, two 20 mm. long, one 15 mm. long, and two 10 mm. long, and in a Macrones seenghala only just over 220 mm. long I obtained three Amphilina (70 mm., 64 mm., and 59 mm. long), while in many other fishes, over 900 mm. in length, I obtained only one or two Amphilina measuring not more than 20–30 mm.

In addition to what I have called the active parasite, there also exist in a still larger percentage of the fishes masses of tissue of various sizes and usually of irregular form which closely resemble the parasite in colour and to some extent consistency (Pl. 3, fig. 2). These masses, which range from minute spheres and ovoids (Pl. 3, fig. 2, *a*) up to large shapeless, often very thick, bodies (Pl. 3, fig. 2, *c–n*), are usually attached to the mesentery, but are also to be found free in the body-cavity, and, although of the same colour, yet can easily be distinguished from masses of fat by their more solid texture. Though evidently consisting of the same kind of tissue as the parasites, yet they can usually be easily distinguished from these, not only on account of their lack of definite form, but by the fact that they cannot be flattened out between glass slides. In some cases the connexion between these masses and the parasites is betrayed by parts of these masses assuming the characters of parts of the body of the active Amphilina paragonopora (usually the anterior or posterior end—Pl. 3, fig. 2, *m*, *n*), and in other cases these masses are almost exactly similar in form to the parasite (Pl. 3, fig. 2, *g*, *h*, *j*, *k*) and can only be distinguished by their total immobility (lack of contractions) when removed from the fish and the fact,

already mentioned, that they cannot be flattened out between glass slides. We must, therefore, assume provisionally that these masses represent as a rule bodies which would, in the ordinary course of events, become transformed into active Amphilina paragonopora. They undoubtedly represent a phase in the life-history of Amphilina paragonopora, and they will be described and discussed in detail in Part II.

(*b*) The Reproductive System.

The general plan of construction of the reproductive system of Amphilina paragonopora (Pl. 3, fig. 8) is similar to those of A. foliacea (vide Pl. 3, fig. 9, adapted from the figures by Salensky and Hein) and A. liguloidea (as described and figured by Janicki), but, as we shall see later, there are a few noteworthy differences. Previous to describing the genitalia of A. paragonopora in detail, it is necessary to decide as to which surface of the animal is to be labelled 'dorsal', since previous authors have been by no means unanimous concerning even this essential preliminary: thus e.g. while Salensky and Wagener consider that the side of the body on which the uterus arises from the ovary is the left, Monticelli assumes that it is the right (the view taken by the present writer), while other authors apparently avoid the subject. In typical mesozoan Cestoda it is the rule that the surface of the body to which the ovary is the more adjacent is to be regarded as the 'ventral' surface; and that the ootype is situated on the 'ventral' side of the ovary. In Trematodes also, it is a rule that the vitelline ducts enter the ovary on its 'ventral' aspect, whatever may be their subsequent course. If we apply these rules to Amphilina paragonopora, then Pl. 3, figs. 8, 10, 11, and 12 represent the organs as seen from the dorsal[1] aspect, as proved by the study of serial transverse sections. It will be observed from these figures that when viewed from the dorsal aspect, (*a*) the uterus arises from the right side of the ovary, runs forward nearly to the

[1] Wagener's ventral aspect.

anterior end of the body and returns on itself forming a loop, then crosses posteriorly to the left side of the ovary, and again returning on itself (making a short posterior loop to the left of the ovary) runs forward and opens on the left [1] side of the proboscis at the anterior extremity, and (*b*) the sperm ducts lie dorsal to the uterus where they cross. These two features are apparently common to all three species of Amphilina, and we may therefore assume for all three species that when the animal is so placed that the proboscis is directed away from the observer and the uterus arises from the right side of the ovary, we are then viewing the animal from the dorsal aspect.

I will first briefly describe the genitalia of A. paragonopora. Pl. 3, fig. 8 shows the general disposition of the genitalia, and the three diagrammatic sections the relative positions (dorsal and ventral) of the various organs relative to each other. Pl. 3, fig. 10 shows the arrangement of the various ducts in the neighbourhood of the ovary. The oviduct (OVD) arises from the ovary towards the right posterior corner and, as shown, soon opens into a small spherical chamber, the fertilization chamber (FC), into which also opens the slight terminal dilatation of the vagina, the receptaculum seminis (RS). The fertilized eggs then escape from the fertilization chamber by an opening situated close to that of the oviduct, which leads into what may be called the zygote or fertilization duct (ZD). The zygote duct shortly receives the common vitelline duct (VD) and then dilates slightly to form the receptaculum vitelli (RV), after which its walls become thickened and glandular to form the shell gland (SHGL), and this portion of the

[1] Wagener figures the opening in A. foliacea as lying on the same side of the proboscis as the first limb of the uterus, but if this was the case his specimen must have been abnormal, since Salensky, Cohn, and Hein all figure it on the opposite side in this species, and the opening lies on what I designate the left side in A. liguloidea, A. magna, and A. paragonopora. It is a pity that Braun (Bronn's 'Thierreich', Bd. iv, Abt. 1 b, 1894–1900, p. 1155) and Benham (Platyhelmia in Lankester's 'Treatise on Zoology', part iv, 1901, p. 100) should have popularized Wagener's figure of A. foliacea.

duct is known as the ootype (OTP). The uterus (U) is continuous with the ootype and is a thin-walled much convoluted duct of wide diameter (capable of great expansion when filled with grown larvae), considerably more than three times the length of the entire body (taking the convolutions into account) and having the characteristic conformation shown in the figures—a conformation which is found in all five species of Amphilina. The ducts lying in the proximity of the ovary—from the receptaculum seminis to the beginning of the uterus—are embedded in a dense cushion of connective tissue (CT), probably protective in function. The common vitelline duct is formed by the junction of the vitelline ducts (VD) of the two sides of the body and all run dorsal to the ovary and the uterus. The vitellaria (VIT) extend, as shown, in a row along each edge of the body for nearly its entire length (Pl. 3, figs. 8, 14).

The uterus does not contain eggs until the worm is at least 30–35 mm. in length, and then unsegmented eggs are only present at the very commencement of the uterus, the rest of the uterus being quite empty (observed in three specimens). In a worm about 41 mm. long about a quarter of the first (proximal) limb of the uterus is filled with embryos in the blastomere stage of development—groups of four, eight, and twelve blastomeres having been observed. In a worm circa 75 mm. long, the uterus is full of eggs in various stages of development, but mature larvae are not present. In the worm which I described as in the act of escaping from the fish through the body-wall and which was more than 130 mm. in total length, mature larvae in large numbers were found in the terminal (third) limb of the uterus, the other two limbs containing larvae in earlier stages of development. I may mention that I have observed the larvae escaping from the uterus to the exterior in an Amphilina not more than 100 mm. in length.

The testes (TES), as shown in Pl. 3, figs. 8 and 13, consist, on each side of the body, of a row of sacs (Pl. 3, fig. 13) opening at frequent intervals into the convoluted vas deferens (SD). They lie just internal to the row of vitellaria, and, like these,

extend over the greater part of the body length. The two vasa deferentia unite to the left of the middle line at about the level of the hind end of the ovary, the right vas deferens crossing the ovary on its dorsal side. The common sperm duct then runs posteriorly to the left of the vagina, and just anterior to the terminal opening becomes invested by a thick coat of muscular tissue [1] (MTD) and then forms the thick-walled ductus ejaculatorius (DEJ). The arrangement of the terminal openings of the ductus ejaculatorius, vagina, and terminal excretory duct in A. paragonopora differs considerably from those of other species of Amphilina and merits careful description. Pl. 3, fig. 11 shows the general rather indeterminate appearance of these ducts as observed in flattened and therefore distorted specimens of A. paragonopora. This figure shows at least that all three ducts possess openings to the exterior which lie very close together (hence the name of the species ' paragonopora ', suggested to me by Dr. Baylis) and which are situated on the small median papilla enclosed in the bay (BAY) at the posterior extremity of the body, but to ascertain the exact inter-relationships of these ducts and apertures it is necessary to study series of horizontal and sagittal sections through this region (Pl. 3, fig. 12). Such series show (1) that the vagina opens on the dorsal side of the base of the papilla when this is extended (see Pl. 3, fig. 1, *l*) ; (2) that the opening of the ductus ejaculatorius to the exterior is terminal on the papilla (PE) ; and (3) that the opening of the terminal excretory duct (TED) is also terminal on the papilla and lies, so far as I can ascertain from sagittal sections and flattened whole-mount specimens, just to the left of the opening of the ductus. In some specimens the opening of the excretory duct appears to be confluent with that of the ductus. Thus all three openings lie very close together—so close that for a long time I thought they were all confluent. I may add that the excretory duct is situated ventrad to the ductus ejaculatorius where the former crosses the latter, and that

[1] This is commonly called a ' prostate gland ', but there is certainly no evidence of its glandular nature in my preparations.

the tip of the ductus is markedly thick and muscular, forming a penis. There is no conspicuous cirrus sac and no penial setae.

I will now briefly note some differences which exist between the genital system of A. paragonopora, as just described, and the genital systems of A. foliacea, A. liguloidea, and A. magna, as described by authors already named. All these three latter species are apparently distinguished from A. paragonopora by the fact that the vaginal aperture lies at a considerable distance apart from the penial aperture: in A. foliacea it is separated by about one-third the distance between the base of the ovary and the end of the body, and is situated on the edge of the left side (the 'right' side of Salensky, who, according to my determination, viewed his specimens from the ventral surface) of the body; in A. liguloidea it is separated by about three-quarters of this same distance, and, according to Janicki, is paired [1]—one opening being median and ventral and the other median and dorsal; in A. magna Southwell figures the vaginal aperture as being separated by about one-fifth of this same distance, and lies in the middle line and it presumably opens dorsally.

In A. foliacea the posterior half of the vagina lies to the left of the ductus, but in the other three species the whole of the vagina lies to the right of the ductus. In A. foliacea the cirrus sac is not terminal but lies midway between the ovary and the opening; in A. liguloidea the sac is figured as terminal; in A. paragonopora there is no distinct cirrus sac. Only in A. foliacea are there penial setae in connexion with the very long penis (setae and length correlated with the lateral situation of the vagina and the distance separating the two openings?). In A. liguloidea alone the vagina carries an anterior blind diverticulum extending anterior to the ovary—the so-called 'anterior vagina'. The testes are stated to be scattered in A. foliacea but in the other three species [2] they are linear in arrangement, lying just

[1] Monticelli only describes a single aperture.

[2] Southwell states that the testes are 'scattered about through the

internal to the row of vitellaria on each side of the body. In all four species the uterus opens at the extreme anterior end on the left side at the base of the protruded proboscis (Pl. 4, fig. 16).[1] Some small differences may exist in the arrangement of the ducts adjacent to the ovary in the different species, but, judging from the (in some cases rather doubtful) figures, the general arrangement found in A. paragonopora is found in all. I may add that the ventral position of the vitelline ducts relative to the uterus shown in Hein's fig. 14 (correctly indicated as dorsal in his fig. 13 however), and relative to the sperm ducts and the ovary shown in Janicki's figs. 5 and 6, is probably an error in both cases—in both cases the vitelline ducts should be shown as dorsal to these organs.

(*c*) The Proboscis: its Musculature and Connexions.

When A. paragonopora is removed from the body-cavity of a freshly opened fish and placed on a slide in body-cavity fluid, the extreme anterior end of the worm is sometimes seen to be protruded into a narrow process which moves vigorously from side to side in groping movements (Pls. 3, 4, figs. 1, *k*, and 15, *a*). More usually, however, this protrusible portion of the anterior end is not visible, it having been tightly retracted inside the body-contour (Pl. 4, fig. 15, *b*). This protrusible portion of the anterior extremity I shall term the 'proboscis', and, as will be seen, it is essentially an introvert in structure. In three or four of my specimens the proboscis chanced to be preserved in a protruded condition (Pl. 4, fig. 16), while in all the others the proboscis was retracted (Pl. 4, fig. 17). From these figs. 16 and 17 it will be seen that the proboscis essentially consists of a thickening of the wall at the extreme

parenchyma' in A. magna, but his figure shows that the arrangement of the testes is linear, as in A. liguloidea and A. paragonopora.

[1] I am unable to understand why Southwell (**8**, p. 327) supposes that A. foliacea has no uterine opening 'at the base of the small anterior end of the worm'.

anterior end of the worm to form a thick-walled invaginable conical bulb (Pls. 3, 4, figs. 1, *k*, and 16). In transverse section (Pl. 4, fig. 18) it can be seen that the cavity of the retracted bulb is star-shaped in outline, and in longitudinal sections of the protruded proboscis it is seen that the portion of thickened wall at the very extremity of the worm forms a kind of terminal cushion (Pl. 4, fig. 16, TT). The lateral walls of this bulb resemble the ordinary body-wall in general histological structure, but there are three very striking and significant differences, and had one of these been observed by previous writers, the true function of the proboscis and the huge so-called ' retractor ' muscle attached to it would have been obvious. The first difference is the total absence of circular and longitudinal muscle-layers, the second is the presence of very distinct large radial muscle-fibres (Pl. 4, fig. 16, RTM) which extend up to the base of the cuticle (Pl. 4, fig. 19), and the third the minute but well-marked serration of the cuticle (SER) which covers the greater part of the outer surface of the protruded proboscis (Pl. 4, figs. 16, 16 *a*) but not the terminal thickening (TT), which is altogether devoid of a cuticle. This serration of the proboscis cuticle has not been previously observed. With regard to the second difference mentioned, the radial fibres (RTM) which originate immediately under the serrated cuticle at first run longitudinally, i. e. parallel with the cuticle (Pl. 4, fig. 16, *a*), but soon bend nearly at right angles [1] and run direct to the surface of the big muscle (BM) shown in Pl. 4, fig. 16 as occupying the axis of the proboscis and then bend again and run parallel to the surface of the big muscle posteriorly, and at the hind end of the proboscis these muscle-fibres diverge, extend posteriorly in the general parenchyma of the central core of the body for a considerable distance, and finally apparently become continuous with the parenchyma. It is difficult to determine how far these fibres extend posteriorly because they are not easily distinguishable from the ordinary

[1] Some anteriorly attached fibres do not bend but run longitudinally in the proboscis wall, crossing the greater number of fibres in so doing. They do not form a layer of longitudinal muscles.

longitudinal musculature of the body. These muscle-fibres constitute the true retractor muscle of the proboscis (RTM), and are very similar in form and disposition to the retractor muscle-fibres of the proboscis of a simple Turbellarian, e.g. Pseudorhynchus bifidus, v. Gr. The terminal thickening (TT) of the proboscis consists mainly of short columnar cells and forms a thin cellular pad covering the anterior extremity of the huge axial muscle just referred to (Pl. 4, fig. 16, BM). It is to be remarked that this introvert proboscis does not possess extraneous radial protractor muscles attached to the body-wall.[1]

Occupying the central axis of the proboscis is the huge muscle (Pl. 4, fig. 16, BM) which is so conspicuous in Amphilina and which previous authors have either labelled 'retractor' or, in view of its disproportionate size to the minute proboscis which it was supposed to retract, have regarded as a bundle of gland ducts! Before discussing, however, the views of previous authors, I will describe the entire apparatus of the proboscis. The huge muscle can be seen to extend posteriorly as far back as the anterior end of the ovary (Pl. 4, fig. 20), its shiny fibres showing plainly in whole mounted specimens of the worm. Anteriorly, i.e. in the anterior fifth of the body-length, it is of considerable thickness and occupies at least the middle third of the body seen in transverse section (Pl. 3, fig. 5), but more posteriorly it becomes attenuated (Pl. 3, fig. 4) and immediately in front of the ovary only consists of a few centrally situated fibres. Each of these fibres (some nearly as long as the worm itself) can be seen, if traced through serial sections, to run parallel with the central longitudinal axis of the body for the greater part of its length, but just before its termination it always bends at right angles (i.e. becomes more or less radially disposed in a transverse section) and becomes connected with one of the remark-

[1] Cohn figured extraneous radiating protractor muscles in his drawing of the proboscis of A. foliacea, but they certainly do not exist in A. paragonopora. Salensky provides what is probably a much more accurate figure and shows no extraneous muscles.

able giant cells which Salensky labelled 'problematic'. The 'problematic cells' of Salensky are then simply the attachment or 'anchor'-cells of the large axial muscle. The mere distribution (as seen under a low-power objective) of these anchor-cells, as I shall call them, is evidence that they have something to do with the fibres of the muscle, since in the anterior fifth of the body in which the muscle-fibres are most numerous, the anchor-cells are most plentiful and extend laterally to the region of the testes (Pl. 4, fig. 21); whereas, more posteriorly, where the muscle-fibres are much fewer in number, the number of anchor-cells is also much smaller and is obviously roughly proportional to the number of fibres in any given zone (Pl. 4, fig. 21). The continuity of the giant anchor-cells (each as big as the egg-filled uterus in transverse section) with the fibres of the muscle can be easily seen both in longitudinal and transverse serial sections, whereas in whole preparations it is not easy to see the connexion, and this accounts for the erroneous supposition of Salensky that the processes from these 'problematic' cells become sooner or later attenuated and indistinguishable from the ordinary parenchyma with which they are connected, since he doubtless did not trace these processes through serial sections. All these anchor-cells are elongated and their long axes in all cases lie in a plane at right angles to the long axis of the body (Pl. 3, figs. 4, 5)—a significant fact. These long axes are, in the case of cells occupying the axial region of the body, disposed vertically (the muscle processes arising from either their dorsal or ventral ends—Pl. 3, fig. 4), but anteriorly, in those cells which are situated nearer the sides of the body, the long axes are often obliquely inclined towards the median axis (Pl. 3, fig. 5). Cytologically the anchor-cells are, as Salensky remarked, not unlike nerve-cells, and, indeed, they may be neuro-muscular in function, though they apparently have no connexion whatever with the main nervous system of the worm. Each cell (Pl. 4, figs. 22, 23) contains a nucleus (with a conspicuous nucleolus) which lies in a small island of chromatophil cytoplasm (very evident in indigo-picro-carmine preparations, in

which the island is stained red and the rest of the cell-plasm green), and the remainder of the cell is packed with conspicuous granules. The cell process grows out from one pole of the cell and at first is packed with granules identical with those in the cell, but at some distance from the cell the substance of the fibres becomes longitudinally striated (the striations being sinuous when the muscle is not fully extended) and much less granular ; in other words, comes to resemble muscle-substance (Pl. 4, fig. 24). Another and very significant fact to be mentioned concerning the fibres of the big muscle is that, in the proboscis and for some distance below it, these fibres, at least in many cases, run obliquely, as is proved by the fact that the fibres are seen to be cut transversely or obliquely in longitudinal sections cut parallel with the long axis. Pl. 4, fig. 25 gives some indication in very diagrammatic form of the general arrangement of the muscle-fibres and their connexion with the anchor-cells—an arrangement also found in A. foliacea and A. liguloidea and probably in A. magna.

What can be the function of this huge axial proboscis muscle ? Its extraordinary size enables us at once to dismiss the idea that it is simply the retractor muscle of the minute proboscis, as supposed by Salensky [1] and other authors, especially in view of the fact that a well-defined retractor muscle (indicated in A. liguloidea by Janicki in his Text-fig. 9) already exists. In no other worm do we find a proboscis, of the size found in Amphilina, associated with a muscle (known to be a retractor) of the dimensions just described. In Turbellaria the small proboscis is always retracted by a small retractor muscle consisting of short diverging fibres ; on the other hand, when, as in the Nemertines, the retractor muscles are long,

[1] Salensky described the fibres of the proboscis muscle as originating posteriorly in two halves from the lateral subcutaneous muscle-layer, and naturally could offer no explanation of the presence of his ' problematic cells '. Hein and Cohn both failed even to find the anchor-cells (the former confusing them with the myoblasts and the latter assuming that they were in part identical with his flame-cells and in part ' Kunstprodukte ' !) though these cells can be easily seen under a magnification of 30 diameters and less.

the proboscis is also long, and even in the Tetrarhynchus scolex in which the four retractor muscles of the four proboscides reach, as in Amphilina, to the hind end of the 'segment', each proboscis is at least one-fifth the length of its retractor muscle.

Wagener (1858) and Lang (1881), on the other hand (neither of whom can be supposed to have recognized the fact that the fibres of the large proboscis 'muscle' are individually connected with the giant cells which Salensky called 'problematic'), adopted the view that the whole of the proboscis 'muscle' is a bundle of elongated ducts connected with 'Speicheldrüsen' (!) lying in the parenchyma, the ducts being supposed to open on the surface of the proboscis. Needless to say, apart from the superficial resemblance of the anchor-cells to gland-cells (and ganglion-cells !), and the necessity of adopting an alternative to the 'retractor' theory, there is no justification whatever for this view, though it has been adopted both by Pintner and Janicki and referred to in such well-known general works as Bronn's 'Thierreich' and Lankester's 'Treatise on Zoology'. The three obvious facts (evident in any series of well-stained longitudinal sections through the proboscis), viz. (1) that there is no trace of a lumen in the individual fibres, (2) that the fibres are distinctly composed of muscle substance, and (3) that the supposed ducts do not reach the surface of the proboscis,[1] are sufficient by themselves to dismiss this gland-complex theory, quite apart from the further facts, already described, of the greater part of the proboscis being covered with serrated cuticle (the terminal cushion provides far too small an area for the openings of such a large number of supposed ducts) and the suggestive twisting of the muscle-fibres anteriorly, and the consideration that it is difficult to conceive the necessity for the existence of such an enormous gland—a gland which, with its ducts, extends throughout nearly three-quarters of the substance of the body.

The proboscis muscle then, being neither a retractor nor

[1] Janicki, in his fig. 9 of the 'vordere Körperspitze', expressly refrains from figuring the supposed ducts of his 'Frontaldrüsenzelle' !

a bundle of gland ducts, can only be associated with some special function of the proboscis. The proboscis, as we have seen, is not an organ of attachment—a sucker—but it is, on the other hand, a very efficient organ of penetration. Amphilina, as already related, normally bores its way through the tough body-wall of the fish in order to liberate its larvae to the outside world, and we know that it also occasionally bores through muscles, the wall of the gas bladder, the kidney, and other organs and tissues. But in order to overcome resistance a penetrating organ must possess (1) some degree of rigidity, (2) some instrument with which to tear tissue, and (3) a powerful muscle to work the apparatus. The large proboscis muscle—which I shall in future term the boring muscle (BM)—fulfils requirements (1) and (3), and the serrated cuticle covering the outside of the protruded proboscis fulfils requirement (2). The anterior thickness of the boring muscle not only supplies the proboscis with a dense more or less rigid core with which the animal can push its way into resistant tissue, but the posterior extension and firm attachment of the muscle to the large anchor-cells firmly embedded in the axial parenchyma (and placed at right angles to the length of the fibres) enables the worm to ' put its whole weight ' into the boring process and to draw the hind portion of the body through the perforation or path made by the proboscis. The slight twisting of the fibres of the boring muscle in and below the proboscis also doubtless serves for a semi-rotary movement of the proboscis, enabling the serrated cuticle to saw its way through the tissue. The proboscis muscle thus serves as a boring muscle, as a notochord for the anterior end of the body and as a means of dragging the hind half of the body along the path excavated by the proboscis, and these important functions amply account for its huge size. Retraction of the proboscis is effected by the retractor muscle, and protrusion of the proboscis probably results merely from the slackening of the retractor (the stiff boring muscle naturally projecting forwards in a position of rest) but possibly also from the contraction of the longitudinal muscles attached to

the body-wall at the base of the proboscis—the proboscis being exposed by this means.

(*d*) The Excretory System.

Salensky has described the presence in Amphilina foliacea of two lateral excretory channels (lying one on each side of the body internal to the nerves) which receive branches from the parenchyma. This description agrees essentially with the plan of excretory system which I have found in A. paragonopora, and I therefore venture to doubt the accuracy of Hein's account (with figures) of an irregular close network of excretory channels occurring in A. foliacea.[1] In A. paragonopora, as in A. foliacea, a large excretory channel extends along the whole length of each side of the body, lying immediately internal to the testes (Pl. 3, fig. 6). Anteriorly (Pl. 4, fig. 17) each channel apparently originates as a narrow channel or loop (equal in calibre to one of the branches or loops given off more posteriorly) in the parenchyma situated at the sides of the base of the proboscis; posteriorly the two lateral channels converge and meet in the middle line in a slight dilatation (situated at about a third of a millimetre from the terminal aperture—Pl. 3, fig. 12, EXB) to form the terminal single excretory duct (TED), which in its turn runs directly to open externally on the papilla (PAP) at the extreme posterior end of the body, the opening lying adjacent to and to the left of that of the penis. In addition to these two lateral main excretory channels there are four series of subsidiary (as regards size) excretory channels, which arise from the two lateral main channels along their entire length. The first that may be mentioned comprises the dorsal transverse channels (Pl. 3, figs. 4, 6, DTC), which put the two lateral main channels into direct communication across the dorsal side of the body, lying between the

[1] I am aware that Cohn speaks of definite lateral channels and a network in A. foliacea, and that Janicki figures a fine network in A. liguloidea.

internal longitudinal muscle-layer and the dorsal anchor-cells and fibres of the boring muscle; the second series comprises the similar transverse channels situated on the ventral side of the body—the ventral transverse channels (Pl. 3, figs. 4, 6, VTC); the third comprises those channels which arise from each lateral main channel on its dorsal side and turn outwards towards the outer edge of the body—the dorsal external channels (Pl. 3, fig. 4, DEC); and the fourth comprises the similar outwardly directed channels which arise from the ventral side of the lateral main channel—the ventral external channels (Pl. 3, fig. 4, VEC). In many and perhaps in most cases, the dorsal external channels and the ventral external channels join together to form a lateral excretory loop (Pl. 3, fig. 6, LEL), but in other cases the two channels do not appear to communicate. Thus, in its main plan, the excretory system consists (1) of two lateral main channels which unite posteriorly to form a single exit channel which opens to the exterior at the posterior extremity, and (2) of subsidiary smaller channels which take the form, roughly speaking, of three series of ' rings '—the axially situated series of flattened ' rings ' formed by the dorsal and ventral transverse channels, and the two lateral series of ' rings ' arising from and lying external to the lateral main channels. It must be mentioned, however, that the upper and lower halves of these ' rings ' very rarely lie in the same transverse plane—they only form a ' ring ' when a considerable thickness of the body is viewed end-on. In one worm measuring about 40 mm. in length I observed that in 5 mm. of this length approximately 21 external channels were given off from one of the lateral main channels—about 150 in the entire length of the worm.

Apart from this system of channels I have been unable to discover any other portion of the excretory system, though I have searched most carefully, in both transverse and longitudinal series of well-fixed sections, for flame-cells. According to Hein and Cohn flame-cells exist in A. foliacea in large numbers, and the former author figures them; but these statements need confirmation, since Hein is possibly mistaken

in his description of the general plan of the excretory system and admits that his preparations were ill adapted to show even the central nervous system; and Cohn, as we have seen, suggested that Salensky's 'problematic' cells were themselves the flame-cells! I may add, finally, that I was also unable to detect cilia in any of the excretory canals.

(*e*) The Central Nervous System.

In 1874 Salensky remarked upon the presence in Amphilina foliacea of two longitudinal nerve-trunks, and Lang (**4**) in 1881 confirmed this, and also described a 'brain commissure' (a band of fibres piercing the boring muscle) and branches given off from the two lateral longitudinal trunks. Cohn in 1904 added the information that the branches given off dorsally and ventrally from each of the two lateral longitudinal trunks meet dorsally and ventrally across the body so as to form a series of nerve-rings throughout the length of the body, but since these rings were not observed by Lang, and are certainly not present in the elongated and more nearly cylindrical A. paragonopora, I doubt their existence. Pl. 4, fig. 29 illustrates the anterior end of the central nervous system in A. paragonopora, and it will be seen that it confirms Lang's description in all respects. Anterior to the 'brain commissure' the two lateral longitudinal trunks extend forwards and end in the margin of the anterior end of the body. Posteriorly, in A. paragonopora, the two lateral longitudinal trunks converge slightly (in accordance with the narrowing of the body) but do not appear to join: they terminate separately at the sides of the posterior inlet or semicircular bay at the posterior end, in much the same way as the trunks terminate anteriorly. In transverse and longitudinal sections each of the lateral longitudinal trunks is seen to give off dorsal, ventral, and internal branches (Pls. 3, 4, figs. 4, 5, 29), and these are distributed to the body-wall muscles and other organs. I have not ascertained the exact numbers of these branches. The trunks are uniform in diameter and there are no special aggregations of ganglion cells.

(*f*) The Histology of the Body-wall and the Body Musculature.

Pl. 4, fig. 26 shows the appearance of the body-wall in transverse section. The cuticle (CUT) is not very thick, and immediately underlying it is the thick 'subcuticula' (SUBC). The outer zone of the subcuticula apparently consists solely of numerous radially disposed thin fibres similar in nature to those which compose the general parenchyma. Three muscle-layers lie in the outer zone of the subcuticula—the thin outer circular muscle layer (OCM), a thin layer of longitudinal muscle-fibres (OLM), and a second thin layer of circular fibres (ICM). Internal to these three muscle-layers lies the inner zone of the subcuticula ('epidermal' layer), consisting of spindle-shaped cells, in between which lie radially-disposed fibres and numerous calcareous bodies (CALC). Underlying the subcuticula is the parenchyma (PAR), in the outer zone of which lies a powerful longitudinal muscle-layer (ILM), and internally to which there is to be seen an attenuated scattered layer of oblique longitudinal muscle-fibres (OBLM). Gland-cells (GC), excretory canals, and nerve-fibres (NFI) are also of course contained in the parenchyma. Included in this Pl. 4, fig. 26 is a drawing of a giant anchor-cell (ANCC) drawn to the same scale, which will give some idea as to the enormous size of this class of cell. Pl. 4, fig. 27 illustrates a longitudinal section through the body-wall, and this shows the indentations of the cuticle and subcuticula due to the outer substance of the body-wall being ridged transversely when contracted longitudinally.

(*g*) Some Stages in the Development of the Larva.

The mature eggs in the ovary which are about to enter the oviduct in an Amphilina paragonopora about 30 mm. long are of the ordinary alecithal type. After having been fertilized and passed through the ootype, the eggs normally become encased, with a quantity of yolk material, in a relatively thin irregularly oval shell, to one end of which is attached

a short filament or ' tag ' (Pl. 4, fig. 30, *f*). Occasionally eggs are shed into the uterus without a shell. Only unsegmented eggs are to be found in a worm about 30 mm. long, and they are situated in the portion of the uterus immediately adjacent to the ovary, the rest of the uterus being empty. In an older A. paragonopora (a little over 40 mm. in length and cut into serial horizontal sections) I have observed early segmentation stages—groups of four, eight, twelve and more blastomeres —and in some of the early morulae it is possible to detect one or two blastomeres which differ from the rest and which are doubtless destined to form the investing membrane of the embryo. In a worm a little over 70 mm. in length the uterus is full of embryos, the oldest stage being a solid morula which fills the shell, the morula being surrounded by an investing membrane (Pl. 4, fig. 31 depicts a very young morula). In another worm (87 mm. long when uncontracted, though it shrank to 32 mm. when placed on a slide) which I cut into serial transverse sections, the third limb of the uterus was full of embryos of the stage represented by Pl. 4, fig. 34, while earlier stages (e.g. that represented by Pl. 4, fig. 32, which I drew on account of the single large internally situated blastomere shown—GBL) were present in the other two limbs. The embryos in limbs 2 and 3 of the uterus all possess a definite ectoderm, while in limb 3 the embryos are further distinguished (1) by the possession of a small group of large glandiform cells (GC) which are drawn out towards one end of the embryo and may be unicellular glands similar to those found in many cerariae, and (2) by the elongation of the terminal cells at the other end of the embryo (Pl. 4, fig. 33) and the secretion by these cells of the ten (I think) calcareous hooklets so characteristic of the Amphilina larva (Pl. 4, fig. 34). Pl. 4, figs. 33 and 34 are drawn from sections. In three worms each about 100 mm. long when uncontracted, the third limb of the uterus was full of larvae still contained in their shells, which they filled and were somewhat longer than the stage represented by Pl. 4, fig. 34. At this stage the larvae are often liberated and are about 100 microns in length. Pl. 4, fig. 35 represents

in optical section the type of larva found in large numbers in the third limb of the uterus in a worm more than 130 mm. long (the worm which was in course of boring its way through the fish body : vide Pl. 3, fig. 3). In these larvae (which were about 200 microns in length—twice the length of other mature larvae observed by me), whether mounted whole or in section, I found it difficult to detect for certain the central group of gland-cells which is so distinctly figured by Salensky (vide his fig. 34) and Janicki (vide his fig. 16) and which I have seen clearly in sections of younger larvae, the probable reason being that the gland-cells are much distended and full of unstained secretion : it is difficult to suppose that the gland-cells have disappeared at this stage. The anterior end of the larva (the end opposite the hooklets) is usually drawn out somewhat and probably carries the fine ducts of the gland-cells. The larva at this stage has usually escaped from its shell and has secreted a thin but definite cuticle, outside which lies the remains of the investing membrane. I could detect no trace of ciliation on any part of the surface.

The foregoing facts were, as I have stated, observed by me in specimens of A. paragonopora ranging from 30 mm. to 130 + ? mm. in length and require no particular comment. In one of my two largest specimens of this worm (both 280 mm. long when living and uncontracted), however, I found, both in horizontal and vertical longitudinal sections, in portions of the body mounted whole and in macerated preparations of the body, two types of products in the uterus (all three limbs of which apparently contained the same or similar products) : (1) oval flattened larvae (Pl. 4, figs. 38, 39) with typical hooklets and possibly with gland-cells (though I could not observe them even in sections ; however, large spheres, not shown in Pl. 4, figs. 3, 8, could occasionally be distinguished deep in the substance) and only as long as the larvae liberated from worms 100 mm. long, i.e. half the length of the larvae represented by Pl. 4, fig. 35 [1] (though the hooklets were the same size in both)

[1] I am unable to say whether the larvae of Pl. 4, fig. 35 are abnormally large (since they were only found in a single worm) or whether the flat

and of much denser consistency, and (2) oval egg-shells but little inferior in size to the larvae and containing only a few large dissociated blastomeres of different sizes (Pl. 4, figs. 37, 39). These practically empty egg-shells were extremely numerous—from five to ten times more numerous than the oval larvae. They are best displayed by macerating portions of the Amphilina, either fresh or preserved, in Marcacci's fluid (equal parts of nitric acid, glycerine, and water) over-night, followed by teasing or, better still, by grinding up on metal gauze suspended in water with a piece of flat wood—the products of the grinding sink through the gauze and can be collected by centrifuging. Lest it be thought that the half-empty condition of the egg-shells was due to the Marcacci's fluid, I may mention that I obtained the same result with simple maceration in water (Pl. 4, fig. 39) and that Marcacci's fluid does not damage even such objects as spermatozoa ; the half-empty egg-shells can also be seen, as already mentioned, in whole-mounted specimens and in serial sections mounted in balsam. I may also mention that it was only by employing Marcacci's fluid that I first detected the filament or tag on the egg-shells, it usually being difficult to see this structure in sections and whole mounts. I suspect that similar maceration would display a tag on the egg-shells of Amphilina magna, Southwell.

The half-empty egg-shells undoubtedly represent larvae that have degenerated. It is conceivable that if for any reason a worm cannot escape from its fish host, the larvae degenerate in consequence of not being liberated into water. Facts cited in Part II afford additional evidence in favour of this view.

(*h*) Re-definition of the Genus Amphilina, and the chief Distinctions between the five Species.

Wagener's definition of the genus Amphilina (**9**) must be amended in order to comprise the facts that in A. paragonopora the body is ribbon-shaped, the posterior end is

larvae (which are the same length as the largest larvae I have seen in utero) are abnormally small.

not pointed, the two surfaces are alike in curvature, and that the proboscis does not bear the openings of a gland complex, the latter being a large boring muscle, the fibres of which extend a considerable distance posteriorly (probably about eight-ninths of the length of the body in the three best-known species) and become connected individually with giant ' anchor '-cells. Thus amended the definition of the genus reads as follows : Body flat and varying in outline from an oval to a narrow ribbon. Anterior end pointed or slightly truncated according to the state of contraction ; posterior end pointed, rounded, or emarginate. A small evaginable proboscis is present at the anterior end, and connected with this is a large boring muscle, the fibres of which end posteriorly in giant ' anchor '-cells situated in the parenchyma. The excretory system consists usually of two main lateral channels connected with a system of smaller channels and opening posteriorly by an approximately median single pore. Testes numerous. Ovary and openings of vas deferens and vagina posterior. Uterus a long convoluted duct consisting of three limbs (N-shaped when viewed from the ventral surface), each extending nearly the entire length of the body, and opening anteriorly at the base of the proboscis and on the left side (i.e. on the side of the body opposite to that on which the uterus arises from the ovary).

I may add that in no species is there an ' acetabulate sucker '.

Some of the more conspicuous distinctions between the five known species of Amphilina are stated in the Table on the next page.

The specific distinctness of A. neritina from A. foliacea needs confirmation.

Three type-specimens (including one of maximum size) of Amphilina paragonopora have been deposited in the British Museum (Natural History) at South Kensington, London.

	A. neritina.	A. foliacea.	A. liguloidea.	A. magna.	A. paragonopora.
Colour	Grey-green	Creamy-white	Grey-white	Milky-white	Creamy-yellow to orange.
Maximum length	18 mm.	26–60 mm.	86 mm. (preserved)	250 mm. (preserved) 381 mm. (living)	170 mm. (preserved) 280 mm. (living).
Approximate ratio of maximum breadth of body (taken as 1) to length	1/2·0	1/1·2–1·30 *	1/3 5–4·0	1/10·6–12·8	1/20·8–28·3 †
Body extremities	Both ends narrow and rounded	Both ends narrow and rounded	Both ends narrow and pointed	Both ends narrow and rounded	Anterior end pointed or rounded, posterior end emarginate.
Host	Acipenser sp. Europe.	Acipenser sp. Europe.	Arapaima gigas, Brazil	Diagramma crassispinum, coast of Ceylon	Macrones aor et seenghala, rivers of North India.
Vagina and its aperture	In both species vagina lies posteriorly to left of ductus and opens on left margin of body about 2 mm. away from the median ductus opening		Vagina to right of ductus and has dorsal and ventral openings in middle line about 3 mm. from hind extremity. Vagina has anterior diverticulum	Vagina to right of ductus and opens dorsally (?) in middle line 2 mm. from hind extremity	Vagina to right of ductus and opening at hind extremity just dorsal to ductus opening in middle line.
Penal setae	?	Penial setae	No penial setae	No penial setae	No penial setae.
Testes	?	Said to be scattered	Linear arrangement	Linear arrangement	Linear arrangement

* Measurements of five drawings from Salensky, Hein, and Cohn.

† In my largest living specimens the ratio was 1/56.

Part II.

On the Irregular-form Stage of Development in the Life-cycle of Amphilina paragonopora.

I have already mentioned in Part I that in addition to the active Amphilina paragonopora there are to be found attached to the mesentery and, in the case of the larger bodies, lying free in the body-cavity of Macrones aor and M. seenghala, large numbers of masses of tissue, varying greatly in size and form and, except in the case of the smaller bodies, rarely showing any approach to a definite shape (Pl. 3, fig. 2), but which, nevertheless, are similar in colour to the active worm, though they differ in consistency, it being impossible to flatten them between glass slides. They are quite distinct from the similarly coloured masses of fat, being denser in appearance and texture. These amorphous [1] masses are stages in the development of the active Amphilina, a fact which is proved by the discovery of stages transitional between the two (vide Pl. 3, fig. 2, *m*, *n*), and they represent a distinct part of the life-cycle of the species. The portion of life-cycle from the formation and escape of the larva from the fish to the first appearance of the amorphous masses in the mesentery of the fish is unknown.

The smallest amorphous masses which can be detected in the mesentery by the naked eye are spherical or ovoid in shape (Pl. 3, fig. 2, *a*),[2] and there exist all transitions from these small masses up to elongated masses 80 mm. or more in length (Pls. 3, 5, figs. 2 and 46). I have cut serial sections of some dozens of these masses and also through portions of infected mesentery, and by close examination of these I have

[1] I use this term, which strictly speaking can only be applied to a gas, for want of a better. So far as I am aware there is no term in use in English to express the meaning of indefinite shape as apart from definite. Professor Platt suggested ' ataxomorphic '.

[2] It is impossible to distinguish these with the naked eye from the numerous Nematode cysts which also abound.

found what are undoubtedly the earliest stages of growth of these masses, and several other facts of interest.

The earliest certain developmental stage of the amorphous mass which I have observed was approximately spherical and measured 29 microns in diameter (Pl. 5, fig. 40, ×500). The mass simply consists of a globule of plasm with a dozen or so nuclei embedded in it, and is surrounded by a relatively thick capsule of loose concentrically arranged fibres; usually capillaries are to be seen near by. I have seen about a dozen of these early stages [1] (one measuring 40·2 microns in diameter is shown in Pl. 5, fig. 41, ×500, and another larger one in Pl. 5, fig. 42, ×500). It will be noticed that these minute masses are smaller than the larvae liberated from the uterus of the worm, and it is therefore evident that the larva must undergo some process of subdivision, probably in an intermediate host, before infection of another fish can occur. I have not been able to trace these masses back to a unicellular stage. Encapsulated masses larger than these early stages are very numerous, and of course are easily distinguishable in section from small Nematode cysts of the same size both by the absence of the worm, the nature of the contents, and by the structure of the capsule wall. As I have said before, all stages of the growth of these masses are to be found, and since it would be profitless to describe the gradual process of histogenesis which commence in masses of about the size shown in Pl. 5, fig. 45, I have only figured sections of three of the later stages, the magnifications stated giving some idea of their relative sizes. Pl. 5, fig. 43 (×112) illustrates an encapsuled mass with tissue still quite undifferentiated, and Pl. 5, fig. 44 (×112) a larger similar mass, and Pl. 5, fig. 45 (×35) shows a later stage of the worm after it has become too long for the capsule and is consequently becoming coiled. The commencing histogenesis is not indicated in the figure. In still later stages

[1] These early stages must be distinguished from sections through nerve-fibres in the mesentery, which are very numerous and are often twisted into lumps of about the same size as the masses, and in some cases are so small as to resemble unicellular bodies.

the worm becomes much more coiled inside the capsule,[1] and at some period escapes from the capsule. Judging from the widely different sizes, both of masses lying free in the body-cavity and of the active Amphilina, the differentiated masses must escape from their capsules at very different stages of growth. It must be understood that although I have only figured a few of the stages of development of these masses, yet all stages transitional between the youngest and the oldest can be observed in sections through infected mesentery. It is evidently unnecessary to give figures of the entire series.

Two other kinds of encapsulated masses must be described. In two of the largest amorphous masses (Pl. 3, fig. 2, *e*, represents one of these masses) I found enclosed in each a histologically well-developed Amphilina of large size and considerable length, the body being tightly coiled on account of the restricted space. Many of the tissues of these encapsulated Amphilina were well differentiated and especially the reproductive system, the uterus being of large size and, to my great surprise, full of large oval flat larvae with their characteristic hooklets and equal in size to the similarly shaped larvae in the uterus of the 280 mm. active Amphilina described in Part I. The larvae, however, were very degenerate (in most cases largely disintegrated) and were only recognizable as larvae on account of their location, general shape, and the characteristic hooklets. The only hypothesis which I can suggest to account for these facts is that for some reason the amorphous mass in this instance had been unable to escape from its capsule, and being compelled to undergo its development inside the capsule, this development was both incomplete (e.g. the proboscis was not developed) and one-sided, the reproductive organs developing at the expense of other organs. The larvae being formed and unable to escape, naturally degenerated. Certain appearances suggest to me that in places the substance of the worm was being invaded by histolytic tissue derived from the walls of the capsule.

[1] Salensky states that he once found a young A. foliacea contained in a capsule on the peritoneum covering the liver of the Sterlet.

In one other case—an isolated instance—I found a small capsule (roughly 500 microns long and 384 microns in mean transverse diameter) also to contain a dozen or so degenerate flat larvae and some disintegrated matter, but in this case no worm was enclosed in the capsule nor could ever have been in a capsule of this small size (Pl. 5, fig. 47). The only explanation of this remarkable situation of the degenerate larvae is that the larvae had been ejected from an Amphilina into the body-cavity of the fish (and we have seen that larvae are sometimes extruded from the uterine pore long before the worm escapes from the body-cavity) and that the larvae having come into contact with the mesentery, a histolytic capsule had been formed by the mesentery tissue to isolate and destroy them (the amorphous masses are themselves surrounded by capsules formed from mesentery tissue, in much the same way that Linguatulid larvae become encapsuled). It will be noticed from Pl. 5, fig. 47 that the walls of the histolytic capsule are very different in construction from the walls of the capsules enclosing normal amorphous bodies.

It is thus of interest to note that larvae, if not liberated soon enough, can become degenerate (1) in an active Amphilina (as e.g. in the 280 mm. Amphilina described above), (2) in an Amphilina permanently imprisoned in its capsule, and (3) when liberated into but unable to escape from the body-cavity of the host (fish). The only fact which it is difficult to understand is why, in the 280 mm. Amphilina, only a small proportion of the eggs had developed as far as the full-grown larva stage, the rest becoming degenerate while still inside their shells. But perhaps this was an anomalous, as it certainly was, in my observations, an isolated, occurrence : in no other instance have I observed the uterus to contain anything but normally developing or developed larvae. I may emphasize that the flat oval type of larva seen in the 280 mm. Amphilina also occurred in the capsule-imprisoned Amphilina and in the histolytic capsule, and possibly this is the fully grown stage of the larva, the type of larva figured by Selensky, e.g., being not fully grown.

Notes on Technique.

I fixed specimens of Amphilina paragonopora either in Zenker's fluid, Mann's fluid, aceto-bichromate, or simply in 6 per cent. formalin. Some of the specimens were first flattened between glass slides, and these, when properly stained, showed the reproductive organs well. Unflattened specimens were either embedded for section-cutting (the specimens being kept straight by being placed between small squares of wire gauze while in the embedding bath) or simply preserved in formalin and glycerine. Whole specimens and sections were usually stained either with Delafield's haematoxylin (diluted with ten times its bulk of water, and immersion over-night) and in some cases followed by eosin or other plasma stain, or with borax carmine. Both stains gave good results.

In conclusion I wish to express my indebtedness to Dr. H. A. Baylis, who very kindly consulted for me, while I was in India, several original papers and checked my references to previous work, and to Dr. S. W. Kemp, who kindly sent to me some volumes from the Indian Museum library at Calcutta while I was in Allahabad. I am also indebted to Colonel G. E. F. Stammers for some assistance in checking references, and to Messrs. B. K. Das, M.Sc., and S. K. Datta, M.Sc., for the considerable assistance they have kindly given to me in obtaining large numbers of Macrones and finding Amphilina.

Summary of Principal Conclusions in Parts I and II.

1. Amphilina paragonopora is parasitic in the body-cavity of the Siluroid fishes, Macrones aor and M. seenghala, from the Ganges and Jumna, India. It is linear in shape and varies in length from about 10 mm. to 280 mm., but full-grown larvae are not formed in the worm until it is at least 100 mm. in length. The ' anterior ' end of the parasite is rounded and carries a small proboscis, which is a boring organ and not a sucker. The uterus opens anteriorly to the left and at the base of the proboscis. The ' posterior ' end of the body is marked by a semicircular inlet or bay, and on

a median papilla in the bay open the ductus ejaculatorius (at the extremity of the papilla in the middle line), vagina (at the base of the papilla on its dorsal side, the opening thus being practically terminal) and terminal excretory duct (immediately to the left of the opening of the ductus and sometimes confluent with it). The parasites when mature (the uterus being filled with larvae in various stages of development) apparently escape from the fish by boring through the body-wall at the base of one of the pectoral fins. In addition to the ' active ' parasite, an inactive stage in its life-cycle is to be found in the form of irregularly shaped masses usually attached to the mesentery.

2. The general plan of the reproductive system is similar to that in Amphilina foliacea, but there are some noteworthy differences, already summarized at the end of Part I.

3. The small boring proboscis (covered with a serrated cuticle) is connected with and manipulated by a huge boring muscle (formerly miscalled ' retractor ' by some authors, and by others interpreted as a bundle of gland ducts—the ' problematic ' cells of Salensky being the glands) which is very thick anteriorly and extends posteriorly, though in an attenuated form, to the region of the ovary. The fibres of this boring muscle end in the giant cells which Salensky called ' problematic ' and which I have renamed ' anchor '-cells. The function of the boring muscle is (1) to give a semi-rotary movement to the proboscis (its fibres being twisted anteriorly), (2) to act as a stout support for the anterior end of the body when engaged in boring, and (3) to drag the hinder portion of the body through the perforation made by the proboscis—and these three functions account for the enormous size of the muscle. A true and distinct retractor muscle lies externally to the boring muscle.

4. The excretory system consists of two lateral main channels which unite posteriorly and form a short straight terminal excretory duct which opens in the median line posteriorly, and a series of smaller loops and branches given off from these two lateral main channels, which appear to form, typically, three series of ' rings ' when the body is viewed end-on (Pl. 3, fig. 6). Flame-cells are absent.

5. The central nervous system is, in the main, that described by Lang for Amphilina foliacea.

6. Some stages in the development of the larva are described, and the larvae, when liberated from the uterus, appear to be similar to those of Amphilina foliacea. Fully grown

larvae found in a 280 mm. specimen of A. paragonopora are oval in shape and flattened, and possibly do not possess the large gland-cells, but these may be degenerate forms.

7. The genus Amphilina is re-defined and the more conspicuous specific differences between the five species of Amphilina are stated.

8. A brief account of the irregular-form stage in the life-history of A. paragonopora is given, from which it appears that the amorphous masses found on the mesentery of the fish and which give rise to the active Amphilina, arise from small spherical multicellular masses (about 30 microns in diameter) enclosed in capsules formed by the mesentery tissue. All transitions can be observed from these smallest masses up to the stages in Pls. 3, 5, figs. 2 and 45, and thence to the active Amphilina.

Occasionally the masses develop into sexual Amphilina inside the capsules which enclose the masses, since Amphilina containing full-grown larvae are occasionally met with inside large capsules.

Occasionally also larvae liberated into the body-cavity become secondarily encapsulated by the mesentery tissue and are apparently disintegrated.

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EXPLANATION OF PLATES 3, 4, AND 5.

N.B.—The magnifications given for all figures are those at which the figures were drawn. The extent to which these magnifications have been reduced in printing can be estimated by comparison of the printed 5 cm. scale with an actual 5 cm. The majority of figures were drawn under the camera lucida.

Plate 3.

Reference Letters in Figures 1–7.

ANCC, giant anchor-cells; BM, boring muscle; CALC, calcareous bodies; DEC, dorsal external excretory channel; DEJ, opening of ductus ejaculatorius; DNB, dorsal nerve branch; DTC, dorsal transverse excretory channel; LEC, lateral main excretory channel; LEL, lateral excretory loop; LLT, lateral longitudinal nerve trunk; P, perforation at base of left pectoral fin; RTM, retractor muscle-fibres of proboscis; TED, terminal excretory duct; TES, testes; U 1, U 2, U 3, first, second, and third limbs of uterus; VEC, ventral external excretory channel; VIT, vitellaria; VNB, ventral nerve branch; VOP, opening of vagina; VTC, ventral transverse excretory channel.

Fig. 1.—*a*, *b*, *c*, *d*, *e*, outlines of young specimens of Amphilina paragonopora (drawn natural size); *f*, *g*, *h*, three specimens of A. paragonopora showing transverse constrictions of body when removed from the host; *j*, an A. paragonopora which measured 280 mm. when alive and uncontracted (contracted to 170 mm. when preserved). The proboscis is evaginated; *k*, the anterior end of the 280 mm. specimen showing the evaginated proboscis as seen under the binocular; *l*, the posterior end of the same specimen showing the opening of the vagina (dorsal) at the base of the papilla and the opening of the ductus at the extremity.

Fig. 2 (drawn natural size) illustrates some of the immobile, mostly amorphous, masses which represent a phase in the life-history of the species; 2, *a* represents the young spherical and ovoid masses; in 2, *m*, one end of a large mass has assumed the form of the anterior extremity of the active worm, and in 2, *n*, the posterior extremity of the active worm is apparent.

Fig. 3 (drawn natural size).—Anterior portion of a large A. paragonopora which has escaped from the body-cavity of the fish through the perforation at the base of the left pectoral fin.

Fig. 4 (×cir. 63).—Transverse section through A. paragonopora about midway in the length of the body.

Fig. 5 (× cir. 63).—Similar transverse section at about the level of the hind end of the anterior fifth of the body.

Fig. 6 (× cir. 7).—Diagrams representing the general arrangement of the excretory channels in the anterior third of the worm and the two main channels throughout.

Fig. 7 (× cir. 950).—Portion of a very fine excretory canal.

Reference Letters in Figures 8–14.

BAY, terminal bay or inlet at hind end of body; BM, boring muscle; CMO, median opening of the ductus ejaculatorius; CT, connective tissue investment of ducts; DEJ, ductus ejaculatorius; EXB, junction of two lateral main excretory channels; FC, fertilization chamber; LEC, lateral main excretory channel; MTD, muscular tissue round ductus; O, ovary; OTP, ootype; OVD, oviduct; PAP, papilla with terminal opening of ductus; PE, penis; PRO, proboscis; RS, receptaculum seminis; RV, receptaculum vitelli; SD, sperm duct; SHGL, shell gland; TED, terminal excretory duct with opening to the left of ductus; TES, testes; U, uterus; U 1, U 2, U 3, first, second, and third limbs of the uterus; UO, opening of uterus; VAG, vagina; VD, vitelline duct; VIT, vitellaria; VOP, opening of vagina; ZD, zygote duct.

Fig. 8 (× cir. 7).—Dorsal aspect of the general reproductive apparatus of Amphilina paragonopora, with three diagrammatic transverse sections in the region of the ovary to show the relative dorsal and ventral positions of the various ducts.

Fig. 9.—Diagram of the general reproductive apparatus of Amphilina foliacea, for comparison with fig. 8.

Fig. 10 (× cir. 660).—The base of the ovary and associated ducts.

Fig. 11 (× cir. 660).—Surface view of the posterior ending of the ductus ejaculatorius, vagina, and lateral main excretory channels.

Fig. 12 (× cir. 63).—Reconstruction from serial longitudinal sections of the structures shown in fig. 11.

Fig. 13 (× cir. 330).—Testes opening into the vas deferens.

Fig. 14 (× cir. 330).—Vitellaria opening into the vitelline duct.

Plate 4.

Reference Letters in Figures 15–25.

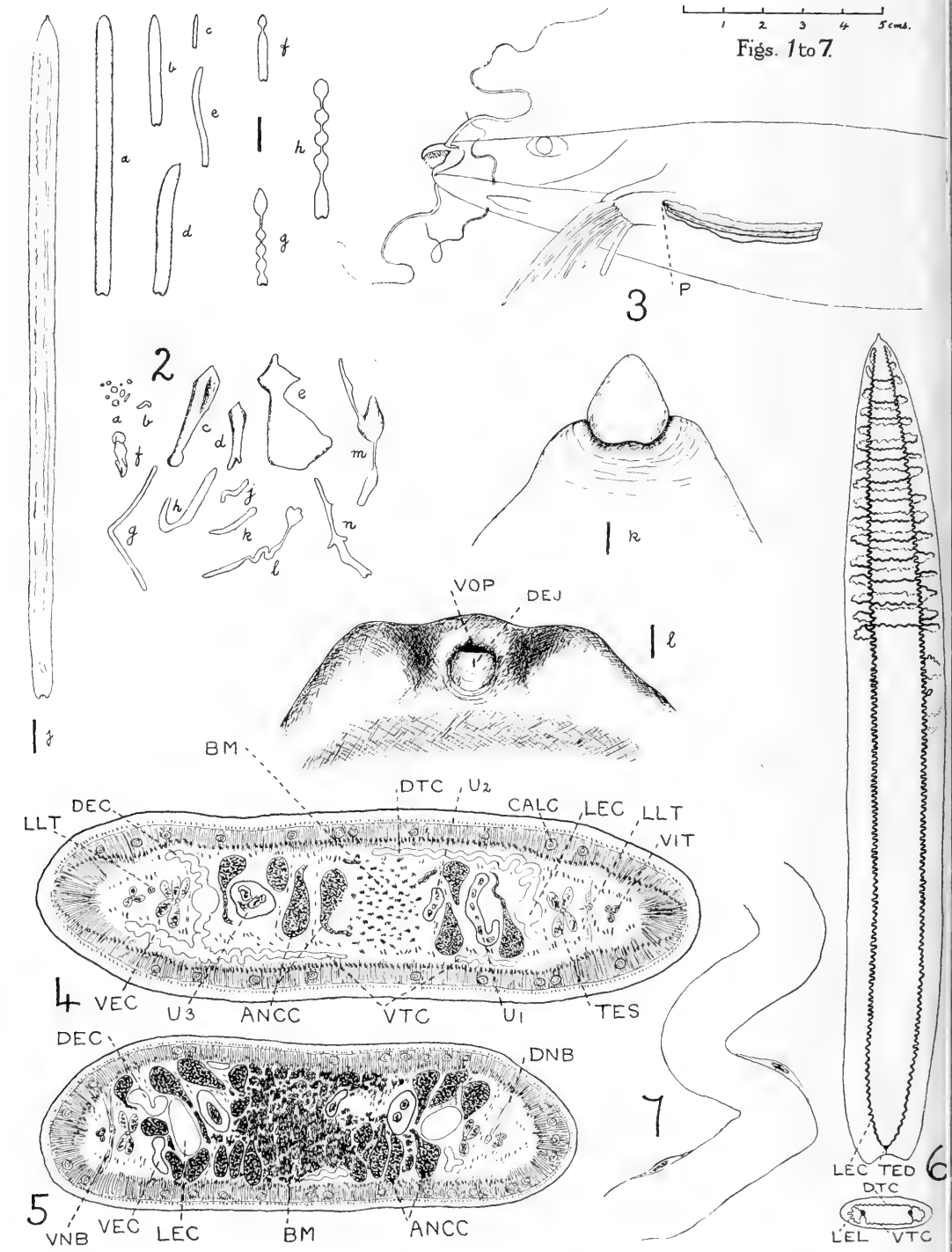
ANCC, giant anchor-cells; BM, boring muscle; CALC, calcareous bodies; LEC, lateral main excretory channel; O, ovary; PRO, proboscis; RTM, retractor muscle of proboscis; SER, serrated cuticle of proboscis; TES, testes; TT, terminal thickening of proboscis; U 1, U 2, U 3, first, second, and third limbs of uterus; UO, opening of uterus; VIT, vitellaria.

Fig. 15.—Sketch of proboscis everted (*a*) and retracted (*b*), drawn from the living animal.

Fig. 16 (× cir. 78).—Everted proboscis in longitudinal section.

Fig. 16, *a* (×1060).—Serrated cuticle covering proboscis.

Fig. 17 (× cir. 78).—Introverted proboscis in longitudinal section (drawn from a whole-mount specimen).



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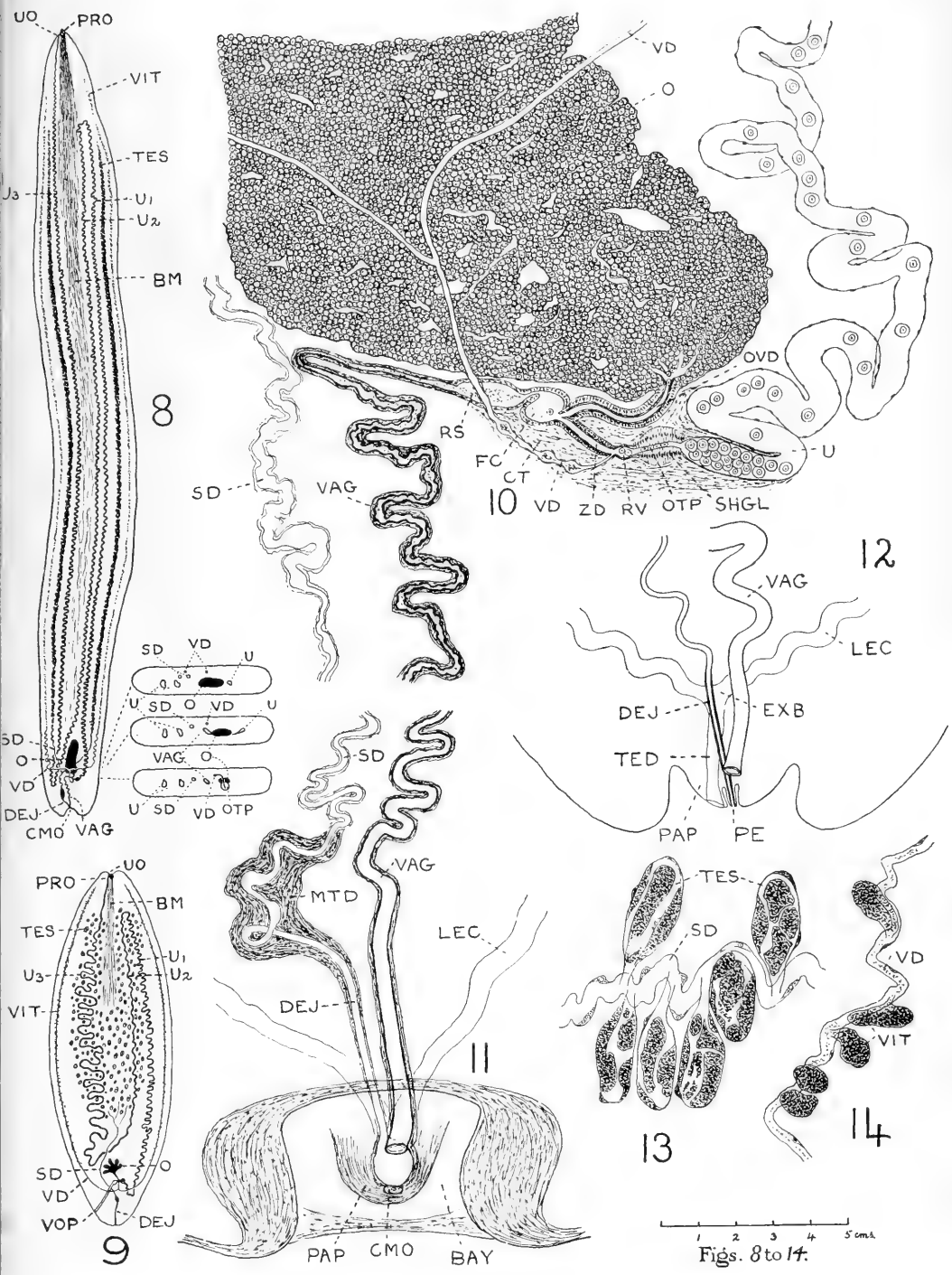
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Figs. 8 to 14.

Fig. 18 (× cir. 150).—Retracted proboscis in transverse section.

Fig. 19 (× cir. 310).—Portion of wall of proboscis in longitudinal section.

Fig. 20 (× cir. 2).—Diagram to illustrate the longitudinal extent of the boring muscle of the proboscis.

Fig. 21 (× cir. 7).—Diagram to illustrate the distribution of the boring muscle-fibres and the giant anchor-cells (Salensky's ' problematic cells '), and the same structures shown in two transverse sections (*a* and *b*) at the levels indicated.

Figs. 22, 23 (× cir. 300).—Giant anchor-cells with boring muscle processes.

Fig. 24 (× cir. 300).—Fibres of boring muscle.

Fig. 25.—Diagram illustrating the connexion of the boring muscle-fibres with the anchor-cells.

Reference Letters in Figures 26–39.

ANCC, giant ' anchor '-cell ; BC, ' brain ' commissure ; BM, boring muscle ; CALC, calcareous body : CUT, cuticle ; EGSH, egg-shell ; F, filament or tag on egg-shell ; GBL, giant blastomere ; GC, gland-cell ; HK, hooklet ; ICM, inner circular muscle-layer ; ILM, inner longitudinal muscle layer ; INB, internal nerve branch ; IVM, investing membrane of larva ; LLT, lateral longitudinal nerve-trunk ; NFI, nerve-fibre ; OBLM, oblique muscle-layer ; OCM, outer circular muscle-layer ; OLM, outer longitudinal muscle-layer ; PAR, parenchyma ; RTM, retractor muscle-fibres ; SUBC, subcuticula ; UO, opening of uterus ; YO, yolk material.

Fig. 26 (× cir. 330).—Transverse section through body-wall of A. paragonopora.

Fig. 27 (× cir. 330).—Longitudinal section through body-wall.

Fig. 28 (× cir. 980).—Early stage of growth of calcareous body.

Fig. 29 (× cir. 63).—Semi-diagrammatic figure of the anterior end of the central nervous system.

Fig. 30 (× cir. 580).—Eggs in commencement of uterus with shells and yolk.

Fig. 31 (× cir. 580).—Morula stage of embryo surrounded by investing membrane.

Fig. 32 (× cir. 580).—Early larva in longitudinal section showing one large blastomere (shell not shown).

Fig. 33 (× cir. 580).—Section through posterior end of larva showing formation of hooklet-cells.

Fig. 34 (× cir. 580).—Early larva in section showing gland-cells and hooklet-cells.

Fig. 35 (× cir. 580).—Late larva seen in optical section (the gland-cells, if present, were invisible).

Fig. 36 (× cir. 980).—Hooklet from posterior end of larva.

Fig. 37 (× cir. 580).—Three of the degenerate larvae in shells, which

occupied most of the uterus of the 280 mm. specimen of A. paragonopora (macerated in Marcacci's fluid).

Fig. 38 (× cir. 580).—An oval flat free larva from the same uterus (i. e. of the 280 mm. Amphilina) seen in section.

Fig. 39 (× cir. 480).—Similar degenerate larvae in shells and free larvae from the same large Amphilina, macerated in water only.

Plate 5.

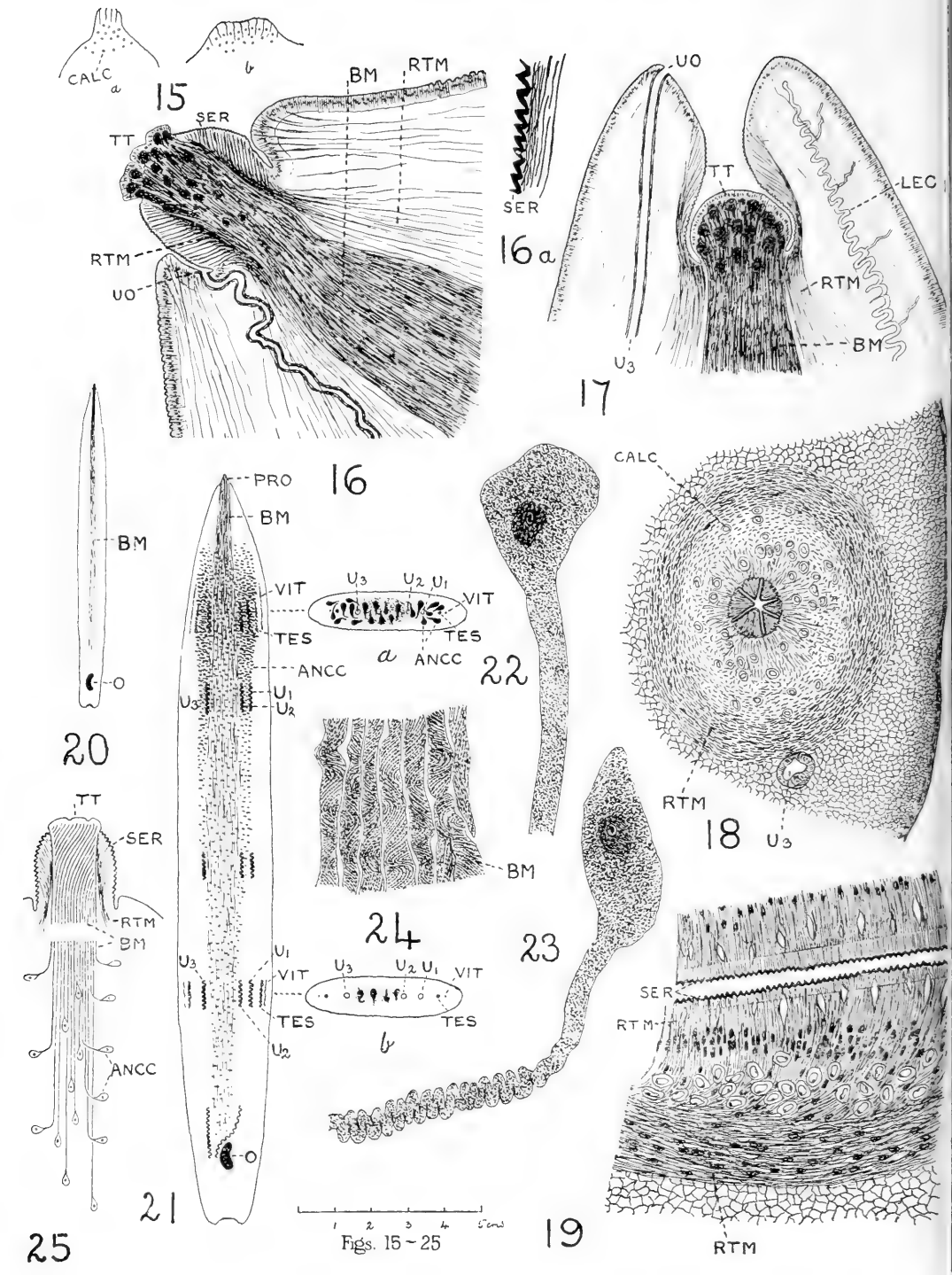
Figs. 40, 41, 42 (×500).—Sections of early stages of development of amorphous masses, from mesentery of Macrones sp.

Figs. 43, 44 (×112).—Sections of later young stages of development of amorphous masses.

Fig. 45 (× 35).—Section of amorphous mass elongated and slightly coiled in capsule.

Fig. 46 (natural size).—An elongated large fully-grown form of amorphous body lying free in body-cavity of fish, which would soon become transformed into an active Amphilina.

Fig. 47 (×112).—Young cyst developed from the mesentery and enclosing degenerate larvae and disintegration products.



Figs. 15 – 25

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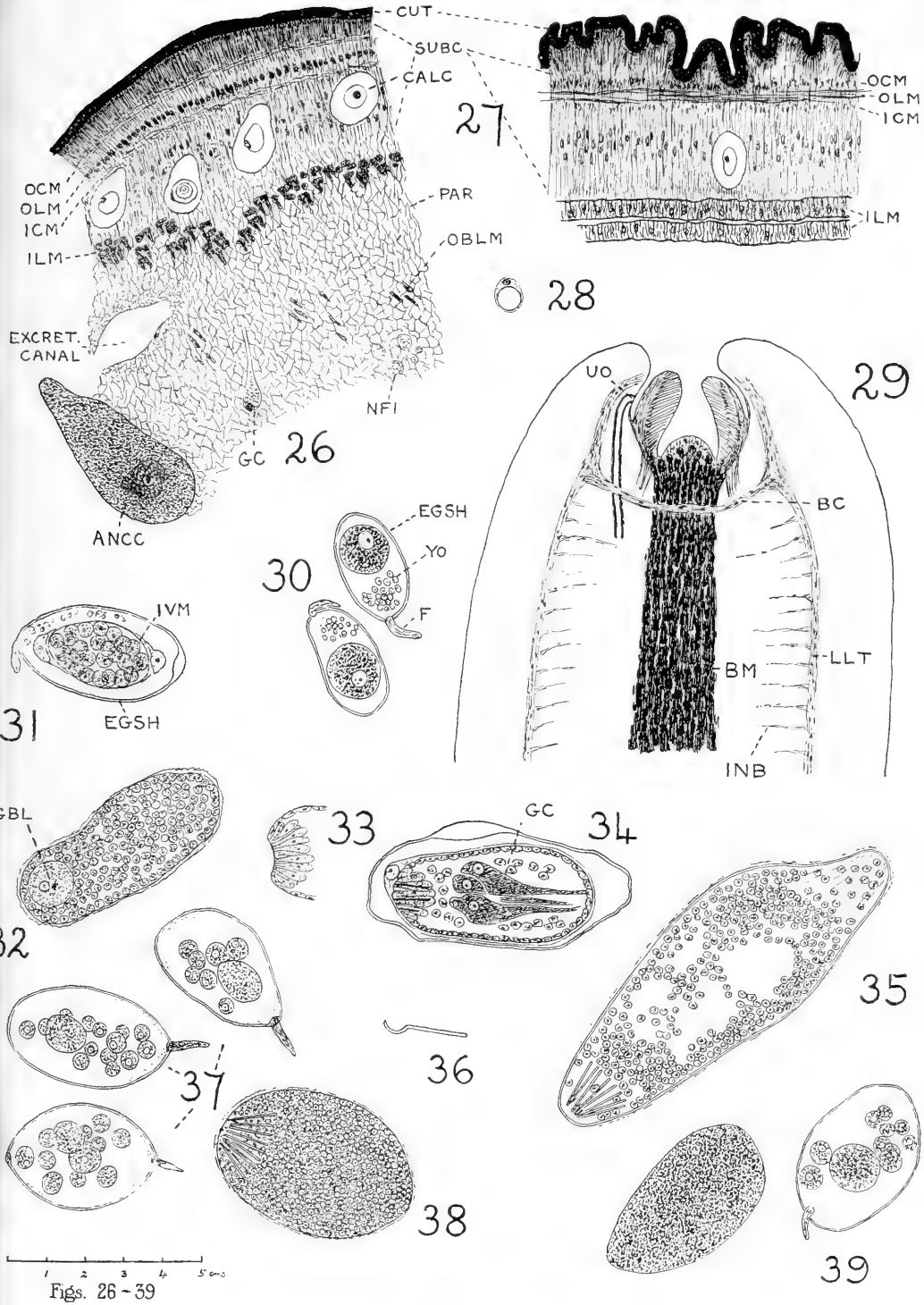
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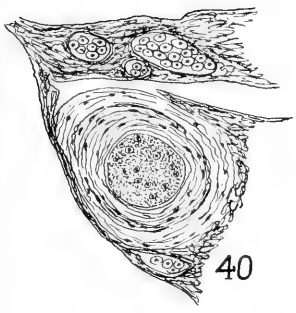
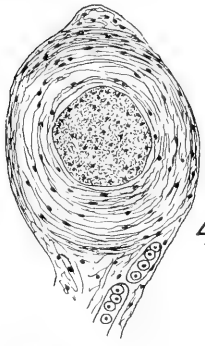
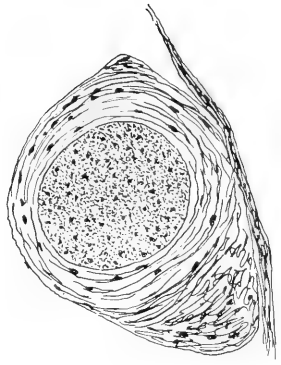
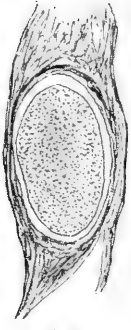
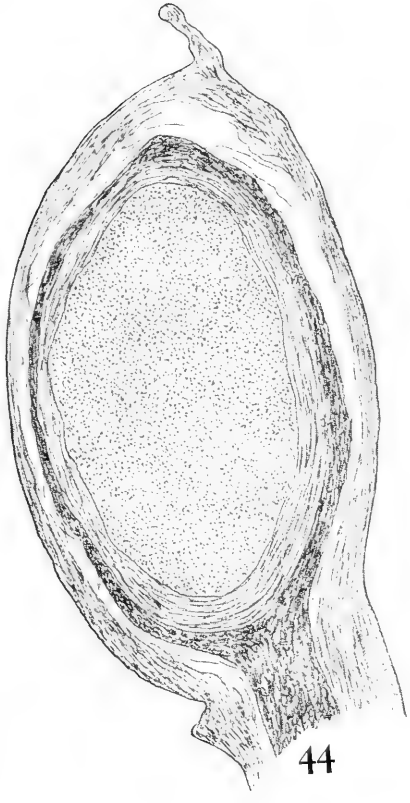
Figs. 26–39

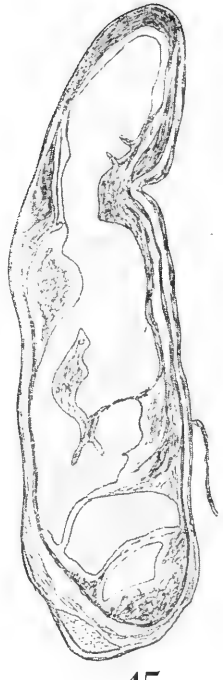


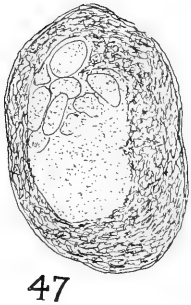


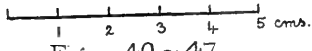












Figs. 40–47

W. N. F. Woodland, del.

On the Strobilization of Aurelia.

By

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With Plate 6 and 3 Text-figures.

1. Introductory and Historical.

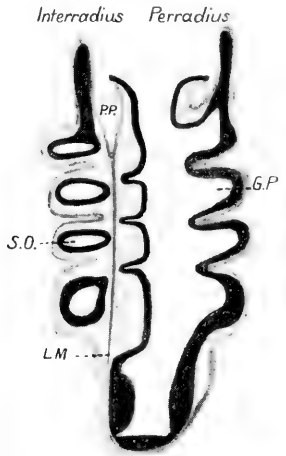
It is well known that in the production of a polydisc strobila the scyphistoma undergoes a series of constrictions as far as the septal longitudinal muscle-strands, commencing just below the wreath of tentacles and continuing downwards towards the foot. There is always a portion at the pedal end which does not undergo constriction, remaining ultimately as a polyp after regeneration of oral disc and tentacles. The animal at this stage is a pile of segments, the oldest and most defined being at the oral end, while the youngest and least defined is near the pedal end. Later, there usually arise from each segment, or ephyra rudiment, eight radiating lobes enclosing diverticula from the enteron, four in the interradii and four in the perradii. At the end of each lobe develops a median outgrowth containing an extension of the enteron, which becomes the tentaculocyst. On each side of the tentaculocyst is a wing-like lappet of ectoderm.

The scyphistoma, just before undergoing the external changes, has four radiating septa perforated each by an ostium beneath the oral disc, forming together a 'gastral ring-sinus'. As the body increases in length new ostia arise below the old ones (Text-fig. 1), and the constriction takes place between the sets of perforations which later take part in the formation of the gastral cavity of the ephyra.

There appears to be unanimity of opinion between previous workers on this portion of the change, but with regard to later developments there is some diversity of view. Perhaps they

are least unanimous on the subject of the origin of the manubrium of the non-terminal ephyra. 'According to Goette it proceeds from a completely new formation after the casting off of the preceding ephyra' (Heric). Claus (**1**) figures Aurelia as having, between two ephyra rudiments, a circular shelf or horizontal fold of the two-layered wall of the connecting tube. The ectodermal portion of the fold is apparently continuous all round, but the endodermal portion is interrupted in

Text-fig. 1.



Early stage in strobilization.

each interradius. Its four radial components appear from within as grooves, the adjacent ends of which grow towards each other and meet between the longitudinal muscle-strand and the ectoderm, thus forming the continuous ring-canal which Claus calls the 'ring-sinus of the proboscis (i.e. manubrial) disc'. As a result of this the septal or longitudinal muscle-strand in the region connecting adjacent ephyrae is surrounded by endoderm, and when the edge of the incipient manubrium becomes split off from the exumbrella of the preceding ephyra, it bends outwards, leaving the septal muscles to act as connecting strands between the two ephyrae. In fact the original

neck connecting one ephyra with its neighbour is now reduced to the four longitudinal muscle-strands, each surrounded by a strip of endoderm, while the wall of the neck has become converted into the manubrium and spread out horizontally.

Heric (**4**), working on Chrysaora, shows the intersegmental folds, but states that they occur only between the septa as four bladder-like outgrowths of both layers. These, on breaking away distally from the exumbrella of the upper ephyra, spread outwards as four semicircular flaps the adjacent edges of which meet and fuse in the interradii, so producing the flat manubrium. Thus, he says, the connecting strands have endoderm on the inner side and ectoderm on the outside (whereas in Aurelia, according to Claus as we have just seen, ectoderm is entirely absent).

Claus and Heric show, then, that the manubrium is undergoing development at the same time as the rest of the ephyra, and that the lining of the manubrium is of endoderm.

Claus also states that the manubrium of the polyp, which remains after strobilization, is lined by endoderm, so that at no time is there a stomodaeum such as is described by Goette. The work of Friedemann (**3**) and Hein (**2**) on the embryological aspect confirms the above as regards the endodermal lining of the manubrium and the absence of stomodaeum.

Claus and Heric state that the gastral filament is derived by the transformation of the columella, i.e. the axial portion (internal to the ostium) of the taeniole containing the longitudinal muscle-strand, which for a time connects the subumbrella and exumbrella of a developing ephyra.

With regard to the septal muscle-strands Goette has described them as hollow structures with the cavity continuous with that of the peristomial pit. Claus (**1**), Friedemann (**3**), and Heric (**4**) state that the muscles are solid structures, and Friedemann figures the peristomial pit of a well-developed scyphistoma as being distinct from the septal muscle.

There does not appear to be any record of an observation on the development of the peristomial pits in a non-terminal ephyra. Claus (loc. cit.), in one figure, indicates a peristomial

pit on one non-terminal ephyra but shows nothing further. Heric concludes that they are new structures.

In the same way there is little information on the regeneration of the oral disc of the polyp which remains after strobilization. Heric indicates that the development of the proboscis is similar to that of the manubrium of the ephyra, but says that the four strands connecting the polyp with the ephyra above pass directly to the wall of the enteron after having become dissociated from the proboscis. According to this the polyp would have no septal ostia, and the oral end of the longitudinal muscle would be absent for a time. Whether this is so or not in Chrysaora I cannot say, but, as shown below, it is not the case in Aurelia. He goes on to say: 'How the oral disc (of the polyp) is connected with the septa can be answered just as little as the question whether the septal muscle, in its course, is transformed by the remainder of the polyp to be replaced by a new muscle originating from the oral disc, or whether it is preserved and enters (secondarily) into connexion with the oral disc'.

A complete ephyra becomes free by the gradual extension of the connecting strands and by their final rupture. Prior to this event the exumbral opening has closed. Heric believes that this closure takes place simultaneously with the breaking of the strands. He did not find any trace of longitudinal muscle in an ephyra immediately after detachment.

2. New Investigation.

The material used in this work was obtained from the Dove Marine Laboratory, Cullercoats, Northumberland, and was killed and fixed in saturated aqueous corrosive sublimate solution with 2 per cent. glacial acetic acid. Delafield's haematoxylin, and iron haematoxylin and eosin were used to stain the serial sections. Delafield's haematoxylin is very useful for general observations, but the latter stains were better for determining the limits of ectoderm and endoderm, especially in the early stages of the manubrium.

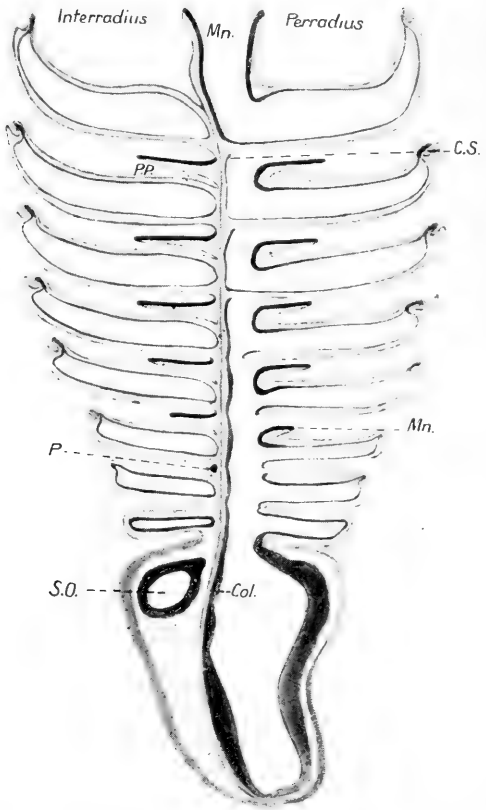
It should be understood that my description of the forma-

tion of an ephyra does not refer to the transformation of the original oral disc of a scyphistoma, but to the fate of the segments formed below the original oral disc.

Formation of Manubrium.—The constriction of a polyp proceeds as far as the longitudinal muscle, the ectodermal groove interradially cutting the septum horizontally. Between the interradii the endoderm is pushed inwards, but the edge so formed does not reach the imaginary line taken between two adjacent longitudinal muscles. This edge is concave internally, so that a transverse section between two ephyra rudiments would show a roughly cross-shaped gastral cavity with the longer diameters lying in the perradii. About this time a groove appears in the subumbral endoderm near the inner edge, beginning in the perradius as a very shallow groove (Pl. 6, fig. 12 *a*) and becoming deeper passes towards the septum or taeniole. The fold of endoderm thus formed pushes into the mesogloea between the subumbral endoderm and ectoderm (Pl. 6, fig. 12 *b*, *Gr.*). The groove, as it approaches the taeniole, divides into two portions at its septal end. One of these portions is a gutter passing round on the inside of the septal muscle to meet its fellow from the other side, and the other portion is a solid plug of cells (Pl. 6, fig. 12, *P.*) which grows through the septum towards the interradius, where it meets and fuses with a fellow from the other side, lying between the septal muscle and the ectoderm. The muscle is thus surrounded, in this manubrial region, by endoderm. The portion of the plug of endoderm cells lying in the interradius causes a bulging of the ectoderm at that point (Pl. 6, fig. 9), but the outer layer does not project all the way round. Four horizontal perradial slits now appear in the connecting tube in the same plane as the ectodermal projections (Text-fig. 2), so that the gastral cavity comes into communication with the outside. Finally, the slits unite across the interradial ectodermal projections and the outer layer of an upper ephyra is cut off from that of the next below. While this takes place, a split occurs in the plug of endoderm cells connecting the two endoderm grooves (Pl. 6, fig. 9, *S.*), which now become continuous

abaxially to the longitudinal muscle. The lip of what is now the rudimentary manubrium, curls outwards involving the subumbral wall of the endodermal groove. Perradially, where

TEXT-FIG. 2.



Determination of number of ephyrae and laying down of form of polyp.

there is practically no groove (Pl. 6, fig. 12 *a*) the curling causes a considerable increase in the diameter of the manubrial opening. This, no doubt, has some influence on the formation of the cruciate cavity of the manubrium. As Claus and Heric have shown, a manubrium formed this way has a lining of

endoderm. It is, for a time, a flattened plate having a central opening into the enteron. This change does not involve the appearance of a ' proboscis ring-sinus ' as described and figured by Claus, but it is possible that the solid rod of endoderm cells and the ring-sinus may be developed under different conditions.

Peristomial Pit.—Associated with the development of the manubrium is the formation of the peristomial pits. As has been stated, the constriction in the interradius brings the ectoderm very close to the longitudinal strand (they are separated by a thin layer of mesogloea), and when the endodermal plug pushes out the ectoderm a small interradial funnel is formed in the angle made by the fold and subumbrella (Pl. 6, fig. 9, *P.P.*); this is the rudiment of the peristomial pit. The subsequent curling outwards and growth of the manubrium and the increase in depth of the ephyra bring about the deepening of the funnel (Pl. 6, fig. 10, *P.P.*).

Longitudinal Muscle.—Contrary to what Claus, Friedemann, and Heric have maintained, transverse sections of a scyphistoma show that the septal muscles may be hollow structures (Pl. 6, fig. 8). The cavity may not be continuous along the whole length, but it generally appears a short distance below the peristomial pit, and, as Friedemann has shown, the end of the muscle and the apex of the pit are not continuous with each other. The muscle-cells are arranged with their tails to the mesogloea and the protoplasmic parts towards and projecting into the lumen (Pl. 6, fig. 8). There are mesogloeal ridges projecting into the cavity and on these are set muscle-cells, and this probably serves to increase the amount of muscular surface. Sometimes the cavity is quite wide, and none would describe such a structure as a solid muscle. Towards the foot the muscle-cells are usually closely packed, and this portion of the strand can be described as solid.

In a strobila, mainly in the lower segments, sections show that some parts of the muscles may be hollow, though the cavities in such cases are short, extending along the depth of an ephyra rudiment. As an ephyra becomes more developed and the circular and radial muscles begin to function, the

longitudinal muscles begin to atrophy and are seen in longitudinal sections as mesogloeal bands striated with degenerate muscle-fibres (Pl. 6, fig. 11, *R.L.M.*). The bands stain more deeply than the rest of the mesogloea when treated with Delafield's haematoxylin.

Gastral Filaments.—About the time when the manubrium of the ephyra has become curled outwards the gastral filaments first appear. They occur in pairs, one pair per interradius (Pl. 6, figs. 1 and 5, *G.F.*). On each side of the columella, and close to the exumbral endoderm, there grows laterally and towards the central stomach an endodermal process (Pl. 6, fig. 1, *G.F.*). Very soon the tip turns towards the oral opening and remains pointing in that direction until the ephyra becomes free (Pl. 6, fig. 5). Occasionally one or both filaments may be suppressed. Thus, contrary to what has been maintained by all writers hitherto, the longitudinal muscle takes no part in the formation of the filaments which may be seen in a strobila in various stages of development.

Separation of an Ephyra from the Strobila.—In passing upwards towards the oldest ephyra the connecting strands are seen to become slightly stretched and the covering epithelium does not stain so deeply as that lower down. They converge to the apex of the exumbrella when the apical opening has become obliterated. The closure of this opening takes place comparatively early in the history of the attached ephyra (Text-fig. 2), and a free disc having such an opening may be considered to have been prematurely detached. Such may happen in the laboratory when a strobila is roughly handled.

Shortly before an ephyra becomes separated it is seen that the peristomial pits are very deep (Pl. 6, fig. 10, *P.P.*), and the depth from the subumbrella to the exumbrella is much greater than that in a younger disc. Also the columella is stretched so that the gastral filaments are brought some distance away from the exumbrella (Pl. 6, fig. 5). The covering of this stretched portion resembles in staining properties and cell-form that of the connecting strand in the same condition. The longitudinal

muscle here is degenerate, and the apical portion of the ephyra is thickened with mesogloea in which the muscle remnants can be seen (fig. 5, *L.M.*). As Heric suggests, the separation is probably brought about by the violent action of the circular and radial muscle-bands. The connecting strands break close to the exumbrella. About the same time the stretched part of the columella breaks, resulting in the carriage of the gastral filaments to the subumbral side. The remnant of the stretched portion is seen as a small papilla of cells between the bases of the gastral filaments (Pl. 6, fig. 3, *R.C.*).

The rupture of the columella brings about a rapid shortening of the peristomial pit, which is now seen to be a small but distinct funnel-shaped depression (fig. 3, *P.P.*) in the subumbrella close to the bases of the gastral filaments. The greater part of the wall of the pit has gone to form a portion of the subumbral surface and the base of the manubrium. The pit, which was close to the base of the manubrium, is now some distance away. The gastral filaments no longer point to the oral opening but come to be roughly in a line at right angles to the interradius.

When an ephyra becomes free the connecting strands are left projecting from the oral opening of the next ephyra (Pl. 6, fig. 10, *C.S.*) and are soon reduced in length. The flat manubrium of this next disc now begins to assume the tubular form, which is not completed until separation and the rupture of the columella (Pl. 6, fig. 5).

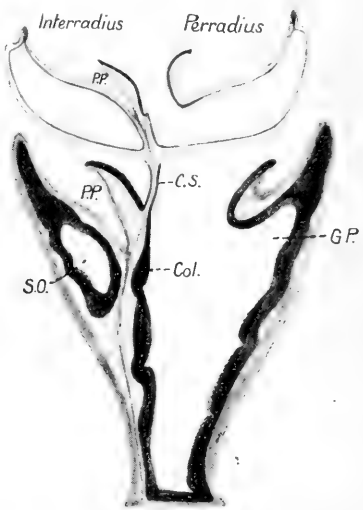
Muscle remnants are to be found in a newly separated ephyra in the thickened apex (Pl. 6, fig. 4) and at the base of the manubrium on the inner side of the peristomial pit. A vestige of the connecting strand and the part of the muscle between it and the apex of the pit can be found at the base of the manubrium (Pl. 6, fig. 3, *R.L.M.*).

Formation of the Proboscis of the Polyp.—A strobila can be divided into two regions, viz. segmented, giving rise to ephyrae, and unsegmented, producing the polyp. Between every two adjacent constrictions lie four septal ostia arranged in a 'ring-sinus'. Below the lowest

constriction are four ostia which usually remain to form the gastral ring-sinus of the scyphistoma.

The proboscis of the polyp develops in a manner similar to that of an ephyra, but there is evidence of the formation of a proboscis ring-sinus such as Claus has described for the ephyra. The edge of the proboscis curls outwards but the opening is much wider than that in an ephyra. The passage of the

Text-fig. 3.



Before liberation of last ephyra. Polyp complete.

connecting strands direct to the gastral wall of the enteron and unconnected with the wall of the oral opening was seen in only one case. Such a condition (which it will be remembered Heric regards as normal in Chrysaora) is probably accidental, and may be described in Aurelia as abnormal. In a case of this kind there are no septal ostia and so no gastral ring-sinus. The upper portion of the enteron is as entire as in the free ephyra. Usually the columellae persist and the resulting polyp possesses all the features of the original scyphistoma from which it has been formed (Text-fig. 3).

As far as has been ascertained the peristomial pit of the

polyp does not arise in exactly the same manner as in the ephyra. It occurs in the same relative position but apparently not as a pit. A specimen showing the earliest trace had a solid strand of cells reaching from the oral disc obliquely inwards and downwards to the longitudinal muscle (Pl. 6, fig. 2, *R.*). At the outer end was a slight depression which was probably the commencement of the formation of a cavity in the strand. Another specimen showed a complete pit the apex of which bore the same relation to the longitudinal muscle as does that of an ephyra (see Text-fig. 3). Probably, then, the strand of cells becomes hollow from outside inwards. This cavity should not be confused with that in the longitudinal muscle, nor do the two ever communicate.

Ciliation of the Ectoderm.—Gemmill (**5**) has shown that, in the ephyra, there are definite currents passing over the ectodermal surface apparently for the purpose of carrying small animals to the lappets, where they are pierced by stinging threads and afterwards carried to the mouth by flexure of the arm. He also states that the scyphistoma captures infusoria in much the same way as the ephyra, the tentacles taking the place of the arm lappets.

Powdered carmine suspended in sea-water will also serve admirably to demonstrate these currents. In the scyphistoma the carmine particles are carried upwards along the surface of the body and become entangled in slime secreted by the ectoderm. Between the bases of the tentacles ropes of particles and slime may be seen carried along the disc and up to the edge of the proboscis. The tentacles, along which the current passes to the tip, sometimes curl over into the proboscis and the material travels into the gastric cavity. Often a slowly revolving ball is formed above the mouth and finally passes slowly down into the enteron. The same applies to the ephyra, in which the passage of the carmine ball into the gastric cavity can be easily seen. Sometimes the ball will be slowly ejected from the enteron immediately after being taken. Gemmill believes that particles are taken in ' by a central inhalant current which is compensatory to exhalant currents produced by ciliary action

in the floor of the mouth angles ', adding ' but this may not be the whole explanation '.

In both ephyra and scyphistoma the use of powdered carmine seems to show that the lining of the proboscis or the manubrium has cilia which may produce exhalant or inhalant currents when necessary. The stream of particles passes down the angles in the perradii as well as along the ridges of the interradii; further, expulsion of material may follow the same channels. In only one case did I observe both currents acting simultaneously, and then the in-currents moved along the interradial ridge and the ex-currents along the perradial angles. A strobila shows a strong current from the foot upwards to the uppermost ephyra. This is due to the fact that the surface is composed of a considerable portion of the aboral surface of each ephyra, on which surface the current is centrifugal to the ends of the lappets. There did not appear to be any passage of carmine in between any two ephyra rudiments. This suggests that the intermediate ephyrae or ephyra rudiments do not feed by means of these currents.

High power examination shows that the currents are caused by flagellated ectoderm cells (Pl. 6, figs. 6 and 7). The ectoderm of the scyphistoma, except on the pedal disc, and of the ephyra appears to consist entirely of flagellated cells. Even the surface of the tentaculocyst is provided with flagella. When death takes place, either naturally or by poison, the flagella usually disappear. Occasionally in a well-fixed specimen they still persist and can be studied by means of sections. In the case of the lining of the manubrium or of the proboscis the flagella are almost always visible in sections, an interesting point which corroborates the histological evidence as to the endodermal nature of this lining.

Remarkable cases of Polyp Formation.—While keeping under observation a young strobila for the purpose of watching the transformation of the oral disc into an ephyra, I was able to observe a number of interesting but unexpected changes. There were five constrictions in the strobila, and the two ephyra rudiments next below the oral disc proceeded

normally to give rise to ephyrae. The two rudiments below these, however, produced eight tentacles each and no lobes. The oral disc did not undergo any change except that it became separated from the rest and lay on the bottom of the dish. There was a large apical opening the presence of which suggested that the disc had been prematurely separated. After some days the hole closed and the tentacles were absorbed. No lobes developed and later a pedal stalk grew out from the apical end, the body ultimately becoming attached to the floor of the dish. Later four perradial tentacles appeared and the whole had the form of a normal scyphistoma. The next two rudiments proceeded normally and produced ephyrae which were subsequently liberated.

The rudiments possessing tentacles then became free and moved over the surface of the dish for about a week by means of the flagellated surface, one of them revolving, the other progressing in a more or less straight line. Later each grew a foot stalk from the apex and became attached to the vessel. In the meantime one had increased the number of tentacles to twelve.

A second young strobila also behaved in an unusual manner. Here the two segments next to the oral disc gave rise each to four tentacles and no lobes. They, along with the oral disc, became separated in a body and remained attached to each other while on the floor of the dish. The oral disc did not appear to undergo any change, but the other two segments gradually absorbed their tentacles and became converted into a long narrow stalk at the end of which was a pedal disc. Attachment with the dish was effected, the stalk became stouter, and the whole body assumed the form of a normal scyphistoma.

The occurrence of segments which give rise to polyps leads one to believe that there is a distinct difference between these and the ephyra rudiments. It may be that they have been derived from ephyra rudiments, the sequence of changes necessary to produce an ephyra having been interrupted or reversed, or they may be different from the start. Again, it may be that up to a certain point the segment may be regarded

as undifferentiated and, in the cases where polyps are produced, they have remained so. It is interesting to note how they agree in general behaviour with the upper part of the base of the strobila, which produces oral disc and tentacles, while the segment above gives rise to an ephyra.

The several ways in which a polyp may be formed are thus :

By direct development from the egg.

By the outgrowth of stolons from a polyp.

By the elaboration of the basal portion of a strobila.

By the separation of an unchanged oral disc.

By the elaboration of an intermediate segment.

I wish here to acknowledge my indebtedness to Professor W. Garstang for the kindly criticism and help which, from time to time, he has afforded.

Summary.

The manubrium of a primarily non-terminal ephyra is formed from the connecting tube between two ephyra rudiments.

The proboscis of the polyp remaining arises in a manner similar to that of the manubrium of the non-terminal ephyra.

The apical opening of an ephyra normally closes before liberation.

The connecting strands are covered with endoderm.

The longitudinal muscles of a polyp may be hollow and the cavities are not in communication with the peristomial pits.

The formation of the peristomial pit is associated with the development of the manubrium.

The gastral filaments are formed as paired outgrowths of the endoderm of the columella. They do not involve the longitudinal muscle in their production. They originate early in the history of an ephyra.

There are definite currents over the ectoderm caused by flagellated ectoderm cells.

The segments of a strobila may give rise to ephyrae or polyps and the oral disc may separate unchanged to continue its existence as a polyp.

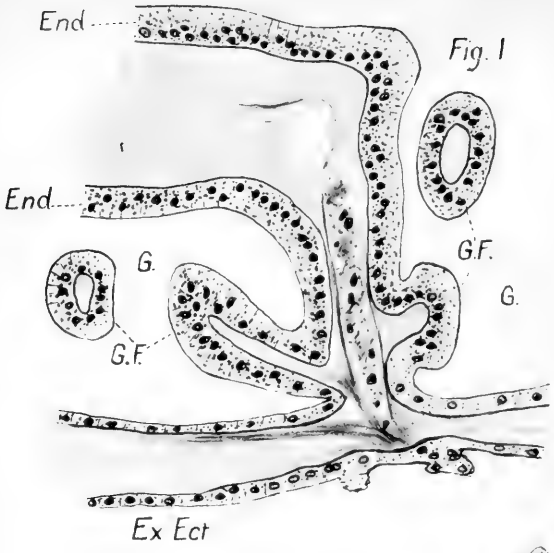


Fig. 1

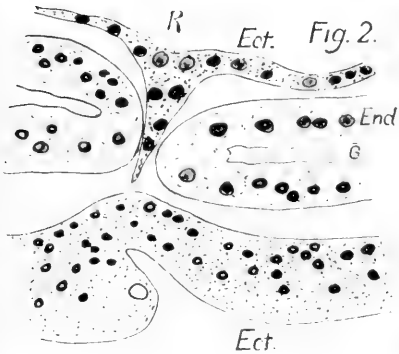


Fig. 2.

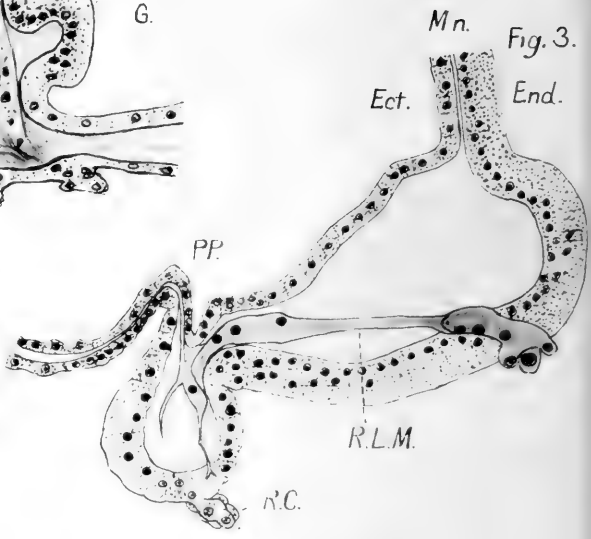


Fig. 3.

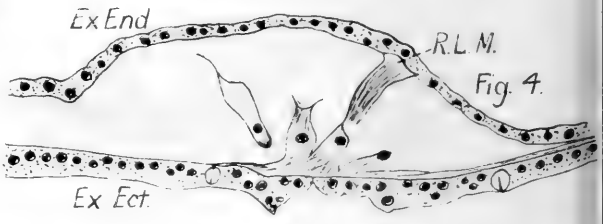


Fig. 4.

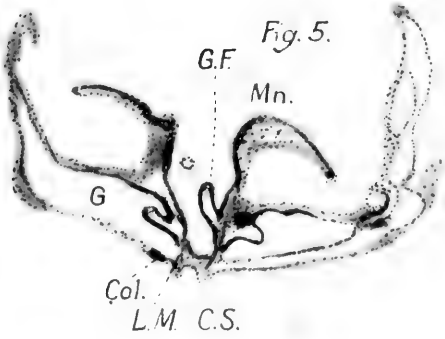


Fig. 5.

Fig. 6

Fig. 7.

b

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Fig. 8.

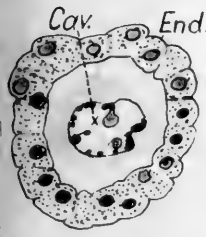
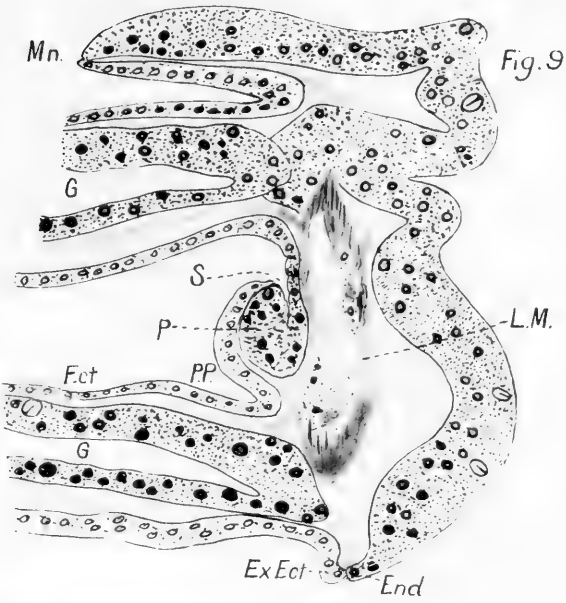


Fig. 9.



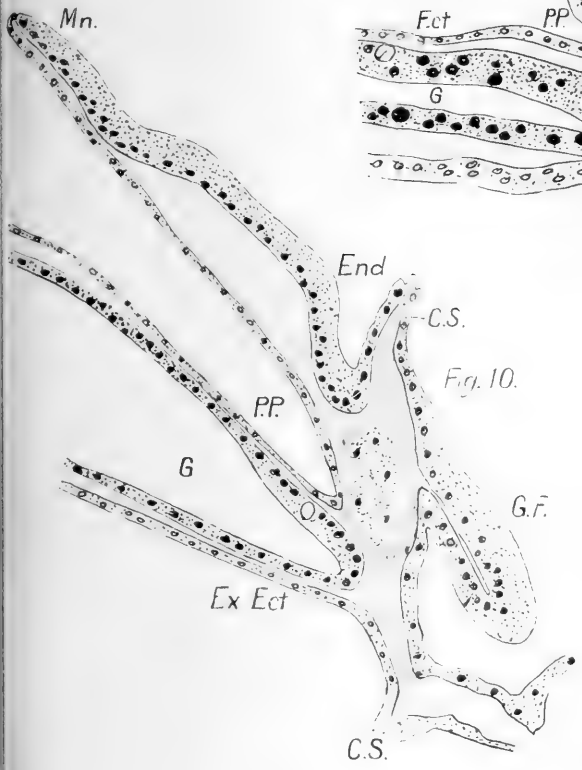


Fig. 10.

Fig. 12.

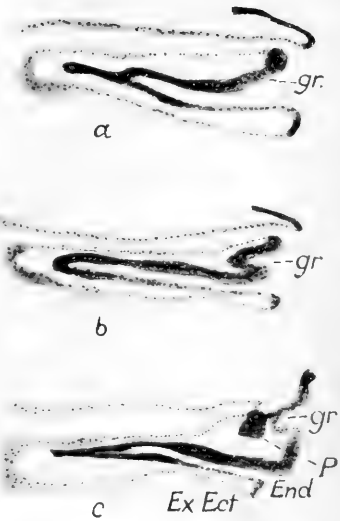
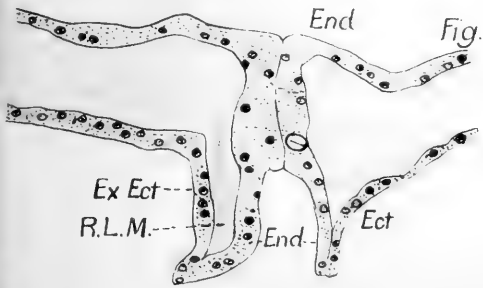


Fig. 11.



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EXPLANATION OF PLATE 6.

Fig. 1.—Longitudinal section of polydisc strobila showing relation between gastral filaments and columella.

Fig. 2.—Tangential section of base of strobila showing portion of solid rod of ectoderm cells passing from oral surface to longitudinal muscle.

Fig. 3.—Vertical section through interradius of newly liberated ephyra showing base of manubrium, peristomial pit, common base of gastral filaments, and remnant of longitudinal muscle.

Fig. 4.—Vertical section through apex of newly separated ephyra showing mesogloeal thickening and muscle remnants.

Fig. 5.—Median vertical section through uppermost ephyra of polydisc strobila.

Fig. 6.—Flagellated cell from ectoderm of scyphistoma.

Fig. 7.—Portion of living tentacle (*a*) extended, (*b*) contracted, showing flagella and cells.

Fig. 8.—Transverse section through connecting strand about half-way along a polydisc strobila, showing cavity of muscle, and muscle fibres.

Fig. 9.—Longitudinal section (somewhat oblique) cutting interradius of two lowermost ephyra rudiments showing origin of manubrium.

Fig. 10.—Longitudinal section (oblique) through interradius of strobila showing relation between peristomial pit and longitudinal muscle of second ephyra down.

Fig. 11.—Longitudinal section through centre of third ephyra down showing closure of apical opening.

Fig. 12.—Three longitudinal section of lower ephyra rudiment in fig. 9 showing form of endodermal groove which provides lining of manubrium (*a*) almost perradial, (*b*) about adradial, (*c*) near interradius and showing commencement of abaxial rod of cells.

Abbreviations.

C.S., connecting strand. *Cav.*, cavity of longitudinal muscle. *Col.*, columella. *Ect.*, ectoderm. *End.*, endoderm. *Ex.Ect.*, exumbral ectoderm. *Ex.End.*, exumbral endoderm. *G.*, gastral cavity. *G.F.*, gastral filaments. *G.P.*, gastral pouch. *Gr.*, groove of endoderm to form manubrium. *L.M.*, longitudinal muscle. *Mn.*, manubrium. *P.*, plug of endoderm cells to form interradial portion of manubrium. *P.P.*, peristomial pit. *R.*, rod of ectoderm cells to form peristomial pit of polyp. *R.C.*, remnant of exumbral end of columella after rupture. *R.L.M.*, degenerate longitudinal muscle. *S.*, split in ectoderm and in endodermal plug. *S.O.*, Septal ostium.

Histology of the Soft Parts of Astraeid Corals.

By

G. Matthai, M.A.,

Mackinnon Student of the Royal Society during the years 1914–17.

With Plates 7 and 8.

The following account of the histology of the soft parts of the Astraeidae is supplementary to the description given in the Introduction to my paper on 'A Revision of the Recent Colonial Astraeidae possessing Distinct Corallites' (**25**, pp. 1–32). It is based on the study of a large number of polyps belonging to various Astraeid species of the Indo-Pacific and Atlantic regions, particularly of Favia favus (Forsk.), Favia hululensis (Gard.), Coeloria daedalea (Ell. and Sol.), Leptoria gracilis (Dana), Eusmilia aspera (Dana). During a short stay at the Carnegie Marine Biological Station at Tortugas (July 16–August 2, 1915), living colonies of all coral species of that locality were kept under observation, but at that time larvae were extruded only from colonies of Favia fragum (Esp.). These were fixed at different intervals during the free-swimming stage—from eight hours to about ten days—in Flemming's fluid, corrosive acetic solution, and Bouin's fluid, and were subsequently sectioned serially to thicknesses of 4 μ, 6 μ, 8 μ, and 10 μ in order to compare their histology with that of adult colonies. No larvae of any species were obtained during a subsequent visit to the Bermudas (Aug. 20 – Sept. 14). Solid embryos lying in the coelenteric cavities of polyps from a colony of Favia fragum, which Dr. Vaughan forwarded to Professor Gardiner from South Bight, Bahamas, and which have been sectioned, were also studied.

The colonies from the Indo-Pacific region were fixed in saturated solution of corrosive sublimate and in formic aldehyde poured into sea-water; those from the Atlantic region were

narcotized in a partly expanded condition in weak solutions of magnesium sulphate before fixation in formalin. They were then brought up to 75 per cent. alcohol for preservation. The decalcification was done in 2–3 per cent. solutions of nitric acid in 75 per cent. alcohol, some of the colonies with hard coralla taking as long as three months to decalcify, but, as a rule, the histological condition of the soft parts has not been affected to any extent by the process.

Various staining methods were employed, chiefly Haidenhain's iron-haematoxylin followed by eosin, aniline blue, and orange G (Mallory), safranin O, borax-carmine followed by picro-nigrosin and picric aniline blue. Well-preserved polyps were subjected to teasing before and after maceration in the Hertwig's osmic-acetic solution; while at the American Biological stations a similar investigation of fresh coral tissue could not be made to my satisfaction owing to pressure of other work, though it was found that the method of teasing was not so suited as that of serial sectioning to reveal the true histological relationships of lowly differentiated tissues like those of the Madreporaria.

I have to thank the Governing Body of Emmanuel College for financial aid in connexion with this research.

The soft parts of the Madreporaria consist of an outer and inner protoplasmic sheet and an intermediate supporting lamina. The two former are described in this paper under the widely accepted terms Ectoderm and Endoderm, which had originally been employed by Allman in 1853 to denote the outer and inner layers of the Tubulariadae (**1**, p. 368). But I have refrained from applying any of the suggested names to the middle lamina since (as will be shown in the course of this study), from its nature and formation, this lamina does not appear to be essentially different from the Mesoderm of the Triploblastica. It may also be stated at once that these laminae in Astraeid corals are not discrete layers, as has been the prevalent view, but are of the nature of three strata in a continuous multinucleated sheet.

Ectoderm.

The ectoderm forms the entire outer lining of the soft parts, i.e. of the oral-discs, tentacles, column-walls of polyps (where it is termed the calicoblastic layer), and edge-zones and coenosarc (the ectoderm of their outer wall is a continuation of the oral-disc ectoderm, while that of their inner wall is a continuation of the calicoblastic layer).

In the oral-disc and outer wall of the edge-zone (figs. 1 and 2) the ectoderm has an even free surface which, in sections, is often seen to be covered with mucous secretion and is of more or less uniform thickness. It has a thin free border containing fine vertical striae and is provided with short cilia. Elongated nuclei are aggregated somewhat along the middle of the ectoderm; smaller round ones which are less numerous lie more or less scattered in its inner half. Both mucous and granular vacuoles are present, the former being more abundant than the latter, and a varying number of nematocysts also occur in it. Outwardly diverging tracts are sometimes visible in the protoplasm between the vacuoles. Fibrils are continuous between the ectoderm and middle lamina. At the base of the ectoderm is a finely granular stratum in which a faint network can be discerned which is probably the result of intercrossing of the basal processes of the nuclei of the ectoderm and the fibrils which pass into it from the middle lamina. This stratum has been usually regarded as nervous, but, on renewed examination, no such nerve elements as those described by the Hertwigs in Actinians could be found in it. In the tentacles (fig. 3 and **25,** fig. 44) the ectoderm is greatly thickened at intervals to form the batteries in which nuclei are considerably increased in number, the elongated ones lying in the upper half of the batteries, smaller oval and round nuclei in the lower half. The granular stratum at the base of the tentacular ectoderm is thicker than at the base of the oral-disc ectoderm.

The calicoblastic ectoderm (figs. 10, 13–15) is very thin except where the column-wall processes are being formed, and has a somewhat irregular outline and granular protoplasm, the

granular appearance being more pronounced than elsewhere in the ectoderm. Nuclei are few and are arranged in a single row; they are large, oval or round, rarely elongated, finely granular, and lie tangentially in a single row at comparatively wide intervals, except near the attachments of mesenteries to the corallum, where the calicoblastic layer is usually thickened and nuclei tend to become irregularly distributed without any crowding. Most of the nuclei contain a brilliantly stained spot (perhaps the nucleolus) which is conspicuous under an oil-immersion lens. The calicoblastic layer persists to the base of polyps, though considerably attenuated.

From this account it will be seen that the ectoderm consists of two histologically different regions, viz. the part covering the exposed surface of colonies (i.e. the oral-disc, edge-zone, and coenosarc ectoderm) and the calicoblastic layer, the differentiation being doubtless in accordance with differences in their functions. In the free-swimming larva of Favia fragum, the ectoderm of the column-wall (fig. 4) is histologically similar to that of the oral-disc of the polyp (the calicoblastic layer being non-existent at this stage), and is, in this respect, comparable to the column-wall of Actinians; at the aboral pole the ectoderm is thickened, presumably for future attachment. Tentacles had not appeared in any of the larvae examined.

Endoderm.

The endoderm forms the lining of the coelenteric cavities, i.e. forms the inner lining of the column-wall, oral-disc, edge-zone, and tentacles, the outer lining of the stomodaeum and the double sheet of the mesenteries. It is usually vacuolated and is apparently without cilia, while in Actinians the Hertwigs found a single long cilium or flagellum on each endoderm cell; the vacuoles are often elongated, their longer axes are more or less perpendicular to the width of the mesenteries, the vacuoles being somewhat broader distally. Nuclei are oval or round, smaller and less numerous than those of the oral-disc ectoderm. The endoderm varies in thickness in different parts of the same

polyp and in different species. In the tentacles it is considerably swollen in some species, almost filling their lumina ; in the column-wall it is thin in the stomodaeal region, but becomes highly vacuolated and reticular below this region, where it contains comparatively few nuclei which are arranged in a row near the free surface ; in the stomodaeal wall the endoderm remains uniformly thin. In the mesenteries the endoderm is usually swollen along the pleatal region and behind the filaments. A narrow constriction is present behind the filament, which is deeper in principal than in subsidiary mesenteries (figs. 7 and 8). Numerous round bodies, usually regarded as symbiotic algae, are present in the oral-disc, edge-zone, and tentacular endoderm, i.e. in the exposed regions of colonies ; in some polyps they are so massed as to fill parts of the endoderm. In the mesenteries these algal bodies occur in varying numbers, chiefly in the exocoelic side, but are scarce in the column-wall. Fibrils are continuous between the endoderm and middle lamina, somewhat as between the latter and ectoderm. In all the larval stages examined, the endoderm has attained histological similarity with that of the polyp, but organic débris containing scattered nuclei are still seen in the coelenteric cavity (fig. 4) and are perhaps remnants of the contents of the earlier solid planula stage (**37,** Pl. ii, fig. 4).

The distinguishing characters of the endoderm are its vacuolated condition, presence of algal bodies, the numerical inferiority and somewhat scattered condition of its nuclei. The endoderm, unlike the ectoderm, has a homogeneous appearance, except for its relative swelling in different parts and the varying number of algal bodies present.

Inner Lining of Stomodaeum (figs. 5 and 17).

In the larva, the stomodaeum is said to be formed by invagination of one of its extremities, while it is found that in colony-formation new stomodaea may be formed by invagination or by the union of the broader mesenteries in diverticula (**30**). In the two former cases, the inner lining of the stomodaeum is a continuation of the surface ectoderm, while

in the latter case it is an endodermal formation ; but, whether ectodermal or endodermal in origin, the inner lining possesses histological identity. It is raised into ridges over the attachments of mesenteries (fig. 5) ; these ridges vary in thickness and breadth in different species and frequently possess median grooves as they approach the enterostome. The free border of the inner lining shows the vertical striation better than in the surface ectoderm. It is conspicuously ciliated, the cilia being longer in the median grooves of the ridges and in the sulci between the ridges ; these cilia would function in the ingress and egress of currents of water.[1] Below the striated border is a somewhat finely granular non-nucleated stratum, beneath which is a much thicker region containing massed nuclei of varying length and size (mostly tapering at both ends). Between the nucleated region and the middle lamina is a fibrillar region which is thicker than all other regions and comprises the lower half or two-thirds of the ridge ; it contains a few small, round or oval nuclei, and the fibrils are continuous with the middle lamina. At the base of the inner lining is the fine granular stratum which shades off into the middle lamina. The striated border and the non-nucleated stratum underlying it are of uniform thickness over the entire stomodaeum, while the nucleated and fibrillar regions are considerably thinner in the intermesenterial parts. Vacuoles are usually present in the stomodaeal ridges and are fewer in the intermesenterial region ; many of them contain granules which vary in their density, being quite fine in some vacuoles. The vacuoles extend to the surface of the ridges and, in sections of some polyps, the granules are seen to have actually passed into the lumina of the stomodaea. Nematocysts are sometimes present in the inner lining of the stomodaeum. At the enterostome the inner lining of the stomodaeum becomes continuous with its outer endodermal lining.

Since numerous stomodaea are present in a Madreporarian

[1] Finely powdered carmine, when put into sea-water containing a live colony of Manicina areolata was passed into the stomodaea and subsequently ejected.

colony, the ectoderm of the free surface is continuous with the stomodaeal lining at frequent intervals. The inner lining of the stomodaeum of the larva is not raised into ridges at the mesenterial attachments, hence both grooves and sulci are absent ; its nuclei are not so crowded together nor so slender as in the polyp, and are arranged along the middle of the layer whose protoplasm is conspicuously granular and opaque above the nuclei, while below them it is vacuolated and translucent ; the condition of the stomodaeal lining of the larva is on the whole intermediate between that of the oral-disc ectoderm and the stomodaeal ridges of the polyp.

Mesenterial Filaments (figs. 7, 8, and 18).

The epithelium of mesenterial filaments has essentially the same structure as the inner lining of the stomodaeum, the median lobe being similar to the stomodaeal ridge and the ventro-lateral tracts to the parts between the ridges. The median grooves of most of the stomodaeal ridges are continued to some distance along the middle of the straight regions of their corresponding mesenterial filaments, the cilia in the grooves being longer than those over the rest of the filaments. A transverse section through a principal filament just below the stomodaeum, as shown in fig. 6, bears striking resemblance to Ashworth's figure of a transverse section through a dorsal mesenterial filament of Xenia Hicksoni (**3,** fig. 19). Granular vacuoles are frequently present in the straight region of the filaments. Nematocysts are few in the straight region, numerous in the convoluted parts, where they often become massed together. Each of the ventro-lateral tracts of a filament is organically continuous with the mesenterial endoderm on its side. Filament-epithelium is present on subsidiary mesenteries (except on the very narrow ones) along the greater part of their length, but is rudimentary in their upper half or one-third, where it contains a few aggregated nuclei or is sometimes entirely absent. Subsidiary filaments are smaller in transverse section than principal filaments. Mesenterial filaments of the free-swimming larva (whether of principal or

subsidiary mesenteries) are similar to the inner lining of its stomodaeum, i.e. nuclei in them are not so closely aggregated nor so slender as in filaments of polyps.

It is obvious that in a subsidiary mesentery of a polyp the filament is formed by modification of the endoderm of the mesentery along its free margin, attaining histological similarity with the inner lining of the stomodaeum and with filaments of principal mesenteries. Stages in this modification are seen in subsidiary mesenteries of varying width. In larvae of Favia fragum, also, filament-epithelium is present along the margins of some subsidiary mesenteries which is undoubtedly formed by modification of the marginal endoderm of those mesenteries. I have previously described the presence of filament-epithelium on the mesenteries of an extra-tentacular bud of Favia hululensis (Gard.) (**27**).

In some species, Favia fragum, Mercilina ampliata, Hyderophora maldivensis, Isophyllia dipsacea, there are regions in the convolutions of mesenteries in which the filament-epithelium is considerably vacuolated and swollen, in which nematocysts i or ii are closely arranged. In dumb-bell-shaped transverse sections of these, the filament-epithelium at each end resembles the intervening endoderm. In other words, histologically identical epithelia are found in stomodaea and mesenteries, whether the inner linings of the former and the filaments of the latter are ectodermal or endodermal in origin. But it is to be noted that algae are absent from the inner lining of the stomodaeum and mesenterial filaments.

The Supporting Middle Lamina.

The middle lamina is found everywhere between the ectoderm and endoderm and forms the median core of mesenteries. Though Bourne could find no trace of structure in the middle lamina of Fungia, he remarked that the use of proper reagents might possibly have disclosed a fibrillar structure (**5**, p. 310). As a result of making careful microscopical preparations this lamina is now found to consist of (1) a homogeneous matrix or clear cementing substance containing (2) fine fibres

and (3) nuclei (figs. 1, 11, and 12). The fibres are of two kinds : those which have a wavy appearance and run in various directions but have chiefly a longitudinal and transverse disposition—such fibres appear to be unbranched and are closely cemented together to form the substance of the lamina ; branching fibres which form a loose plexus in the lamina—these are brought to view by carefully staining sections of not more than 6 μ thickness. The apparently homogeneous appearance of the middle lamina is due to the thinness and close cementing of the fibres. Nuclei are comparatively few and lie scattered in the lamina ; they become evident in tangential or oblique sections through the thicker regions. Each nucleus is oval in shape, containing a conspicuous spot (the nucleolus), and lies in thin finely granular protoplasm from which irregular processes usually radiate into the substance of the lamina ; not infrequently a narrow clear space can be detected around the protoplasm. In several West Indian species of coral Duerden noted the presence of ' migrant connective-tissue cells, such as occur in the larger Actinians ' (**9**, p. 22).

The middle lamina is thickened in the mesenteries and is raised on one side into longitudinal pleats whose breadth and thickness vary in the different species (fig. 9). In the stomodaeal region the pleats extend over part of the width of mesenteries to a varying distance from their column-wall attachments, while below the stomodaeum they cover almost the entire width of mesenteries. The lamina is usually considerably thickened where mesenteries join stomodaea and column-walls. While at the stomodaeal attachment the thickening is restricted to the ridge, at the column-wall insertion the thickening usually spreads a short distance into the adjacent middle lamina, these lateral thickenings appearing, in transverse section, like two arms. The middle lamina is thickened to a less extent in the tentacles and oral-disc ; in the former, outer longitudinal pleats are present which are less conspicuous than those of mesenteries. Processes arise from the middle lamina over the entire extent of the column-wall to attach the soft parts to the corallum, and are more numerous

and larger at the insertions of mesenteries. These processes are composed of fibres and cementing substance, and are the homologues, in the column-wall, of the pleats in mesenteries and tentacles.

The superficial longitudinal fibres in the pleats of mesenteries and tentacles are specially thickened. These specialized fibres, which vary in thickness, appear to be composed of fibrils, but had usually been supposed to be similar to the muscular elements described by the Hertwigs in Actinians, Faurot in 1895 being the first to doubt their muscular nature. In teased preparations and in sections, nuclei are not found in these fibres nor is there any morphological or physiological evidence for regarding them as muscular. Specialized fibres are present on the exocoelic side of mesenteries (but not so thick nor so close together as on the entocoelic side), although pleats are absent from that side or only a few feebly developed ones are present near the stomodaeal attachment. The striae in the processes of attachment appear to be specialized fibres which have a radial disposition.

In preparations of mesenteries with the endodermal lining scraped off, the specialized longitudinal fibres of the middle lamina could be seen running along its entire length. When parts of the living tissue of Isophyllia were isolated from expanded colonies, teased in sea-water, and stained in methylene blue, the middle lamina took a purple or violet tinge and its fibrous texture became quite apparent. The fibrous condition could also be unmistakably seen in properly preserved tissue which had been teased after maceration and removal of the protoplasmic sheets, as well as in such tissue cut to 4 μ and 6 μ thicknesses. The branching fibres were best seen by staining in safranin O and picro-nigrosin. The specialized fibres of the middle lamina, whether in the pleats or in the processes of attachment, were similarly coloured with different stains, e.g. dark in iron haematoxylin, purple in aniline blue and orange G, slaty blue in borax-carmine followed by picro-nigrosin, such results suggesting identity of texture of both sets of fibres. The absence of muscular fibres in the

soft parts of the Astraeidae would also explain the absence of a nervous system—central or peripheral.

In the larval stage the middle lamina is present everywhere and is fibrous, though thinner than in the polyp. Pleats are hardly recognizable in mesenteries, but specialized fibres are present. H. V. Wilson found that in the development of Manicina areolata the middle lamina appears in the solid planula stage (**37,** fig. 5). This observation is corroborated by my study of the solid embryonic stages occurring in the coelenteric cavities of polyps of Favia fragum.

The middle lamina of Actinians as seen from a study of serial sections of young polyps of Sagartia bellis, Metridium senilis, and Corynactis viridis (which in alcohol measured 2 mm. × 1 mm., 12 mm. × 3 mm., and 4 mm. × 3·5 mm.), obtained from Plymouth, is in essential points similar to that of Astraeid corals. In the former two species, the middle lamina has a swollen somewhat loosely spongy core containing many nuclei which is bounded by closely arranged unbranched fibres; the plexus of the spongy core consists of branching fibres which are more abundant than in coral polyps (fig. 16). In Corynactis viridis the meshwork is closer, approaching the condition in the Astraeidae. The principal difference is that, in the column-wall of Actinians, the middle lamina is considerably thickened. The fibrous condition of the middle lamina of various Zoantharia had been previously observed by Kölliker, Schneider, and Rötteken, von Heider, Jourdan, the Hertwigs, and Faurot.[1] As early as 1875, Allman remarked that the 'hyaline lamella' (= middle lamina) of Myriothela consisted of 'two layers—internally a perfectly transparent, thin, structureless membrane, and externally a layer of fibrillae, which adheres closely to the structureless membrane' (**2,** p. 554, fig. 6).

The histology of the middle lamina of the Madreporaria resembles that of mammalian connective tissue, the massed

[1] Hickson in 1883 described the middle lamina of Tubipora as consisting of a 'homogeneous matrix' in which might be found 'cells and fibres' (**17,** p. 11).

wavy unbranched fibres, the network of branching fibres, and the nuclei with the granular protoplasm in which they lie, are comparable respectively to the white fibres, branching fibres, and the so-called connective-tissue ' corpuscles ' ; the various elements lie in a clear matrix in both cases. It is probable that the branching fibres in the middle lamina of coral colonies are elastic like those of connective tissue.

The possibility has not been excluded that the fibrous strands of the ' epithelio-muscular ' or ' myo-epithelial ' cells, so commonly figured in connexion with the histology of Coelenterates, might only be fibres of the middle lamina torn apart with the adjacent parts of the ectoderm and endoderm in the process of teasing. Such results are to be expected ; the ectoderm, middle lamina, and endoderm are organically continuous. (In teasing, protoplasmic parts are sometimes dissociated from the fibres of the middle lamina, as in Hickson's figures 28 *b–e* of Alcyonium digitatum.) It is not improbable that, as in the Astraeidae, these strands may be of the nature of connective-tissue fibres, for, in figured examples of epithelio-muscular cells, the nuclei, unlike their position in plain muscular fibres of mammals, lie in the protoplasm extrinsic to the strands (**16,** Pl. vi ; **18,** Pl. xxxix ; **3,** Pl. xxvi).

Bourne, H. V. Wilson, Duerden, and others regarded the middle lamina of the Madreporaria as a secretion of one or both of the protoplasmic layers and ' not formed by the direct metamorphosis of the ends of ectoderm or endoderm cells ' (**37,** p. 198). The appearances in my various preparations, however, suggest that the middle lamina is formed by modification of part of the protoplasm of the ecto-endoderm into cementing substance and fibres, all or some of the nuclei in the modified part of the protoplasm becoming the nuclei of the lamina. The formation of the middle lamina is well seen in the case of the processes of attachment which are formed in the calicoblastic layer, stages in their development being abundantly present in my preparations (figs. 13–15). Where such a process is to be formed, the calicoblastic layer is raised

into a short, somewhat irregular eminence which may or may not contain a nucleus. Subsequently, the protoplasm of this projection becomes modified from its periphery inwards to the middle lamina of the column-wall and specialized fibres appear in it. At the attachments of mesenteries to the corallum, the calicoblastic projections are usually larger and each of them often contains more than one nucleus ; when their protoplasm has been modified, the attaching structures become connected with the middle lamina usually by means of narrow necks, while elsewhere they are smaller and arise directly from the middle lamina. In my preparations there is no indication that these processes are at first formed in cellular elements or ' desmocytes ' which become subsequently connected with the middle lamina by the modification of neighbouring ' cells ' of the calicoblastic layer, as Bourne described (p. 329), but they are the result of a continuous change in the multinucleated calicoblastic layer, the transformation of the protoplasm beginning from its periphery and gradually extending inwards to join the middle lamina. The processes are the parts that project beyond the outer margin of the calicoblastic layer and in which the specialized fibres lie. These fibres are pronounced towards the periphery of the processes, gradually becoming fainter as they reach the middle lamina, and probably they merge into the fibrils of the latter.[1]

Part of the middle lamina is formed entirely in the ectoderm, viz. the processes of attachment in the calicoblastic

[1] According to Bourne in Caryophyllia Smithii, ' where a desmocyte is about to be formed, one, two, or three nuclei become surrounded with a mass of darker, finely granular protoplasm. The next phase is the appearance of a band-shaped or ovoid body in the centre of the granular protoplasm which already shows faint signs of striation . . . usually one nucleus remains in close association with this body; the others (if more than one combine to form the granular protoplasmic mass) appear to be concerned in the formation of the mesogladal process which will join the desmocyte to the mesogladal lamina. The striations next become more defined, and the desmocyte, which was at first separate from the mesoglada, becomes attached to it by a process developed, as it seems, at the expense of neighbouring cells ' (pp. 528–9). (A desmocyte containing more than one nucleus he regarded as a cell.)

layer, part of it arises in the endoderm, viz. the median core of mesenteries, and the remainder is contributed to by both the ectoderm and endoderm, viz. the middle lamina of the column-wall, oral-disc, edge-zone, and stomodaeum. While Bourne in 1899 held that the processes of attachment, which were essentially similar to and became part of the middle lamina, were formed by modification of elements in the calicoblastic layer or ' desmocytes ', i.e. were intra-protoplasmic formations, he had in a previous paper (**5**) inferred that the middle lamina itself was a secretion of ectoderm and endoderm, i.e. was an extra-protoplasmic product.

The middle lamina appears to be essentially a supporting stratum, i.e. has the function of connective tissue of Vertebrates and, like the latter, has a fibrous texture. It is best developed in mesenteries, since they support the oral-disc with the tentacles and keep the stomodaeum in position, the longitudinal pleats giving additional strength to the mesenteries. Owing to the presence of a calcareous skeleton to support the column-wall, the middle lamina in the Madreporaria is very thin, whilst in Actinians the absence of such a skeleton has necessitated a considerable thickening of the middle lamina in the column-wall (being best developed in this region), which, when the column-wall is folded longitudinally as in Metridium senilis, is swollen into longitudinal ridges within the folds or rugae (fig. 16). The column-wall processes are analogous to tendinous structures in Vertebrates, since, doubtless, they attach the soft parts to the corallum. This function would account for their sucker-shape, usually concave attaching surface, and comparatively small size, combined with their numerical abundance ; the specialized fibres in them presumably impart additional toughness to these processes.

Since the middle lamina has a spongy texture, the infilling of its meshes with fluid would help in the distention of polyps, which is, however, mainly effected by the ingress of sea-water into the polyp cavities, while the general contractility of the middle lamina would help in the retraction of polyps.

General Considerations.

In the tissues of the Astraeidae, cell-limits cannot be discerned, the nuclei lying immersed in the general protoplasm. This is particularly the case in the surface ectoderm, inner lining of stomodaeum, and in mesenterial filament. While in the endoderm, nuclei tend to lie between vacuoles, in the middle lamina nuclei are few and the protoplasmic areas containing them are not definitely circumscribed but appear to be organically connected together by means of their radiating strands. Sections of 4 μ and 6 μ thicknesses treated with silver nitrate failed to show any cell-limits, nor is a cellular structure seen in sections cut in gelatin with Aschoff's CO_2 freezing microtome, nor again in celloidin sections of polyps. Duerden observed that in Siderentsea radians the endoderm of the wall of the polyp lining the uppermost parts of the skeleton is ' a syncitium showing no signs of cellular divisions ', and that the calicoblastic layer ' in the growing areas of the skeleton shows no evidence of cell limitations ' (**9,** pp. 30, 31). Gardiner, too, could not find definite cell outlines in Coenopsammia and Flabellum (**13** and **14**). Such outlines, so frequently represented in figures of the ectoderm and endoderm of the Anthozoa, are doubtless conventional and arbitrary.

The products of teasing of the tissues, whether before or after maceration, cannot be regarded as separated units of structure or ' cells ', but are really bits of protoplasm inevitably torn apart with the nuclei in the mechanical process of teasing. Hence, it is generally found that such pieces of protoplasm possess neither regular nor uniform contour and are sometimes torn apart with fibres of the middle lamina. If an appearance of cellular strands is noticeable in some preserved tissues, it is due to the shrinkage of protoplasm around the nuclei, which probably act as centres of force. H. V. Wilson regarded the endodermal mass of the solid planula stage of Manicina areolata as a ' plasmodium which was subsequently broken up into cells ' (**37,** p. 200) ; he regarded the earlier blastosphore as composed of cells, although their inner ends were ' not

distinctly marked off from the solid endoderm' (p. 197). In many of Bourne's figures of the soft parts of the Anthozoa definite cell boundaries are not visible, although such limits have been presupposed in the descriptions. Indeed, in Caryophyllia, Euphyllia, Madrepora, and 'several other' corals Bourne could not find any cell outlines in the calicoblastic layer (**7,** p. 532), the latter being an irregular multinucleated sheet of protoplasm. When more than one nucleus was present in a mass of protoplasm, it was assumed to be a coenocyte formed by the fusion of uninucleated cells; for example, in referring to scleroblasts or spicule-forming cells of Alcyonaria, Bourne remarked that they were 'often coenocytes containing two, three, or more nuclei' (p. 509). It would appear to be more likely that the scleroblastic tissue was of the nature of a syncytium in which spicular bodies formed.

The mucous and granular vacuoles in the outer lamina of the Madreporaria have also been regarded as cells, but nuclei are not definitely related to them, some of them having more than one nucleus while others show none at all. The only cellular elements in the soft parts of the Astraeidae are nematocysts, algal bodies, and the reproductive elements; these are all characterized by their definite and uniform outline. Nematocysts are secondary formations in the ectoderm for special purposes. Algal bodies are restricted to the endoderm, but little is known of their life-history; it is doubtful if they are symbiotic organisms, as is generally supposed, since they are found in newly hatched larvae and even in earlier embryonic stages (**37,** Pl. ii, fig. 4). Ova and spermatozoa lie in spaces in the middle lamina (**25,** figs. 9, 10, and 49).

The laminae of Astraeid corals are therefore to be regarded as syncytial, and since, as has been seen, there is organic continuity between them, i.e. the ectoderm is everywhere directly continuous with the middle lamina and the latter with the endoderm, and, further, the ectoderm passes into the endoderm by way of the inner linings of the stomodaeum, the tissues form one nucleated continuum which has undergone

partial differentiation into three strata. Towards the base of the column-wall, the middle lamina is absent in places, the calicoblastic layer and the endoderm merging into each other. In such places the appearance is that of one sheet of nucleated protoplasm with an outer granular stratum containing large oval nuclei tangentially placed at intervals, which represents the calicoblastic layer, and an inner stratum whose nuclei are smaller but more numerous and placed vertically, which represents the endoderm (fig. 10).

Since the middle lamina of the Astraeidae is nucleated, formed early in development, and is of the nature of connective tissue, it is comparable to the mesoblast and mesoderm of other animals.[1] Bourne in 1887 restricted these terms to denote the intermediate layer of the triploblastica (which he apparently identified with the coelomata), on the view that the middle lamina of Coelenterates was neither embryonic nor ' cellular ', to which he gave a new name, mesoglaea (**5,** p. 311). This nomenclature was subsequently accepted by most authors—Haddon, van Beneden, Hickson, Ashworth, McMurrich, Duerden—who regarded the nuclei occurring in the middle lamina of Coelenterates as belonging to cells which secondarily migrated into the gelatinous secretum from one or both of the protoplasmic laminae—a view to which my studies on the Madreporaria lend no support. Moseley, von Heider, O. and R. Hertwig, and other earlier zoologists had described the intermediate layer of the Anthozoa under the term mesoderm. This prior usage was resumed in 1895 by Faurot, who, from his comparative study of many Actinian species, disagreed with Bourne in regard to its supposed extra-protoplasmic formation and structureless consistency and the need for a new terminology. It is also clear from the embryological

[1] Bourne states that ' by mesoblast is meant a layer of undifferentiated cells, developed in the embryo before the differentiation of other organs or tissues from either one or the other or both of the primary germ-layers, the epiblast and hypoblast. By mesoderm and its adjective mesodermic are meant all such tissues in the adult as are clearly derived from the mesoblast ' (**5,** p. 314).

studies of Jourdan on Actinia equina and Balanophyllia regia, of E. B. Wilson on Renilla, and of H. V. Wilson on Manicina areolata, that the middle lamina appears early in development—in the solid planula stage. I have found this to be the case in the solid embryos of Favia fragum. Jourdan distinguishes between a 'membrana propria' and a granular mass; while the origin of the former was uncertain, the latter was said to be formed by the severance and fusion of the inner ends of the ectoderm cells of the body-wall, which subsequently become fibrous.[1] E. B. Wilson, who made a more or less similar distinction, also found that the middle lamina of the body-wall was formed by the separation and fusion of the swollen inner ends of ectoderm cells, though he somewhat arbitrarily termed the process 'a peculiar form of cuticular secretion' (**36**, p. 759). Bourne's account of the formation of the middle lamina in Heliopora (**6**) is not different from those of Jourdan and E. B. Wilson.

Although a discussion of the highly controversial subject of the history and homology of the germ-layers of the Metazoa does not lie within the scope of this paper, it will be seen from the foregoing account that there was not adequate reason for withholding the application of the term mesoderm to the middle lamina of the Anthozoa.

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[1] The mode of formation of the middle lamina of certain Alcyonarians described by Kowalevsky and Marion (**23**) is essentially similar to that of Jourdan, but in their subsequent discussion they, however, came to the conclusion that the middle lamina of Coelenterates was not homologous with the mesoderm of Coelomates.

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EXPLANATION OF PLATES 7 AND 8.

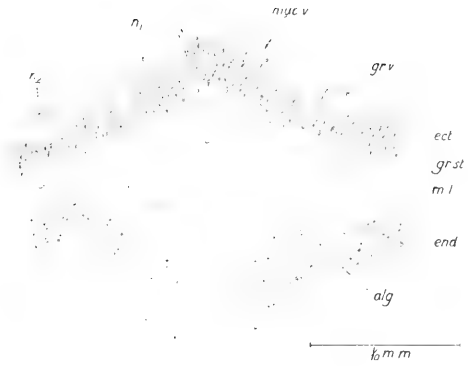
Lettering employed.

alg., algal bodies. *c.l.*, calicoblastic layer. *ect.*, ectoderm. *end.*, endoderm. *gr.st.*, granular stratum at base of ectoderm. *gr.v.*, granular vacuole. *lg.f.*, longitudinal fibres of middle lamina. *m.f.*, mesenterial filament. *m.l.*, middle lamina. *muc.v.*, mucous vacuole. n_1, type I nematocyst. n_2, type II nematocyst.

Fig. 1.—Coeloria daedalea (Ell. and Sol.). Part of somewhat oblique section through oral-disc and mesentery.

Fig. 2.—Eusmilia aspera. Part of vertical section through edge-zone. The endoderm is crowded with algal bodies.

Fig. 3.—Coeloria daedalea (Ell. and Sol.). Part of transverse



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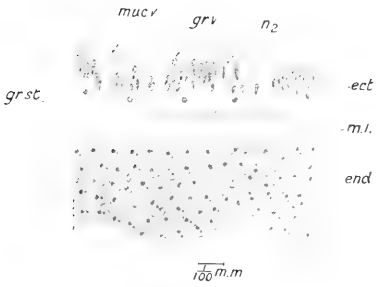

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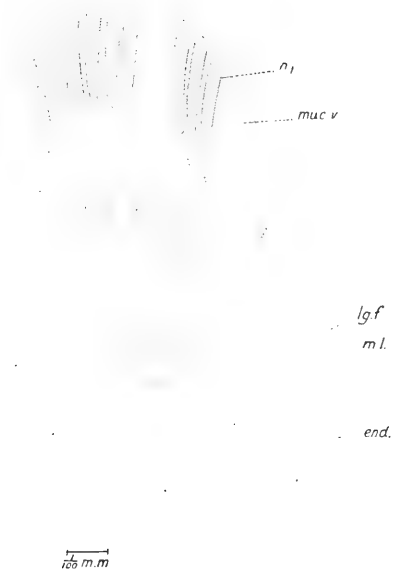

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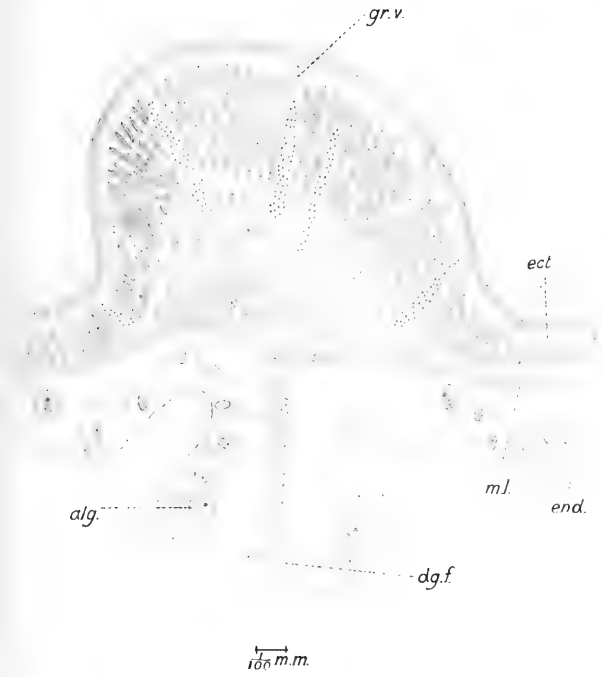
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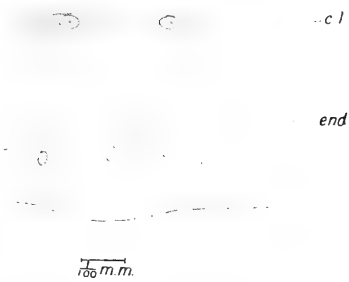
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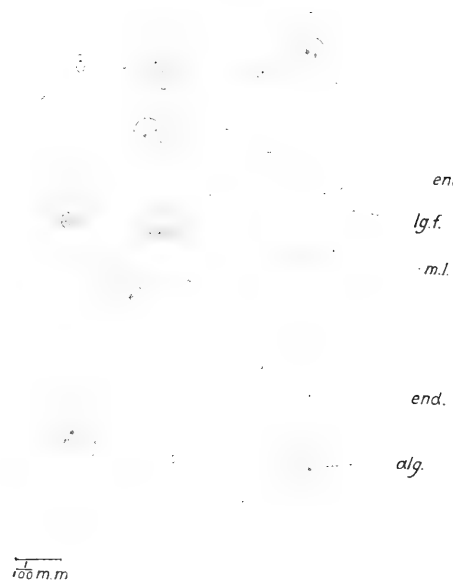
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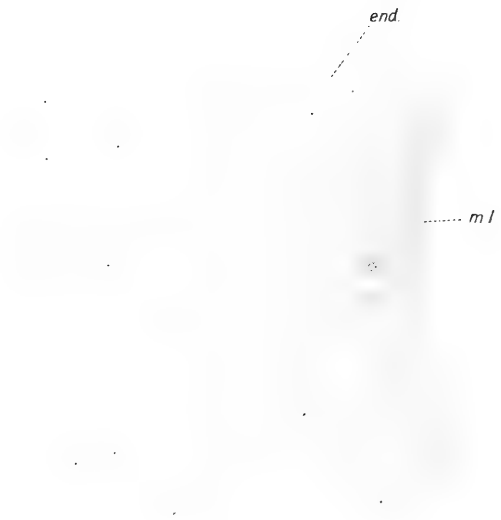

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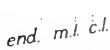

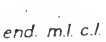

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18

section through a tentacle showing a sub-terminal battery. Since the section was cut somewhat obliquely, the middle lamina and its specialized longitudinal fibres (*lg.f.*) appear thicker.

Fig. 4.—Larva of Favia fragum (Esp.) ten hours after extrusion. Part of transverse section through column-wall. Note fibrous condition of middle lamina and protoplasmic remains in coelenteric cavity.

Fig. 5.—Coeloria daedalea (Ell. and Sol.). Part of transverse section (slightly oblique) through stomodaeum, showing a ridge and adjacent intermesenterial areas.

Fig. 6.—Ibid. Part of transverse section through a principal mesentery just below stomodaeal region, showing continuation of median groove on filament.

Fig. 7.—Ibid. Part of transverse section through a principal mesentery below stomodaeal region, showing straight region of filament and the two endodermal lobes.

Fig. 8.—Ibid. Part of transverse section through a subsidiary mesentery, showing straight region of filament and the two endodermal lobes.

Fig. 9.—Ibid. Part of transverse section through pleatal region of a principal mesentery in stomodaeal region.

Fig. 10.—Favia hululensis (Gard.). Part of transverse section through column-wall at base of polyp. The middle lamina is thin and somewhat discontinuous at this level.

Fig. 11.—Coeloria daedalea (Ell. and Sol.). Longitudinal fibres of the middle lamina of a mesentery after maceration in osmic-acetic solution and staining in borax-carmine and picro-nigrosin.

Fig. 12.—Ibid. Part of tangential section (6 μ thick) through a mesentery, showing the network of branching fibres of the middle lamina. Note the mass of unbranched fibres and nuclei in the middle lamina.

Figs. 13–15.—Showing stages in the formation of column-wall processes in the calicoblastic layer.

Fig. 13.—Leptoria gracilis. Part of transverse section through intermesenterial region of column-wall at level of stomodaeum.

Fig. 14.—Ibid. Part of transverse section through column-wall at attachment of a mesentery in stomodaeal region.

Fig. 15.—Coeloria daedalea (Ell. and Sol.). Part of transverse section through column-wall at attachment of a mesentery in stomodaeal region.

Fig. 16.—Metridium senilis. Part of transverse section through column-wall, showing a ridge. In the middle lamina note (1) the swollen loosely spongy core which is less open towards the ectoderm and endoderm, the network itself consisting of branching fibres; (2) circularly arranged unbranched fibres bounding the spongy core, the fibres being massed against the endoderm; (3) nuclei in the spongy core; a thin granular protoplasmic area can be seen around most of them. The points marked

in the meshwork are probably transverse sections of longitudinal fibres, being more numerous where the meshwork is less open.

Fig. 17.—Larva of Favia fragum (Esp.) ten hours after extrusion. Transverse section through stomodaeum. Four mesenteries have joined stomodaeum ; their stomodaeal attachments are shown in figure.

Fig. 18.—Larva of Favia fragum (Esp.) ten hours after extrusion. Part of transverse section through a primary mesentery, showing filament.

The Yolk-Sac and Allantoic Placenta in Perameles.[1]

By

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With Plates 9–11 and 4 Text-figures.

Contents.

[1] The present communication was awarded the University Medal when presented to the University of Sydney as a thesis for the degree of Doctor of Science. The absolute necessity, in the present time, of reducing publication costs to the minimum, has resulted in the omission of some of the original figures, these being reduced from fifty-two to the present limit.

1. Introduction.

It is proposed in the present communication to initiate the publication of a series of papers dealing with marsupial embryology. The facts and interpretations embodied will be based on result of an investigation into an amount of marsupial material now collected in the Biological Laboratory of the University of Tasmania and fairly representative of the Tasmanian fauna.

It is evident that any attempt to solve the phylogenetic problems presented by the mammalian group without having at our disposal a large number of the facts relating to the morphology and embryology of the Metatheria will be extremely unsatisfactory.

Yet in the not very distant future, with the spread of settlement and the almost incredible devastation caused among these animals by fur-hunters and trappers—a depletion but little compensated for by restrictive legislation—it may be considered certain that the connected stages necessary for the elucidation of their intra-uterine development will be practically unobtainable.

In this connexion it may be instructive to quote the words of Hubrecht (1909), who says, in discussing placental arrangements in the Marsupialia, that 'the early ontogenetic events and the different phases in the mutual relation of the blastocyst and mucosa ought to be fully known in order to furnish us with all the data that can be brought to bear on this important question. And it is to be fervently hoped that those genera that are very rapidly diminishing in their native land, some of them even on the verge of disappearance, may yet be fully investigated before they have been exterminated, and have thereby become as mute on this important point as are their fossil predecessors.'

Of such disappearing genera two may be mentioned, Thylacinus and Sarcophilus, both certainly primitive in the morphology of their genital organs, and for that reason alone possessing important possibilities with regard to their

intra-uterine nutritional arrangements. Both genera are absolutely on the verge of extinction. The intra-uterine development of Thylacinus will probably never be known, and it is extremely unlikely that detailed investigation will be possible in the case of Sarcophilus.

Under these circumstances it is a pleasure to acknowledge gratefully any help extended towards an investigation of the ontogenetic and phylogenetic relationships of this fast disappearing fauna.

First, in this way, I must express my most grateful thanks to the Trustees of the John Ralston Bequest and particularly to their chairman, Dr. L. G. Thompson, these gentlemen having placed at the disposal of the University of Tasmania a sum of money to be used chiefly in marsupial investigation. It is mainly by their financial aid and unswerving support that the important collection of embryological material in the University of Tasmania has been brought together.

Secondly, I am indebted to the Committee of the British Association for the Advancement of Science, who, at their Australasian meeting in 1914, placed at my disposal a grant with the object of procuring material for the study of the brain and embryology of marsupials.

The preliminary portion of the examination of the material of the present paper was carried on in my own laboratory, but lack of facilities for consulting almost all the literature, and other disadvantages of isolation in Tasmania, led me to consult my old friend Professor S. J. Johnstone as to the possibility of accommodation in one of the laboratories of the University of Sydney.

This was arranged and the work was carried on under these altered conditions.

Still more recently, by the courtesy of Professors Hill and Watson, I have been accommodated at University College, London. I am indebted to the Senate of the University of Sydney and to the Research Committee of that University for a grant from the McCaughey Bequest to defray the cost of the plates illustrating this paper.

Finally, I must not omit to pay a tribute to the friendly interest which has been shown in my work by my former teacher, Professor W. A. Haswell, who has been always ready to help with kindly criticism and advice. To him, also, I am indebted for the loan of literature otherwise inaccessible to me.

2. Review and Criticism of Previous Work.

The discovery by Hill of a true allantoic placenta in the two forms, Perameles obesula and nasuta, was of the greatest importance in regard to the phylogeny of the marsupial group and its relationship to the remainder of the mammalia. The actual placental material, upon which Hill was able to base his conclusions, consisted of three stages. The first of these, Stage C, concerned an embryo 7 mm. long. This was followed by a Stage D describing the placental connexion in embryos 8 to 8·75 mm. long. In a subsequent paper Hill described the similar phenomenon in a 12·5 mm. embryo which was also designated D. For the sake of clearness, when speaking of these stages, I will designate them by their length in each case.

In his earliest (7 mm.) stage the fixation of the embryo was already completed and the allantoic placenta was well on the way towards being fully established.

From an examination of these three stages Professor Hill showed that the epithelium of the uterus becomes converted into a vascular syncytium, the nuclei of which arrange themselves in groups in the lower portion of this layer. 'At the same time maternal capillaries pass up between the syncytial lobules, penetrate the syncytial protoplasm, and form a network on and just beneath the surface' (1897, p. 387).

The fixation of the embryo is brought about in the usual way by means of the chorion. The ectodermal portion of this membrane, by which actual approximation of the foetal membranes to the uterine wall is first achieved, consists of a single layer of large cells by means of which the attachment is brought about.

With the somatic mesothelial layer of the chorion, the

splanchnic mesoderm of the allantois fuses, and the vessels ramifying on the surface of the allantois thus come to lie immediately below the layer of true chorion. Up to this point the development of the placenta may be regarded as being fairly typical, but, from now on, the development of this organ, according to Hill's account, results in the appearance of such structural peculiarities and modifications as to give rise to the general impression that the placentation of Perameles, at any rate as concerns its more intimate development, is without parallel in the whole mammalian group.

The allantoic placenta is completed ' by the gradual degeneration and resorption of the enlarged chorionic ectoderm cells over the placental area proper. These cells thus take no further share in placental formation.' The result of this is the close apposition of maternal and foetal blood-vessels, the two blood-streams being ' now only separated by their thin endothelial walls and perhaps a thin layer of syncytial protoplasm ' (p. 388).

A yolk-placenta is present, formed by the close apposition of the vascular area of the yolk-sac to the highly vascular uterine syncytium outside the allantoic placental area.

It is not necessary at this stage to enter into any discussion as to the significance of the presence of an allantoic placenta in Perameles other than to indicate that it has been definitely accepted by most embryologists, that there is now no reason to doubt the common origin of Metatheria and Eutheria from a primitive placental stock. Hill says it is ' exceedingly improbable that an allantoic placenta should have been twice independently acquired and in such a fundamentally similar manner within the limits of the mammalian class ' (p. 433).

Now, outside the intrinsic interest of the presence of an allantoic placenta in a member of a group formerly regarded as aplacental, it would be expected, on a priori grounds, that the occurrence would be of importance in elucidating the phylogeny of the mammalian placenta or, at least, in giving us some means of arriving at a definite idea of the method of placental formation in the original protoplacental group.

It need hardly be said that in these respects the placentation of this animal, as interpreted by Hill, is extremely disappointing.

The combination of a persistent uterine syncytium with a degenerating chorionic ectoderm is without parallel in the Eutheria, and any attempt at a comparison of the placentation of the two groups results in a deadlock.

Although Hill's results have been accepted by many, nevertheless Hubrecht (1909) ventures to question whether, morphologically, the placenta of Perameles will not prove on further investigation to be more comparable with some one or other of the various placental styles found in the Eutheria. And that there is some foundation for Hubrecht's opinion is undoubtedly apparent from an examination of Hill's figures alone.

Before passing to a consideration of this question it will perhaps be necessary to have before us a short account of some special details of the placentation of this marsupial as described by Professor Hill.

His most important and peculiar point concerns the history of the foetal ectoderm. The portion of this which is concerned in the fixation of the embryo is said to disappear almost completely, being represented in the 12·5 mm. stage by a few scattered cells in the original ectodermal position. The disappearance is stated to be of the nature of a degeneration, this, apparently, not being inaugurated until the allantoic attachment has taken place and the allanto-chorionic fusion completed. In the earliest placental stage (Stage C, 7 mm.) the allantoic placenta is already on the way towards full establishment, the allantoic vesicle being already attached to the chorion by its placental face. Excellent figures are given of this stage (1897, Pls. xxix and xxx, figs. 5 to 12).

At a later stage (D, embryos 8–8·75 mm.) the ectoderm has, apparently, almost completely disappeared. In the description of Stage C, it is stated that the cells towards the central portion of the foetal ectoderm are ' of a very varying size and shape, and, in places, through the disappearance of the outlines between adjacent cells, *large multinucleate cells* have been

formed. . . . In many of the ectoderm cells shown in fig. 9 the nuclei are also seen to be in various states of disintegration. Many of them stain only slightly; the nuclear membrane is becoming indistinct, while the chromatin is found broken up and diffused in the form of small granules throughout the delicate nuclear reticulum. Eventually the position of the nucleus is only marked by a few straggling irregularly thickened remnants, which finally become diffused through the protoplasm and lost to view' (p. 404). [Italics mine.]

In this stage (embryos 8–8·75 mm.) the ectoderm is represented centrally, 'only by more or less isolated degenerating cells' (p. 413).

In the 12·5 mm. embryo Hill states that 'over the placental area usually single, much degenerated, and deeply-staining chorionic ectoderm cells are still to be found' (1899, p. 9).

From the above it appears that one of the causes of the disappearance of the ectoderm is the loss of the chromatic constituents of the nucleus, these being absorbed into the surrounding cytoplasm.

As to the other possible reasons for the presumed degeneration and disappearance of this ectodermal layer, Hill states further that, in some cases, 'the inner ends of the cells are greatly vacuolated, a fact which suggests that a process of vacuolation may also play a rôle in the retrogression of the chorionic ectoderm' (1897, p. 404).

Further, referring again to this layer, he is 'inclined to believe that the allantoic capillaries, so closely related to its inner surface, are by no means the least active agents in effecting its removal. *Of direct fusion of the degenerate ectoderm with the underlying syncytium there can be no question. All the facts negative such a view*' (1897, p. 404). [Italics mine.]

From the above it appears that Hill is of the opinion that the layer of chorionic ectoderm disappears, and that the following processes mutually assist in causing this removal:

(*a*) Degeneration in situ with or without vacuolation.

(*b*) Removal by allantoic capillaries with or without previous degeneration.

Thus it is definite enough, according to Hill's views, that the chorionic ectoderm takes no share in the formation of the placenta proper.

The completion of the placenta is brought about by the allantoic capillaries coming into intimate relationship with the maternal capillaries ramifying on the surface of the maternal syncytium.

The above is, I am inclined to think, an accurate précis of Hill's results. His interpretation of the facts and his strongly-expressed opinion that there is nothing of the nature of a fusion between the foetal ectoderm and maternal tissue has given rise to the idea—almost generally accepted—that the placentation of Perameles is of 'a peculiar type not met with anywhere else' (Jenkinson, 1913, p. 216).

The correctness of Hill's conclusions has been questioned by A. A. W. Hubrecht in his famous essay on 'The Early Ontogenetic Phenomena in Mammals' (Hubrecht, 1909).

Hubrecht's criticism:—

The opinion of this gifted investigator was arrived at evidently on somewhat theoretical grounds, but he was confirmed in his ideas by an examination of material placed at his disposal by Professor Hill. It is important to understand Hubrecht's standpoint thoroughly, because, in my opinion, it is to some extent justified. He believes that the foetal ectoderm, so far from disappearing, penetrates into the maternal syncytium to form a 'mixed syncytium', corresponding to what Schoenfeld has described (1903) for the dog. 'The Perameles placenta may be said to be a somewhat simpler—because thinner—form of placenta than that of the Carnivora, but at the same time to approach more closely to that type; whereas amongst the Insectivora, Sorex provides us with an example of a yet more extensive proliferation of the material uterine epithelium before the allantoic attachment of the blastocyst comes about than even Perameles. At all events, the placentation of Perameles, characterized by so intimate a fusion between foetal and maternal elements, should never be classified amongst those forms of placenta

which are either primarily primitive (as yet unknown to us) or secondarily simplified (Ungulates, Lemurs, Cetacea, &c.)' (1909, p. 117).

With similar material before each of them hardly could two authors come to more different conclusions. Whereas Hill is of the opinion that there is no interfusion of maternal and foetal tissues other than that caused by the intergrowth of allantoic tissue—which is comparatively insignificant—Hubrecht is just as emphatic that an invasion of the maternal syncytium by the foetal chorion does occur and that therefore the type of placentation in Perameles corresponds closely with that of some of the Eutheria, notably the Carnivora.

Interpretation of Professor Hill's Figures:—

Without as yet adducing any evidence from my own material, I may be permitted to say that from the evidence of Hill's figures alone there appears to be a considerable defence for Hubrecht's standpoint in this matter.

According to Hill's account (1897, 1899) the histology of the placental area is extremely simple. With the exception of the syncytial nuclei, endothelial nuclei, and leucocytes, the only elements of this region are the cells of the foetal chorion, which in no case enter into the constitution of the placental thickening but degenerate and disappear.

With this interpretation before us it will be possible to proceed to examine his figures, particularly those of Pls. xxix and xxx, figs. 7, 8, and 9 (1897), representing portions of the placental area with the chorionic ectoderm attached. These figures are all representative of Stage C (7 mm.).

As Hill has shown, there is a marked difference in the condition of the chorionic layer dependent more or less on its distance from the centre of the fixed area. In general terms it may be stated that the farther from the centre of the attached area the less alteration is evident.

Examining Pl. xxix, fig. 7, representing the edges of the placental area of Stage C, it will be seen that the ectoderm is

almost intact throughout. At certain localized points, however, the ectodermal cells are deeper than the average. Some cells have become multinucleate and the nuclei are in many cases situated basally, such cells being deepened considerably compared with their original condition. Below the ectoderm the syncytial nuclei are arranged in their lobules. These nuclei are rounded, and although membranate the chromatic contents are spare. Each nucleus contains usually a well-defined nucleolus. Examination of the syncytial nuclei of the extra-placental area shows that the suppression of the chromatin contents and the presence of a single rounded nucleolus is a general characteristic. Even with iron-haematoxylin staining only a very faint network can be made out. It is quite otherwise with the nuclei of the cells of the ectoderm layer. A reference to Hill's Pl. xxx, fig. 9, will serve to show that the nuclei of these cells contain quite a well-developed chromatin network with a number of karyosomatic aggregations, even as many as half a dozen in some cases. Further, these nuclei are seldom spherical, but irregularly elliptical, ovalish, or lenticular, and generally of large size.

The difference in shape and histological characteristics between these and the syncytial nuclei is most marked, and in my preparations they can immediately be distinguished from one another.

Bearing in mind, then, the difference between the rounded, bead-like, maternal syncytial nuclei—' typical resting nuclei '—now congregated in groups in the syncytial lobules whether within or without the placental area—and the more robust layer of irregularly-shaped nuclei of the foetal ectoderm, each with its network of easily-stained chromatin, it will be possible to follow out their migration and rearrangement in a general way.

In Hill's Pl. xxix, fig. 7, which represents a marginal portion of the placental area with the chorion attached (embryo 7 mm.), the foetal ectoderm (*ch.ect.*) is apparently complete and unbroken, while on the lower side of the figure are the syncytial lobules containing groups of syncytial nuclei. Between these two sets of elements are to be seen a number of

nuclei of the origin of which nothing is said by Hill. Apparently he leaves it to be inferred that they are of the nature of syncytial nuclei which have not yet reached the lobules.

In my opinion it is quite definitely indicated that these nuclei originate from the foetal ectoderm through its active proliferation, and such centres of proliferation are to be seen in this figure. It needs, I think, no other evidence than that of Hill's fig. 7 to show conclusively that such a process of proliferation is in progress.

Any possible doubt, however, must be dispelled by an examination of figs. 8 and 9 of Pl. xxx of the central portion of the placental area. These are drawn at a greater magnification than fig. 7, and show the features I have indicated above with more certainty. I cannot see that any other conception than the one I have suggested can be possible. Particularly is this evident in the case of fig. 9. Here a most active proliferation and migration of the chromatically rich trophoblastic nuclei is quite apparent. They have so far advanced as to invade the syncytial lobules. In the latter position the original maternal epithelial nuclei are being overwhelmed by the advancing ranks of foetal nuclei. In some cases but one or two trophoblastic nuclei have entered the syncytial nests; in extreme cases maternal syncytial nuclei appear to be entirely absent, their place being taken by the newly-arrived, evidently phagocytic, foetal nuclei. One result of this is that the original chorionic ectoderm is now no longer a perfectly discrete layer. Another is the inclusion of the maternal capillaries by the cytoplasm of the advancing trophoblast and their consequent approach towards the stratum of allantoic capillaries.

The above statements being granted, it will be easy enough to apply the new interpretation to the remainder of Hill's stages and figures. To the further consideration of this I will return in the descriptive portion of the present paper.

Gland Alteration.—There is still, however, one point to which I would like to draw attention at this stage. It concerns a drawing of a gland in Pl. xxxi, fig. 18, one of Hill's many figures in which a gland is depicted. In the whole of the paper,

no mention is made of any gland alteration, nor would any such be likely to occur under Hill's conception of a degenerating chorion with a passive syncytium—yet distinct traces of such alteration is evident in this figure. It will be seen that up to the point where the gland enters the presumable syncytium its epithelium consists of the somewhat low cubical cells so characteristic of many of the glands of this stage. From this point to the opening of the gland, however, there is abundant evidence of degeneration. Apparently this consists of a syncytialization similar to—though not as marked as—that occurring in the Carnivora. It was the evidence of this phenomenon in my own material which first drew my attention to the possibility of the occurrence of a more complex process in Perameles than was described by Hill, and in itself lends sufficient colour to the view I have expressed above that there is something more to be reckoned with in the placentation of this animal than a simple degeneration of the foetal ectoderm.

Accepting the fact that the trophoblast of the placental area in Perameles proliferates and that the uterine epithelium after a preliminary preplacental extension remains afterwards passive, then we can come to the conclusion that placental phenomena in Perameles can now be brought more or less into line with similar phenomena in the Eutherian mammals. Here, further, I may be allowed to state that I will be able to show by the aid of my own material that the chorionic ectoderm after attachment proceeds to form by proliferation two structures :

(*a*) a plasmodiblast, plasmoditrophoblast, or plasmodium,

(*b*) a cytoblast or cytrophoblast,

and that Hill failed to recognize the presence of the plasmodiblast nuclei, the structure which he calls the chorionic ectoderm being really only the cytoblastic portion of that layer.

3. Material.

At my disposal for the examination of the foetal membranes of Perameles, I have two intra-uterine stages both of which

are important. The younger one, a specimen of Perameles obesula, shows the first fixation of the chorion; the older one, which belongs to the Tasmanian form, P. gunni, is a stage in which the first attachment of the allantois is in progress.

Both specimens were preserved in Hill's fluid (picro-nitro-aceto-osmic). Sections were stained, sometimes with Ehrlich's haematoxylin, sometimes by the iron-haematoxylin method, counter-stained in each case by means of eosin.

In addition, I have been able, by the courtesy of Messrs. L. Harrison and E. A. Briggs of the Zoological Department of the University of Sydney, to refer to several excellently-preserved sections in the collection of that department and representing some of the material mounted by Dr. Hill of the stages described in his paper. This very important collection is as follows:

Perameles obesula, 7 mm. stage, one microscope slide containing one representative section of the uterine wall, showing the placental and extraplacental areas and the attachment of the allantois.

Perameles obesula, 12·5 mm. stage, one slide with five sections similar to the above.

Perameles nasuta, post-partum stage, one slide with one section.

All the above are stained with haematoxylin and eosin.

Hill's Stage D, representing embryos 8–8·75 mm., is, unfortunately, not represented in the Sydney University collection.

4. Terminology.

The expressions 'omphalopleure', 'vascular omphalopleure', and 'bilaminar omphalopleure' first used by Hill are so convenient and expressive as to need no apology for their continued employment.

The term 'chorion' or 'true chorion' will be used by me in the same sense as by Minot and Hill to indicate that part of the extra-embryonic somatopleure which remains after separation of the amnion.

I will follow the example of most embryologists in using

Hubrecht's term 'trophoblast' for the outer ectodermal layer of the mammalian blastocyst without, however, associating it with the more recent theoretical and hypothetical meaning with which Hubrecht has invested it.

I shall also use Minot's expression 'trophoderm' for that portion of the trophoblast which proliferates and enters into relationship with the maternal epithelium. As will be seen later, this, in Perameles, undergoes changes comparable to those occurring in the Eutheria. Similarly we find a complementary structure, the maternal 'trophospongia', used to indicate (Hubrecht, 1909) 'maternal cell proliferation, specially intended for the fixation of the blastocyst'.

5. Descriptive Account of Material.

Stage 1. Perameles obesula, 6·1 mm.

This is perhaps the most important stage which has yet been examined in the placentation of Perameles. Its investigation definitely shows the fundamental connexion between the placentation of this animal and that of the Eutheria.

The specimen of Perameles obesula, on the examination of which the following account is based, was trapped by myself some miles from a small town in the Tasmanian midlands. It had apparently been dead in the trap for about an hour, but no sign of post-mortem change was to be detected. It was dissected on the spot and both uteri were found to be swollen. Conditions at the time made any dissection of the uteri inadvisable. They were opened slightly and placed in fixing solution (Hill's fluid). Later detailed examination showed that the uterus of the right side was pregnant, but, unfortunately, the delicate foetal membranes had been somewhat damaged. The general condition was that the chorion was already attached to the uterine syncytium over a small area, but that the allantois had not yet come into relation with the conjoint layer so formed.

Pregnant Uterus.—This was found to contain an

embryo of 6·1 mm. direct length, attached to the uterine epithelium by a portion of the true chorion.

In the uterus the allantoic placental area at this stage is distinguishable by the fact that its surface is marked by folds noticeable at once by their depth and distinctness.

To one of these folds the foetal trophoblast is attached.

Sections show that the uterine epithelium has become converted by loss of cell outlines and by proliferation and migration of the nuclei into a syncytium as described by Hill.

I will proceed first to give a description of the maternal structures afterwards passing to those more concerned with the embryo.

Morphology of the Syncytium.—This varies greatly in character according to the locality in the uterus. Over the main wall of the uterus the syncytium is thin, 0·035 mm., while in the region of attachment of the trophoblast it measures as near as can be judged about 0·07 mm.

In the allantoic placental region it is that the complexity of the syncytium has reached its maximum. Here, as Hill has already shown and as happens over the remainder of the uterus to a less degree, the nuclei of the original epithelium have proliferated and migrated to the deeper portion of the layer, which has now markedly thickened. The result is the formation of a syncytium in which the deeply-situated nuclei assume a particular form and arrangement. These nuclei become aggregated mainly in rounded masses or nests situated in lobular projections of the syncytial protoplasm. The lower surface of the syncytium has a wavy appearance due to the presence of these lobules.

The syncytial nuclei at this stage are rounded with a well-defined membrane, a distinct nucleolus, and indefinite chromatin network which, however, is slightly more evident than it is in later stages. Their lack of staining qualities makes them easily distinguishable from the newly-formed trophoblastic nuclei. Careful investigation of the arrangement of the epithelial nuclei in each lobule shows that, when finally at rest, they are more or less definitely arranged round a central cavity. This arrange-

ment is, in many cases, somewhat irregular, as is shown in figs. 1 and 2 (*cav.*), but a glance at the further fig. 3, which depicts quite a common arrangement, will show that in many cases the syncytial nuclei come to form a more or less definite layer round the central space.

The latter becomes filled by infiltrated lymphatic material (fig. 3, *inf.*), which in fact is copiously distributed throughout the syncytium in and between the lobules. At this stage also the syncytium is well vascularized, each capillary being enclosed in its delicate endothelial layer. Endothelial cells and leucocytes are a well-marked feature of the syncytium. They will be referred to in more detail later on.

The syncytium outside the allantoic placental area gradually decreases in thickness. In the region opposite the bilaminar omphalopleure the arrangement of the nuclei is essentially in groups similar to those of the placenta area, yet these aggregations are not so distinctive nor so individual as in that area.

This portion of the uterine epithelium is also extremely well supplied with capillaries, many of which reach the surface. Lymph also finds its way into the uterine lumen through the thin portions of the uterine epithelium between the ill-defined syncytial nests.

Leucocytes are also present in this region but not nearly so plentifully as in the allantoic placental area.

Remainder of the Mucosa.—The main portion of this consists of the much-branched connective-tissue cells, the branches of which are extremely delicate and contain in their meshes abundant lymph material. In the stroma are contained glands and blood-vessels, and around these the connective tissue is condensed to form a thin investing layer.

One feature of this stage is that the glands in the allantoic placental region are narrower and more closely packed than in the remainder of the mucosa. No doubt proliferation of the glands has occurred, their length has increased, and their courses become more tortuous without there being as yet a sufficiently accommodating increase in the thickness of the mucosa. They measure in this region, on the average, ·05 mm.

in diameter while outside this region the average width is 0·065 mm. A result of this is that the mucosa of the former region has a more compact appearance than in the latter.

Another feature to which attention should be drawn is the presence of at least one branched gland, a photograph of which is shown in fig. 10 and an outline, obtained by superimposing a number of sections, in Text-fig. 1. This figure by no means represents all the branches of the gland. This, I believe, is the first record of any but simple glands in the marsupial uterus.

Text-fig. 1.

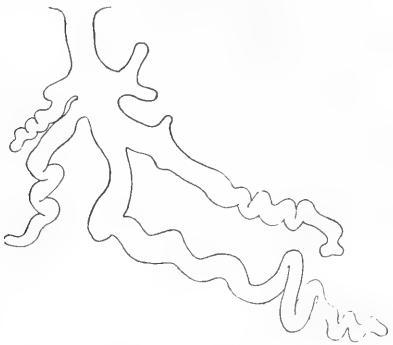


Diagram of a branched gland from the uterine mucosa of Perameles.

In all cases the glands in their lower portions are narrower and more coiled than in the upper, where in general they widen out and retain their epithelium unchanged up to the point of opening into the uterus. This latter characteristic, however, is not shown by glands opening into that portion of the syncytium to which the chorionic ectoderm is attached.

The gland epithelium is of the usual character, consisting of a single layer of cells with peripherally situated dark-staining nuclei. The secretory activity of the glands is most marked, particularly in the allantoic placental area. Migrating through the glandular epithelium, to be added to the secretion, are numbers of leucocytes.

Abundant in the connective tissue of the placental regions are

cells containing pigment in the form of black streaky and granular deposits. These cells occur throughout the whole wall of the uterus. They are very abundant in the serosa and are found distributed through the muscularis, the connective tissue, and the glandular epithelium. Such pigmented cells have often been noted in the virginal and pregnant uteri of Eutherian mammals.

Foetal Structures.—In general the arrangement and histology of the foetal membranes are in agreement with the description given by Hill, and I find I can add nothing of importance to his description of these structures.

Allantois.—The vesicular portion of this is a somewhat flattened body, taking, however, a curved shape corresponding to the dorsal curvature of the trunk of the embryo. In surface view it is somewhat elliptical, measuring 5 mm. by 3·1 mm. The point of attachment of the stalk is placed a little nearer the posterior than to the anterior end of the vesicle. The difference in thickness and texture between the placental and coelomic surfaces of the allantois is easily seen with the naked eye. The coelomic surface is an extremely tenuous sheet bearing the larger blood-vessels, while the outer or allantoic surface is more opaque and abundantly supplied with a network of capillaries derived from or supplying vessels which pass round the margin in the manner described by Hill. For a full description of these allantoic vessels I would refer the reader to Hill's account. The allantoic stalk has the usual relations and structure.

Fixation of the Embryo.

The importance of this stage rests on the fact that, over a very small area, the trophoblast is now attached to the thickened maternal syncytium (trophospongia). This portion of the foetal ectoderm is, of course, the outer layer of the chorion, which consists, in addition, of somatic mesoderm. The latter is a thin mesothelial layer consisting of flattened cells with oval, somewhat deeply-staining, nuclei.

The chorionic ectoderm typically consists also of a single cell-layer as it undoubtedly does in the marginal free portions.

Over the area of fixation to the uterine syncytium, however, an important and highly significant alteration has been impressed upon the ectoderm by which it becomes converted into a typical trophoderm (Minot) or ectoplacenta (Duval) so characteristic of this layer in the Eutheria.

At certain points in this portion of the trophoblast, cell proliferation takes place. The cells of the original layer divide to give rise to nucleated groups in which the cell outlines have disappeared. These cytoplasmic aggregations possess an irregular contour due to the presence of pseudopodial processes, so there is distinctly present here a layer definitely homologous with the plasmodial structure (plasmodium, plasmodiblast, or plasmoditrophoblast) so characteristic of the placentation of the Eutheria. The appearance of the plasmodiblast at this stage is shown in figs. 4, 5, and 6. At various points the plasmodial nuclei invade the uterine syncytium. The soldering of the foetal trophoderm to the maternal syncytium is brought about by the above-mentioned pseudopodial processes, in the meshes of which numerous spaces are enclosed. The remaining basal cells of the trophoblast layer form the cytoblast or cytotrophoblast. This is by no means at first a definite cell-layer. It is apparently not till a little later that the basally-situated nuclei divide in a regular way to form the more definite cellular layer known as the cytoblast.

A point of the greatest significance, and one to which I shall later refer, is the fact that the localities of proliferation are determined by the presence of the syncytial nests, and it is into these that the plasmodial masses pass. Fig. 4 shows this phenomenon, while it is also indicated in fig. 8, in which, however, the nests are not quite cut centrally. The effect of the growth of the attached trophoblastic cells on the maternal structures is shown in figs. 4 and 7. In fig. 4 the chorionic ectoderm cells show but little departure from their original linear arrangement, but have already begun to give off pseudopodial processes which immediately phagocytically attack the syncytial nuclei of a neighbouring syncytial lobule, only part of

which latter is shown in the drawing. Only the nuclei of the nest outside the range of attack are seen to preserve their original shape and structure, the others being in the state of degeneration. Apparently this consists in the loss of contour through the breaking down of the nuclear membrane followed by loss of chromatin and virtual disappearance. Figs. 5 and 6 show stages in the proliferation of the chorionic ectoderm cells. In all cases they give the impression of thrusting forward wedge-shaped plugs which penetrate into the aggregations of maternal nuclei.

There happens to be but one gland present in the small area to which the trophoblast is attached at this stage. This is shown in fig. 7, which depicts a section through the actual opening of the gland. Its uterine mouth is seen to be blocked by the overlying chorion, the ectodermal portion of which may now be regarded as forming two distinct portions, a basal portion, the cytoblast (*cyt.*), and a plasmodial portion, the plasmodiblast. The growth of the plasmodium has extended a considerable distance, particularly on one side, where the protoplasmic processes have caused degeneration in the gland epithelium cells similar to that which occurs in the maternal nuclei. Here, as before, the nuclei have lost their contour, their position in the cell-line, and their chromatin. They decrease in size and disappear, being evidently ingested. Further degeneration of the gland-cells is foreshadowed by the presence of protoplasmic processes involving them. Also in this figure will be seen remnants of syncytial nuclei and other remains, haematids and leucocytes.

That the cells of the plasmodiblast are phagocytic cannot be doubted. Their effect on the syncytial and gland nuclei is some evidence of this, but the presence of numerous rounded granules such as those shown to the left of fig. 7 (*ing.*) and other cellular débris makes the matter certain.

Pigmented cells are present in the trophoblast. This pigment is black and is arranged either as minute granules or as an aggregation of streaky lines, usually in the neighbourhood of the nucleus.

From the above account it will be evident that the method of fixation of the embryo to the uterine wall in this marsupial is fundamently similar to the general type occurring in the Eutheria. If, for example, the figures of this stage which I have given (figs. 4, 5, 6, 7, 29, and Text-fig. 4) be compared with the essentially similar drawings of the comparable phenomena recorded by Schoenfeld for the dog (1903), (Pl. xxii, figs. 14 to 17) (see Text-fig. 3), it will be seen that the difference in the two forms rests mainly, in the early stages, on the behaviour of the uterine epithelium. This, in the dog, remains as a distinct layer until it undergoes degeneration as a result of the inroads of the plasmodiblast.

The Non-pregnant Uterus.—This was examined in sections and found to have undergone changes corresponding to those which had occurred in the right. The ovary of this side, however, had a well-developed corpus luteum. I will, therefore, not enter into a detailed description of the structure of this uterus further than to mention the following:

The epithelium has developed into a syncytium abundantly supplied with capillaries, many ramifying on the surface, some of which discharge their blood by extravasation into the lumen. A point of interest is the fact that, on the dorso-mesial side of the uterus, the syncytium is somewhat thickened and the mucosa is here developed into deep folds. At its edges this thickening passes off gradually into the rest of the syncytium. This thickened portion possibly represents the maternal trophospongia. Throughout the whole uterus the glands show no degeneration. They are in an active state of secretion and the inner ends of the gland-cells are torn and frayed out through abundant breaking off of cellular secretion. Cilia, therefore, are not to be found.

(*a*) Significance of the Uterine Syncytium in Perameles.

Proliferation and syncytialization of the uterine epithelium is a well-marked feature of maternal preparation for allantoic placentation in many Eutheria. In many cases the

preparatory proliferation is soon interfered with by the destructive action of the blastocyst on the uterine epithelium.

Hill (1897, p. 393) institutes a comparison between Perameles and Sorex in the matter of the proliferation of the uterine epithelium. As a consequence, however, of his belief in the degeneration of the chorionic ectoderm and the persistence of the uterine epithelium in Perameles he could not carry the comparison far enough.

In Sorex, Hubrecht states that over the future allantoidean and omphaloidean placental areas the epithelium undergoes a tremendous proliferation and development into a cell aggregate of relatively great thickness.

The history of this maternal formation in the allantoic placental area of Sorex is, I think, worthy of particular consideration and of comparison with what happens in Perameles. Hubrecht shows (1894, p. 492 seq.) that, in the shrew, the nuclei of the epithelial proliferation become arranged in fan-shaped groups at comparatively regular distances, the centre of each group being without nuclei (1894, fig. 69).

'In the following stage', says Hubrecht, 'this arrangement becomes converted into a functionally more important one. The centre of the fan-shaped structure becomes an open crypt, the protoplasm breaking up and the peripheral nuclei forming the epithelial lining of the crypt. The uterine epithelium breaks away from under the crypt and the inner lining of the crypt solders with the surrounding epithelial surface at the lower border' (p. 493).

It is quite easy to see the resemblance between the shrew and Perameles in respect of the phenomenon here described. In both there is intense epithelial proliferation, particularly in the placental area. The resulting nuclei or cells are in both arranged in nest-like groups. While in the bandicoot these groups remain practically unaltered, in Sorex they are transformed into epithelial crypts. Nevertheless, as I have shown above, the proliferated nuclei in Perameles take on a more or less definite arrangement as

a layer bounding a central space. This is indicated in figs. 1, 2, and 3, whilst in other figures, 4, 7, and 9, I have drawn attention to the fact that the trophoblastic proliferations bear a definite relation to the groups. Particularly in the next stage, it will be seen that the foetal nuclei invade and fill the nest with consequent more or less complete disappearance of the maternal nuclei. Under these circumstances it does not require any extraordinary stretch of imagination to recognize in this highly characteristic and important phenomenon the remains of a much more elaborate system of placental formation. The conclusion is certainly obvious to me that here in Perameles, in the formation of the peculiar syncytial groups, there is to be recognized an abortive attempt at the formation of crypts such as occur in the placental area of Sorex, and further, while in the latter crypt-building is confined to the placental area, in Perameles the comparable phenomenon occurs at all points of the uterine epithelium, although in a lesser degree opposite to the omphalopleure than in the placental area.

(*b*) General Remarks on the Fixation of the Embryo.

Here it will be convenient to interpolate a few remarks on the method of fixation of the blastocyst and on the general terms used to express the nature of the structures taking part in it.

Fixation is brought about in Perameles as in others by the junction of a circumscribed portion of the trophoblast, the chorion, with a corresponding area of preplacentally proliferated maternal tissue, the trophospongia.

There is a fundamental difference in the character of the two uniting layers—the foetal being an active, the maternal a quite passive layer. To a mobile, virile formation of the former type—of foetal origin—the general term plasmodium is applied, while the corresponding multinucleate structure, of maternal origin, usually acting as a pabulum for the foetal plasmodium, is known as a syncytium (see Schoenfeld, 1903).

It is evident enough that in all cases of placentation where the uterine epithelium is not immediately destroyed, the area of fixation consists for a longer or shorter time of a conjoint layer of foetal and maternal epithelia, each having the characteristics outlined above. Development of the former and degeneration of the latter proceed in Perameles side by side, and it would be convenient if a single expression could be coined to denote the composite layer consisting of the two. No convenient term seems to exist, and I propose to use the name 'diploplasma' to indicate the conjoint layer consisting of foetal chorionic ectoderm and maternal trophospongia.

The diploplasma consists in Perameles of three zones. Along the line of junction of foetal and maternal tissues, the syncytium is undergoing degeneration and resorption by the plasmodium. Such a degenerating syncytium is called a symplasma (Schoenfeld, 1903), a term which can be correctly applied only to maternal structures of a degenerate nature contained in the plasmodium. Recently, however, Willey has suggested its use to indicate the junctional portion where there is an intimate mixture of active foetal elements and degenerating maternal material.

Accepting this suggestion (Willey, 1914), the three zones of the diploplasma in Perameles consist of the following: a middle junctional layer composed of mixed foeto-maternal tissue (symplasma) with, on one side, a pure layer of foetal, and on the other side, pure maternal material.

The foetal portion differentiates early into a basal layer, the cytoblast, and a plasmodial layer, the plasmodiblast. Contrary to what has been stated by Willey for other mammals, the cytoblastic layer in Perameles is well in evidence before the time of attachment of the allantois.

The plasmodiblast has a twofold duty concerned with (*a*) attachment, (*b*) nutrition. Both functions are performed with the aid of root-like pseudopodial processes which attack the maternal elements converting them into symplasmatic débris which is ingested. Such nutritional material is passed

on to the cytoblast, the cells of which exercise a secretory and selective rôle.

The cytoblast appears to have as one of its duties the amplification of the area of attachment. Cell outlines are always distinct between the cytoblastic cells due to radial divisions. These result in the formation of new cells and consequent increase of the area of attachment. As a result, new marginal zones of plasmodial formation are brought into being, and these attack fresh sources of nutrient material in the maternal trophospongia. This areal increase is subject, of course, to certain limitations, and in Perameles, as will be seen later, the growth in thickness of the plasmodial formation is determined also by the thickness of the proliferated maternal epithelium. In many mammals, however, as is well known, the plasmodiblast extends much further, even in some cases as far as the muscularis, causing the degeneration and disappearance of practically all the structures in its track.

A second function of the cytoblast cells is, as indicated above, the elaboration of a secretion which is passed into the extra-embryonal coelome. They therefore exercise a certain selective capacity on the material passed to them by the plasmodium.

Abundant evidence of this secretory activity is to be seen in sections, the actual secretion being easily observed. From the above it will be seen that structurally and physiologically the proliferating trophoblast of Perameles over the allantoic placental area is quite comparable with the corresponding layer in the Eutheria.

Stage 2. Perameles gunni, 6·6 mm.

The specimen on which the following description is based was brought to me by a trapper on September 17, 1919, about two hours after being trapped. Both uteri were swollen and found to be pregnant. The left uterus after being opened was fixed in Hill's fluid, and the whole uterus with embryo in situ was later sectioned.

The right uterus was examined in salt solution. It was

carefully slit open along the ventral side without any injury to the foetal membranes. It was noted that although there was a close apposition of the yolk-sac wall to that of the uterus, the folds of the former fitting into the hollows of the latter in a very intimate way, yet there was absolutely no sign of fusion or organic connexion. It will be remembered that Hill, at first of the opinion that there was a protoplasmic connexion between the yolk-sac wall and the uterine syncytium, found later that this was not the case.

In the case of P. gunni it was possible by means of careful manipulation to make out the details of the yolk-sac circulation. This, at this stage, is in a state of considerable activity, a condition no doubt to be correlated with the fact that the allantoic placental connexion and circulation is now just on the way towards completion and definite establishment. By removing the lower portion of the bilaminar omphalopleure and reflecting the remainder over the embryo a full view of the vascular area was obtained, and the whole course of the vessels could be made out in detail. After these had been sketched and photographed the lower portion of the yolk-sac was removed, whereupon it was found that the connexion between allantoic vesicle and uterus was so slight that the whole embryo with its allantois could be removed intact. The point of attachment was visible in the case of this embryo as a somewhat central raised area on the placental surface of the allantoic vesicle. The elevation of this area was due to the adherence of a slight amount of maternal tissue brought from the uterine wall.

This being the only pregnant uterus of P. gunni so far known, I may be excused from entering with some detail into the description of the relation of the embryonic and maternal structures.

General Relations of Embryo and Uterus.

Foetal Membranes.—These agree essentially in their arrangement with what Hill has described for other species of Perameles. For that reason I will concern myself in the

following account only with those features which seem to be peculiar to the Tasmanian form.

The Allantois.—This agrees in all respects with that of other species.

In the vesicle the distinction between the outer or placental wall of the allantoic vesicle and the inner or coelomic wall is obvious. A feature worthy of note is the somewhat dense look of the allantoic vesicle in surface view. This is due to the thickness of its walls, the mesodermal layer of which is much denser and thicker than in the species examined by Hill.

The Vascular Omphalopleure.—The vascular omphalopleure (fig. 23, *v. omph.*) is essentially similar in structure to that of other species. It is, however, as well to point out that the tenuity of the ectodermal layer of this area, on which Hill laid so much stress, is not so pronounced a feature in P. gunni as in the forms examined by him. It is, however, still much thinner than in Didelphys (Selenka, 1886–7). In P. gunni the ectoderm cells are somewhat columnar with ovalish nuclei, basally situated. The free end of the cell-body is frayed out into pseudopodia-like processes. In the vascular omphalopleure is contained a large proportion of the extra-embryonal vascular system which in the stage under discussion forms a vascular absorptive organ of some complexity and size. No marsupial so far described appears to have a yolk-sac circulation of such an elaborate type as is characteristic of this stage of P. gunni.

Bilaminar Omphalopleure (fig. 23, *bil. omph.*).—This of course consists of two layers, trophoblastic ectoderm and yolk-sac entoderm. The histological details of a portion of this wall are shown in fig. 22. It will be seen that the ectodermal layer consists of cells which are large and are remarkable for the immense amount of vacuolation which occurs in their cytoplasm.

These vacuoles are of all sizes occupying in the aggregate almost the whole of the interior of each cell, separated from one another by thin bridges of protoplasm in a special condensation of which the nucleus is contained. The outer ends of the

cells are often rounded, but in other cases possess an irregular profile suggesting the presence of pseudopodial processes.

No doubt the vacuolation of the ectodermal cells of this region of the blastocyst is to be associated with an active absorption on the part of these cells of carbohydrate (? glycogen) in process of being transferred to the embryo per medium of the yolk-sac. The cells closely resemble the glycogenic cells figured by authors for certain other mammals (e. g. Jenkinson, 1902, I. vi).

The entoderm of this region consists of a layer of cells somewhat darkly staining with haematoxylin. These are sometimes rounded, sometimes flattened. I have found the entoderm cells occasionally slightly vacuolated in the manner described by Hill for his species.

It is a well-known fact that the vacuolation of cells concerned in embryotrophic processes varies greatly at different stages. Hill found that the entoderm cells of the yolk-sac, but slightly vacuolated in early stages, became greatly so in his 12·5 mm. specimen. This he regarded as a forerunner of degeneration, but I rather think it is to be associated with the active absorption and internal transmission by the yolk-sac wall of substances (probably carbohydrate) secreted by the mother and destined for the nutrition of the foetus.

The Yolk-sac Placenta.—As stated above, this is brought about by the intimate apposition of the vascular omphalopleure to the extra-placental portion of the uterine wall. Fig. 23 shows the details of this circulation and fig. 24 is a somewhat diagrammatic representation of the area with regard to the blastocyst wall and the embryo.

In fig. 23 most of the bilaminar omphalopleure has been removed and the remainder of the blastocyst wall has been reflected over the embryo. The vascular area is therefore seen from its inferior aspect. The anterior end of the embryo is contained in the proamnion (*proa.*), here large and persistent.

The vitelline artery (*vit.a.*) is a very large and thick trunk which, after leaving the yolk-stalk, keeps at first a little to the left side of the body and passes over the surface of the tail to

reach the right side. Here it passes into the outer leaf, i. e. from the yolk-sac splanchnopleure to the vascular omphalopleure. In the course of its passage along the body it gives rise to the numerous fine and extremely characteristic vessels which extend in their peculiarly parallel manner into the yolk-sac splanchnopleure which they supply. These branches are extremely long and pass for some little distance over into the vascular omphalopleure where they alternate with corresponding venous factors to the vitelline vein.

Immediately on entering the vascular omphalopleure the vitelline artery divides to form the sinus terminalis (*s.t.*). Each portion of the sinus passes forward and at first has the usual course. Instead, however, of passing directly forward in the usual way, each branch, at about the level of the forelimb, takes a sudden turn ventrally so that the anterior portion of each lateral branch forms another curve with a ventral convexity. The artery on each side unites with its fellow in front of the head by anastomosis.

The sinus gives off to the area vasculosa a wonderfully rich plexus of branches quite as complex or even better developed than in any marsupial so far described.

The peculiar arrangement of the sinus terminalis results in the division of the vascular area on each side into two regions, anterior and posterior, the former being somewhat smaller in area than the latter. Each of these areas is drained by a separate factor of the vitelline vein on each side. The posterior factor from the posterior area is the larger, and after receiving numerous fine capillaries and branches travels along the dorsolateral aspect of the vascular omphalopleure, parallel to and some little distance from its dorsal edge till the vein reaches the height of the neck flexure of the embryo, where it passes over into the yolk splanchnopleure. Here it receives the anterior factor which drains the anterior area. The branches and capillaries which go to make up the latter are extremely rich. Each lateral vitelline vein (*vit.v.*) formed of the factors just mentioned passes down and unites with its fellow just before entering the body. Between them in this region is the

permanently non-vascular area spoken of by Semon and by Hill.

It should be mentioned that the posterior factor of each lateral vitelline vein receives on its inner side branches from the yolk-sac splanchnopleure corresponding to the fine branches from the vitelline artery supplying that membrane. These pass upwards in the splanchnopleure, and then continue outwards into the omphalopleure to join the posterior factor as stated.

The measurements of the vascular area are as follows :

Across anterior portion	7·5 mm.
Across posterior portion	9·9 mm.
Greatest length, anterior portion . .	4 mm.
Greatest length, posterior portion . .	5 mm.

Formation of the Allantoic Placenta in Perameles gunni.

Various stages of this are shown in figs. 13–16, 19.

Maternal Structures.—The wall of the uterus is divisible, as stated above, into two general portions, placental and extra-placental regions.

Placental Region.—The mucosa varies greatly in thickness, from 0·60 to 1·1 mm., due to folds in the uterine wall. The glands are numerous, long, and tortuous, measuring in diameter from 0·037 to 0·051 mm. They are of the usual type, being unbranched and lined by a single layer of columnar cells, with deeply-staining nuclei, peripherally situated. I am not able to detect any trace of the cilia which Hill and O'Donoghue have observed in Perameles. The glands are in a highly active state of secretion, their basal portions being filled with cellular and other material.

The inter-glandular tissue is extremely thin and tenuous, but condensed where it immediately surrounds glands and blood-vessels. Distributed through the connective tissue is an abundance of lymph material. This is particularly evident just below the uterine epithelium, in which position there is a space up to 2 mm. wide filled with lymph which bathes the lower surface of the uterine syncytium. Here and there this

space is crossed by glands and blood-vessels. I have called this space the sub-epithelial lymph-space. Owing to the presence of this coagulum and the sparsity of cellular elements the whole stroma presents a very homogeneous appearance. Scattered through it, however, are numbers of leucocytes, and here and there are erythrocytes which have escaped from the capillaries by extravasation.

Attached to and intimately united with the epithelium of this area is the embryonic chorion.

Epithelium.—The condition of the epithelial elements of the allantoic placental mucosa shows, unquestionably, that in P. gunni the same process of syncytialization has occurred as in other species, and that here is a similar aggregation of the syncytial nuclei into nests arranged in lobular masses of the syncytial protoplasm. The distribution, as I have suggested above, I believe to represent an abortive attempt at the formation of simple uterine crypts. Between these nests the capillaries and leucocytes of the remainder of the mucosa gain access to the syncytium.

The Extra-Placental Region.

Mucosa.—In all essential respects this resembles the mucosa of the placental area, being highly vascular and containing a large quantity of lymph. Here are to be found leucocytes and red corpuscles, but neither are so numerous as in the placental portion. The glands are in an active secreting phase and their contents—cellular detritus and other nutritive substances—are poured into the uterine cavity to be received into the trophoblastic layer of either region of the omphalopleure.

The sub-epithelial lymph-space, as such, becomes less evident the farther we proceed from the placental area and the glands are somewhat smaller in diameter.

Syncytium.—This has been formed by a similar process to that which has resulted in the modification of the epithelium of the placental area. The difference is mainly one of degree. Thus the extra-placental syncytium is thinner, being about

0·043 mm. opposite the vascular omphalopleure and 0·023 mm. opposite the bilaminar. Similar proliferation and migration of the nuclei have taken place but the lobular nests are much smaller and less individual, and, further, more and more nuclei remain outside the nests until in some places lobules and nests as such are scarcely distinguishable. The nuclei are quite similar to the syncytial nuclei of the placental area being rounded, bead-like, and vesicular with little chromatin.

Chorion.—This shows, both in the amount of area of attachment and of proliferation, a considerable advancement on the condition found in the last stage. The fusion with maternal tissue is so complete that the resulting layer is one in which occasionally there is some difficulty in distinguishing maternal and foetal cytoplasm. Here, as before however, we have the infallible test of the difference in structure, shape, and fate of the maternal and foetal nuclei.

The Diploplasma.—The thickness of this layer measures now from 0·135 to 0·190 mm.

This increase is due mostly to the great growth of the chorionic ectoderm, which consists of a well-marked basal cellular layer, the cytoblast, and a much thicker portion in which cell outlines are not visible, the plasmodiblast. The maternal portion of the diploplasma consists of the remains of both the maternal syncytial protoplasm and of the syncytial nuclei. When unaltered the latter have the characteristics which have been before noted, viz. small rounded vesicles in which the chromatin is suppressed but containing a more or less prominent nucleolus (see figs. 13, 14, and 19, *syn.n.*). They have an exactly similar appearance to the nuclei of the extra-placental area.

At this stage, however, nests are seldom found intact. In almost every case they have been invaded by plasmodiblast nuclei (*pb.n.*), so that by this time a fair proportion of maternal nuclei have degenerated and disappeared.

The Trophoderm.—This is divisible as stated above into cytoblast and plasmodiblast.

The Cytoblast (*cyt.*) is a remarkably definite layer of

cells with distinct cell outlines. The cytoplasm is granular and the nuclei large and plump. Their chromatin contents are in the form of an extremely rich network with a number of karyosomes. A nucleolus may be present, and if so is of large size. At the edge of the placental area the cytoblast (*cyt.*) passes over into the ectoderm of the marginal chorion, whose cells gradually decrease in size till they attain, at the junction with the vascular omphalopleure, the normal dimensions and appearance of trophoblastic ectoderm cells. Some idea of the difference in size of the cells of this layer may be obtained from the fact that, at the margin of attachment, the cells are twice, while in the centre of the area of attachment they are eight times the height of the ordinary trophoblastic ectoderm cell.

The cytoblast cells, therefore, have already the appearance, particularly in the centre of the placental area, of typical placental megalokaryocytes.

Intense proliferation is to be seen in the cells of the cytoblast of the whole of this area. I have not been able to observe mitotic figures, but it must be remembered that in the case of my material as well as that of Hill, fixation was not possible until a couple of hours after death.

Proliferation of the cytoblast (*cyt.*) and its results are shown in figs. 13–16, 19. The plasmodiblast nuclei (*pb.n.*) resulting from this division are usually more darkly stained than the original cytoblastic nuclei and for this reason are easily distinguishable, particularly from the original syncytial nuclei (*syn.n.*). The plasmodiblast nuclei are occasionally isolated, but more often are arranged in clumps to form multinucleated masses (*g.c.*) of sometimes large size. These resemble the giant or monster cells which have been found in similar positions in connexion with the placentation phenomena of higher Eutheria. The main result is that the plasmodiblast makes its way into the nests of syncytial nuclei, in which one or two or, in most cases, a large number of these nuclei can be distinguished. In such cases the syncytial nuclei have to a great extent disappeared, the tendency being to replace them in their nests by the newly-arrived foetal nuclei.

The pseudopodial processes characteristic of the plasmodium of the previous stage are greatly in evidence in this stage also. The result is that spaces occur in the diploplasma giving it a very uneven appearance. The arrangement of these plasmodial spaces suggests that new nuclei are formed in a somewhat spasmodic manner in waves one after the other.

One result of the advance of the plasmodium is the inclusion of the maternal capillaries which pass up between the remains of the syncytial lobules and ramify below the layer of cytoblast. Each capillary is contained in its delicate endothelial layer. Further than this in the symplasmatic zone of the diploplasma are to be found remains of maternal nuclei, maternal blood corpuscles and various granules. These are all obtained phagocytically. An evidence of this action in the plasmodiblast is the strong affinity which some of its nuclei have for pigment. In this stage much of the pigment found in other cells of the previous stage has disappeared. It is now almost entirely confined to the cytoblast and plasmodiblast. Here it appears usually as black granules deposited round the nucleus, but in other cases as narrow irregular lines in the cytoplasm (figs. 25–27). A similar pigment (related to haematoporphyrin?) is found in Ungulates, and is regarded by Jenkinson and Duval as being the remains of ingested haematids whose iron has been passed on to the embryo. The result is that the pigment remains in the trophoblast increasing in amount up to the time of parturition.

Leucocytes.—These are extremely characteristic of the uterine mucosa, particularly in the placental regions. They are found in the connective tissue, from which they migrate through the epithelium of the glands and mingle with their secretion. They also pass into the diploplasma, from which they reach the extra-embryonal coelome. They can be seen abundantly present in all sections of this region at this stage. In the connective tissue they are small, taking the form of small mononuclear leucocytes. In the diploplasma, however, and particularly when they reach the cytoblast, they have increased greatly in size, forming large mononuclear leucocytes or macro-

cytes. In this position they occasionally displace cytoblast cells. All the leucocytes carry well-defined pigment. Isolated patches of pigment occur occasionally in the plasmodium.

The coagulable material (proteid, lymph) passing through the epithelium of the uterus is particularly abundant in the placental area. The secretion of the cytoblast cells is visible on their coelomic faces as small rounded swellings which break off to form spherical bodies floating in the coelomic fluid.

I can find no evidence of fat secretion either in the cytoblast cells or in the gland cells, but it must be remembered that no special means have been employed for the detection of fat, glycogen, or iron.

The chorionic mesoderm, where it can be made out, is a thin mesothelial layer consisting of flattened cells with ovalish darkly-staining nuclei. I must confess that I find it impossible to discern this layer over most of the placental area.

The free margin of chorion forms the connecting link between the fixed chorion on the one hand and the vascular omphalopleure on the other. The mesoderm here is similar to that of the central portion of the chorion, but the trophoblastic layer consists of somewhat flattened cells near the vascular omphalopleure, these increasing greatly in height as they approach the fixed portion.

Gland Alteration.—A word about the condition of the glands in this stage. The body of the gland is lined by an epithelium which is somewhat lower than in the preceding stage. In its upper portion the gland swells out and becomes somewhat barrel-shaped. The whole of this portion enclosed in the diploplasma suffers a degeneration of its epithelium. The result is that here the gland appears to be a mere space lined only by plasmodiblast. The gland-mouth is closed by a layer consisting of the cytoblast plus a certain amount of plasmodiblast. The latter by no means takes the form of a plug as in the last stage, but passes down on either side apparently causing the disorganization of the gland epithelium on its way. There appears to be a struggle here between the downward force of the growing plasmodiblast and the upward

pressure of the gland secretion, evidenced by the swelling of the gland and the formation of lateral fissures in the plasmodium. Such appearances are characteristic of all the glands in this stage.

When such glands are sectioned obliquely or transversely they appear in sections of the diploplasma as irregular spaces without any definite epithelial bounding layer.

Attachment of Allantois.—At this stage this has occurred over an approximate area of 0·5 by 0·21 mm. The attachment is due to an intimate fusion of the splanchnic mesoderm of the allantois with the somatic mesoderm of the chorion. Apparently the allantois spreads its area of attachment very rapidly, and one of its almost immediate results is a quickening of the proliferating activity of the cytoblast cells.

In the centre of the area of attachment the distinction between the plasmodial layer and the cytoblast to a great extent breaks down, the cell outlines of the latter disappearing and its nuclei being converted into plasmodiblast nuclei. It is in these positions that apparently the union of the allantois with the chorion is best effected. This dissolution of the cytoblast allows maternal capillaries to come closer to the surface of the diploplasma, and this can be seen in some cases even before the allanto-chorionic fusion is in being (fig. 14).

Stage 3, Perameles obesula, 7 mm.

This is Hill's Stage C, being his earliest placental stage. It is represented in the Department of Zoology of the University of Sydney by a single slide containing one section. The specimen, however, is well stained and mounted, and shows all the details of the relations of the foetal membranes to the uterine wall.

The excellent general account given by Hill of the foetal membranes of this stage renders any further description unnecessary. I will therefore confine myself to a consideration of the maternal and foetal structures associated with the allantoic placentation.

The attachment of the allantois may now be said to be com-

plete, the allanto-chorion being united with the maternal epithelium over an area whose diameter, following the well-marked uterine folds, is some 13–14 mm.

The area of attachment is not co-extensive with the whole of the chorion since there is a marginal zone of the latter quite free, as in the 6·6 mm. stage.

Structural Details of the Allantoic Placenta.

As would have been expected from the description of the last stage there is by this time a most intimate fusion of the maternal and foetal tissues in the diploplasma, the proliferation of the trophoblastic ectoderm and its invasion of the maternal syncytium having advanced considerably beyond the condition found in the 6·6 mm. embryo of P. gunni.

Here again, therefore, in the trophoblastic proliferation there can be recognized two portions, a basal cellular layer corresponding to the cytoblast or cytotrophoblast of higher mammals, and externally to this a proliferating plasmodial portion, the plasmodiblast or plasmoditrophoblast.

The cytoblast is throughout its extent fairly distinct. Over a fair area towards the centre, however, it has already begun to lose its integrity—having disappeared in many places as a cellular layer and become converted here into plasmodiblast (fig. 18). It was upon this condition of the cytoblast in its central portion that Hill based his suggestion of the degeneration of the chorionic ectoderm.

In the marginal portion (fig. 21) the cytoblast still consists of high columnar cells separated from one another by distinct cell walls. Externally, however, the boundaries of these on their plasmodial aspect are indistinct. Where intact, they have somewhat elongated nuclei arranged at right angles to the surface of the uterus. These nuclei stain well and are rich in chromatin. They do not, however, stain so darkly as the more granular plasmodial nuclei to which they give origin.

At the edge of the placental area the development of plasmodiblast from cytoblast is in its minimum condition of activity, as is shown in the figure (fig. 21).

Here it can be seen that the arrangement of the syncytial nuclei of the extra-placental area is similar to that of the preceding stages. The presence of an internal space, free of nuclei, in the syncytial nest should be noted.

Passing from this region into the placental area the gradually increasing activity of the proliferating chorionic ectoderm becomes evident. More and more of the foetal nuclei occupy the nests. The plasmodial nuclei in a large number of cases form multinucleate groups. In many cases there are but two nuclei in each group, these being comparable to the binucleate cells 'diplokaryocytes' described for some Eutheria (for example in Ungulates, Assheton, 1906). More often three or more nuclei are contained in one plasmodial mass.

An examination of a more central section shows that in this portion of the placental area many nests are now quite filled with the plasmodiblast nuclei (fig. 18). So closely are these packed in the nests that instead of being oval or irregularly shaped they take a polygonal form due to mutual pressure. The condition of the original epithelial nuclear nests can be gauged from the fact that of twenty-seven observed in one central portion of the allantoic area four were untouched; eleven were partly, twelve completely filled by invading nuclei, the nests having the appearance of solid multinucleated masses.

In such positions also it is that the original cytoblast layer has practically disappeared. It is evident, therefore, that a large proportion of the maternal epithelial tissue has been replaced by intruding foetal material. One effect of this is that the maternal capillaries of the allantoic area become enclosed by the advancing plasmodium, and in many cases are now to be found at the surface of the diploplasma, where they directly underlie the allantois and even come into contact with the allantoic vessels.

Pigment is not so common as it is in the previous stage but it is still to be found, particularly in leucocytes and in the cytoblast cells. Here and there in the plasmodium are to be seen isolated patches of pigment pointing to an active ingestion

of maternal cellular material. Haematids are often to be found contained in the plasmodium in process of being absorbed.

Glands.—Most of the glands of the allantoic placental area have undergone certain characteristic changes. Instead of being narrow and tortuous, they have now become generally straighter and wider.

The necks of the glands are particularly spacious, and often the gland on its entrance into the diploplasma shows a barrel-like enlargement. The aperture of the gland in the placental area is closed by the cytoblast, while the plasmodiblast has disappeared from its mouth but bounds this portion of the gland on either side where it has been responsible for the disorganization of the gland epithelial cells (fig. 28). The cells of the body and neck of the glands have now been transformed into a low cubical epithelium, and their nuclei instead of being oval are rounded. The gland-cells throughout are ciliated. Occasionally some of the glands swell to a relatively enormous size. A further feature of note is that a number of glands have penetrated into the muscularis.

The Allantois.—For the general description of this I would refer the reader to Hill's account. At this stage the splanchnic mesoderm of the allantois is found fused with that of the chorion over the full extent of the placental area. There is but a small amount of penetration of allantoic tissue into the plasmodiblast, and this only becomes possible where gaps have occurred in the disorganized cytoblast. The complex 'interlocking system'—by which apt phrase Hill describes the mutual apposition of foetal and maternal capillaries—is brought about by short finger-like downgrowths of the allantoic mesoderm combined with the opposite (inward) tendency of the maternal capillaries. This mutual process becomes more and more easy the more the basal cytoblastic layer loses its integrity and becomes converted into plasmodiblast. Where the cytoblast remains practically intact as at the margins (fig. 23), the approximation of foetal and maternal vessels does not occur. The maximum penetration of the allantois appears to be a little more than the thickness of the cytoblast. One

result of this penetration is the tendency to form occasional cytoblastic islands, containing usually one or two nuclei, isolated by the growth round and behind them of the allantoic capillaries (fig. 24).

Stage 4. Perameles obesula, 8–8·75 mm.

Of this stage I have no material available, so will content myself by stating that Hill's figures (1897, figs. 15 to 21) show that the various processes of allantoic placental formation which have been initiated in the case of the younger embryos can be recognized as being continued in this.

The growth of the plasmodiblast has gone on apace, with a corresponding diminution of the amount of maternal tissue contained in the diploplasma. This results in a very homogeneous appearance of the tissue of the placental area. In Hill's figures very few syncytial nuclei are recognizable with certainty. On the other hand, it can be seen unmistakably that, by this time, most of the cytoblast has been converted into plasmodiblast, and that in this formation giant multinucleate cells are a very prominent and characteristic feature (see especially his fig. 17).

Here again the greatest activity is being shown towards the centre of the placental area. Of the still remaining basal cytoblast cells Hill says (p. 414), ' in some cases they are multinucleated . . . or the single nucleus is also hypertrophied and vesicular,' a statement which well accords with the facts to which I have already drawn attention in the preceding pages.

Many of these remaining cytoblast cells have the appearance of diplokaryocytes. Some of them plainly show a tendency towards plasmodial formation, as can be seen in Hill's fig. 21, where the cell marked *ch.ect.* has very much the appearance of a giant cell with plasmodial processes.

Stage 5. Perameles obesula, 12·5 mm.

Of this stage I possess five consecutive sections stained in haematoxylin and eosin.

Here, as before, I will confine myself to a description of the

structural modifications associated with the formation of the allantoic placenta.

A section through the very folded allantoic placental area reveals the presence of an extremely homogeneous granular layer with lobed lower margins, indicating the positions of the original syncytial nests.

Unfortunately, the gap between this and the preceding stage is too wide to make it possible to closely follow and be certain of the histolytic changes which have taken place in the placental region.

Within the lobulated portions of the diploplasma are very irregular groups of nuclei corresponding in position to the groups of the original syncytium.

An examination of such an aggregation at this stage shows that it consists mainly of plasmodiblast nuclei which are in a marked stage of degeneration. One simple group is shown in fig. 26 of this paper, while many others are depicted by Hill (1899, Pl. xliii, figs. 6 and 7).

One particular type of degeneration change in the case of a plasmodial nucleus is shown in fig. 17. This corresponds closely with what has been recorded by Jenkinson (1902, figs. 24–5) for the megalokaryocytes of the mouse. The original robust nucleus loses its contour through shrivelling of the nuclear membrane and escape of the nuclear sap. The chromatin becomes irregularly arranged. Thereupon the whole nucleus flattens and becomes of the nature of an extremely thin rod, which by further absorption is seen as a few darkly staining particles in the general ground-mass, finally disappearing.

There are to be found also degenerating vesicular elements which no doubt are the remnants of the formerly numerous maternal epithelial nuclei. On this important point, in view of the paucity of the material available, I regret I am not able to make any certain statement.

If, however, this interpretation be correct, then Hubrecht's suggestion (1909) that the placenta of Perameles consists in its final stage of a ' mixed syncytium ' is not very far from the truth.

It is difficult to suggest a cause for the degeneration and resorption which has taken place in the case of these nuclei, but it is a significant fact that associated with the degenerating groups are to be found abundant leucocytes of the small and large mononucleate types.

Situated just internally to the allantois are to be found occasional large trophoblastic cells, being remains of the cytoblast. These also are undergoing degeneration.

In places the homogeneous nature of the ground-tissue is less evident, and here multinucleated masses of protoplasm are still in evidence. Apparently the foetal chorionic cells have performed their function, viz. the fixation and a portion of the preliminary nutrition of the embryo, and are now in a process of degeneration and disappearance.

The maternal vessels are extremely numerous, and their finer branches now ramify at the surface of the diploplasma, where they come into intimate apposition with the vessels of the allantois in the way Hill describes.

The allantoic mesoderm has penetrated but little into the trophodermic layer. No spaces are formed in which maternal blood flows, all maternal vessels being contained in definite endothelial walls. The two blood-systems, however, are elaborately interlocked, and very often foetal and maternal blood-streams are separated merely by the thickness of two endothelial walls.

As regards the glands of the placental area, they are now characterized by the possession of a low cubical epithelium. Their cavities are wide, particularly near the opening of the gland. The cells are ciliated. The mouth of each gland is sometimes closed by the allantois with its numerous vessels, at other times by degenerating remains of the trophoblast. The condition of the portion of the gland contained within the diploplasma is similar to that characteristic of the preceding stage.

Outside the placental area the maternal nuclei of the epithelium still take the form of rounded vesicles in which it is difficult to distinguish even a nucleolus. The glands of this portion of

the uterine wall possess the usual columnar epithelium and are also ciliated. They are narrower than the glands opening into the placental area.

Seeing that the newly-born young of Perameles measures but 14 mm. in length, the condition of the allantoic placenta in the 12·5 mm. stage may be accepted as being practically that of the full-term placenta.

Stage 6. Perameles nasuta, post-partum.

With regard to this stage the only way in which I can supplement Hill's description is by pointing out that the amount of foetal tissue left behind in the uterus is considerably more than would be the case if Hill's conception of the placentation of this animal were correct. Instead of consisting merely of the allantois—with the addition of a few remaining foetal cells left behind after the degeneration of the chorionic ectoderm—there is really comprised in the contra-deciduate portion, in addition to the allantois, the whole thickness of the diploplasma, of which undoubtedly the greater part is foetal.

6. Summary of Conclusions.

The conclusions arrived at in the preceding pages may be summarized as follows:

1. Allantoic placenta.

(*a*) The fixation of the embryo is brought about by means of the chorionic ectoderm at a time when the embryo measures about 6 mm. direct length.

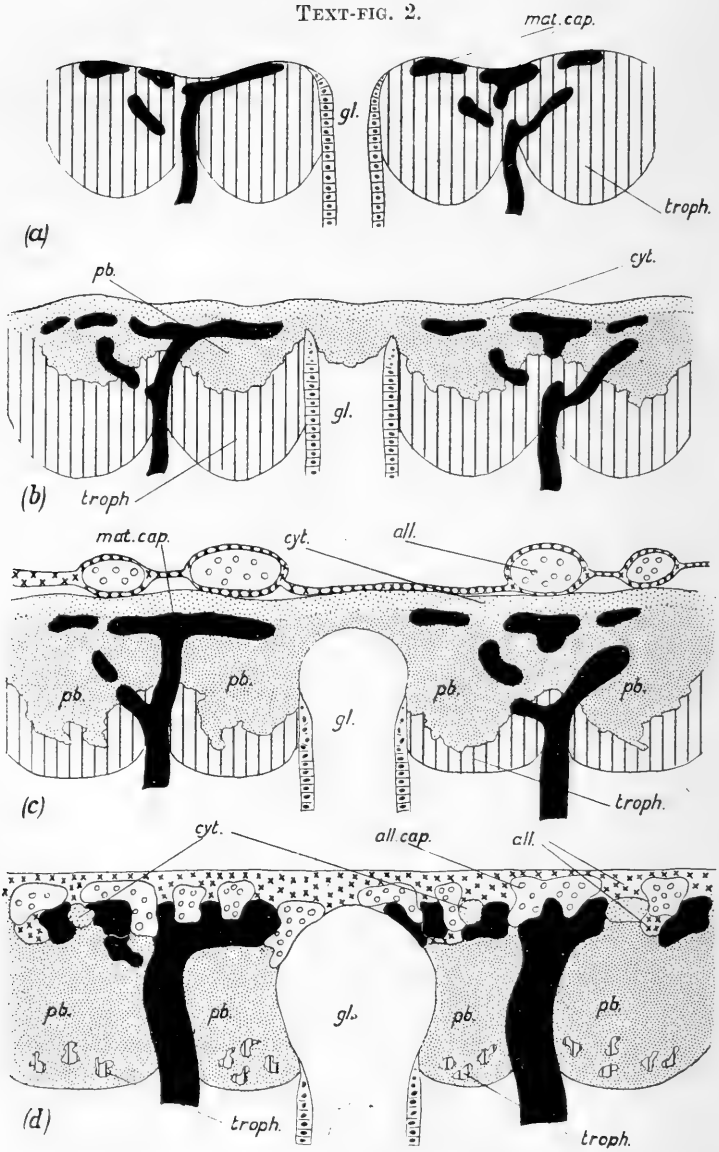
(*b*) The chorionic ectoderm develops directly into two portions, (1) a basal cellular cytoblast which by proliferation gives rise to (2) a plasmodiblast.

(*c*) The plasmodiblast phagocytically attacks the maternal tissues, particularly the maternal nuclear aggregations or nests.

(*d*) The proliferating foetal ectoderm and maternal syncytium are thus intimately fused to form a structure, the diploplasma.

(*e*) Sooner or later most of the cytoblast layer disappears, being converted into plasmodiblast.

TEXT-FIG. 2.



Diagrams showing the development of the allantoic placenta in Perameles.

(*a*) Shows the character of the preplacental maternal trophospongia with its lobules, between which vessels and glands penetrate. The capillaries ramify at the surface.

(*b*) Chorionic attachment has now taken place and the chorion has proli-

(*f*) The outgrowth of foetal plasmodium does not extend any farther than to involve the proliferated maternal epithelium.

(*g*) The attachment of the allantois is effected when the embryo has attained a length of approximately 6·5 mm.

(*h*) The outward migration of the basal cytoblast cells (when converted into plasmodiblast) gives opportunity for the maternal and foetal vessels to come into intimate apposition.

(*i*) In the final stage the foetal nuclei in the placenta are found to be in a state of degeneration.

(*j*) Remains of the maternal epithelium still probably exist in the full-term placenta.

(*k*) The uterine glands persist throughout gestation, but the portion of their epithelium within the diploplasma disappears.

(*l*) All maternal vessels have definite endothelial walls: hypertrophy of the endothelial cells does not occur and lacunae are not formed.

(*m*) An allantoic placenta is recorded for P. gunni.

2. Yolk-sac Placenta.—A virtual yolk-sac placenta is present in P. gunni as in other species of Perameles, brought about by the intimate apposition of the complex system of vessels in the vascular omphalopleure with the highly vascular portion of the uterine syncytium just beyond the placental area.

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ferated to form a basal cytoblast and an external plasmodiblast. The proliferations of the latter are related to glands and nuclear nests. The capillaries ramify just below the cytoblast layer.

(*c*) Allantoic attachment is now in its first stages. Further proliferation of the foetal trophoblast has taken place and the plasmodiblast now occupies the greater part of each nest. Within the diploplasma the gland epithelium has disappeared and the gland is closed in above by cytoblast plus plasmodiblast. Capillaries still ramify below the cytoblast.

(*d*) The foetal plasmodium now occupies the whole of each nest with the exception of (possibly) a few remains of maternal tissue. The cytoblast layer has broken down, having mostly been transformed into plasmodiblast. A few islands of trophoblastic tissue are, however, still left in the cytoblast position. The very much branched and interlocked foetal and maternal vessels have now come into intimate apposition. The gland epithelium in the diploplasma has entirely disappeared, and the glands are closed in above by all antoic vessels alone.

all., allantois; *all. cap.*, allantoic capillary; *cyt.*, cytoblast; *gl.*, gland; *mat.cap.*, maternal capillary; *pb.*, plasmodiblast; *troph.*, trophospongia.

Further, considerable vacuolation of the cells of the bilaminar omphalopleure at certain stages mark it as being an absorptive organ of some importance in embryotrophic processes.

7. Concluding Remarks.

Now that we have some clear idea of placentation in Perameles, the question arises what relation exists between this and the placentation of other marsupials and of Eutheria generally? Further, can any light be thrown on the phylogeny of the mammalian allanto-placenta by a consideration of this question?

Before, however, entering on a discussion of these, it is, I think, necessary to get a clear idea of the morpho-physiological conception of the term 'Placenta'.

(*a*) The Placental Conception.

It is inevitable that the conception of placentation which has arisen in the minds of investigators should, until fairly recently, have been associated with the very complex and highly advanced structure developed in the intimate fusion or apposition of the foetal membranes of the commoner and best-known Eutheria with the uterine mucosa.

It might, however, be taken for granted that—in the case of an organ so prominent in mammalian developmental processes and rightly regarded ontogenetically and phylogenetically as of the highest importance—there would be no two opinions as to its definitive structure or its physiological significance. But such a supposition would be wrong, and a very superficial examination of the writings of the more recent investigators soon shows that they hold radically different views as to what is understood by the term 'placenta'.

All the important recent works treating of the comparative anatomy of the placenta to which I am able to refer (Strahl, 1905; Grosser, 1910; Jenkinson, 1913) are insistent that the fundamental idea of placental formation lies in the apposing of two blood-streams—one foetal, one maternal—to form

a structure by which the physiological processes intended for the well-being of the embryo can be carried out.

Such a conception makes no allowance whatever for the work of the bilaminar omphalopleure of marsupials—itself non-vascular but physiologically of considerable importance.

Even less acceptable is the suggestion of Professor Hubrecht (1909), who insists that 'fusion of embryonic with maternal tissue is a conditio sine qua non, and so we must admit a placenta in the case of Didelphia (Perameles) and deny it to certain Monodelphia (Equus, Sus, Nyticebus, Galago, and others)'. As Assheton has pointed out, under this scheme 'the sheep is a placental, a cow a non-placental mammal'.

Reaction between mother and embryo, or rather dependence of the latter on the mother for food, oxygen, and the removal of its waste products, may be said to commence from the time of the first appearance of the ovum in the uterus. 'The mammalian ovum,' says Hill (1910, p. 113), 'already in the monotremes greatly reduced in size as compared with that of reptiles, and quite minute in the Metatheria and Eutheria, contains within itself neither the cubic capacity nor the food material necessary for the production of an embryo on the ancestral reptilian lines. We accordingly find that the primary object of the first developmental processes in the mammals has come to be the formation of a vesicle with a complete cellular wall capable of absorbing nutrient fluid from the maternal uterus.'

Our knowledge of the physiology of the early stages of mammalian intra-uterine development is admittedly as yet very incomplete. Nevertheless, it is quite certain that the amount of nutrient material present in the ovum is absolutely insufficient for even the most elementary developmental processes, and has to be supplemented, from the very beginning, from outside sources.

Even a cursory consideration of the above will serve to indicate that in the uterine development of the viviparous mammals there occur two distinct phases, differing entirely in the means by which the necessary physiological processes

of the embryo are arranged for. In the first of these, extending over the time of cleavage and of blastocyst formation, absorption and exchange are performed solely by means of the trophoblast.

When blood-vessels appear and are functional, quite a new phase is inaugurated, lasting to the end of pregnancy, during which these vessels come to the aid of the trophoblastic layer in more quickly and efficiently performing the necessary embryonic services.

Grosser's (1910, p. 94) terms 'embryotrophic' and 'haemotrophic' could have been conveniently employed to indicate these periods, but, unfortunately, his use of the word 'exclusively' in the definition of the latter term has made it applicable alone to haemochorial placentae, and it is even doubtful if the definition would be strictly correct in their case.

Nor does Resink's (1902) arrangement suit the case any better. This author regarded the intra-uterine life of the hedgehog as falling into two well-defined periods as follows:

(*a*) Preplacental period, during which maternal and foetal preparation for the allantoic placentation takes place; the trophospongia and ectoplacenta are formed and the embryo fixed. Broadly, this period may be said to occupy the earlier portion of intra-uterine existence up to the time of attachment of the allantois.

(*b*) Euplacental period, in which the allantoic attachment is made and the placenta completed.

The weakness of this arrangement as applied to mammals generally is to be found, in my opinion, in the extreme importance given to the allantoic placentation and the inclusion of the yolk-sac (vascular) placentation in the first of these periods. Such a scheme becomes difficult of application, particularly to the Metatheria, in which an allantoic placenta occurs, so far as is known, in but one genus, various methods of trophoblastic attachment in others, in many no attachment whatever, a yolk-sac placenta in all.

It is therefore apparent that allantoic placentation is only of the greatest importance in one group of mammals, the

Eutheria, and it is due to the fact that the most detailed investigation has been expended on this group and to the prominence of the allantoic placenta in it that other features of embryonal intra-uterine life have been for so long overlooked.

Viewed in the light of what we already know of the morphology and physiology of the foetal membranes in the two groups of viviparous mammals, it is evident that placentation, as generally understood, is but part of a much larger conception which has to do with the whole physiological intimacy, during intra-uterine life, between foetus and mother.

Thus I am fully in accord with Assheton's suggestion (1909) that the term 'placenta' should be applied to all organs consisting of an intimate apposition or fusion of the foetal membranes with the uterine wall for the purpose of carrying out physiological processes destined for the well-being of the embryo.

Such a conception would include the following types of placenta:

(*a*) That in which the trophoblast is vascularized from the allantois—allantoplacenta.

(*b*) That in which the trophoblast is vascularized from the yolk-sac—omphaloplacenta.

(*c*) That in which no foetal blood-vessels are concerned. This is the case of the bilaminar omphalopleure of marsupials whether there is a fusion of part of this with the uterine mucosa (Dasyurus, Phascolarctos), or merely, as is more usual, intimate apposition. For this type of placenta I propose the term 'metrioplacenta'. These may be illustrated by referring to Perameles, the genus which is the subject of investigation in the present paper.

(*b*) Placental Phases in Perameles.

Preliminary Phase.—During this period the blastocyst is formed and the physiological processes are carried on by means of the trophoblastic cells. There is no union in

Perameles between the trophoblast and the uterine wall, and there is no absorption by means of foetal blood-vessels.

The work of this phase is carried on in later intra-uterine life by the bilaminar omphalopleure.

Intermediate Phase.—This is the stage of the vascular yolk-sac placenta. It comes into being with the functional formation of the vascular area. There is a close apposition between the foetal and maternal blood-vessels. This phase reaches its most active condition before the attachment of the allantois, and although, maybe, less efficient, endures, with the existence of the vascular area, until the end of pregnancy.

Final Phase.—The allantoic attachment takes place and the allantoic placenta is completed.

It is evident that in marsupials, with the exception of Perameles, the preliminary and intermediate stages are the more important, in fact the only ones present, while in general, in Eutheria, the preliminary and the final phases are of the greater value.

Under these circumstances we can denote the placental periods in Perameles as metricoplacental, omphaloplacental, or allantoplacental, according to the type of placenta which is the dominant one for the period concerned.

(*c*) Placental Phenomena in Marsupials generally.

In reviewing these I will commence with the most specialized groups.

Macropodidae.—The works of Owen (1834–7, Macropus major), Semon (1894, Aeprymnus rufescens), and Hill (1895, M. parma, M. ruficollis, M. robustus, and M. major) emphatically show that in these forms the allantois throughout life remains small, buried in the splanchnocoele. From my own observations I am able to state that this is also the case for Potorous tridactylus and Bettongia cuniculus. It is possible that in some Macropods the allantois reaches the chorion, although Cald-

well's statement (1884) that there is such a union in the case of Halmaturus ruficollis, as well as his testimony of a fusion between the bilaminar omphalopleure and the uterine wall, have not yet been confirmed. An omphaloplacenta is well developed in Macropods.

Phalangeridae.—In Trichosurus vulpecula (Hill, 1889) and Petaurus sciureus (Semon, 1894) the allantois is similar to that of Macropods. I am also able to state that this is the case for Pseudochirus cooki. Here again the embryo depends on the work of the trophoblast both of the vascular omphalopleure and of the bilaminar omphalopleure.

Phascolarctus.—This genus is particularly interesting in possessing, according to Caldwell (1883) and Semon (1894), a respiratory allantois. There is a well-developed omphaloplacenta and also a union in the metrioplacenta between an annular zone of the bilaminar omphalopleure (just outside the simus terminalis) and the uterine mucosa.

Didelphys.—The allantois does not meet the chorion. The omphaloplacenta is well developed. Certain portions of the ectoderm of the bilaminar omphalopleure are stated by Selenka to form absorptive proliferations similar to those found in certain Eutheria, for example Manis (Weber), and Equus (Ewart).

Dasyurus.—The allantois shows interesting stages in degeneration. At a particular stage it becomes applied to the chorion which is itself in intimate association with the uterine mucosa. Later the allantois withdraws from the chorion and degenerates considerably, its vascular system practically disappearing. An omphaloplacenta is present as well as a similar annular fusion of the bilaminar omphalopleure with the uterine wall as occurs in Phascolarctus.

Perameles.—This is a most primitive form possessing a well-developed allantoic placenta and an omphaloplacenta, and there is considerable evidence of absorption in the bilaminar omphalopleure.

From the above abstract it will be seen that we can, as yet, hardly be said to have a detailed knowledge of the structure,

physiology, and ontogeny of the foetal membranes of most marsupials. Particularly in such primitive genera as Thylacinus and Sarcophilus, it may be expected that investigation will help to shed a clear light on the phylogeny of the placenta in this group.

Another point of importance (of greater value, I think, than Assheton would have had us believe) lies in the behaviour of the uterine mucosa. Of this our knowledge in the marsupials is particularly meagre. Yet it is extremely important, since there is naturally a mutual reaction of embryo and uterus. An investigation of the modifications of the uterine mucosa during pregnancy would, there is not the slightest doubt, be of great value in shedding a light on ancestral placental arrangements in marsupials.

Pseudochirus cooki is instructive in this regard. Preliminary investigations which I have already made in the case of this diprotodent marsupial have shown that the uterine epithelium in a very early stage of pregnancy consists of a single layer of very high columnar cells with correspondingly elongated deeply-staining nuclei. Below the epithelium the connective tissue is condensed to form a layer in which run the capillaries. This stage can be recognized as being very similar to one occurring in many Eutheria.

At a later stage of gestation, cell outlines have disappeared and a vascular syncytium is formed similar to that of Perameles, except that it is composed apparently not only of the epithelial cells but of those of the sub-epithelial capillary layer. These capillaries now ramify at the surface as is the case in Perameles.

Here without doubt can be recognized the remains of an ancestral trophospongial proliferation.

From the consideration of the above facts, particularly as regards the condition of the foetal membranes in Perameles, Dasyurus, and Phascolarctus, bearing in mind the complementary modifications of the uterine wall where they are known, it must be evident that these conditions in marsupials represent a degeneration from a more complex system

of placentation which undoubtedly obtained in the original protoplacental group.

Into a full treatment of this there is no need for me to enter. It has been ably discussed by Hill and the facts and conclusions embodied in the preceding pages can only be regarded as confirming and strengthening his expressed opinions.

(*d*) The Relation of the Allantoic Placentation of Perameles to that of the Eutheria.

This question I will discuss but briefly, reserving its full treatment for some future occasion when adequately fixed and preserved late gestation stages of Perameles may perhaps be available.

It is with some pleasure that I have been able to bring the method of allantoplacental formation of Perameles into line with that occurring in the simpler Eutherian forms. In fact it may be said in general that the only difference between the two is one of degree. There are the same characteristics of passivity of the uterine epithelium and activity of the trophoblast with a division of the latter into a cytoblastic and plasmodial layers. After preliminary diploplasmatic preparation the allantois becomes fixed and an apposition of the two blood-streams becomes effected. I might here briefly refer to the resemblances between the earlier stages of allantoplacentation in Perameles and the dog and rabbit. In Text-fig. 3 I have indicated the main points of Schoenfeld's fig. 14 (1903) representing an early stage of chorionic invasion in the dog. A somewhat comparable stage in Perameles is represented by Text-fig. 4. The agreement in the method of foetal invasion is evident. In the dog, however, according to Schoenfeld, the uterine epithelium does not form a syncytium.

In the rabbit, on the other hand, as in many other Eutheria, such a maternal syncytium is formed, and here the early stages show an even more significant resemblance to those occurring in Perameles. Particularly I may refer to Schoenfeld's (1903) figs. 4, 5, and 6, Pl. xxi, and those of Maximow (1900, figs. 1 and 2, Pl. xxx).

The phylogenetic importance of the presence of large multinucleate masses of foetal origin in the allantoplacenta of Perameles, the dog, the rabbit, and others cannot be overestimated.

Bearing in mind the accepted origin of the Metatheria

Text-fig. 3.

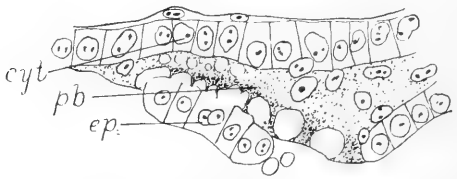

Fig. 3.—An early stage of development of the dog showing chorionic attachment (after Schoenfeld). *cyt.*, cytoblast; *pb.*, plasmodiblast; *ep.*, uterine epithelium.

Text-fig. 4.

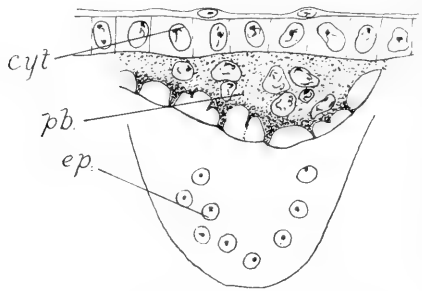

Fig. 4.—A stage in Perameles comparable with that of the dog in Text-fig. 3. Lettering as in Text-fig. 3.

and Eutheria from a primitive diphyodont protoplacental stock (Hill, 1897, p. 432), it is possible to state with certainty that, in that early group, the same conditions of passivity of the uterine epithelium and active phagocytic quality of the trophoblast were already in existence; with the further differentiation of the latter layer in its placental portion into two distinct layers, respectively cellular and plasmodial.

The foregoing facts make this conclusion inevitable. This being so, those Eutheria, particularly the Ungulata,

in which there is no union between the trophoblast and mucosa in the allantoic placental region, must have reached this condition, in the course of their phylogeny, by a process of secondary simplification.

It is evident enough then that the attempt made by Strahl (1906) to group Perameles with the Ungulates and others in the Semiplacenta breaks down. At the same time it is not easy to suggest any arrangement by which Perameles will take its proper place in placental classification.

The only possible course at this stage appears to be to examine briefly what is the relation of the allantoic placentation in Perameles to some one or other of the groupings at present in use.

Assheton's suggestion to divide the Placentalia into Placentalia cumulata and plicata seems to be the most promising, since in addition to having a structural basis these divisions are to some extent physiological. Assheton has given a table of the characteristics of the two groups as he conceived them (1909), and even a cursory glance at these will indicate that the allantoplacenta of Perameles structurally and physiologically occupies a place somewhere between the two but more primitive than either.

Thus in the 'heaping up' of the trophoblast—a very fundamental point—it agrees with the cumulate type, while in many other features, absence of lacunae and the mildness of attack on maternal tissues, it approaches the plicate type. The secretion of the placental glands appears to be at first of minor importance in Perameles, but, later, absorption by the allantoic vessels is direct, increasing the value to the embryo of glandular secretion tremendously.

It is apparent that if we regard the above grouping as a rational one, the allantoic placenta can be regarded as being of a central primitive type from which development in either direction might easily have proceeded.

And the very real relation of the allantoplacentation in Perameles to that of the Carnivora—the relation of the simple to the slightly more complex—shows that the Carni-

vora, as suggested by Hubrecht and upheld by Assheton, exhibit, of the Eutheria, the most undifferentiated arrangement. The trophoblastic 'heaping up' is common to the allantoplacenta of both Perameles and the Carnivora. This is fundamental, and is sufficient evidence of the fact that the cumulative type of placenta is the more primitive.

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9. DESCRIPTION OF FIGURES.

The outlines of all figures have been drawn with the aid of Zeiss's camera lucida, then enlarged by means of the pantograph and details filled in by freehand.

List of Reference Letters.

all., allantois. *all.cap.*, allantoic capillary. *all.ent.*, allantoic entoderm. *all.mes.*, allantoic mesoderm. *all.pen.*, penetration of the allantoic mesoderm. *all.st.*, allantoic stalk. *all.ves.*, allantoic vesicle. *amn.*, amnion. *ana.*, anastomosis of the two portions of the sinus terminalis. *bil.omph.*, bilaminar omphalopleure. *cav.*, cavity contained in syncytial nests. *ch.mes.*, chorionic mesoderm. *ch.ect.*, chorionic ectoderm. *coel.w.*, coelomic wall of the allantoic vesicle. *cyt.*, cytoblast (cytotrophoblast). *emb.*, embryo. *ex.coel.*, extra-embryonal coelome. *ex.syn.*, maternal syncytium of the extra placental area. *ex.syn.n.*, syncytial nuclei of the extra-placental area. *g.c.*, multinucleate giant cell. *gl.*, gland. *gl.ep.*, gland epithelium. *inf.*, infiltrated material (? lymphatic) contained in the cavity of a syncytial nest. *ing.*, material ingested by the plasmodiblast. *leuc.*, leucocytes. *mat.cap.*, maternal capillary. *m.ch.*, marginal chorion. *muc.*, uterine mucosa. *musc.*, muscularis. *pb.*, plasmodiblast. *pb.n.*, plasmodiblast nuclei. *pgm.*, aggregations of pigment in the plasmodiblast. *plac.*, allantoic placenta. *pl.syn.*, maternal syncytium of the placental area. *proa.*, proamnion. *syn.n.*, nuclei of the maternal trophospongia. *troph.*, maternal trophospongia. *vasc.omph.*, vascular omphalopleure. *v.omph.*, vascular omphalopleure. *vit.a.*, vitelline artery. *vit.v.*, vitelline vein. *yk.cav.*, yolk-sac cavity. *y.s.*, yolk-sac. *yl.spl.*, yolk-sac splanchnopleure.

EXPLANATION OF PLATES 9–11.

Plate 9.

Figs. 1–8, 10–12, Perameles obesula, 6·1 mm. Fig. 9, Perameles obesula, 12·5 mm.

Figs. 1, 2, and 3.—Sections showing the arrangement of syncytial nuclei as a more or less irregular layer round a central cavity. Note the vesicular shape and chromatic characteristics of these nuclei. Note also intruding leucocytes.

Figs. 4, 5, and 6.—Phases in the earliest growth of the plasmodiblast. In fig. 4 the syncytial nuclei are already undergoing degeneration under the effect of the plasmodial advance processes.

Fig. 7.—Plasmodial attack on a gland. Note the breaking down of the gland epithelium on one side. Here also ingested material is evident in the plasmodiblast.

Fig. 8.—Another stage in plasmodiblast formation. The cytoblast is a well-defined layer. Note presence of leucocytes.

Fig. 9.—Shows the vesicular and degenerate appearance of foetal nuclei at this stage with close apposition of maternal and foetal vessels.

Figs. 10, 11, and 12.—Photomicrographs of sections through a branched gland just outside the area of the first fixation of the chorion (see Text-fig. 1).

Plate 10.

Figs. 13–16, 19, Perameles gunni, 6·6 mm. Figs. 17, 18, Perameles obesula, 12·5 mm. Figs. 20, 21, Perameles obesula, 7 mm.

Fig. 13.—Placental area towards the centre, showing the commencement of the disorganization of the cytoblast allowing maternal capillaries to approach the surface. The very superficial position of one of these capillaries is, however, very exceptional for this stage. Note the penetration of the plasmodiblast nuclei into the syncytial nests.

Fig. 14.—Section of somewhat more peripheral portion of the same area. A distinct cytoblast is present and a plasmodiblast in which giant cells and ingested material are outstanding features. This figure should be compared with Maximov's fig. 1 of the rabbit.

Figs. 15, 16, and 17.—Show attachment of allantois and disorganization of cytoblast to allow of the apposition of foetal and maternal blood-vessels.

Figs. 17 *a*, *b*, *c*, *d*, *e*.—Stages in the degeneration of a foetal nucleus.

Fig. 18.—Section of the central portion of the placenta showing the following: breaking down of cytoblast, almost complete filling of syncytial nests by foetal nuclei, penetration of allantoic capillaries and almost final apposition of maternal and foetal blood-streams.

Fig. 19.—Placental area showing section through a gland. Note disappearance of gland epithelium, the enlargement of the gland lumen at the apex and the tendency to form lateral slits in the plasmodium. This section shows well the intruding leucocytes of the large mononucleate type. The plasmodiblast shows a particularly noteworthy giant cell, pigment patches, and ingested material.

Fig. 20.—Section of placental area towards the margin. Cytoblast is intact. The nests are well filled with plasmodiblast nuclei.

Fig. 21.—Section of placental area at the margin. For description see text.

Plate 11.

Figs. 22–27, Perameles gunni, 6·6 mm. Fig. 28, Perameles obesula, 7 mm. Fig. 29, Perameles obesula, 6·1 mm.

Fig. 22.—Section showing structure of the bilaminar omphalopleure. Note the extreme vacuolation of the ectoderm cells.

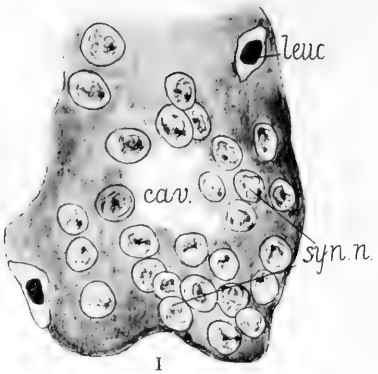
Fig. 23.—The yolk-sac circulation. For description see text.

Fig. 24.—Diagram showing the relation of the embryo to its membranes.

Figs. 25, 26, and 27.—Pigment-bearing cells: fig. 25 of the serosa, fig. 26 from the connective tissue, and fig. 27 from the trophoblast.

Fig. 28.—Section through the mouth of a gland.

Fig. 29.—Shows the relation of a plasmodial proliferation to a group of syncytial nuclei.



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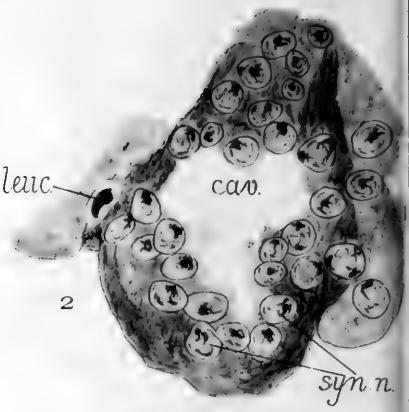

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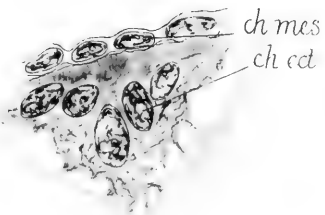

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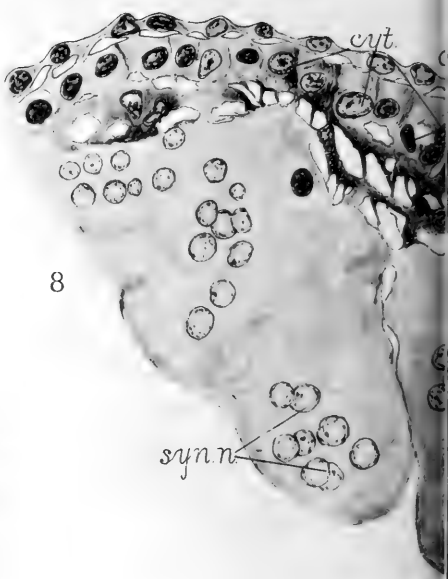

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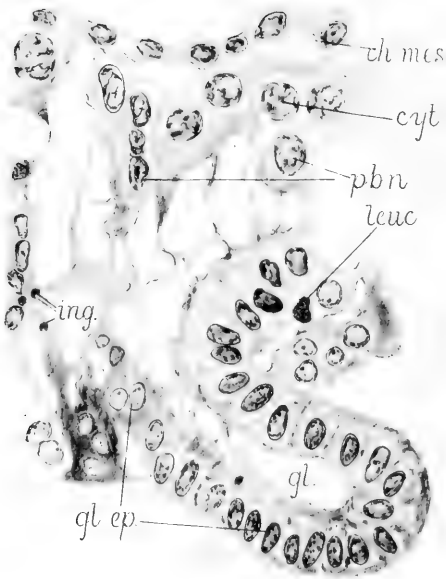

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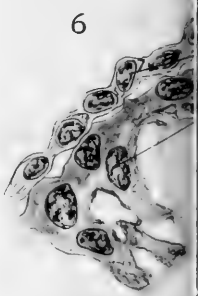

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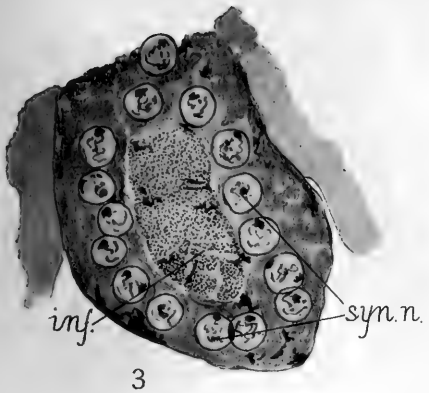
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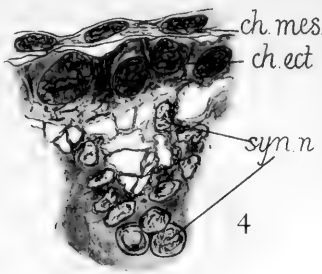
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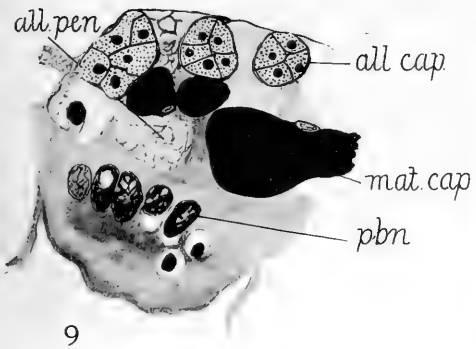
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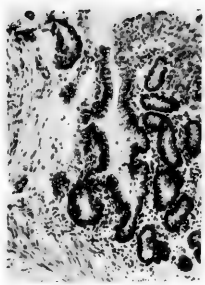

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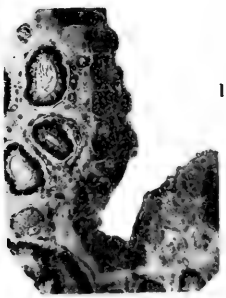

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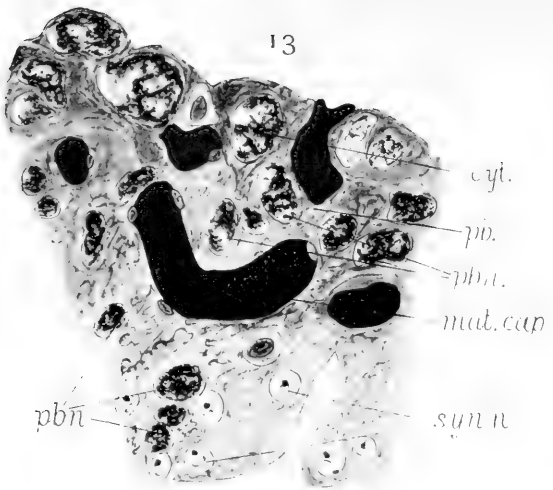
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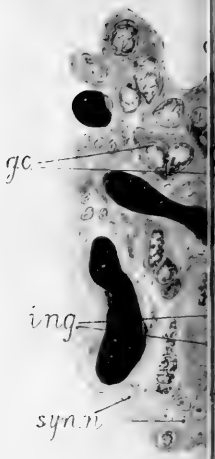
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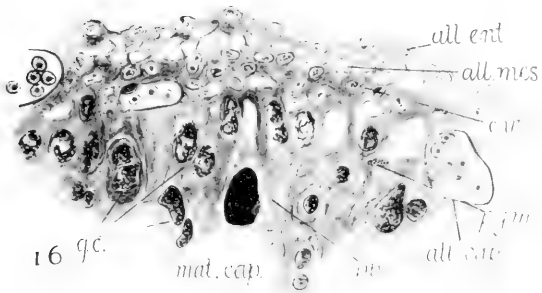
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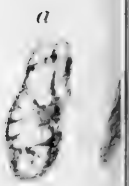
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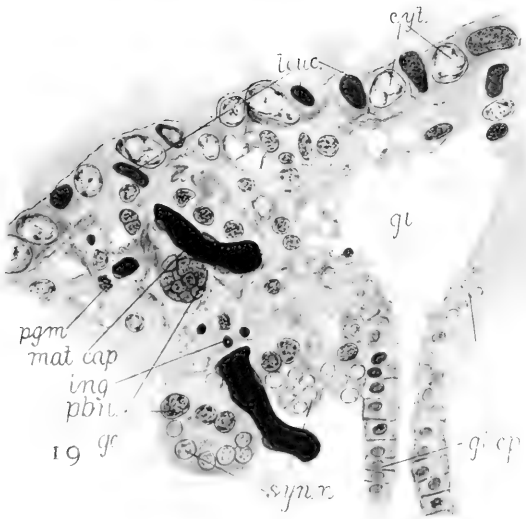
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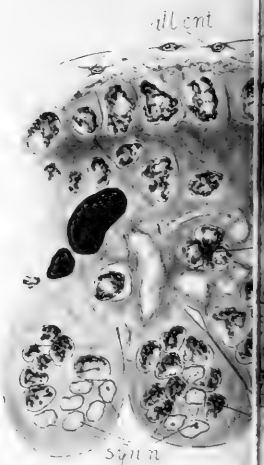
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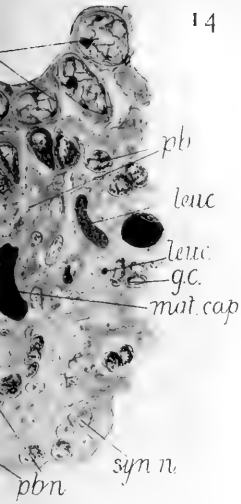

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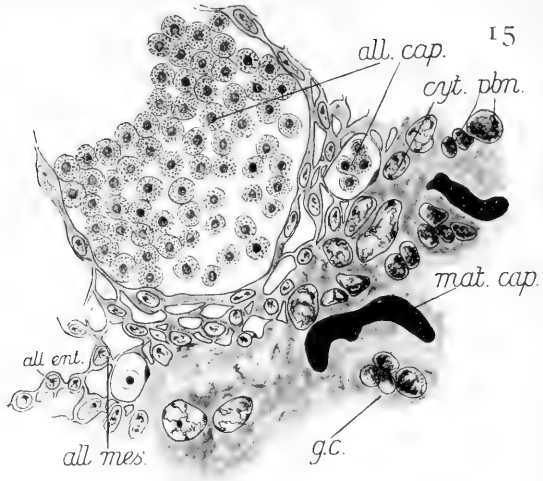

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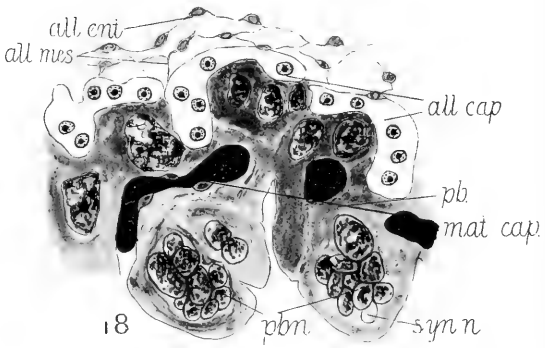

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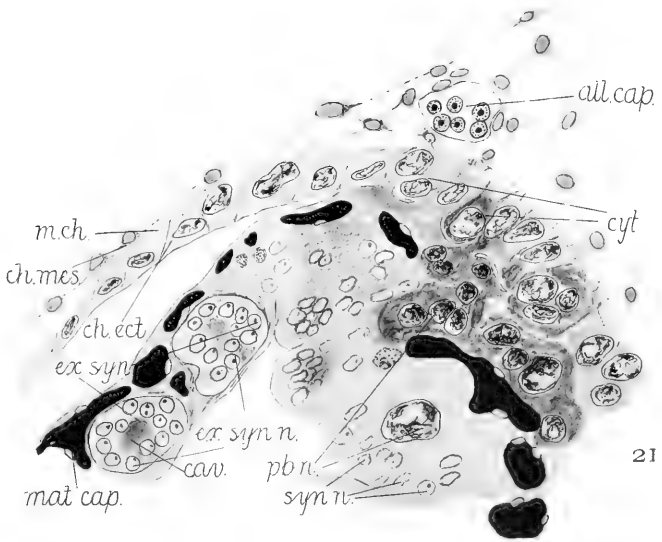

21

The Male Meiotic Phase in two Genera of Marsupials (Macropus and Petauroides).

By

W. E. Agar, F.R.S.,

Professor of Zoology in the University of Melbourne.

With Plates 12–14.

A VERY slight experience of cytological research is sufficient to impress the worker in this field with the different facilities for accurate research afforded by different organisms, and also with the importance of discovering the most favourable objects for such research. Consequently I have been making a cytological survey of such groups of Australian animals as seemed most likely to afford useful cytological material, paying at first particular attention to the Marsupials, for two reasons. The first is the well-known technical difficulties presented by the Eutherian mammals on account of the usually rather large number and tendency to clump of their chromosomes, and the second is that Jordan's work on the American opossum (1911) showed that in this marsupial the number of chromosomes is comparatively small. Jordan determined the number as seventeen (male), but Painter (1922) has raised it to twenty-two. Up to the present we have made in this laboratory a preliminary survey of some fourteen species of Marsupials, and this paper presents an account of the more important features of two of these which have been worked out in more detail.

At present the most interesting feature of this work is undoubtedly the determination of the conditions of the sex chromosomes, a problem which has always presented in mammals considerable elements of dubiety. Of primary importance is probably the confirmation of the occurrence of

Y-chromosomes in the Mammalia—first clearly established by Painter (1922) in Didelphys. In Macropus this element is very minute, and, though I had shown it in several figures, I failed to interpret it properly until we received Painter's paper, which arrived at a moment when Mr. Greenwood (whose results are to be published in this journal) was paying attention to a very minute chromatic body occurring in several other species of Marsupials at which he was working. It immediately became apparent that this body was the Y-chromosome. Comparison with his work, and with Painter's description of Didelphys, quickly established that the small body already observed in Macropus is also a Y-chromosome.

It is a pleasure to acknowledge my indebtedness to several people for assistance in obtaining this material. Dr. T. L. Bancroft, of Eidsvold, Queensland, has sent me in the last two years a great number of specially preserved testes of Marsupials, Monotremes, Ceratodus, and many other Australian animals. For living material utilized in the present paper I have to thank Dr. Colin MacKenzie, Director of the Australian Institute of Anatomical Research, and Mr. W. H. D. Le Souef, of the Zoological Gardens, Melbourne.

Material and Methods.

The two species dealt with in the present communication are Macropus ualabatus and Petauroides volans. These genera belong respectively to the Diprotodont families Macropodidae and Phalangeridae.

The material was mostly preserved in Flemming, Bouin, and Allen's modification of Bouin (1913, 1915). I have found the latter method excellent, especially when followed, as Allen recommends, by anilin and bergamot oils in place of the higher alcohols and xylol. For general purposes I have found this method the best I have yet tried for mammals.

The standard stain used was Heidenhain's iron haematoxylin, though safranin, methyl green, and acid fuchsin, and others, were used as controls.

As in former work, I have found thick sections much more

valuable than the thin ones usually used by cytologists. Most of the work has been done with sections of 15–20 μ in thickness, mounted between two coverslips instead of in the usual way between a slide and a coverslip. This allows any nucleus to be examined from both sides. For convenience of examination the lower coverslip is temporarily attached to a microscope slide by a drop of immersion oil.

A. Macropus.

Number of Chromosomes.—This animal is remarkable among mammals for its small number of chromosomes. The chromosome formula is of the type first clearly established for mammals in the case of Didelphys (Painter, 1922), the males being of the XY type. The diploid chromosome number in the male is 10+XY, or twelve, and in the meiotic division there are five autosome bivalents and the XY bivalent. This very small number allows of great certainty in counting the chromosomes, Macropus being obviously far more favourable for this purpose than any other known mammalian genus.

Although counts of the meiotic chromosomes leave no doubt that the number is as just stated, it is only rarely that there are twelve separate chromosomes in the spermatogonial mitoses. As a rule there are quite indubitably only eleven (Pl. 12, fig. 2), of which one, usually occupying the centre of the ring, is very minute. This is the Y-chromosome. In a small percentage of cases, however, there are equally plainly twelve (Pl. 12, fig. 3), the extra one being smaller than any of the others except Y. This is obviously the X-chromosome, for the meiotic phase shows that X is much smaller than the autosomes. In the 11-chromosome spermatogonial mitoses X is presumably attached to one of the autosomes, though I have not been able to identify with certainty the chromosome to which it is joined. Owing to its small size it would not add much to the length of one of the longer chromosomes. I have frequently found a constriction near the end of one of the longer chromosomes, but in view of the widespread tendency of chromosomes to develop such constrictions it would be unjustifi-

able to assume that this represents the point of attachment of the X-chromosome.

As will be described in more detail below, similar conditions are found in the meiotic division. The XY bivalent is possibly sometimes independent, but more often it is attached to one of the autosomes. In this phase, however, the XY is easily identifiable even when attached to an autosome.

In the fact of its usual attachment to an autosome but occasional independence, both in spermatogonial and meiotic mitoses, the X-chromosome in Macropus resembles that of Ascaris megalocephala (Edwards, 1910). In many male Orthoptera also the X-chromosome is temporarily or permanently united to an autosome (McClung, 1905; Wilson, 1911).

In the female (Graafian follicle cells) the small Y-chromosome, so characteristic of the spermatogonia, is not present. I have never been able to find more than ten separate chromosomes, and here, as in the male, the small number of chromosomes makes it easy to find a large number of dividing nuclei in which every chromosome is distinct (Pl. 12, figs. 4, 5). Since there is no Y, and since in the male, X is generally attached to an autosome, it is quite safe to interpret the ten chromosomes of the female as 10+XX, and the two X's attached to autosomes. The condition here is again comparable to that found in A. megalocephala, where Frolowa (1912) found that the two X-chromosomes are generally attached to autosomes in the female.

The Meiotic Phase.—The spermatogonial nuclei (Pl. 12, fig. 1) contain a very scanty chromatic reticulum and a large central nucleolus. This is apparently a plasmosome impregnated with chromatin, for it stains densely with iron haematoxylin, but in well-balanced methyl green and acid fuchsin preparations it takes up the fuchsin. In the early prophase of the spermatogonial mitoses this nucleolus loses its chromatic staining reaction even with iron haematoxylin, and becomes a typical plasmosome. This nucleolus is the only compact body in the resting spermatogonial nucleus, so unless they are somehow

incorporated with the plasmosome, it is clear that the sex chromosomes at this stage are in a diffused condition like the autosomes.

The earliest stages of the primary spermatocytes which are distinguishable from the spermatogonia are early leptotene stages—a rather later leptotene nucleus being shown in fig. 6. The large nucleolus is shown by its staining reaction to be a plasmosome, and is still the only compact body in the nucleus. The X-chromosome is therefore at this stage in the leptotene condition like the autosomes. The same presumably applies to the Y-chromosome, though this is too small to permit of definite statement.

The synizetic contraction, though unmistakable, is not very pronounced (Pl. 12, fig. 7).

The process of syndesis is difficult to follow in this animal. It begins about the stage shown in fig. 6, and is completed by the stage illustrated in fig. 7, which is a pachytene nucleus. About the stage of fig. 6 frequent duplicity and parallelism of threads can be observed, from which parasyndesis may be inferred. The direct evidence for this mode of syndesis is, however, certainly not strong in this species, but the indirect evidence is very convincing. Firstly, this mode of syndesis can be observed in Petauroides, and the general course of meiosis is so similar in the two genera that it is incredible that the mode of syndesis should not be the same. Secondly, as we shall see below, there is no doubt that the components of the definitive bivalents in Macropus are derived from the pachytene threads by the longitudinal splitting of these, and not by their doubling over. That being so, it follows that the mode of syndesis must have been by longitudinal fusion, unless one of the most fundamental hypotheses of modern cytology—that is to say the individuality of the chromosomes—is false.

In the early pachytene nuclei (Pl. 12, fig. 7), as in still earlier stages, the sex chromosomes are not visibly different from the other chromosomes. As the pachytene threads begin to contract, however, X soon becomes visible by reason of its much more rapid condensation, so that it soon comes to form

a compact rounded mass in sharp contradistinction to the still thread-like autosomes (Pl. 12, figs. 8–10). From its first appearance onwards it is attached to the end of one of the autosomes. At first it is not possible to identify the minute Y, but later, as the autosomes lose in staining capacity, Y becomes conspicuous by reason of its denser stain. At first it is distinct from X, but they soon fuse to form a bivalent (Pl. 12, fig. 9).

A large pale plasmosome makes its appearance at the time that the sex chromosomes are uniting and in close contact with them. The nature of this plasmosome, and its relation to the plasmosome of the earlier stages, is discussed below.

In the late pachytene stage (Pl. 12, figs. 9–10) the staining capacity of the autosomes becomes greatly diminished, and their outlines become somewhat blurred by the development of outgrowths and anastomoses between the different chromosomes. The compact XY bivalent is now very conspicuous, owing to the fact that the general decrease in staining capacity does not affect it nearly so much as the autosomes.

Fig. 11 represents the diplotene stage. This is perhaps chiefly interesting on account of the confidence with which its mode of derivation from the pachytene stage can be determined. In many late pachytene nuclei, such as about the stage shown in fig. 10, the five pachytene bands can be counted with ease and certainty, and one can follow step by step in great detail the conversion of each of these bands into one of the diplotene bivalents by the appearance and gradual widening of a longitudinal split down its middle. The three stages figured (Pl. 12, figs. 10, 11, 12) will, however, probably be enough to carry conviction that the gemini of the diplotene nucleus are derived from the pachytene bands in this way, and not by their doubling over as is required by the theory of telosyndesis.

Fig. 13 shows a stage in the contraction of the diplotene loops into the definitive bivalents. The nuclear membrane has by now disappeared. The great increase in bulk of the chromosomes which has taken place between the stages shown in figs. 11 or 12 and that shown in fig. 13 is remarkable. A

rough estimate of the relative volumes of the total chromatin content at these two stages made by means of plasticene models showed that the volume of the chromatin is more than twice as great in the later as in the earlier stage.

The XY bivalent is visible as before, attached to an autosome. It is now, however, seen to be attached to one limb only of the bivalent.

Figs. 14–16 (Pl. 13) represent metaphases of the first meiotic division, to show the relations of the sex chromosome. In fig. 14 they form a compact body attached to one end of one of the autosome bivalents. In fig. 15 they are similarly attached, but somewhat drawn out towards the equator of the spindle. At this stage no distinction between X and Y is visible, but as the metaphase progresses the two components begin to separate, as shown in figs. 16 A and B. The minute Y is very characteristically pulled out along the spindle-fibre at this stage.

Fifty metaphase I's were examined especially in respect to the mode of attachment of XY.

In fourteen cases it was attached as in fig. 14.

In twenty-five cases it was attached as in fig. 15.

In eleven cases it was apparently free, forming an independent bivalent. In many of these cases, however, it was probably attached as in the manner of fig. 15, but by a longer and finer thread. There is also little room for doubt that an attachment as in fig. 14 indicates an early metaphase, and that later the relations shown in fig. 15 are always assumed.

I have not been able to determine whether the autosome to which XY is attached is always the same one, but it appears probable that it is (note the distinctive shape of this chromosome in the two groups figured in fig. 13, and in figs. 14 and 15). The bivalent in question is, however, certainly always one of the larger ones.

The first meiotic division is the differential division for X and Y (Pl. 13, figs. 16, 17). During anaphase Y becomes still further pulled out along the line of the spindle-fibre, and presents in the late anaphase the characteristic appearance

illustrated in fig. 17. In Heidenhain preparations both X and Y are at this stage slightly paler than the autosomes.

Two kinds of secondary spermatocytes are therefore produced in the well-known manner, one with the X-chromosome and the other with the Y. There is a complete, and apparently prolonged, resting stage between the two divisions. The difference between the two kinds of secondary spermatocytes is conspicuous in the young nuclei, one member of each pair containing a dense chromatic body (presumably X) which is lacking in the sister nucleus or represented by a very much smaller speck (Pl. 13, fig. 18). A group of fully resting secondary spermatocytes, presenting the same dimorphism, is shown in fig. 19.

Fig. 20 shows a prophase for the second division in a secondary spermatocyte containing the larger chromatic body (X) which is seen attached to one of the chromosomes.

The expected two types of second division are easily found. An early, and rather irregular anaphase with the Y-chromosome is shown in fig. 21. Here Y has just divided. A later anaphase of the other type of second division is illustrated in fig. 22. Here we have apparently only five chromosomes present in each group; this must clearly be interpreted as a division in which X is present and fused, as usual, with an autosome.

It is noticeable that there is no trace in Macropus (nor in Petauroides) of the second pairing of chromosomes to give a quarter of the diploid number which has so often been described for the second division in birds and mammals.

Jordan (1911) described such a second pairing for Didelphys, but Painter (1922) found that it did not occur in his material.

B. Petauroides Volans.

Number of Chromosomes.—The determination of the number of chromosomes in this species presents more difficulty than in the case of Macropus, owing to their greater number. The number countable in the spermatogonial mitoses is generally twenty-two, forming typically a ring of twenty, with two smaller, slightly unequal ones, in the centre.

These are presumably X and Y, the latter being much larger than the corresponding element in Macropus, and being, indeed, but slightly smaller than X. I have, however, found some spermatogonial mitoses with apparently only twenty-one chromosomes, and yet containing this pair in the centre. I am therefore in doubt, from the spermatogonial mitoses, whether the number is 20+XY or 20+X. I have some quite unequivocal counts of polar views of the first meiotic metaphase, and some of these show eleven and some twelve separate elements. When twelve are present, one is always distinctly smaller than any of the others. Presumably, when the number is eleven, there are ten autosome bivalents and the XY bivalent. When the number is twelve, X and Y have dissociated. Side views of the meiotic metaphase (of which I have never found one that could be counted) show that one of the bivalents (? XY) commonly dissociates much in advance of the others. The conditions in the early pachytene nuclei also point to the presence of two sex chromosomes. It appears, therefore, to be fairly well established that the formula for the male Petauroides is 20+XY, or twenty-two in all. This corresponds with Painter's enumeration for Didelphys. It will also be noticed that the number of autosomes is double that of Macropus. None of my ovarian material proved suitable for chromosome counting.

The Meiotic Phase.—The spermatogonial nuclei of this animal differ from those of Macropus, in that the place of the fine reticulum of the latter genus is taken by a number of irregular blocks of chromatin, united by anastomoses. In the young spermatogonia these chromatic bodies are in approximately the diploid number, and from a study of the spermatogonial pro- and telophases it appears probable that these blocks are of the nature of 'prochromosomes', being the undiffused remains of the telophase chromosomes, and passing directly into the chromosomes of the following prophase. When the spermatogonia pass into a more profoundly resting stage the number of these bodies becomes more difficult to determine, owing to their becoming broken up and their

fragments scattered, till at last a stage is reached where the chromatin is finely distributed throughout the nucleus except for three or four masses representing the remains of the larger blocks which have escaped complete fragmentation. Very often, however, the number of the chromatic masses remains at approximately the diploid number throughout the whole interphase from the telophase to prophase.

The interstitial nuclei of the two genera present a similar difference, those of Macropus being finely reticular, and those of Petauroides containing about the diploid number of irregular chromatic masses.

The leptotene stage develops from a primary spermatocyte having the same structure as a spermatogonium, i.e. containing a number of massive chromatic bodies (Pl. 13, fig. 23). Each of these becomes the centre of a process of thread formation (Pl. 13, figs. 24, 25), to produce in the aggregate the leptotene nucleus (Pl. 13, fig. 26). This process resembles that by which the leptotene stage in certain insects is developed from a nucleus containing the diploid number of chromatic bodies (Wilson, 1912). In Petauroides, however, the evidence that these blocks represent each a single chromosome, though strong, is not complete.

The massive centres from which the thread-spinning starts persist for a long time, and indeed appear to form the basis of the synizetic knot. Synizesis is more pronounced in Petauroides than in Macropus—at least in my material (Pl. 13, fig. 27).

Parasyndesis occurs during the synizetic contraction (Pl. 13, figs. 27, 28), but not with the regularity observable in those animals in which the leptotene nucleus is orientated into a bouquet. In the nucleus shown in fig. 27 it is proceeding over one length of the thread, but appears to be already completed over the rest of the nucleus. That syndesis concerns, not the chromosomes as a whole but their constituent chromomeres, is beautifully shown in Petauroides (Pl. 13, figs. 27, 28). In this animal the chromomeres are unusually distinct and large at this, and some other, stages.

Figs. 27 and 28 show that, (1) the chromomeres in a single chromosome differ greatly in size, (2) the series of chromomeres in a pair of conjugating chromosomes closely corresponds, (3) conjugation takes place between the corresponding (homologous) chromomeres. It will be noticed also that the final union of chromomeres appears to be very intimate, all external trace of duplicity having disappeared in the most completely fused pairs.

Syndesis is followed by the usual pachytene stage (Pl. 14, figs. 29–33), during the early part of which two compact bodies, presumably X and Y, are conspicuous (Pl. 14, fig. 31). These soon unite into a single body, one or both of them often being pulled out into irregular shapes during the process (Pl. 14, fig. 32). The autosomes remain filamentar and suffer a temporary diminution of staining capacity. An important feature which is very conspicuous in Heidenhain preparations subjected to the right amount of extraction is that the stain is retained much more tenaciously by certain of the chromomeres than by others (Pl. 14, fig. 32). The general significance of chromomeres is discussed below.

As the chromosomes regain their staining powers towards the end of the pachytene stage, the chromomeres again become very conspicuous (Pl. 14, fig. 33), as they are also in the diplotene nucleus (Pl. 14, figs. 34, 35). It is interesting to compare the chromosomes shown in detail in fig. 35 with those in fig. 28, the latter representing the conjugation of the chromosomes, the former their separation. The correspondence between the chromomeres of homologous chromosomes is still evident in the diplotene stage, but now they are beginning to run together on the contracting chromosome, ultimately to give rise to the smooth chromosomes shown in fig. 36.

I have not been able to trace with certainty the movements of the sex chromosomes in the first meiotic division. In regard to the second division, all that can be said is that the number is clearly about ten or eleven, showing that there is no second numerical reduction.

The dimorphism of the secondary spermatocytes is not so

conspicuous as in Macropus, probably because the X and Y chromosomes are more nearly of the same size. Indeed, for a long time I thought there was no visible difference between the two types, but closer examination has shown that such a distinction exists. All secondary spermatocytes have a compact chromatic nucleolus, but in the case of young sister nuclei, whose relation to each other can still be seen by the persistent spindle remains, one of them constantly has a distinctly larger nucleolus than the other. Sometimes, as in the pair figured (Pl. 14, fig. 37), this is expressed by one of them being bilobed and the other single. In other cases it is merely a difference in size. The difference between the two classes of secondary spermatocytes is thus very small, but once recognized it is seen to be constant.

Discussion of some Special Problems raised by the foregoing Descriptions.

(1) Chromomeres.—Many cytologists maintain that chromomeres are purely artefacts, due to unequal contraction of the chromatic thread under the influence of the fixative, or else, in the case of smaller chromomeres, are mere optical effects of angles, &c., in the thread. At any rate, according to this view, they do not represent any real local differentiations of the substance of the chromosome.

The strength of the criticism that the chromomeres are artefacts depends much upon the exact meaning attached to that word. It is of little importance whether or not chromosomes which are beaded when fixed appear smooth in life (a very difficult observation in any case!) For the sake of argument it may be granted that the beading is produced by the action of the fixative. The important question is: Is the beading of such a nature that it could be produced mechanically by precipitation in and contraction of a homogeneous thread, or is it the expression of a pre-existing though perhaps invisible differentiation in the living chromosome? Doubtless so-called chromomeres have been described which might have been

produced by the action of the fixative on a homogeneous thread, but the view that the chromosomes are composed of differentiated chromomeres cannot be disposed of by demonstrating mistaken interpretation in individual cases. On the contrary, we have now a large accumulation of observations where the answer to the above question seems undoubtedly to be in the negative; observations, that is to say, which lead to the conclusion that whether the beads exist as such in life, or whether they are produced by the fixative at the moment of death, they must be expressions of local differentiations of the substance of the chromosome—and that is all, of course, that is required by the theory that connects the chromomeres with the linear arrangement of hereditary factors in a chromosome.

The reasons which seem to exclude the view that such chromomeres are produced mechanically, and so to speak, accidentally, on a homogeneous thread which is contracting unequally under the influence of unequal stresses in different parts are:

(1) The chromomeres in a single chromosome may differ very greatly as regard size (Pl. 13, figs. 27, 28).

(2) Had they been produced by unequal contraction of parts of a homogeneous thread, larger chromomeres would be separated from each other by longer intervals of connecting thread than those which separate the smaller chromomeres. A glance at fig. 28 shows that this rule does not hold.

(3) There is a close correspondence between the chromomeres of homologous chromosomes, both during syndesis and the diplotene stage.

(4) Wenrich (1916) has described the constant arrangement of the principal chromomeres on a given chromosome—a constancy which is maintained not only in all the nuclei (of the same stage) in a single animal, but even in different animals. Unless this observation be doubted, it supplies conclusive evidence that the chromomeres as seen in fixed nuclei correspond to definite local differentiations of the substance of the chromosome.

(5) It is now well known that the shape assumed by the long type of chromosome common in many forms of mitosis is

characteristic and constant for any given chromosome. This shape is commonly some form of V, with equal or unequal limbs. The point at which the chromosome bends to form the V—whether in the middle or towards one end—(which is also the spot to which the spindle-fibre is attached) varies from chromosome to chromosome, but is constant for any given chromosome. Similarly, the transverse constrictions which develop across the chromosomes of so many organisms are constant in position for a given chromosome. This constancy in position is proof of the existence of a constant differentiation, in a lengthwise direction, of the substance of the chromosome.

The tendency of certain chromosomes to develop transverse constrictions at spots constant for each particular chromosome is specially significant in estimating the value of the criticism that chromomeres are 'artefacts'. In Lepidosiren (Agar, 1913) the long somatic chromosomes usually show no trace of a transverse constriction. Each chromosome is a smooth curved rod or V of approximately uniform thickness. When the chromosomes become shorter and thicker, as happens regularly in the meiotic phase, and occasionally, from unknown reasons, in somatic tissues also, the transverse constrictions develop in a spot characteristic for each particular chromosome (which is also the point at which the apex of the V is situated, when the chromosome is in this shape).

This shortening and thickening of the chromosomes was produced in several plants by Sakamura (1920) by the action of various reagents, such as chloral hydrate and chloroform. These artificially shortened chromosomes showed well-marked and characteristically placed constrictions, though the position of these is barely indicated in normally fixed tissue. The constancy in position of these constrictions shows that they can only be called 'artefacts' in the sense that they make visible a pre-existing heterogeneity of the chromosome substance, which is concealed from view in the 'typical', well-fixed, and apparently uniform chromosome. Indeed, to deny that the chromomeres correspond to pre-existing local differentiations

of the chromosome substance on the ground that they only appear in tissues treated in a certain way, would be as illogical (granting that it is true) as to deny the distinction between a plasmosome and a chromatin nucleolus because the difference only becomes visible under the action of appropriate stains.

We conclude, therefore, that the chromomeres which appear in certain stages of mitosis in fixed tissues correspond to real local differentiations of the substance of the chromosome, though the actual shape which they assume (namely, bead-like swellings on a fine thread) may be assumed, or at least exaggerated, under the stress of the fixative.

(2) Crossing over.—Whether or not the phenomenon of crossing over occurs in mammals is still in doubt. Castle (1921) has described such a case in rabbits for the linked genes, English and non-English, and short-haired and Angora. Three individuals were tested, two males and a female, and crossing over was found in all of them. If this is established, it will show that the phenomenon in mammals is not quite comparable to that in Drosophila and Bombyx, where it occurs only in the sex which is homozygous for the sex chromosomes. By analogy with these, crossing over is to be expected in the female mammal alone. Considering the cytological evidence only, it would certainly seem that the conditions supposed to be necessary for crossing over are provided in the male diplotene nuclei of both these genera.

This is specially clear in Petauroides, because of the chromomeres. As fig. 27 shows, fusion of chromomeres in syndesis is intimate. Indeed, no sign of duplicity may remain. In fig. 35 (diplotene stage) the intertwined chromosomes are still held together at certain of the crossing places by unsplit chromomeres, and in view of their intimate union it is not difficult to imagine that when they finally do separate they may do so in such a way that the portions of the two chromosomes on either side of the point of union have been interchanged.

(3) The Relation between the Sex Chromosomes and the Plasmosomes in the Meiotic Phase.—The

condition of the plasmosome in Macropus has already been described. In Petauroides a plasmosome can occasionally be seen in the primary spermatocytes, but usually none can be identified with certainty—probably because, as in Macropus, this plasmosome is partly chromatic in the resting nucleus, and therefore does not stand out clearly from the other chromatic bodies. In the leptotene, synizetic, and early pachytene nuclei of Petauroides, a plasmosome can sometimes be identified, but is usually concealed among the dense tangle of chromatic threads. In the later pachytene stages, two plasmosomes are plainly visible (Pl. 14, fig. 32). One is considerably darker than the other, and has no relation to the sex chromosomes. This one I take to be the plasmosome of the earlier stages. The other plasmosome is in close relation to the sex chromosomes, and indeed appears to be formed out of their substance. It makes its appearance as a large pale body at the time that the sex chromosomes are uniting into a bivalent, and at first is an elongated structure closely attached to the bivalent. In some cases its shape and relations suggest that it is the persistent part of the bivalent from which the chromatin has flowed away into the rounded mass which forms the condensed sex bivalents: this appearance is strengthened by the fact that sometimes rounded granules or drops of chromatin are left embedded in the plasmosome. In other cases it is pear-shaped and is attached by its neck to the bivalent, irresistibly suggesting that it has been squeezed out of the contracting chromosomes like a viscid fluid from a narrow aperture. These appearances are illustrated in figs. 32 and 38. In later stages this plasmosome becomes approximately spherical and often becomes detached from the sex bivalent, though always lying close to it.

For a time the two plasmosomes—one presumably the remains of the pre-leptotene nucleolus, and the other apparently formed out of material (plastin or linin ?) derived from the sex chromosomes—coexist, the former being the first to disappear.

In Macropus the larger pale plasmosome which appears in close connexion with the uniting sex chromosomes has also the

appearance of being formed out of their substance, though no figures were discovered quite so striking as those illustrated for Petauroides.

(4) Chromatoid Bodies.—In Petauroides one or two bodies staining densely with iron haematoxylin appear suddenly in the cytoplasm in the pachytene stage. Their origin and fate have not been determined, but they seem to be distributed capriciously at cell-division. In fig. 37 they have all passed to one daughter cell (that containing the sex chromosome), but this mode of allocation is not invariable. In Macropus chromatoid bodies are either absent or inconspicuous.

Summary.

Macropus ualabatus has twelve chromosomes, namely 10 + XY in the male and 10 + XX in the female.

In Petauroides the number is almost certainly twenty-two, the male being of the formula 20 + XY. No female counts were obtained for this animal.

In the male Macropus X is generally attached to one of the autosomes in spermatogonial mitoses. Y, which is exceedingly minute, is free. During the pachytene stage, while the autosomes are still elongated, X and Y condense into a bivalent. In the first meiotic division this bivalent is attached to an autosome.

As a result of the first meiotic division the usual two classes of secondary spermatocytes are formed one with X and the other with Y. In the second meiotic division, those with X show only five separate chromosomes, showing that X, as usual, is fused with an autosome. The other class of second divisions shows five autosomes and the minute Y.

In the female Macropus the sex chromosomes were never found free from the autosomes in the ovarian follicle cells, which therefore show only ten separate chromosomes.

In Petauroides the sex chromosomes cannot be distinguished with certainty from the autosomes. An unequal pair of small chromosomes usually situated in the centre of the

spermatogonial metaphase plates probably, however, are X and Y. Early pachytene nuclei show two compact bodies which unite into one, presumably the sex bivalent.

The second reduction of the chromosome number to one-quarter of the diploid total in the second meiotic division, which has been described for several species of birds and mammals, does not take place either in Macropus or Petauroides.

Chromomeres are very prominent in Petauroides in the zygotene and diplotene stages.

Probably in Macropus, and more convincingly in Petauroides, the cytological conditions to permit of 'crossing over' are present in the male.

The plasmosome which appears in the pachytene stage is probably formed from the plastin or linin basis of the contracting sex chromosomes.

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EXPLANATION OF PLATES 12, 13, AND 14.

The scale given on Pl. 12 applies to all the figures except 19 and 38, which are on a smaller scale.

Lettering.

P, pre-pachytene plasmosome. Px, plasmosome which appears at the time that the sex chromosomes are uniting. X, Y, the sex chromosomes.

Figs. 1–22, Macropus ualabatus; Figs. 23–38, Petauroides volans.

Plate 12.

Fig. 1.—Resting spermatogonium.

Fig. 2.—Metaphase, spermatogonial mitosis, eleven chromosomes.

Fig. 3.—Metaphase, spermatogonial mitosis, twelve chromosomes.

Figs. 4, 5.—Metaphase, ovarian follicle cells, ten chromosomes.

Fig. 6.—Late leptotene nucleus.

Fig. 7.—Synizesis.

Fig. 8.—Early pachytene nucleus showing condensation of X.

Fig. 9.—Later stage, showing pairing of X and Y.

Fig. 10.—Late pachytene nucleus.

Fig. 11.—Early diplotene nucleus.

Fig. 12.—The chromosomes of an early diplotene nucleus shown separately.

Fig. 13.—Contracting bivalents in two adjacent cells.

Plate 13.

Figs. 14, 15.—Two metaphases of the first meiotic division, to show modes of attachment of XY to an autosome.

Figs. 16 A, B.—Autosomes, with attached XY bivalent, from more advanced metaphases, to show separation of X and Y.

Fig. 17.—Anaphase of first division.

Fig. 18.—Pair of young secondary spermatocytes, still connected by the spindle remains, one with large compact chromatic body, the other without.

Fig. 19.—A group of secondary spermatocyte nuclei to show the dimorphism. Half with large and half with small chromatic body (presumably X and Y).

Fig. 20.—Early prophase of a second division with the X-chromosome.

Fig. 21.—Anaphase of a second division with the Y-chromosome. A small cyphlasmic inclusion is shown at the bottom right-hand corner.

Fig. 22.—Anaphase of a second division with the X-chromosome (indistinguishably fused with an autosome). To avoid overlapping the two groups have been slightly shifted laterally in drawing.

Fig. 23.—Resting primary spermatocyte.

Figs. 24, 25.—Fragments of developing leptotene nuclei to show conversion of the massive blocks of the resting spermatocyte into the leptotene threads.

Fig. 26.—Leptotene nucleus.

Fig. 27.—Synizesis and syndesis.

Fig. 28.—Three short lengths of conjugating chromosomes from three different zygotene nuclei.

PLATE 14.

Fig. 29.—Syndesis complete.

Fig. 30.—Early pachytene nucleus, synizesis loosening out.

Fig. 31.—Pachytene stage, with two compact bodies, presumably X and Y.

Fig. 32.—Later stage. X and Y, one of them greatly pulled out, uniting into a bivalent.

Fig. 33.—Late pachytene nucleus showing evidence of commencement of diplotene stage. Chromatoid body in the cytoplasm.

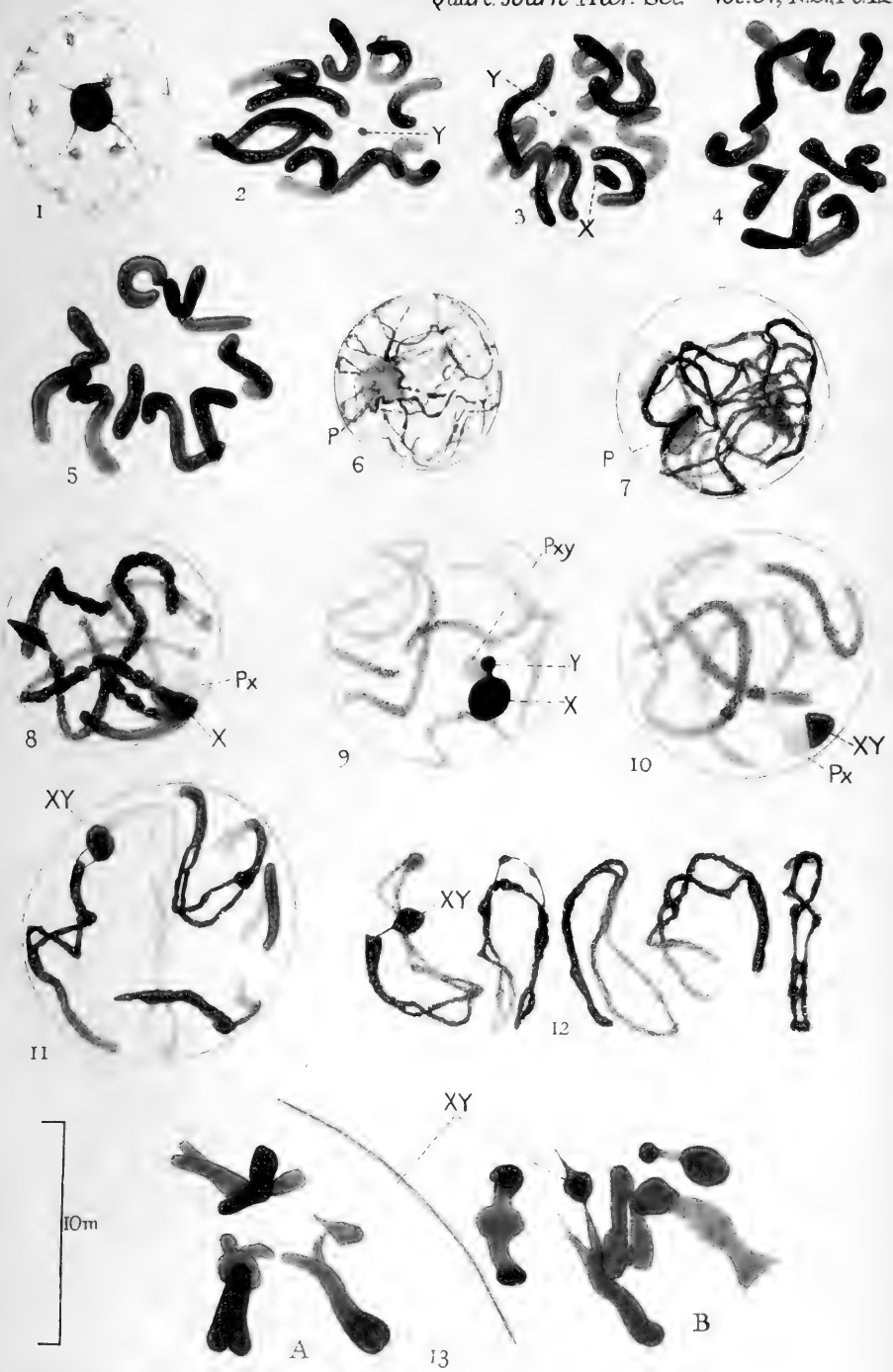
Fig. 34.—Early diplotene nucleus. Chromatoid body in cytoplasm.

Fig. 35.—Three bivalents from an early diplotene nucleus.

Fig. 36.—Late diplotene nucleus, chromatoids in cytoplasm.

Fig. 37.—A pair of young secondary spermatocytes, still connected by the spindle remains. Note larger and bilobed chromatic body in the upper nucleus. Chromatoids in the cytoplasm of one cell.

Fig. 38.—Outline drawings of four nuclei, about the stage of fig. 32, to show relations between the sex chromosomes and the plasmosome.



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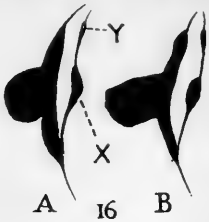

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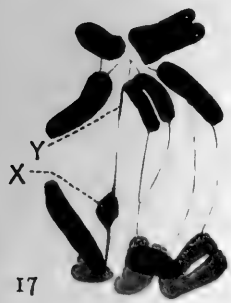

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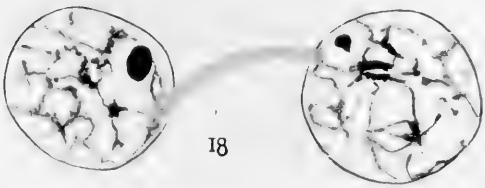

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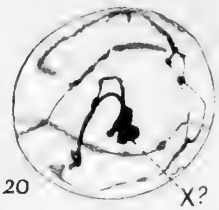

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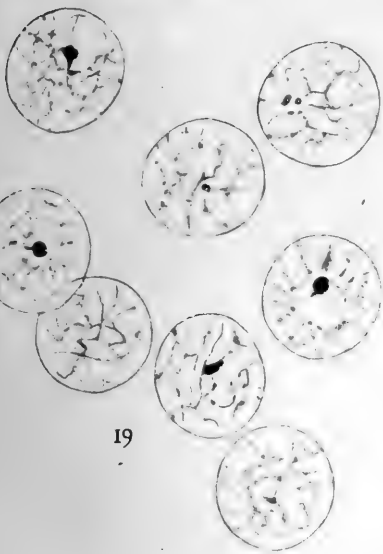

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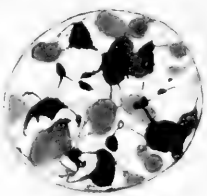

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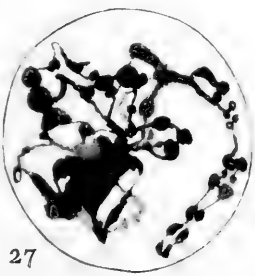

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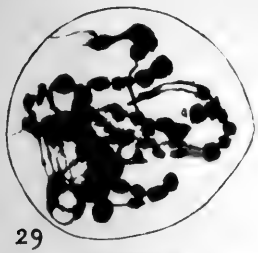

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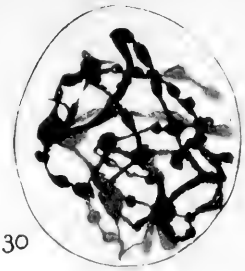

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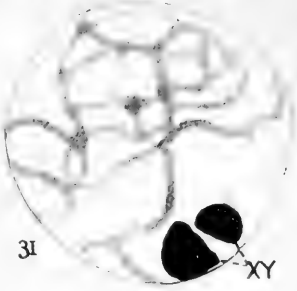

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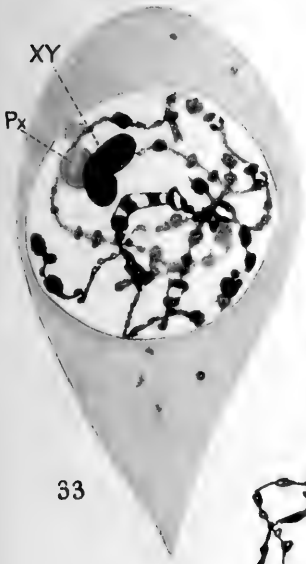

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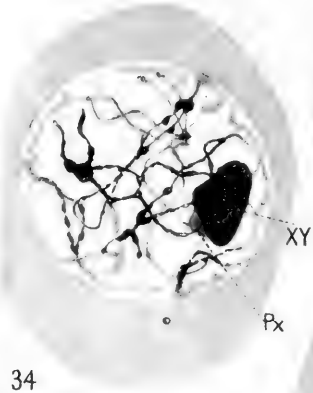

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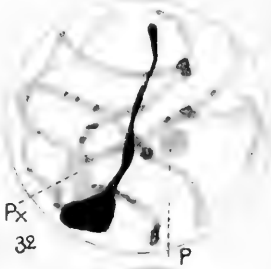

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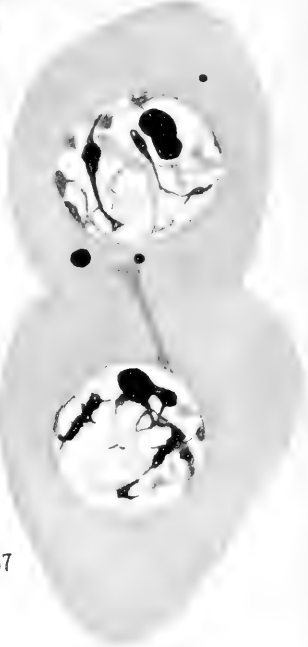

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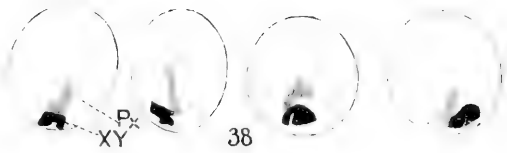

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W. E. Agar del.

Marsupial Spermatogenesis.

By

A. W. Greenwood, B.Sc.,

University of Melbourne.

With Plates 15 and 16.

Introduction.

This work is a contribution to the cytology of the Marsupials, a group which, owing to the small number of their chromosomes, affords peculiar advantages over other mammals for this kind of study. The sex chromosomes are particularly clear in this group, and Painter's discovery of a Y-chromosome in the American opossum (Didelphys) has been fully confirmed in several species of Australian Marsupials examined in this laboratory. In the present paper I give the results of the study of three species of Marsupials belonging to two different families, two species belonging to the family Dasyuridae of the sub-order Polyprotodontia, and one to the family Phalangeridae of the sub-order Diprotodontia.

The form examined in most detail is Phascolarctus cinereus. In the other forms I have done little more than determine the number and the behaviour of the sex chromosomes.

The following work was undertaken under the guidance, and with the assistance, of W. E. Agar, F.R.S., Professor of Zoology in the University of Melbourne.

Spermatogenesis of Phascolarctus cinereus.

Material.

The animals were obtained through the courtesy of Mr. Kershaw of the National Museum, from the National Park at

Wilson's Promontory. The first set of testicular and ovarian material was obtained by Professor Agar, who killed and fixed the material. All the other animals obtained were killed and the gonads fixed at the University laboratory. The animals were received at different times during the year, namely in the months of January, April, May, July, and August. In all testis, except those obtained in April, the tubules were filled with various stages in spermatogenesis; in the latter the tubules contained a relatively large number of Sertoli cells, but other stages in spermatogenesis were few in number.

Fixing.

Various methods of fixing the material were employed—Bouin, Allen's modification of Bouin, cold Flemming, and corrosive acetic. The only satisfactory fixatives were the Bouin fluids and the Flemming. For early prophases the Flemming-fixed material gave the best results, but the Bouin fixatives gave very clear figures of the division stages.

Staining.

Sections of 10 μ and 20 μ were cut. Some were mounted in the ordinary way on glass slides, and others were mounted between coverslips so that the nuclei could be examined from both sides.

Heidenhain's iron haematoxylin with iron alum was used for staining the sections. Staining with safranin and gentian violet was also tried, but the results were not very satisfactory.

Number of Chromosomes.

The diploid number of chromosomes in Phascolarctus is sixteen. This number was obtained from numerous counts in both male and female material. Female counts were obtained from the prophases of large cells of the corpus luteum. One such cell is figured (Pl. 15, fig. 1). Altogether sixteen chromosomes can be seen. In the female it should be noted that no chromatic dot is present, such as is shown so clearly in the

spermatogonial metaphase plates (Pl. 15, figs. 2 and 3). In the female there are the comparatively large autosomes (14) and two much smaller chromosomes. These two smaller chromosomes, similar in size and shape, are the sex chromosomes. The chromosome complex of the female is therefore 14+XX.

In the male counts were obtained from equatorial plates of the dividing spermatogonia, and from first and second meiotic division figures. The number of chromosomes obtained from these stages was sixteen. In the spermatogonial plates the chromosomes are arranged in a circle around a central clear space. Inside the circle of chromosomes a chromosome much smaller than the others is seen; also near this small chromosome a small chromatic dot is to be seen (Pl. 15, figs. 2 and 3). From their subsequent behaviour these are identified as the X- and Y-chromosome respectively. The chromosome formula of the male is therefore 14+XY.

Structure of Testes.

The testes have the typical mammalian structure of numerous convoluted tubules. Close to the wall of the tubules are situated the Sertoli cells, spermatogonia, and the early meiotic prophases. Passing in towards the lumen of the tubule, the later stages of the maturation divisions occur, leading up to the formation of the spermatozoa. These are found nearest the lumen of the tubule. There appears to be a definite layering of the cells of the different stages, although some overlapping of the inner layers occurs.

Spermatogonia.

The nuclei of the early spermatogonia are oval in shape, and are frequently lobed. The nuclei of the later generations of spermatogonia are much smaller and are approximately circular in shape. In the resting condition the spermatogonial nucleus contains a well-defined nucleolus which stains very densely with the iron haematoxylin. This is evidently of the nature of a plasmosome impregnated with chromatin. The chromatin

of the resting cell occurs in the form of rather faintly stained blocks connected by fine strands. The blocks of chromatin appear as loose masses, frayed out at the edges, and the number present is approximately the same as the diploid number of chromosomes.

The onset of the prophase is marked by an increase in the staining capacity of the chromatin blocks which now give rise to short irregular threads. These threads increase in length, probably at the expense of the nucleolus, which now shows up as a large pale body with a few deeply staining granules embedded in it. The long irregular threads begin to contract, ultimately giving rise to the thick chromosomes marking the end of the prophase. The nucleolus has given rise to a large, oval, faintly stained body, a typical plasmosome.

Meiotic Phase.

The origin of the leptotene stage has not been determined with absolute certainty, but it appears that the telophase of the last spermatogonial division does not pass into a complete resting stage, but the chromatin remains in the form of blocks situated close underneath the nuclear membrane. These blocks are present in approximately the diploid number. They are more compact and stain more deeply than the chromatin blocks seen in the resting spermatogonial nucleus. The leptotene stage appears to be derived from this by the formation of long threads from these chromatin blocks. The early leptotene nucleus consists of a tangle of fine threads. On the threads chromomeres can be seen. These are spaced rather far apart and vary considerably in size. In the centre of the nucleus the pale plasmosome can be seen. Following this stage the threads begin to contract away from one side of the nucleus, and, at the same time, begin to contract in length. This is the earliest indication of synizesis (Pl. 15, fig. 4). Although the exact time of syndesis could not be determined, it is probable that it begins to take place now.

It is significant that the leptotene nucleus entering synizesis shows that the threads contract away from that side of the

nucleus which is opposite to that on which the archoplasmic mass is found, and it appears as if this body exerts some influence, if it is not wholly the cause of the synizetic contraction.

In the early leptotene stage no sign of a compact X- or Y-chromosome could be seen, so that they are evidently threaded out like the autosomes at this stage.

Fig. 5 shows a much later stage in syndesis and synizesis. The nucleus is not complete, but shows very clearly the pairing of the chromomeres in homologous threads. The chromomeres exhibit great variability in size. In one of the threads syndesis appears to be nearly complete. In the centre of the nucleus can be seen the compact mass of the synizetic contraction.

The leptotene stage is followed by the pachytene stage. Fig. 6 shows an early pachytene stage consisting of thick, looped chromosomes which have emerged from the synizetic contraction. These chromosomes are seen to be distinctly double in composition, the presence of the chromomeres showing up as darkly stained bodies in the more lightly stained thread. The threads are now very thick. The ends of the threads at this stage are directed towards the archoplasmic mass. Later, these threads lie scattered through the nucleus and all trace of duplicity is eventually lost. The X-chromosome makes its appearance in the early pachytene stage. It appears first as a thin, deeply stained thread (Pl. 15, fig. 7), but contracts down to form a round mass, which is typical of the X-chromosome in later prophase. I have been unable to identify the minute Y-chromosome at this stage. Whether the Y-chromosome fuses with the X-chromosome to form a bivalent could not be determined with certainty owing to the minuteness of the Y-chromosome and the presence in the nucleus at this stage of several deeply staining granules.

Always in contact with the X-chromosome there is a large pale plasmosome (Pl. 15, figs. 7, 8, 9). This varies considerably in shape, and usually contains a number of deeply staining granules. In later pachytene stages two plasmosomes are visible, each containing one or more deeply staining granules

(Pl. 15, fig. 9). One of the plasmosomes (Px) remains in contact with the sex chromosome, the other (Px′) appears to be formed from a division of this plasmosome.

The early pachytene stage is followed by a late pachytene stage in which the chromosomes show a marked diminution in staining capacity. They become diffuse and furry in appearance, the X-chromosome or possible XY bivalent alone remaining as a deeply stained body. The onset of the diplotene stage is marked by the recovery of the staining power of the chromosomes. The chromosomes begin to split so that two long, thin threads are formed. The chromomeres can be distinctly seen on these threads occurring in pairs (Pl. 15, fig. 10).

The thin threads now begin to contract, but never entirely separate from one another, remaining in contact at two or more points (Pl. 15, fig. 11). At this stage the nucleus, which has been increasing in size from the onset of the meiotic prophase, has now attained its maximum volume. Following this stage the nuclear membrane breaks down and the chromosomes lie free in the cytoplasm.

In a few cases during the diplotene stage I have seen the X-chromosome apparently attached to the end of one of the autosomes, but this condition appears to be exceptional. In most cases it lies free. It is in the metaphase of the first meiotic division that the Y-chromosome can first be identified with certainty. Figs. 12 and 13 are of metaphase plates. The first is a cell just after the nuclear membrane has disappeared. The seven large bivalents and the separate X- and Y-chromosomes can be seen attached to one another by threads. Fig. 13 is a rather later view. In Phascolarctus the X- and the Y-chromosome do not usually form a bivalent. In division figures they are always found separate. In the meiotic prophase they may possibly be in the form of a bivalent, but, as mentioned before, this point could not be determined.

Fig. 14 shows a metaphase side-view. In this it will be seen that the X- and Y-chromosomes are on opposite sides of the mass of chromosomes at the equator of the cell, and are travelling to opposite poles of the cell ahead of the other chromosomes.

In many cases, on the other hand, the sex chromosomes lag behind on the spindle. This is shown in fig. 15 of an anaphase. In this figure it will be noted that the X- and the Y-chromosome are attached to the same spindle-fibre.

The first division is therefore the reductional division, and gives rise to two daughter secondary spermatocytes which are dimorphic, one containing the X-chromosome and the other containing the Y-chromosome. This dimorphism of the secondary spermatocytes is shown in fig. 16 (Pl. 16). That these are two daughter spermatocytes is shown by the remains of the spindle-fibres connecting the two cells. In one of the cells can be seen a rather large, deeply stained body in the nucleus which I take to be the X-chromosome. In the other cell nucleus there is a much smaller body not so deeply stained, which probably represents the Y-chromosome.

Second Meiotic Division.

Before the onset of the prophase of the second meiotic division, the secondary spermatocyte undergoes a prolonged resting stage and increases greatly in size. From the resting stage with its faintly staining network, the onset of the prophase is shown by the recovery of the staining power of the chromatin in patches. From this the deeply stained, irregular threads of the prophase are formed (Pl. 16, fig. 17). Right through the meiotic stages the X-chromosome has retained its staining capacity and does not thread out, except in the early prophase of the first meiotic division. During the prophase of the second division the X-chromosome remains compact and does not thread out. The division follows on as before, but this time the sex chromosomes divide, one half going to each pole of the cell. The second division is therefore equational. No further reduction in the number of chromosomes takes place during this division.

Fig. 18 shows an anaphase of the second division with the X-chromosome divided and lagging behind on the spindle. Fig. 19 shows a late anaphase of the same division showing the presence of the Y-chromosome at both poles of the cell.

Cytoplasmic Inclusions.

So far in my description of the spermatogenesis of *Phascolarctus* I have made no mention of cytoplasmic structures. Beyond noting their occurrence in the germ cells I have done little to determine their nature.

In the cytoplasm of the spermatogonia and meiotic stages a large round body is to be seen (Pl. 15, fig. 6). This varies greatly in size and staining capacity at different stages. In some stages it is quite deeply stained, notably in the spermatogonia, leptonema, and early pachynema. Later it stains capriciously, and eventually, during the first meiotic division, becomes quite pale (Pl. 15, figs. 12 and 13). It does not divide during the division but passes indiscriminately to one or other of the secondary spermatocytes (Pl. 16, fig. 16). It occasionally is found in the early spermatid nuclei, but I have been unable to find it at any later stage.

This body, I believe, is probably the same as that figured by Gatenby in his work on the ' Cytoplasmic Inclusions of Germ Cells ', as an excretory granule.

Another cytoplasmic inclusion seen in all stages of spermatogenesis is a very pale, somewhat sausage-shaped body lying close up against the nuclear membrane (Pl. 15, figs. 6 and 10). It is towards this body that the synizetic contraction takes place. It is found in all the secondary spermatocytes and spermatids, although I have never found any sign of its division during any of the stages in spermatogenesis. From its behaviour I believe this to be the archoplasmic mass.

Other cytoplasmic inclusions are the chromatoid bodies. These are conspicuous in the sections fixed with Flemming, but are pale and inconspicuous in those sections fixed in Bouin.

The leptotene nucleus always contains one or more deeply staining granules. In the pachytene stage also the nucleus often contains a deeply stained granule usually lying close beneath the nuclear membrane (Pl. 15, fig. 7). These granules appear to give rise to the chromatoid bodies seen in the cytoplasm of the germ cells at different stages (Pl. 16, fig. 28).

These chromatoid bodies vary in size and in number. During the first meiotic division they are distributed indiscriminately between the two daughter cells. Further than this they have not been traced.

The Sertoli Cell in Phascolarctus.

In Phascolarctus the Sertoli cell nucleus is very large (Pl. 16, fig. 20). It is about three times as large as the nucleus of the primary spermatocyte when it has attained its maximum volume. The nucleus lies at the foot of the Sertoli cell close to the wall of the tubule. The outlines of the cell could not be distinguished. The chromatin of the nucleus is in the form of a coagulum distributed through the nucleus. In the centre of the nucleus there is a clear space surrounding a granular mass. This granular mass is probably formed by the degeneration of the nucleolus.

The cytoplasm of the Sertoli cell contains, usually lying close up against the nucleus, a varying number of refractive, rod-like bodies. These are a constant feature of the Sertoli cell in Phascolarctus, and I found them present in all the material examined by me whatever time of the year the material was obtained. Usually in close association with the rods, a pale yellow, fluffy mass can be seen. This probably consists of deutoplasmic material. This shows up well in sections which have been fixed in cold Flemming before staining with the iron haematoxylin.

In some of the outside tubules of the sections, especially in the Flemming-fixed material, instead of the bundle of rods a mass of over-lapping, very pale plates can be seen (Pl. 16, fig. 21).

The rods appear pale yellow in those sections fixed in the Bouin, but often in the Flemming-fixed material are quite black. The rods are found together, lying approximately parallel. They vary in length, some of them consisting of small pieces lying end to end and probably formed by the fracture of one of the longer rods. Although the rods are usually comparatively straight, in many cases they are seen to possess a very wavy outline.

Much has already been written regarding these rod-like bodies present in the cytoplasm of the Sertoli cells in Phascolarctus. I do not propose to discuss at much length the theories already advanced, but will give my conclusions arrived at by a study of these bodies, and the results of experiments undertaken by me to determine if possible their nature. The experiments were undertaken with the view to ascertaining whether the rods served a nutritive function. The fact that these rods were found only in the Sertoli cells led me to believe that possibly these rods were a source of nutriment, or connected in some way with the supply of nutriment to the developing spermatozoa.

Below I give the results of some digestion experiments undertaken to prove this point if possible.

Fresh material was cut by means of a freezing microtome. Owing to the difficulty of picking up the rods in unstained material, the tissue was stained. The stains used were neutral red and methyl green.

The sections after staining were submitted to the action of a weakly acidic mixture of pepsin and glycerine. Cells with rods were picked out and their position marked by means of a micrometer eyepiece. Then the microscope was placed in an electric oven and kept at a constant temperature of 30° Centigrade. The progress of the action was watched from time to time. The accumulation of the products of digestion after a time tended to stop the action and only partial digestion took place. In all cases of partial digestion the rods were still visible. With the use of more of the digestive fluid complete digestion of the cytoplasm occurred. In this case, with complete digestion, the cells moved about in the fluid and were difficult to find again. In the majority of the experiments the rods were identified even after complete digestion, but in some cases they could not be found.

The same experiment was carried out, using an alkaline mixture of zymine and glycerine. Again, here the rods were still visible after partial digestion ; but in the case of complete digestion, owing to the difficulty of picking up the rods in the

resulting fluid, I could not be absolutely certain whether the rods were dissolved or not, although in the majority of cases the rods were still visible.

Bardeleben refers to these bodies as crystals of haematoidin. He also describes the presence of similar bodies in the great blood lacunae in the material of the testis. With regard to the composition of these bodies I have not been able to determine whether they are composed of haematoidin or not.

Fresh tissue was boiled in chloroform. The tissue was then embedded and sections cut, stained, and mounted. Upon examination it was found that in no case did the chloroform have any action on the rods in the Sertoli cells.

This does not of course confirm Bardeleben's view that they are crystals of haematoidin, but goes to show that they are, at any rate, not a blood derivative which is an acid.

With regard to the occurrence of similar bodies in the blood lacunae of the testis, in all the material I have examined I have not found any bodies comparable with the rods found in the Sertoli cells. The presence of somewhat similar rods has been described by several authors. Montgomery has shown that the Sertoli cells in man are derived from the spermatogonia, and that the nature of the resultant cell is determined by the presence of a rod-like body in the cytoplasm, i. e. all spermatogonial cells containing the rod-like body give rise to the Sertoli cells. This, however, does not appear to be the case in Phascolarctus. Although I am convinced that the Sertoli cells in Phascolarctus arise from a division of the spermatogonia, I have never been able to find any trace of the rods until the nucleus of the cell, by its peculiar structure, is definitely defined as a Sertoli cell. No Sertoli cell divisions have been found in Phascolarctus, but in Perameles (in this animal the Sertoli cells are comparable with Phascolarctus in point of size and nuclear structure) I have found an apparent diplotene nucleus which from its size and position in the tubule appears to have originated from a Sertoli nucleus. From this it seems safe to assume that the Sertoli cell has been derived from the same cells as give rise to the germ cells.

Spermatogenesis of Sarcophilus ursinus.

Both male and female animals of this species were obtained. The technique followed was the same as in Phascolarctus, viz. fixatives used were Allen's modification of Bouin, and cold Flemming; this was followed by staining in Heidenhain's iron alum haematoxylin. In the testis the same more or less definite layering of the germ cells in the tubules is noted as in Phascolarctus.

Number of Chromosomes.

In the female the number of chromosomes was obtained from metaphase plates of dividing follicle cells surrounding the ovum. The number found was fourteen. Of this number twelve are large and the other two are much smaller. These two smaller ones, similar in size and shape, are the sex chromosomes. The chromosome formula of the female Sarcophilus is therefore 12 + XX (Pl. 16, fig. 22).

In the male chromosome counts were obtained from spermatogonial plates and first meiotic division stages.

From the metaphase plates of the spermatogonial divisions the number of chromosomes was found to be fourteen. Here, as in Phascolarctus, the presence of a small X-chromosome and a minute Y-chromosome, usually in the centre of a circle formed by the twelve larger autosomes, was again noticed (Pl. 16, fig. 23). In fig. 24 the spermatogonial chromosomes are dividing or have already divided. Two of the chromosomes as yet show no sign of splitting (Pl. 16, fig. 24, *a* and *b*). The division of the Y-chromosome is very clearly shown in this figure.

The chromosome formula of the male Sarcophilus is therefore 12 + XY.

Meiotic Phase.

I have made no attempt to follow out in detail the phenomena of the meiotic phase, but stages similar in appearance to those of Phascolarctus are found in Sarcophilus.

The side-view of an early anaphase of the first meiotic division is shown in fig. 25. The X- and the minute Y-chromosome are on opposite sides of the central mass of chromosomes, and are travelling towards the poles of the cell ahead of the other chromosomes. The first meiotic division therefore acts as the reductional division and the two daughter secondary spermatocytes produced are dimorphic, one containing the X-chromosome and the other containing the Y-chromosome. Satisfactory second meiotic division figures have up to the present not been obtained.

Spermatogenesis of Dasyurus maculatus.

Only the male of this species was obtained, and so a check count of the number of chromosomes of the female could not be obtained. However, very good counts from spermatogonial metaphase plates were obtained leaving no doubt as to the number of chromosomes present. The number of chromosomes in this animal is the same as in Sarcophilus, a member of the same family. Of the fourteen chromosomes, twelve are the autosomes, one is the small X-chromosome, and the other the minute Y-chromosome (Pl. 16, figs. 26 and 27).

From figures of the first meiotic division, the separation of the X- and the Y-chromosome is seen to take place as in the other animals (Pl. 16, fig. 28).

Summary.

In the three animals studied the total number of chromosomes in the male is as follows :

Phascolarctus	16 (14 autosomes + XY).
Sarcophilus	14 (12 autosomes + XY).
Dasyurus	14 (12 autosomes + XY).

In the female the number of chromosomes is as follows :

Phascolarctus	16 (14 autosomes + XX).
Sarcophilus	14 (12 autosomes + XX).

In all animals dealt with in this paper the Y-chromosome is very minute in size compared with the other chromosomes ;

also the X-chromosome is much smaller than any of the autosomes.

Chromomeres are conspicuous during syndesis, early pachytene, and early diplotene stages.

The early pachytene stage is followed by a late pachytene stage in which the threads become diffuse and lose their capacity for taking up the stain.

Except in the early meiotic prophase the sex chromosome remains compact and deeply stained and does not thread out like the autosomes.

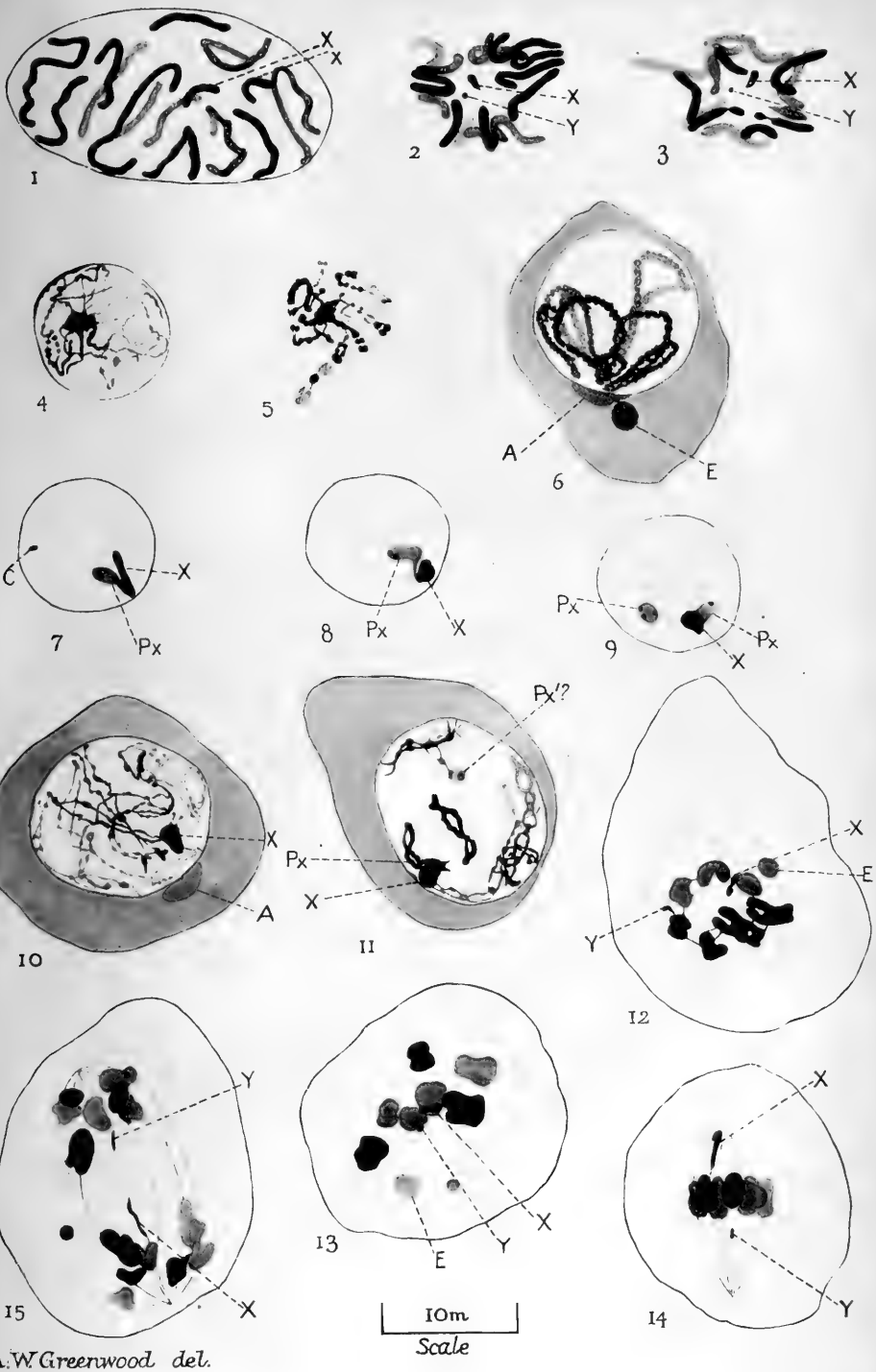
In all the above animals the first meiotic division is reductional, separating the X- and the Y-chromosomes, and the second division is equational, in each cell the sex chromosome dividing. The spermatozoa are therefore of two kinds, one containing an X-chromosome and the other containing a Y-chromosome.

No further reduction in the number of chromosomes takes place during the second meiotic division.

The Y-chromosome could not be identified during the meiotic phase until the metaphase of the first meiotic division. At this stage in Phascolarctus the sex chromosomes are separate and do not form a bivalent.

The archoplasm seems to exert some influence on the chromatin threads at synizesis and during the early pachytene stage. In the former case the contraction takes place to that side of the nucleus at which the archoplasmic mass is situated ; in the latter the chromosomes are in the form of thick loops with the ends of the chromosomes pointing towards the archoplasmic mass.

In Phascolarctus the Sertoli cells are very large and possess peculiar rod-like bodies, the origin and function of which was not arrived at. The result of experiments seem to show that the rods are not affected by the action of digestive fluids.



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EXPLANATION OF PLATES 15 AND 16.

Reference Letters.

A, archoplasmic mass. *E*, excretory body. *D*, deutoplasm. *P*, plasmosome. *Px*, Plasmosome associated with X-chromosome. *Px'*, plasmosome derived from *Px*. *Chr*, chromatoid body.

Plate 15.

Phascolarctus cinereus.

Fig. 1.—Female. Nucleus of a cell from the corpus luteum. The two sex chromosomes (XX) are much smaller than any of the fourteen autosomes. (Bouin.)

Fig. 2.—Spermatogonial plate with fourteen autosomes surrounding the X- and Y-chromosomes. (Bouin.)

Fig. 3.—Spermatogonial plate. The constrictions seen at the end of some of the chromosomes are not usually present. (Bouin.)

Fig. 4.—Late leptonema, and beginning of synizesis. Only some of the threads are shown. Chromomeres are distinct on some of the threads. (Flemming.)

Fig. 5.—Late synizesis and syndesis. Incomplete nucleus. Showing the pairing of the chromomeres on homologous chromosomes. Central mass representing the synizetic contraction. (Bouin.)

Fig. 6.—Early pachynema. Thick-looped chromosomes still showing some duplicity. (Flemming.)

Fig. 7.—Showing the condensation of the sex chromosome, also the associated plasmosome containing deeply staining granules. *C*, chromatic granule in nucleus which probably gives rise to chromatoid body. (Bouin.)

Figs. 8, 9.—Showing the presence of the second plasmosome (*Px'*). (Bouin.)

Fig. 10.—Early diplonema. Compact X-chromosome or XY bivalent. Paired chromomeres distinct.

Fig. 11.—Later diplonema. (Bouin.)

Fig. 12.—Metaphase plate of first meiotic division just after nuclear membrane has disappeared. (Bouin.)

Fig. 13.—Later metaphase plate. (Flemming.)

Fig. 14.—Metaphase (side-view). X- and Y-chromosomes travelling to opposite poles of the cell ahead of the other chromosomes. (Bouin.)

Fig. 15.—Anaphase of first meiotic division. X and Y lagging behind on the spindle. (Bouin.)

Plate 16.

Phascolarctus cinereus.

Fig. 16.—Daughter secondary spermatocytes (dimorphic) with remnants of spindle-fibres between the two cells. One cell nucleus contains a deeply stained body—the X-chromosome. (Bouin.)

Fig. 17.—Prophase of second meiotic division. Irregular threads. X-chromosome compact. (Flemming.)

Fig. 18.—Anaphase of second meiotic division showing division of the X-chromosome. (Bouin.)

Fig. 19.—Anaphase of the second meiotic division showing division of the Y-chromosome. (Bouin.)

Fig. 20.—Sertoli cell nucleus with associated rods and deutoplasm. (Flemming.)

Fig. 21.—Sertoli cell nucleus with accompanying pale plates. The chromatin of the nucleus is represented semi-diagrammatically. (Flemming.)

Sarcophilus ursinus.

Fig. 22.—Female. Metaphase plate of follicle cell. 12+XX chromosomes. (Flemming.)

Fig. 23.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 24.—Splitting of chromosomes during spermatogonial mitosis. All chromosomes except two (*a* and *b*) have divided. (Bouin.)

Fig. 25.—Metaphase (side-view) of first meiotic division showing separation of the X- and the Y-chromosomes. (Bouin.)

Dasyurus maculatus.

Fig. 26.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 27.—Spermatogonial plate. 12+XY chromosomes. (Bouin.)

Fig. 28.—Metaphase (side-view) of first meiotic division showing the separation of the X- and the Y-chromosomes. (Flemming.)

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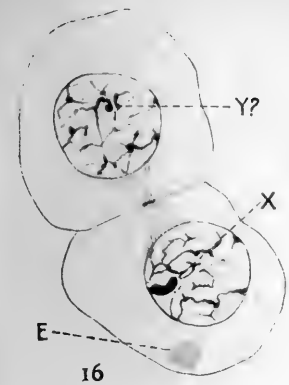

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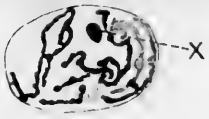

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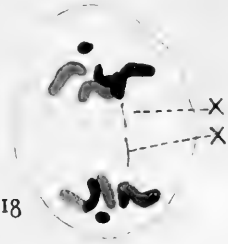

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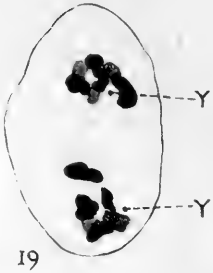

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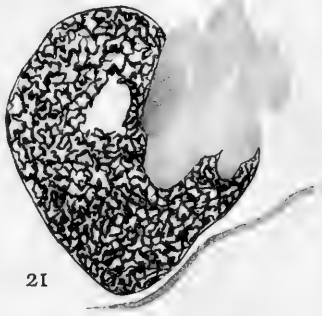

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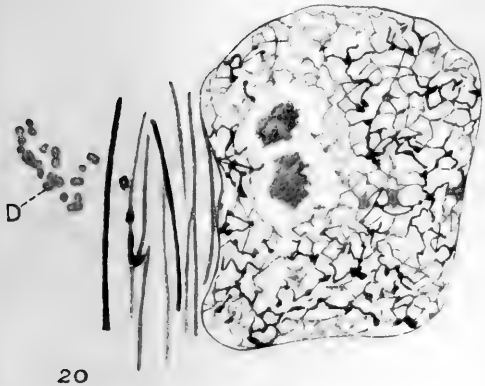

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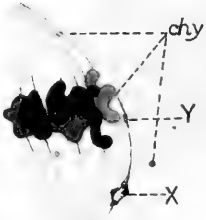

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A. W. Greenwood del.

On Sexual Differentiation in the Infusoria.

By

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With Plate 17 and 1 Text-figure.

The object of this preliminary note is to describe a very curious type of conjugation which may throw light on the process of sexual differentiation in the Infusoria—a type leading from conjugation between two hermaphrodite individuals to copulation between male and female specimens. In his very lucid account of the genetics of the Ciliata, C. Dobell (1914),[1] with good reason, points out that in cases of typical conjugation both the conjugants are to be looked on as hermaphrodites which are performing cross-fertilization. Only in Vorticellids there are males and females, and the sexual process changes to copulation, while the dwarf-like male perishes after fertilizing the larger female. In other Ciliata there are no well-proved cases of sexual differentiation of the conjugants, although some hints at it are to be found in the works of Cull, Doflein, and Enriques. Doflein states that the conjugants of the same pair in Paramecium putrinum differ very much in size, a feature which he is ready to attribute to sexual differentiation. Enriques relates of Chilodon that the conjugants, which are very much alike at the beginning of conjugation, become differentiated into a longer and a shorter one during the process of conjugation. The meaning of such conjugants—termed by Enriques ' male and female hemisexes '—remains obscure, as also the extensive discussion of the author on the subject of sexual differentiation. The observations of Cull on ' incipient

[1] ' Journ. of Genetics,' vol. iv, p. 131, 1914.

sexuality ' in Paramecium proved to be erroneous (Jennings and Lashley), and need no further mention. But in this paper I propose to describe a case of conjugation in the Ciliata where the members of a pair show very marked differences, which may have a relation to sex.

The species of ciliate which I have studied belongs to the genus Ophryoscolex (order, Oligotricha ; family, Ophryoscolecidae). The representatives of this family lead a parasitic mode of life in the stomach or the intestine of different Ungulata. Ophryoscolex janus, the form described, is a new species, found in the stomach of African antelopes, Bubalis cokei and Madoqua sp. The antelopes were shot by me during my expedition to British East Africa in 1914, on the shores of Lake Naivasha.

In order to make my description more comprehensible I am obliged to describe in some detail the morphology of O. janus. The ordinary or, as we shall call them, neuter individuals of O. janus have the following structure. The body (Pl. 17, fig. 1) is oblong, and approximately cylindrical, its posterior half being a little wider than the anterior one. The anterior end of the body bears the mouth, lying on the top of the oral cone : the posterior end is provided with a long and slender terminal spine. The ciliary apparatus consists of an adoral zone (*Adz*) and a dorsal crescent (*Dz*) of membranellae. This crescent in O. janus is removed very far backwards, lying a little behind the middle of the body. The mouth leads into a long pharynx, with a very complicated structure. It begins with a rather narrow oral cavity, which farther backwards widens into the pharynx proper ; the pharynx follows the right side of the body to its hinder end, growing gradually narrower backwards. Close to the posterior end of the body the pharynx communicates with the endoplasm. This latter is so well defined by a thin continuous membrane that we can term it (in agreement with other authors) the mid-gut. The mid-gut sends out anteriorly a long conjugation outgrowth, which lies parallel to the pharynx and during conjugation serves as a bridge for the migration of the male pronucleus.

A short tube-like rectum ends with a circular anal aperture at the base of the terminal spine. As a characteristic feature of O. janus we can mention the very strong development of the inner skeleton. It is represented by a thin plate (Pl. 17, fig. 1, *skp*) of alveolar structure, lying under the tough superficial cuticle of the body. Anteriorly the skeletal plate surrounds the body, forming under the cuticle a sort of stiff collar, whose opposite ends meet on the dorsal side of the animal to form a suture-line. In the posterior half of O. janus the right side of the plate retains its superficial subcuticular position, while the left one separates from the cuticle and dips into the interior, forming a wing-like outgrowth, which surrounds and supports the hinder part of the pharynx. There are special myonemes, described in other Ophryoscolecidae by Sharp and Braune, closely connected with the pharynx. They form a continuous thin layer on the sides of the pharynx supported by the skeletal plate, to whose inner surface the myonemes are attached. The myonemes begin at the posterior end of the pharynx, pass forwards, and, after reaching the anterior third of the body, detach themselves from the skeletal plate and converge to the centre of the body, following the wall of the pharynx. In so doing this muscular layer forms a sort of oblique diaphragm (fig. 1, *D*) which is concerned with the ingestion of food particles.

The strong development of the skeletal collar removes the nucleus and the contractile vacuoles far backwards. The macronucleus is usually lemon-shaped and lies on the right side of the animal, somewhat nearer to the ventral surface of it than to the dorsal one ; in a cup-like depression of the macronucleus the small ball-shaped micronucleus is situated (Pl. 17, fig. 1, *Mi*). A little dorsally from the macronucleus lie both the contractile vacuoles (V_1, V_2), the anterior being considerably larger than the posterior one.

Besides the neuter individuals I met (in both the antelopes investigated) with conjugating pairs of O. janus. The great majority of pairs proved, to my great astonishment, to consist of individuals widely different in dimensions and several other

morphological features—differing in several points not only from one another but also from the neuters. Let us call them micro- and macroconjugants, without giving to these names the significance of sexual differentiation. The macroconjugant (Pl. 17, fig. 2, left-hand individual) differs less than the microconjugant from the neuter individuals. The macroconjugants are individuals 82–112 μ long and 42–55 μ broad (at the level of the macronucleus), the great bulk of them measuring from 90 μ to 100 μ. The posterior part of the body is somewhat shortened and inflated in comparison with the neuters. The most prominent differences from the neuter individuals, however, consist in the lack of the hind contractile vacuole and in the character of the terminal spine. Long and slender in neuters, the spine is short and thick in the macroconjugants, being about one-third as long as in the neuter specimens.

The microconjugants are even more strongly modified (Pl. 17, fig. 2, right-hand individual). They are 60–75 μ long and 20–5 μ broad. It is easy to see (Pl. 17, figs. 2 and 12) that the general aspect of the body becomes quite different from that of the macroconjugants, the body being long, slender, and somewhat vermiform. The difference will appear still greater if we compare the respective volumes of both types of individual. Thus for a microconjugant of 75 $\mu \times 25$ μ we shall have an approximate volume of 34,000 cubic microns, while a macroconjugant of 90 $\mu \times 45$ μ will give a total of 137,000 cubic microns—that is, about four times the volume of a microconjugant.

Notwithstanding its small size the microconjugant possesses a long and slender terminal spine, of just the same length as that of the neuter specimens. The skeletal plate, so characteristic of O. janus, is wholly lacking. This absence of the endoskeletal plate does not remain without influence on several other internal characters of the microconjugant. The outline of the anterior half of the body becomes folded and wrinkled, while in the macroconjugant it appears smooth and even. The pharyngeal myonemes, having lost their line of insertion, hang loosely back and form a sort of long muscular cone

(Pl. 17, fig. 2). The muscular diaphragm which is usually seen in neuters and macroconjugants becomes obliterated. Finally, there is a difference in the shape of the macronucleus. In the macroconjugants and neuters the macronucleus resembles a lemon. In the microconjugants (Pl. 17, fig. 12) it is more elongated, being at the same time rounded at both ends. Both the micro- and macroconjugants differ from the neuters in having only one contractile vacuole instead of two.

Taken altogether, the differences between the conjugants are so great that the individual members of a pair could be considered as belonging to different species, were they not found in a state of conjugation.

The conjugants adhere one to another with their anterior ends, diverging at an acute angle from the point of conjunction. I could follow on my slides all phases of the nuclear changes characteristic of a typical conjugation. A cross-fertilization takes place—both individuals interchanging their migratory nuclei. Furthermore, I have found conjugating pairs with a syncaryon in both partners, and a large number of exconjugants of both kinds. The latter show different stages in the reconstruction of a normal nuclear apparatus.

At first we find in the exconjugants two pronuclei surrounded by a plasmatic halo (described by Prandtl in Didinium, and present, I believe, in most of the Infusoria). The old macronucleus persists in the microconjugant after separation from its partner, while in the macroconjugant it is already dissolved at the moment of disjunction. The double syncaryon (Pl. 17, figs. 3 and 4) forms the first division spindle, and the reconstruction of the nuclear apparatus is very simple, following the type represented by Chilodon. The first division spindle by its fission gives rise to a pair of nuclei which become respectively a new macro- and micronucleus. An interesting feature of this division is its heteropolarity—from the diaster stage onwards. One of the polar swellings of the dividing syncaryon is smaller and stains more intensely with nuclear stains than the other one. It ultimately becomes still more condensed, and develops into a micro-

nucleus ; while the larger continues to swell and becomes the new macronucleus (Pl. 17, fig. 5, *Ma*).

Several particularly successful preparations help us to elucidate the further fate of the micro-exconjugants. They complete their reorganization and return to the type of the neuter individuals. Specimens with the nuclear apparatus not fully reconstituted show the rudiments of a new and very thin skeletal plate, which is finely alveolar—its alveoli corresponding to those ultimately constituting the fully formed plate. At the same time the fibres of the pharyngeal muscular mantle apply themselves to the inner surface of the plate, and in so doing form the pharyngeal diaphragm. Further modification produces tiny neuter specimens, of the same length as the microconjugants but with fully developed skeletal plates. It might be objected that the stages described can be interpreted in an inverse sense, i.e. that the tiny neuters by losing their skeletal plates may become microconjugants. But there can be no such alternative, for the formation of microconjugants takes place in quite a different way. They arise as a result of an unequal fission of neuter individuals. O. janus possesses two different kinds of fission. One of them, the ordinary one, leads to the formation of neuters. The other one, which may be called progamic, results in the formation of two preconjugants which differ in size and other morphological characters. The beginning of the fission is in both cases manifested by the elongation of the posterior half of the body and of the macronucleus as well, while the micronucleus assumes the shape of a short spindle. At the same time the first rudiments of the new adoral and dorsal zones of membranellae (Pl. 17, fig. 6, Dz_2) appear under the cuticle.

In cases of ordinary fission (Pl. 17, fig. 6) the phase just described clearly indicates the initial stages of the building up of a new skeletal plate in the posterior individual (Pl. 17, fig. 6, skp_2). Further phases lead to the complete formation of the posterior individual, with fully developed skeleton and all the characters of the neuters. As a result of fission we have two neuters with long terminal spines. During the whole process

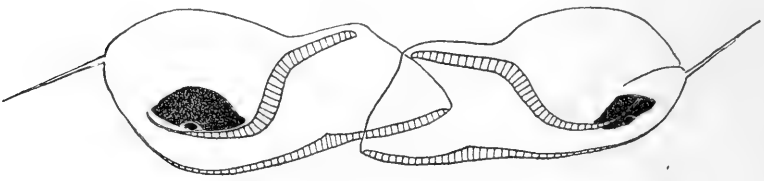
of fission the micronucleus retains its small dimensions; and freshly separated daughter micronuclei are always connected by a long fibrous strand, whose terminal swellings (i.e. groups of chromosomes) stain deeply with all nuclear stains. In cases of progamic fission (Pl. 17, fig. 7) the posterior individual does not show any signs of a skeletal plate from the beginning to the end of the fission—it is the microconjugant in statu nascendi, and gets its long and slender terminal spine from its neuter parent. The anterior individual, which gets the skeletal plate of its parent, differs from the latter by the shortness and thickness of its newly developed terminal spine.

The progamic fission can be easily recognized, moreover, by the behaviour of the micronucleus, which swells to enormous size, becoming at the same time very feebly stainable with nuclear stains (Pl. 17, fig. 7, *Mi*). The micronuclei of both the daughter individuals remain in this condition from the moment of their disjunction to the beginning of conjugation. Such individuals may be termed preconjugants (Pl. 17, figs. 8 and 9). From what has just been said it follows that in a population of O. janus one can easily distinguish the preconjugants from other individuals (neuters) which are incapable of conjugating.

The conjugation of O. janus becomes still more interesting from the fact that this species exhibits a certain percentage of isogamous pairs, exclusively of the macroconjugant type. The number of such isogamous pairs amounted, in both the antelopes examined, to about 20 per cent. of the whole number of pairs, the remaining 80 per cent. being of the anisogamous type. On examining such isogamous pairs more closely, it is seen that about 60 per cent. of them present different stages of nuclear changes characteristic of conjugation (Pl. 17, fig. 10) from the first maturation spindle to the stage of migrating pronuclei. In the remaining 40 per cent. of isogamous pairs the micronuclei remain very small and lie embedded in a flat depression of the macronucleus (Text-fig. 1) without showing any preparation to fission. As a further peculiarity, the conjugants of such pairs appear to assume a position somewhat

different from the normal one. The anterior end of one conjugant is sometimes engulfed by the introverted pharynx of its partner (Text-fig. 1), while both the conjugants lie on the same level without forming the characteristic angle of about 40 degrees. The long slender terminal spines of both the conjugating individuals indicate that they are neuters. The formation of such pairs might, however, be explained in a different way. It might possibly be the result of a fortuitous snapping of one individual at another in its endeavours to engulf food particles; but such an interpretation does not appear to me very plausible. It might also represent an abortive attempt to conjugate on the part of neuter individuals

TEXT-FIG. 1.



A conjoined pair of neuters.

which are unable to conjugate successfully. In all the 60 per cent. of macroconjugants conjugating inter se with success, the terminal spine is short and thick and the posterior contractile vacuole is wanting; and it is thus evident that these specimens have gone through the preparatory process of progamic fission.

Such are the most important results of my investigation of the conjugation of O. janus. A detailed general discussion of many interesting questions arising therefrom will be given in my full account of conjugation in the Ophryoscolecidae, but I may now venture to draw some conclusions regarding the more striking features of the processes described.

First of all, the question arises as to which type of conjugation is primary in O. janus—the isogamous or the anisogamous? Comparison with the other Ophryoscolecidae and with the great majority of free-living Infusoria seems to

indicate isogamous conjugation as the more primitive mode of sexual process.

In other species of Ophryoscolex the conjugants always possess a fully developed skeleton. It is therefore natural to suppose that in O. janus also the primary type of conjugation was that between two macroconjugants formed by equal fission of a neuter individual. At the present time this old mode of conjugation is retained by about 20 per cent. of pairs only, while in the rest it has been superseded by a manifest anisogamy.

Secondly, what causes have evoked this change in the sexual process? This problem is extremely difficult to solve and permits of many different explanations. One that seems to me to be a probable one depends upon the consideration that the change mentioned procures for the preconjugants the advantage of being able to conjugate as rapidly as possible. A fission accompanied by the building up of the whole complex skeleton in the posterior individual and by the growing of the latter to the size of a neuter, would perforce require much more time to perform than a fission which is confined to a simple cutting off of the posterior third of the body. The presence of only one contractile vacuole in the preconjugants also speaks in favour of this supposition. In ordinary fission the anterior individual gets the first vacuole, the posterior individual the second; while those which are wanting are soon afterwards formed anew. There, as we have seen, each of the conjugants possesses only one vacuole, which it obtained at the progamic fission: no reconstruction of the missing vacuole takes place. Admitting that conjugation occurs in critical circumstances menacing the existence of the population, we could thus understand the tendency to abridge the preparations for this process.

Has the observed differentiation of two kinds of conjugants the significance of sexual differentiation, and can we thus regard the micro- and macroconjugants as males and females? It appears that notwithstanding all the differences in size and structure both kinds of conjugants act as hermaphrodites.

Ample evidence for this is afforded by the cross-migration of 'male' pronuclei, and by the reconstruction of the nuclear apparatus and skeleton in microconjugants. Of course, we could suppose that during the progamic fission the material of the micronucleus is distributed unevenly, so that the male elements are taken by the microconjugant, the micronucleus of the macroconjugant getting only the female ones. If so, then before and during the conjugation one specimen acts as a male, another as a female, returning only after conjugation to the hermaphrodite state. But there is no real evidence in favour of this view, and the possibility of conjugation between two macroconjugants (in 20 per cent.) speaks against it.

But even so, we can still see in the complex processes of conjugation in O. janus the first hint of approaching sexual differentiation. Indeed, conjugation seems to have become impossible between two microconjugants, and this circumstance raises them physiologically to quite another category in comparison with the macroconjugants. This loss of the faculty to conjugate inter se speaks clearly in favour of a male tendency in the microconjugants. Then, again, certain abnormal cases of conjugation also confirm my view of the possible future transformation of microconjugants into males. Among about a hundred micro-exconjugants which came under my inspection there happened to be the following abnormal specimens. One exconjugant, instead of old macronucleus + synkaryon (or its derivatives), possessed only the old macronucleus. Another one, instead of having two pronuclei or a syncaryon, showed only one small nucleus of micronuclear type and the remains of the old macronucleus. A few abnormal pairs of conjugants permit us to guess how such exconjugants arise. In one of the pairs (Pl. 17, fig. 11), for instance, the microconjugant contains only a macronucleus, while the macroconjugant has got the macronucleus and four micronuclei (Pl. 17, fig. 11, Mi_1–Mi_4). One of the latter (Mi_4) is lying in the hind half of the body, another one (Mi_1) just at the line of junction of the conjugants, on the way to the partner, while the remaining micronuclei (Mi_2 and Mi_3) lie

in the endoplasmatic conjugation outgrowth of the macroconjugant. The only possible interpretation of such a pair is the following one. The conjugants have reached the stage of pronuclei. Both the pronuclei of the microconjugant have migrated into the larger partner, while the corresponding process in the latter was retarded so that the ' male ' pronucleus of the macroconjugant has not yet succeeded in penetrating into the partner. And we must say that, in the pair under discussion, a further migration of the male pronucleus (Mi_1) into the smaller partner would become absurd, as the corresponding female pronucleus of the microconjugant (Mi_2) has penetrated into the larger individual. Let us imagine that the separation of such a pair is accomplished, and we should then have before us a micro-exconjugant provided with only one nucleus, the old macronucleus (as in the above-mentioned abnormal exconjugant), and a macro-exconjugant with a double set of nuclei. A strong confirmation of such an interpretation is supplied by a macro-exconjugant in the stage of reconstruction of the nuclear apparatus. In this specimen there are two micronuclei and two macronuclei instead of one micro- and one macronucleus. There, as I believe, the pronuclei of the microconjugant penetrated into its partner (as in the case mentioned above) and copulated with the corresponding pronuclei—thus producing a pair of syncarya. The latter then divided, giving rise to a double set of micro- and macronuclei. The micro-exconjugant with an old macronucleus and a small micronucleus (see above) is to be thought of as a specimen whose ' male ' pronucleus has migrated into the larger partner, while the corresponding migration of the macroconjugant's ' male ' pronucleus did not take place ; and the exconjugant represents, therefore, an individual with its old macronucleus and a ' female ' pronucleus.

These and some other abnormal cases give us grounds enough for framing the following hypothesis. If analogous anomalies become more common, a time may come when in conjugating pairs only the ' male ' pronucleus of the microconjugant will migrate, the reciprocal process being suspended.

The macroconjugant, transformed into a fertilized female, will thus go on living and multiplying, while the microconjugant, now to be regarded as a dwarf male, is predestined to die after conjugation.

It is very interesting to note that the sexual phases of O. janus remind us of the reproduction in a group of Metazoa, namely, in some of the Cirripedia. It is well known that several representatives of the Cirripedia possess, besides the large hermaphrodite individuals, so-called complementary dwarf males. I cannot help comparing the macroconjugants of O. janus to hermaphrodite Cirripedia, while the microconjugants are on the way to become complementary males.

Another point worthy of special mention is the progamic fission. Several species of Infusoria (Dileptus, Didinium, Paramecium, &c.) are known to undergo, before conjugation, several 'hunger-divisions'. R. Hertwig, postulating a causal connexion between these divisions and conjugation, says that hunger-divisions may correspond with the maturation divisions of multicellular organisms. Still, it is uncertain how far these divisions are indispensable for the beginning of conjugation, and how far the preconjugants differ from the ordinary (neuter) individuals. All the Ophryoscolecidae examined, and O. janus more than the rest, prove that the fissions preceding conjugation have a peculiar character. They have a close connexion with the commencement of sexual reproduction and must bear the special name of progamic fissions. The individuals resulting from these fissions are the preconjugants, which differ in several points from the neuters. Only the preconjugants are able to conjugate; and this compels us to consider the progamic fission as a process which may be compared with the attaining of puberty by multicellular organisms. The individuals which are formed by the progamic fissions are sexually mature. On the other hand, the reduction divisions of the micronucleus during conjugation are the real homologues of the maturation processes of sexual cells in the Metazoa.

The same rule—that conjugation is possible only between

the preconjugants—is applicable, I believe, to all the rest of the Infusoria. We have already seen the very marked differences which characterize the preconjugants of O. janus in comparison with the neuter specimens. The same differences, though less evident, exist in other Ophryoscolecidae (in all the three species studied by me): and some further indications of the existence of preconjugants in other forms are scattered here and there in different papers on Infusoria; but I do not intend to discuss them in this preliminary note.

The conception of sexual puberty preceding conjugation casts a new light on the question of experimental induction of conjugation by means of different external stimuli (hunger, &c.). In all such cases the specimens treated in the experiment evidently remain still unable to conjugate, whatever be done to them; but the stimuli applied to the Infusoria make them begin the progamic fission, which produces sexually mature individuals ready for conjugation. It is noteworthy that the cases of 'reconjugation' prove this sexual maturity to persist, in some exceptional cases, even after conjugation has occurred.

There is a further point to be discussed, although I do it with some reserve. I refer to the heightened viability of the conjugants as compared with the rest of the population. The high mortality amongst exconjugants is already known. In my material also dying exconjugants are often to be found; in Ophryoscolecidae they are easily recognized by many striking characters, as I shall show in another note. The same symptoms of death are observed in many (17 per cent.) of the neuters. The conjugating pairs, on the contrary, were never found in a dying condition. This circumstance makes me believe that the stages of sexual puberty and conjugation are the most viable. If this is so, we can easily understand why ciliates acquire a tendency to conjugate in bad conditions of life; for the processes of progamic fission and conjugation would enable the animals to acquire, for a couple of days, a heightened resistance to harmful external conditions. It remains to test this experimentally on some free-living Infusoria. If we accept this hypothesis the cases of reconjugation are easily explained:

since under bad life-conditions, continuing for a longer space of time, the exconjugants of the first conjugation would hasten to reconjugate, so as to become again more resistant to their surrounding medium.

EXPLANATION OF PLATE 17.

All figures depict Ophryoscolex janus n. sp. and were drawn with the help of Abbe's apparatus, under a Zeiss 2 mm. homogeneous immersion objective with compensating ocular no. 4. The figures were reduced to three-quarters in reproduction.

Fig. 1.—A neuter individual.

Fig. 2.—Conjugation between a macro- and microconjugant. Each individual has a single macronucleus and two micronuclei.

Fig. 3.—A micro-exconjugant with a synkaryon and the remains of the old macronucleus.

Fig. 4.—A macro-exconjugant with synkaryon; the old macronucleus is dissolved.

Fig. 5.—A micro-exconjugant with old macronucleus (*Oma*), new macronucleus (*Ma*), and new micronucleus (*Mi*).

Fig. 6.—An ordinary fission of O. janus giving rise to a pair of neuters; note the skeletal plate (Skp_2) in the posterior individual.

Fig. 7.—A progamic fission of O. janus; note the absence of a skeleton from the posterior individual and the large size of the micronuclei (*Mi*).

Fig. 8.—A micro-preconjugant.

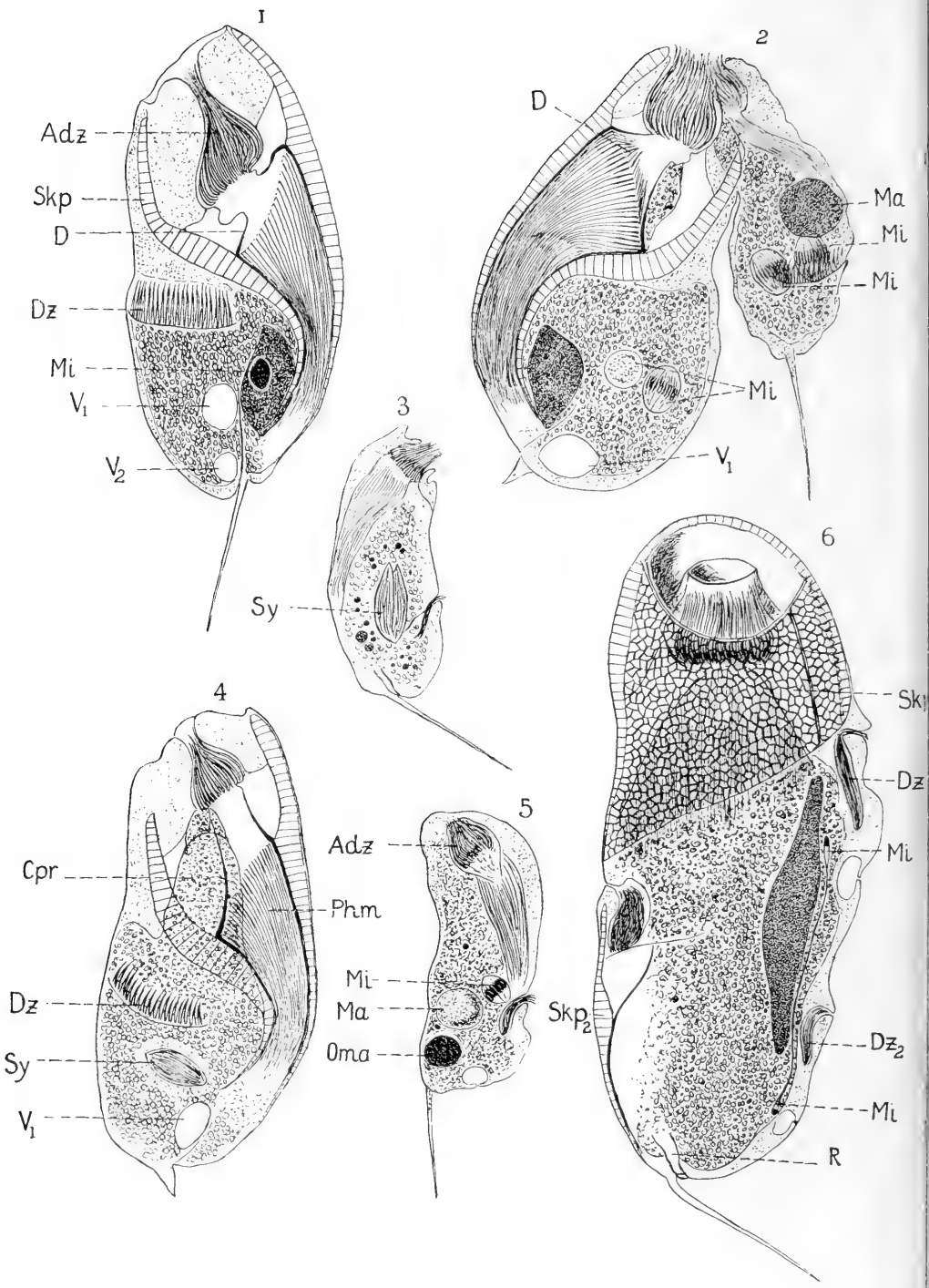
Fig. 9.—A macro-preconjugant.

Fig. 10.—Conjugation between two macroconjugants.

Fig. 11.—An abnormal case of conjugation; all the micronuclei (Mi_1–Mi_4) lie in the macroconjugant, the microconjugant retaining only the old macronucleus.

Lettering.

Adz, adoral zone of cilia. *Cpr*, conjugation process of endoplasm. *D*, oblique diaphragm. *Dz*, dorsal crescent of membranellae. *Ma*, macronucleus. *Mi*, micronucleus. *Oma*, old macronucleus. *Phm*, pharynx. *R*, rectum. *Skp*, skeletal plate. *Sy*, synkaryon. V_1, anterior vacuole. V_2, posterior vacuole.



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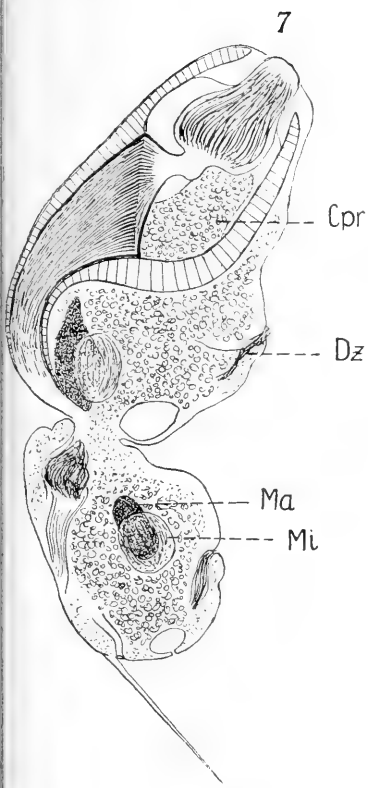
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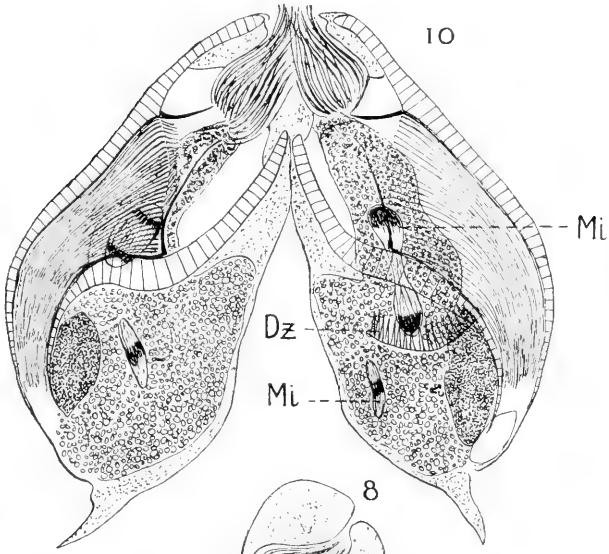
Ma
Mi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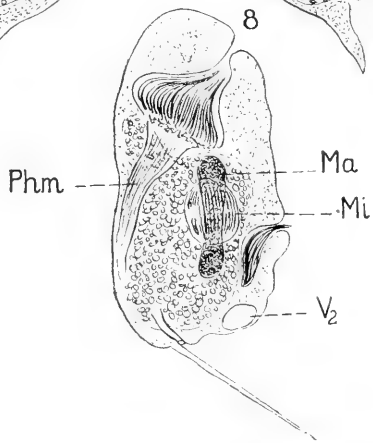
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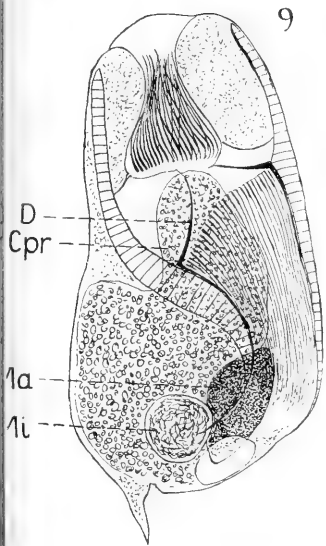
Ma
Mi
V2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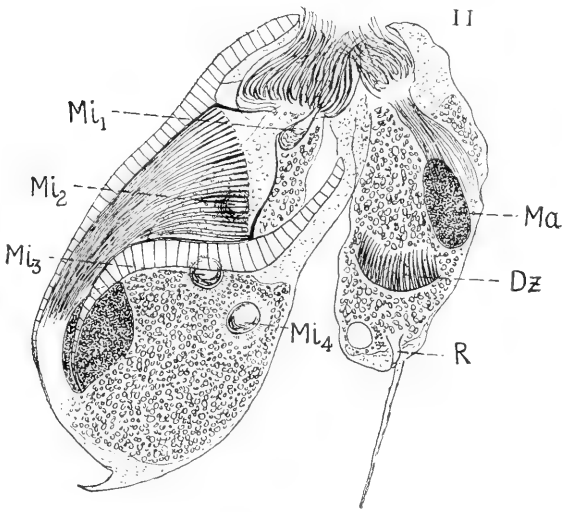
11
Mi1
Mi2
Mi3
Mi4
Ma
Dz
R

Sanguinicola from the Sudan.

By

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With Plate 18.

In 1908 [1] the description by Dr. Marianne Plehn of Sanguinicola as the only known example of a Cestode (Cestodarian) infesting the blood aroused considerable interest, and though the subsequent conclusion by Odhner in 1911 [2] that Sanguinicola was rather to be regarded as an extreme form of suckerless haematobic Trematode somewhat detracted from the significance of the discovery, yet on account of its plesio-Turbellarian structure and possible Turbellarian affinities, Sanguinicola still remains a zoological type of importance.

So far as I am aware, Sanguinicola has, up to the present, solely been found in Germany in the blood of Cyprinidae,[3] and it was therefore a source of gratification to me to discover in the large collection of slides of Helminth material made by the late Dr. A. J. Chalmers when Director of the Wellcome Tropical Research Laboratories at Khartum and kindly presented to the Wellcome Bureau by his successor, Major R. G. Archibald, some thirty specimens of Sanguinicola obtained from the blood in the heart of the Nile Siluroids,

[1] "Ein monozoischer Cestode als Blutparasit (Sanguinicola armata u. inermis, Plehn)", 'Zoologischer Anzeiger', Bd. xxxiii, p. 427, 1908–9.

[2] T. Odhner, "Sanguinicola M. Plehn—ein digenetischer Trematode!" ibid., Bd. xxxviii, p. 33, 1911.

[3] Max Lühe, "Parasitische Plattwürmer II: Cestodes" in Brauer's 'Die Süsswasserfauna Deutschlands', Heft 18, 1910.

Synodontis schall, Bloch-Schneider 1801, and Auchenoglanis occidentalis, Cuv. and Val. 1840. Since I have not examined personally the Sanguinicola armata and S. inermis of Plehn, I am unable to say, especially in view of the somewhat diagrammatic figures and, in the case of certain organs, discordant accounts of the anatomy of these two species, whether the Sanguinicola from the Sudan is a distinct third species or not, but its structure is evidently sufficiently similar to enable me to venture to correct and amplify in some respects the descriptions supplied by Plehn and to furnish some additional evidence in connexion with the possible affinities of this organism.

My material consisted in all cases of specimens already stained and mounted in balsam. Twenty were from Auchenoglanis occidentalis and fourteen (including one very young form, three cut into horizontal and transverse sections, and four which I lost during restaining but which I had previously examined) from Synodontis schall. In length the adult or nearly adult specimens varied between 647·4 and 1,228·4 microns in length, and all were of the 'armed' (i.e. bearing spinelets on the edge of the body) type.

One external feature which Dr. Plehn has not described for S. armata and S. inermis is the presence on the surface of the body which does not carry the genital openings of a furrow or groove, shallow over the greater length of the body but deep posteriorly and terminating just anteriorly to the hind end of the animal in a distinct pocket (Pl. 18, figs. 3, 7, z, y, x, w, v). This furrow or groove is formed by the inturning and posterior fusion of the edges of the body, is apparently a permanent formation judging by the posterior pocket, and is similar in form to the similar body grooves to be found in such Trematodes as Hemistomum clathratum and the male Schistosoma, but differs in that it does not contain the sexual apertures and therefore cannot be of use in copulation. The edges of the body which border this furrow contain the spinelets [1]

[1] If mounted specimens of Sanguinicola be not well flattened out, spinelets will appear to be absent on the 'edges' of the body and can only

or ' Häkchen ' described by Plehn, except posteriorly where the edges turn mediad in order to unite (Pl. 18, fig. 1, SP). The spinelets are distinct rods of the form shown in fig. 9, and are almost entirely embedded in the subcuticula and parenchyma save for their outer extremities, which project slightly. These spinelets attain a maximum length of about 27 microns, and they are apparently identical in nature with the chitinous spines found in the skin of many Malacocotylea.

For the reason to be supplied later when describing the genital apparatus, I shall consider the side of the body bearing the furrow as the dorsal surface, from which it follows that the genital openings are situated, as in most Turbellaria and Trematoda, on the ventral surface.

In general shape (Pl. 18, fig. 1) the Sudan Sanguinicola resembles the Sanguinicola described by Plehn. As Plehn describes, and as is evident even in preserved material, the body is highly contractile and in much contracted specimens may be a broad oval in outline.

As regards the internal organization, the two chief systems of organs to be described are the gut and the genital apparatus ; I have nothing to add to Plehn's description of the nervous and excretory systems and the general histology.

The gut, as shown in figs. 1, 4, 5, 6, is in a much reduced condition as compared with the gut in most Turbellaria and Trematoda ; but that it is a gut and not a ' frontal gland ' is shown not only by the presence of a well-marked muscular sucking pharynx (PH) and by the non-glandular character of the wall of the gut sac (GS), but also by the occasional presence of distinct blood-corpuscles in its lumen (seen in two of my specimens). The anterior mouth-opening (M) is, as in all

be detected by careful focusing ; also in young specimens the spinelets are smaller than in full-grown forms and in very young forms are absent. I hardly like to suggest that a conjunction of these two conditions is responsible for the description of the S. inermis of Dr. Plehn, but it appears to me that the suggestion might be applicable. The difference of size between the two species S. armata and S. inermis quoted by Lühe is of no specific value.

blood-sucking animals, extremely minute, and this leads into a short, very narrow, though distensible channel opening into the thick-walled (when empty) elongated pharynx (PH) which extends posteriorly as far as the transverse nerve-commissure (NC). This pharynx is capable of great distension (Pl. 18, figs. 4, *c*, *d*). Its wall, when not distended, is of considerable breadth and is transversely striated, and I believe is covered externally by a layer of cytoplasm containing relatively few nuclei (judging from the appearance seen in one of my specimens, on which fig. 5 is based) ; but of this I am not quite certain, and Dr. Plehn may be correct in stating that nuclei are absent in this region of the gut. In most preparations it is difficult to distinguish such nuclei from the nuclei of the layer of muscle-cells (Pl. 18, fig. 1, PMUS) which is connected with the outer wall of the pharynx. From the posterior end of the pharynx to the gut sac is a narrow oesophagus (OE), with a wall much thinner than that of the pharynx and covered externally with a layer of nucleated cytoplasm. The oesphagus apparently lies ventral to the nerve commissure. The gut sac (GS), situated at the hind end of the anterior third of the body, is somewhat irregular in shape, but is more or less compact and possesses none of the distinct four or five lobes figured by Plehn. Its wall consists of a thin, occasionally nucleated, layer of granular cytoplasm. Dr. Plehn, in her first description [1] of Sanguinicola as a Turbellarian, naturally regarded the anterior canal and sac as a gut ; but she states that it only contains a ' feinen Brei ' and that blood-corpuscles are never found in the lumen, because the mouth and anterior canal are too narrow to admit of their entrance. But the mouth and all parts of the canal are capable of considerable distension, as is shown in my specimens, and in two cases I have found distinct rows and small masses of evident fish blood-corpuscles in the anterior end and hind part of the pharynx and in the sac. There is thus no question concerning the gut-nature of the anterior canal and sac, and this fact definitely settles the non-Cestode nature of Sanguinicola.

[1] "Sanguinicola armata und inermis (n. gen. n. sp.), n. fam. Rhynchostomida. Ein ento-parasitischer Turbellar im Blute von Cypriniden ", ' Zoologischer Anzeiger ', Bd. xxix, p. 244, 1905-6.

Sanguinicola is hermaphrodite and its reproductive organs have been well described by Dr. Plehn in her first paper,[1] but, judging by my specimens of the Sudan Sanguinicola, she has erred in several respects in her second revised description of Sanguinicola as a monozoan Cestode, as I shall indicate later.

The ovary (ov) in the Sudan Sanguinicola is bipartite in form and is extensive, occupying the margins of nearly the whole of the anterior two-thirds of the body, external to the testes, gut sac, and pharynx (Pl. 18, fig. 1). The oviduct (ovd) is median and, according to the convention I have already adopted, dorsal in position, i. e. on the side of the body bearing the longitudinal furrow (Pl. 18, fig. 2). Ovarian follicles are to be found anteriorly at the sides of the pharynx (interspersed with the muscle-cells surrounding this organ), and they extend posteriorly, on each side, opening into the median oviduct by some eight or more transverse ducts (hidden under the testes in fig. 1). Posteriorly to the last pair of transverse ducts, the oviduct, in a dorsal view (Pl. 18, fig. 1, shows the ventral aspect), turns to the right above the median vas deferens; then, becoming much dilated, bends again to the left and somewhat posteriorly, passing ventral to the vas deferens, and finally, becoming narrow, runs posteriorly on the left-hand side, to open by a narrow aperture into the distinct spherical fertilization chamber (otp) on its posterior aspect. From the anterior side of the fertilization chamber and by a similar small opening, arises the vagina (vagn), which is short, narrow at first, and wider after (vagb), and opens on the ventral surface by a circular vaginal pore (vagp), which lies at the end and at the bottom of an elongated ridged muscular oval groove (vagr), directed to the left and posteriorly, the vaginal groove (Pl. 18, figs. 1, 7, 7y, 7x, 8). The course of the oviduct I have above described is constant in all my specimens. I may also mention that the oviduct, fertilization chamber, and vagina are all distensible structures, but that I have never observed the openings into and from the fertilization chamber to be otherwise than narrow.

[1] Ibid.

The fertilization chamber is a quite distinct permanent spherical dilatation, sharply demarcated from both the oviduct and the vagina by the narrow openings just referred to, and with distinctive thick walls. It usually contains a large cluster of eggs, and eggs of course are usually to be seen in the oviduct, both anteriorly and posteriorly, but I have not been able to detect either eggs or spermatozoa in the vagina, though the eggs must make their exit by this duct and spermatozoa must enter. The eggs (Pl. 18, fig. 10) in the fertilization chamber (which measure in diameter from 5·2 to 5·6 microns) and oviduct appear to be fully mature, and are certainly not the mere accidentally injected yolk-cells postulated by Dr. Plehn. One trifling and yet in a way important point to mention is that the muscular walls of the dilated portions of the oviduct, situated just below the ovaries, as well as the walls of the vas deferens, show a marked longitudinal striation, and this striation, seen in optical or actual section in slides stained with haematoxylin, bears a superficial resemblance to a mass of spermatozoa, and it was this appearance I imagine which led Dr. Plehn to assume that the portion of the oviduct behind the ovaries represented a vagina. Starting from this assumption, Dr. Plehn was logically led into the further assumptions: (1) that a second female duct was present—the duct she labelled 'Uterus' and supposed by her to be continuous in the young animal with the duct she labelled 'Dottergang' (Pl. 18, fig. 11);[1] (2) that an ootype must be situated between the bases of the two ovaries, but, it not being visible, it was necessary to assume (3) that the animal was not mature, and in view of this immaturity, (4) that the eggs plainly to be seen in the oviduct and in my fertilization chamber were only yolk-

[1] Dr. Plehn says in her second paper that she had previously failed to observe the 'ganz typische Cestodenvagina' full of spermatozoa, but a comparison of the figures in her first and second papers proves that the 'Dottergang-uterus' is the new second female duct figured and not the 'vagina'. In her first paper the 'vagina' (i.e. the oviduct) is shown clearly (and correctly) in all its winding course and full of eggs, not spermatozoa; whereas, in her second paper, it is represented as of the same form but devoid of eggs.

cells which had become prematurely or accidentally shed into the ' uterus '.

Concerning the existence of vitellaria as distinct from ovarian follicles, I am unable to speak with certainty from actual observation, since, as Dr. Plehn admits, they are not in any way distinguishable, and I am willing to allow that, if vitellaria exist, the upper part of the oviduct may function as a vitelline duct, but as regards the existence of a separate female duct, i.e. combined ' Dottergang ' and ' Uterus ' (Pl. 18, fig. 11) in addition to the oviduct figured by Dr. Plehn in her original communication (and by me in fig. 1) and relabelled ' vagina ' in her second paper, I am absolutely certain that, in the Sudan Sanguinicola, such a second female duct does not exist (as I can demonstrate in both whole-mounted specimens and in horizontal and transverse sections) and, since there is no shell-gland and Dr. Plehn did not observe this second female duct when first describing the genitalia, I feel tolerably certain also that it does not exist in the German Sanguinicola.[1] Further, the non-existence of a separate vitelline duct almost implies the non-existence of vitellaria, as also does the presence of globules of presumably some sort of food material in the periplasm of the eggs (Pl. 18, fig. 10). As regards the existence of an ootype, a true ootype, i.e. a chamber into which the ducts of the vitellaria and shell-gland open, obviously cannot exist, since both vitellaria and a shell-gland are absent ; but if it did, it would presumably be found next to the fertilization chamber which I have described and not between the ovaries. Finally, I can see no reason to suppose that the eggs found inside the fertilization chamber are not mature : their position alone certifies their maturity.

In short, the female reproductive system of Sanguinicola is in essentials constructed upon the plan found in many Rhabdocoelida and Polycladida, in which vitellaria are also absent and in which the oviduct is also long and opens directly to the exterior, its end part being differentiated into a fertilization

[1] If this second female duct does exist in the German Sanguinicola, then this is radically different from the Sudan form, but this I am unable to credit without further evidence.

chamber or passage and ootype when present and the ' antrum femininum ' serving as vagina, and Dr. Plehn's first idea of regarding Sanguinicola as an aberrant and much modified Turbellarian has still much to be said in its favour, both from the points of view of the genitalia and the gut. Odhner's comparison of Sanguinicola with certain Malacocotylea was of course based on Dr. Plehn's second but, as I believe, erroneous description of the genitalia of Sanguinicola.

The testes (TES) are bounded anteriorly by the gut sac, and laterally and posteriorly by the ovaries (Pl. 18, figs. 1, 2). They consist of large ovoid or spherical capsules connected with the main vas deferens in the median line by transverse ducts. The main vas deferens (VD) is conspicuous in stained preparations by reason of its longitudinally striated muscular walls, and anteriorly it lies ventral to the oviduct. In occasional specimens there appear to be two or three main longitudinal trunks of the vas deferens anteriorly instead of one. Immediately posterior to the ovaries the vas deferens lies below the oviduct, but turning to the right (viewed dorsally) it passes a b o v e the dilated oviduct, then, becoming considerably dilated, bends sharply to the left (lying parallel with and close behind the dilated limb of the oviduct), and then, as sharply bends again to the right, where it enters the penis which lies posteriad and to the left, and opens to the right of and slightly behind the opening of the vagina. The directions in which the penis and the vagina lie are approximately at right angles to each other, and if we imagine two Sanguinicola to apply their ventral surfaces together, then the direction of the penis of one Sanguinicola will coincide more or less with the direction of the vagina of the other, one (or both) of these Sanguinicola perhaps holding on to the wall of the blood-vessel by the spiny-edged furrow of the opposite surface.

During copulation—a process which I assume to result in a mutual exchange of spermatozoa between two Sanguinicola—the spermatozoa must, in each animal, traverse the vagina and reach the fertilization chamber, where fertilization occurs. After fertilization I assume that the eggs are extruded through the vaginal pore into the blood. Since these eggs are devoid

of shells, it seems to be evident that the eggs must be removed from the blood by the agency of some such external blood-sucking parasite as (in the case of the German Sanguinicola of the carp) the carp-louse, Argulus foliaceus, or (in the case of the Sudan Sanguinicola) other species of Argulidae, or perhaps a leech. In Odhner's sketch of the possible life-history of the German Sanguinicola he omitted to offer any suggestion as to how the fertilized eggs reached the Molluscan intermediate host postulated by him from the fish, other than stating that he found a ' mature ' egg, containing a miracidium, in the kidney of a carp, but to me it is difficult to believe, without further evidence, that a miracidium can develop from a shell-less egg in blood and thence take to water. All animals (except perhaps sponges and other very low forms of aquatic life) which extrude eggs into water, even when the animals themselves are living in water, and even when the eggs are not fertilized until after contact with the water, protect the eggs with shells or envelopes of one kind or another, and much more should this be the case in parasitic forms (like Schistosoma) which have to extrude the eggs first into vertebrate blood and then into open water.

As regards the external vaginal groove, this is possibly connected with the insertion of the penis, though it is difficult to understand on that hypothesis why its direction should be at right angles to that of the vagina and not in line with it.

Finally, I must state that I have adopted the convention of regarding the surface of the body bearing the longitudinal furrow as the dorsal side, because it is usual in both Turbellaria and Trematoda for the sexual openings to be situated ventrally. The fact that in certain Trematodes a similar furrow is situated on the ventral side is but of little importance in this connexion because it is probable that the furrow in Sanguinicola is a special structure adapted to life in blood-vessels, and I am by no means convinced, in view of the Turbellarian conformation of the genitalia in Sanguinicola, that Odhner is right in regarding Sanguinicola as a Malacocotylean, despite the analogies (of the gut and habitat) with Aporocotyle and Deontacylix, though it is true that the genitalia of Sanguinicola may have become

secondarily simplified. The evidence of Looss[1] to the effect that a cercaria stage occurs in the life-history of Sanguinicola requires confirmation before a decision can be arrived at, and if it be confirmed, it will be of interest to know how the naked eggs liberated into the blood of the fish are transported to the intermediate Molluscan host.

DESCRIPTION OF PLATE 18.

♂, male aperture. ♀, female aperture (vaginal pore). 'DG', 'dottergang'. EE, narrow opening from fertilization chamber into vagina. EGG, ripe eggs. GS, gut sac. M, mouth. NC, nerve commissure. OE, oesophagus. OOO, narrow opening of oviduct into fertilization chamber. OTP, fertilization chamber. 'OTP', hypothetical ootype. OV, ovary. OVD, oviduct. OVDB, dilated oviduct. OVDN, narrow portion of oviduct. PEN, penis. PH, pharynx. PMUS, muscle-cells surrounding pharynx. SOL, solid posterior extremity of body behind dorsal furrow. SP, spinelets. TEP, terminal excretory pore. TES, testes. 'UT', 'uterus'. VAG, vagina. 'VAG', 'vagina'. VAGB, dilated part of vagina. VAGN, narrow part of vagina. VAGP, vaginal pore (♀). VAGR, vaginal groove on surface. VD, vas deferens. VF, dorsal body furrow. V–V, W–W, X–X, Y–Y, Z–Z, planes of sections across fig. 7 corresponding approximately to the figures of actual sections in figs. 7, V, W, X, Y, Z.

Fig. 1 (× cir. 87).—Sanguinicola viewed from the ventral aspect.

Fig. 2 (× 180).—Transverse section across Sanguinicola behind the gut sac (the convex surface is ventral).

Fig. 3 (× 39).—Sanguinicola in dorsal surface view.

Fig. 4 A (× cir. 120).—Unusually small contracted pharynx.

Fig. 4, *b*, *c*, *d* (× cir. 120).—The undistended (*b*) and distended (*c*, *d*) pharynx in three Sanguinicola.

Fig. 5 (× 530).—The undistended pharynx.

Fig. 6 (× 530).—The gut sac and opening of oesophagus.

Fig. 7 (× 260).—The posterior genital ducts and openings from the ventral aspect. V–V, W–W, X–X, Y–Y, Z–Z represent approximately the planes of the sections shown in fig. 7, V–Z (× 180).

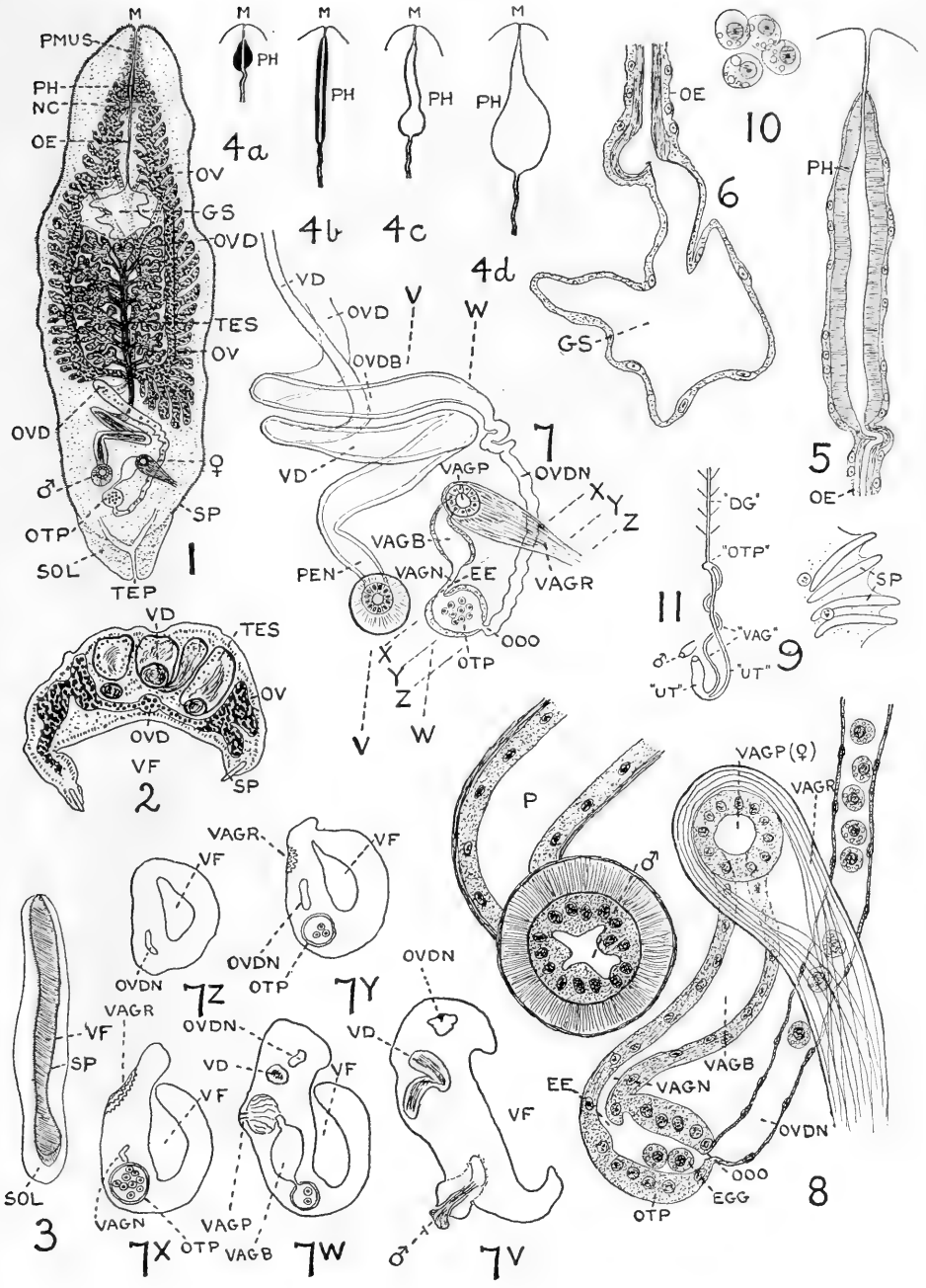
Fig. 8 (× 530).—The posterior genital ducts and apertures magnified to show the character of the walls of the former.

Fig. 9 (× 840).—The spinelets embedded in the edge of the dorsal body furrow.

Fig. 10 (× 840).—Eggs contained in the fertilization chamber.

Fig. 11.—Diagram to show the genital ducts as figured and described by Dr. Plehn in her second paper (1908), for comparison with fig. 1.

[1] Quoted by Odhner.



W. N. F. Woodland, del.

On Centropygus joseensis, a Leech from Brazil.

By

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With 10 Text-figures.

During the Percy Sladen Expedition to Brazil in 1913 Professor J. P. Hill collected six specimens of land leeches, and while I was on leave from the Australian Imperial Force in France, and acting as Demonstrator in his department, he requested me to describe them. I was glad of this opportunity, for these leeches, themselves earthworm-like in appearance, were easy to determine as close relatives of Lumbricobdella, that interesting leech from Brazil, which has so closely copied the form and habit of an earthworm.

I have determined these specimens as

Centropygus joseensis (Grube et Oerstedt, 1859).
Syn. Centropygos joseensis (Grube et Oerstedt, 1859).
Centropygos jocensis (Grube et Oerstedt, 1859).
Cylicobdella lumbricoides (Grube, 1871).
Nephelis tergestina (R. Blanchard, 1892).
Liostomum joseense (Grube et Oerstedt, 1859; R. Blanchard, 1896).

In view of the want of any note (save Weber, 1914) on this leech, made in its natural state, the following extract from Professor Hill's diary is of value:

State of Rio, Brazil, at Government Orchard, Macieiras, altitude 1,500 metres. Found two species (?) of leeches, first

one under clod of earth coiled knot-like, bright red in colour darkening to the posterior end; second specimen under a stone. A third specimen found in earth on dislodging a buried log, darker in colour, and probably belongs to the same species as a fourth specimen, a much larger leech and slaty black in colour. On following day found two more land leeches similar to the first.

I have laid stress on this description, for the next year Weber (1914), describing certain leeches from Columbia as '*Centropygus joseensis*', for the first time mentions their blood-red colour.

The specimens collected by Professor Hill consist of five small and one larger leech. The former measure from 40 to 80 mm. long by 3 to 3·5 mm. in diameter—the latter single specimen is 130 mm. long by 7 mm. diameter.

Historical.

The genus *Centropygus* was first established by Grube in 1859 to contain a leech which Blanchard thinks came from San José near Panama, the generic name being given because the anus was erroneously supposed to open in the centre of the posterior sucker. In 1871 Grube described the genus *Cyclicobdella* in which he placed *C. lumbricoides*, a leech from Desterro.

Blanchard, who has examined these two types, found them to belong to the same species, and in this species placed also a leech described by him as *Nephelis tergestina*, and gave the generic name *Liostomum* priority.

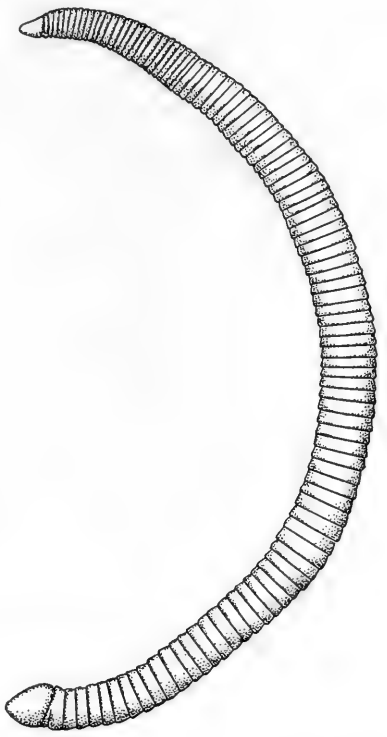
Later, he restored the name *Centropygus* to the genus which contains *C. joseensis* from Trinidad, and another characterized by its blood-red colour described by Kennel in 1886 as *C. coccinea*, the colouring of *C. joseensis* being still unrecorded.

Kennel (1886) gave an excellent account of this species, and separated it from *C. lumbricoides* on certain anatomical details which I will mention later.

Weber (1914) was the first to record the colour of living specimens of *C. joseensis*, and for want of better informa-

tion he described the smaller of these specimens as C. coccinea. He laid stress on the fact that apart from size he could distinguish no difference in the specimens: the form, number of annuli, and the position of the genital pores were the same, small variations occurring in the number of annuli, as was already mentioned by Kennel (1886) and Blanchard (1896).

Text-fig. 1.



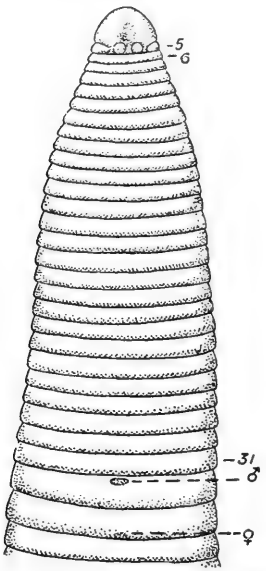
Lateral view of C. joseensis from a preserved specimen 42 mm. long.

Kennel (loc. cit.) separated the species C. coccinea from C. joseensis for the following reasons: in C. coccinea the size is smaller, the ovaries lie ventral to the gut, between it and the nerve-chain, and the anterior part of the mid-gut has no blind sac at its transition into the mid-gut, whereas in C. joseensis as well as the greater size of the leech, the ovaries lie under the lateral blood-vessel and a blind sac is present. He admits that all the specimens of C. coccinea he examined were sexually immature, but does not think that this would account for the differences enumerated. To determine the position of the specimens I dissected one, and cut serial sections of another, and I will be able largely to confirm Kennel's work and bring it up to date, especially as regards the relations of the ganglia and annuli, and to add certain new details.

Distribution.

Blanchard (1896), describing members of this species collected by Borelli in Paraguay and Uruguay, says: 'This species is widely distributed in Central America, and has been collected in Rio de Janeiro, São Paulo, Pará, the basin of the Xinqu,

Text-fig. 2.



Anterior end of C. joseensis from the ventral surface to show the annulation and genital openings.

from San Bernardo, Paraguay, from Chiriqui (Central America), from Caracas, Puerto Cabello, Venezuela, Santa Catharina (Brazil), and Rio Grande do Sul.

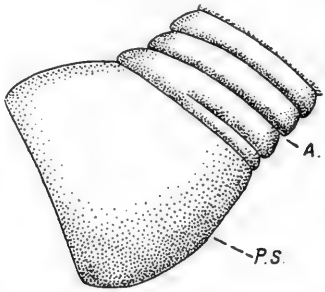
'This leech also extends very far towards the East. It is recorded from Antisana, Ecuador, that is to say to the very middle of the Eastern foothills of the Andes. Does this species cross the mountains, and is it found on the Western slope? Nothing is known to this effect yet, but it is not impossible since other species can live on either slope of the Cordillera.'

Annulation and Measurements.

In dealing with the annulation of these leeches it is necessary to define precisely the method adopted in the enumeration of the annuli. The earlier workers counted as first that annulus which completely surrounded the body, so that the male pore was given as opening between the 26th and 27th annuli.

To make their enumerations tally with those of recent workers, there must be added the four annuli on the dorsal surface of the anterior sucker and the annulus which is immediately below

Text-fig. 3.

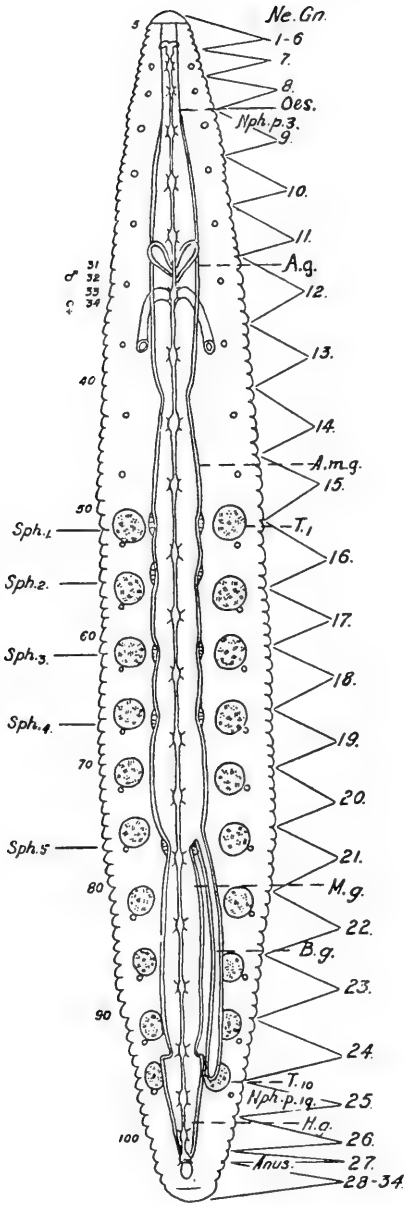


Lateral view of the posterior end of C. joseensis. *A.*, position of anus. *P.s.*, posterior sucker.

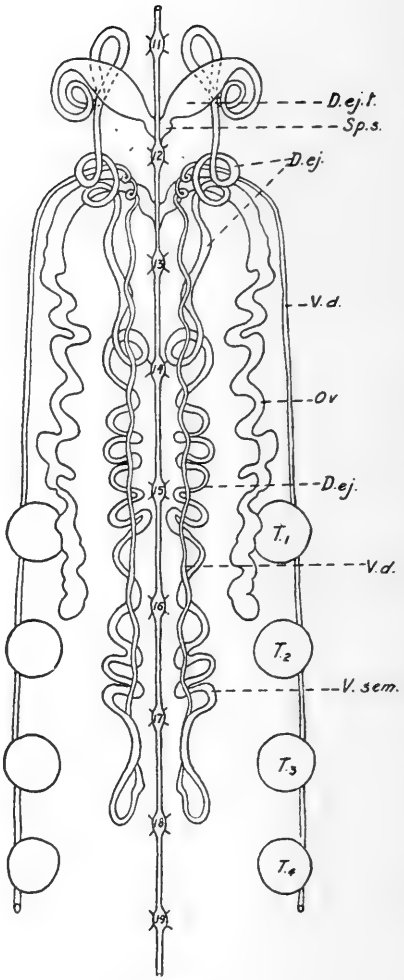
these divisions but is incomplete ventrally, where it forms the lateral boundaries of the mouth cavity.

Again, in regard to the position of the anus, which both Blanchard and Kennel show as between the annuli which are second and third from the posterior sucker, I find that in only two of my specimens is there any indication of the last annulus which marks the dorsal surface of the posterior sucker (Text-fig. 3).

I have shown in Text-fig. 4 the relations of the nerve ganglia to the annuli, and defined the somites on a neuromeric basis. This figure is drawn from the dissection of a specimen 80 mm. long, and certain details added from the serial sections of a specimen 60 mm. in length. The six specimens which I examined ranged in length from 40 to 130 mm., and in breadth from 3 to 7 mm.



TEXT-FIG. 4.



TEXT-FIG. 5.

TEXT-FIG. 4.

Diagram of Centropygus joseensis showing the annuli and their relation to the nerve ganglia, the somite limits, the alimentary reproductive and nephridial systems as seen from the dorsal surface of a dissected specimen. *Ne.gn.*, nervia ganglia and somite limits. *Nph.p.* 3, nephridiopore 3, &c. *Nph.p.* 19, nephridiopore 19. *Oes.*, oesophagus. *A.g.*, anterior gut. *A.m.g.*, anterior portion of mid-gut. *M.g.*, mid-gut. *H.g.*, hind-gut. *B.g.*, blind pouch of anterior portion of mid-gut. *Sph.*$_1$, &c., sphincters of anterior part of mid-gut. *T.*$_1$, testes, first pair. *31*, *32*, male opening. *33*, *34*, female opening.

TEXT-FIG. 5.

Diagram of the reproductive system of Centropygus joseensis as seen from the dorsal surface of a dissected specimen. *T.*$_1$, first pair of testes, &c. *V.d.*, vas deferens. *V.sem.*, vesicula seminalis. *D.ej.*, ejaculatory canal. *D.ej.t.*, terminal portion of ejaculatory canal. *Sp.s.*, spermatophore sac. *Ov.*, ovary. *11*, *12*, &c., nerve ganglia.

The total number of annuli varies from 102 to 104. In all the male pore is placed between the 31st and 32nd annulus, and the female between the 33rd and 34th annulus (Text-fig. 2).

Alimentary System.

The mouth placed at the base of the spoon-shaped anterior sucker is bounded in preserved specimens by two well-marked lobes, which abut on the ventral ends of the 5th annulus (Text-fig. 2). These lobes are a very characteristic feature in all the preserved specimens I have examined; but an examination of sections leads me to believe that they are partly of an oedematous nature—several longitudinal folds furrow the concavity of the anterior sucker.

The pharynx has a well-developed musculature of longitudinal circular and radial fibres, and from it the oesophagus extends and passes into the anterior gut which in its turn gives place to the anterior portion of the mid-gut in the 14th somite. There is a narrowing of the lumen at this point caused by convolutions of this part of the gut. The anterior part of the mid-gut extends to the 21st somite; its relations to other structures, the sphincters surrounding it, and the blind-gut which arises from it are shown in Text-fig. 4 (*Sph.*$_{1\text{-}5}$, *B.g.*).

These last two features—the sphincters and the blind-gut—are of considerable importance in distinguishing C. joseensis from C. coccinea. There are five sphincters, one placed at the end of each somite from the 15th to the 18th; the 5th sphincter is in the 21st somite immediately behind the blind-gut (Text-fig. 4, *Sph.*$_{1\text{-}5}$). Each consists of a layer of circular muscle-fibres surrounding the gut; in preserved specimens, being relaxed, they produce little constriction. The sphincter in the 21st somite is particularly well developed.

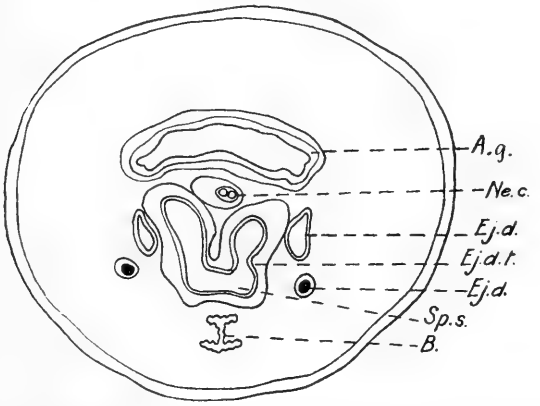
Another feature of systematic importance is the development of a single blind-gut which occurs in C. joseensis but not in C. coccinea. This is given off at the termination of the anterior portion of the mid-gut in the 21st somite; it extends to the 24th somite, lying to the right of the mid-gut. Kennel (1886) figures the blind-gut as coming off on the right

of the anterior part of the mid-gut, but I have seen it coming off on the left in a specimen of 60 mm.

In structure the blind-gut resembles the anterior part of the mid-gut, of which it appears to be a backward prolongation, but no lymph spaces surround it and its circular musculature is better developed (Text-fig. 9, *B.g.*).

The mid-gut extends from the 20th somite to the beginning of the 24th somite; it is not surrounded by a lymph space

TEXT-FIG. 6.



Transverse section of Centropygus joseensis at the level of the male opening, showing the junction of the two horns of the terminal portions of the ejaculatory duct to form the spermatophore sac and parts in relation thereto. (× 30.) *A.g.*, anterior gut. *Ne.c.*, nerve-cord in ventral lacuna. *Ej.d.*, ejaculatory duct. *Ej.d.t.*, terminal portion of ejaculatory duct. *Sp.s.*, spermatophore sac. *B.*, male bursa.

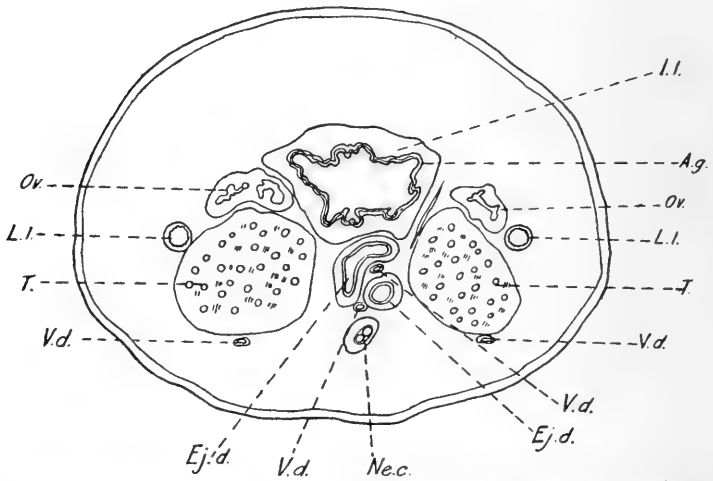
like its anterior part. At its termination on the 24th somite there is a sphincter which separates it from the hind-gut. The anus is placed above the 27th nerve ganglion.

REPRODUCTIVE SYSTEM.

There are ten pairs of testes, a pair being placed within the 1st and 2nd annulus in each somite from the 16th to the 25th (Text-figs. 4, 5, $T._1$, &c.). Kennel (loc. cit.) states that he has found twelve pairs of testes, but in my serial sections of a

60 mm. specimen and in a dissection of an 80 mm. specimen there are only ten pairs. Still, it is not an uncommon thing in leeches for a pair or more testes to be wanting, and Kennel shows in his figure that the 1st and 3rd testis of the right side are wanting. Each vas deferens lies ventral to the testes and runs anteriorly from somite 25 to somite 12; here it turns medially and then runs posteriorly to the 18th somite, where

Text-fig. 7.



Transverse section of C. joseensis at the level of the 16th somite, showing the relation of the various systems. (×30.) *I.l.*, intestinal lacuna of the anterior portion of the mid-gut. *Ov.*, ovary. *L.l.*, lateral lacuna. *T.*, testes, first pair. *V.d.*, vas deferens ascending part under testis, descending part ventral to ejaculatory duct. *Ej.d.*, ejaculatory duct. *Ne.c.*, nerve-cord.

it turns and passes forward as the vesicula seminalis, a convoluted tube packed with sperms, which lies with the vas deferens in connective tissue dorsal to the ventral lacuna. The vesicula seminalis develops muscular walls and becomes the ejaculatory duct in somite 15. The ejaculatory duct of either side pursues a convoluted course until it reaches its glandular terminal portion; this part joins with its fellow and their distal ends form the spermatophore sac which opens into the male bursa (Text-fig. 6, *Sp.s.*).

The ovaries (Text-figs. 5, 7, *Ov.*) are placed in a connective tissue which lies close to the testes. They are much convoluted and extend on either side from somite 12 to somite 16; anteriorly they unite at the female atrium.

This reproductive system shows certain affinities with that of other Herpobdellids, but there is not seen the conducting tissue as described by Brumpt (1899) for H. atomaria.

Nephridia.

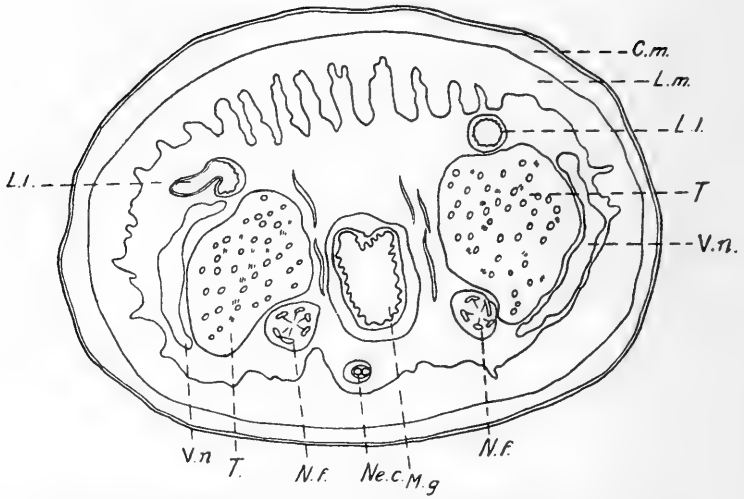
There are nineteen pairs of nephridia placed in somites 7 to 25. As usual the nephridiopores open on the latero-ventral aspect of the annulus preceding that in which the nerve ganglion lies.

The general structure of the nephridium follows that seen in other members of the Herpobdellidae.

Ciliated funnels have not previously been found in Centropygus joseensis. Kennel was unable to find them; he says that 'in spite of careful examination of serial sections I have never seen a picture which would allow one to conclude the presence of a widely open funnel, and if any opening exists it should be fairly narrow'. These ciliated organs are found in each somite, and in the testicular region one is placed in a dilatation of a branch of the lateral lacuna (lateral blood-vessel of early authors) which lies immediately ventral to each testis (Text-fig. 8, *N.f.*).

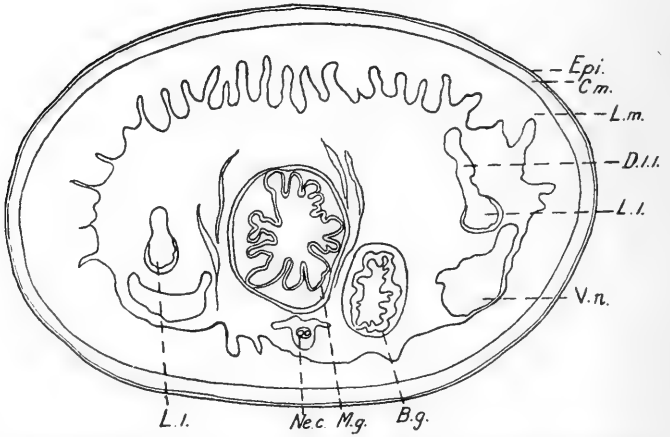
The structure of the ciliated organ in this leech has some resemblance to that of Nephelis as figured and described by Graf (1899). A comparison of a transverse section of a ciliated funnel of C. joseensis, as shown in Text-fig. 10, with Graf's figures of the ciliated organ of Nephelis quadrostriata, shows the similarity of these organs. The terminal vesicle of each nephridium is particularly well developed and lies on either side at the level of the nerve ganglion in relation to it; portion of the lumen of the vesicle is taken up by the bulging into it of the testis.

TEXT-FIG. 8.



Transverse section of C. joseensis, in the 20th somite, showing the position and relative size of the nephridial funnels. (× 30.) *C.m.*, circular muscle layer. *L.m.*, longitudinal muscle layer. *L.l.*, lateral lacuna. *T.*, testes, fifth pair. *V.n.*, terminal vesicle of nephridium. *N.f.*, nephridial funnel. *M.g.*, anterior portion of mid-gut. *Ne.c.*, nerve-cord in ventral lacuna.

TEXT-FIG. 9.

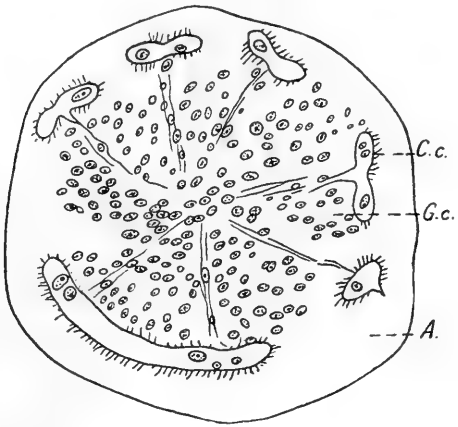


Transverse section of C. joseensis in the 22nd somite, showing the relation of the blind-gut to the middle-gut. (× 30.) *C.m.*, circular muscle layer. *Epi.*, epidermis. *L.m.*, longitudinal muscle layer. *D.l.l.*, dorsal branch of lateral lacuna. *L.l.*, lateral lacuna. *V.n.*, terminal vesicle of nephridium. *B.g.*, blind-gut. *M.g.*, middle-gut. *Ne.c.*, nerve-cord in ventral sinus.

Coelomic System and its Modifications.

The lateral lacunae (lateral blood-vessels of authors before Oka (1912)) extend throughout the body, being much reduced at either end; in each somite there are regularly arranged communications dorsally between the lateral lacunae and ventrally with the ventral lacuna (Text-fig. 9, *L.l.*). There is no dorsal lacuna and this is absent also in other Herpobdellids, and the ventral lacuna is small and contains only the

Text-fig. 10.



Transverse section of a ciliated funnel of C. joseensis, showing the ciliated crown cells and the granular cells. *C.c.*, ciliated crown cell. *G.c.*, granular cells. *A.*, ampulla.

nerve-cord and ganglia and forms no expansion anteriorly to contain the reproductive organs.

Sense Organs.

These consist of a series of flask-shaped organs chiefly arranged along the edge of the oral sucker. They have been described and figured by Kennel (1886), and I have nothing to add to his description.

I wish to thank Professor J. P. Hill, of University College, London, for his kindly assistance whilst engaged in this work in his department.

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Some Observations on the Hypophysis of Petromyzon and of Amia.

By

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With 34 Text-figures.

Contents.

I. Petromyzon.

Introduction.

The hypophysis of Petromyzon has been the object of research of many observers, more indeed than will be referred to in this paper since complete bibliographies are to be found elsewhere, and it might almost seem as though any further work on the same subject could not but be repetition. It seemed to me, however, when considering the problems presented by the hypophysis in the light of more recent

research that there are a few points which deserve further attention.

The main outlines of the structure and development of the hypophysis of Petromyzon have been known since the work of Dohrn, Götte, and Scott, and the problems of the hypophysis then presented themselves, but they have been answered in many contradictory ways by them and the many other observers who have devoted their attention to them. Shortly, the main problems are the following :

(1) What is the primitive position of the hypophysis, and has it any primitive relations with the mouth or the nose or any other organs ?

(2) What is the primitive method of its development given the great diversity which exists in the vertebrates, and especially the great difference presented by Cyclostomes as compared with almost all other vertebrates ?

In this paper I am concerned with these two questions and am led to discuss a few more, such as the relations of the hypophysial cavity to the glandular portions of the pituitary body and the possible presence of one of these portions in Petromyzon corresponding to the pars tuberalis of higher vertebrates.

I wish to express my thanks to Mr. Huxley and Dr. Hogben, not only for stimulating my curiosity in some of these questions but also for useful criticism. To Professor Goodrich also my gratitude is due for helpful advice.

Methods call for no special comment ; as complete a series of embryos as possible was studied, cut into serial sections in the transverse, sagittal, and horizontal planes. Stains such as picronigrosin and Lichtgrün were useful in detecting boundaries and limiting membranes where such were obscure. The material of Petromyzon planeri was collected during a stay at Naples, and some stages were supplemented by the kindness of Professor Goodrich. The work was done in the Department of Zoology and Comparative Anatomy at Oxford.

The origin of the hypophysis in Petromyzon from the anterior surface of the head and close to the rudiment of the olfactory organ is familiar in every text-book, after the researches of

Dohrn (1883), Götte (1883), Haller (1896), von Kupffer (1893), Scott (1888), Stendell (1914), Sterzi (1904), and Woerdemann (1914). (To Sterzi's work I have unfortunately been unable to gain access.) It is described as an invagination giving rise to a cavity into which in the adult the olfactory organs open and which extends posteriorly for a considerable distance between the brain and the gut.

In those cases where the hypophysis arises within the stomodaeum (Selachians and Amniotes) it may develop either as a hollow invagination (Rathke's pocket) or as a solid ingrowth of a mass of cells within which the hypophysial cavity may make its appearance later. Where it arises outside the stomodaeum as in Amia (Reighard and Mast 1908; the writer, see part ii of this paper) or in Amphibia (Götte 1875, Atwell 1919) no invagination occurs, but the rudiment is a solid plate pushing in from the ectoderm. Now the hypophysis of Petromyzon certainly arises outside the stomodaeum, but it is situated next to the region which enlarges enormously during development, viz. the upper lip. It was my first object to decide whether the so-called invagination is due to the growth of the parts composing its walls or to actual invagination, or to both causes. Pending decision on this point I shall call it the hypophysis depression.

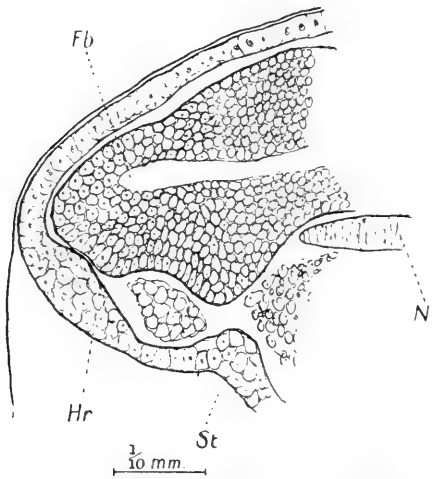
The Hypophysis Depression.

In the earliest stage at which any rudiment of the future organ can be discerned (Text-fig. 1) a thickening of ectoderm on the antero-ventral surface of the head marks the region where the hypophysis and olfactory organs will develop. On the supposed primitive nature of the connexion between these organs I have little to say except that I see no reason for regarding it as anything more than a topographical one, two organs both developing from the ectoderm at the same time and in the same restricted region, viz. the antero-ventral surface of the head of necessity arising together. There is at this stage (just before hatching) no trace of invagination. The stomodaeum is indicated and is separated from the hypophysis

rudiment by a certain distance, occupied by flat ectoderm; there is as yet no upper lip.

In an embryo twelve days old from the time of fertilization (Text-fig. 2), which has consequently hatched, the anterior region of the brain has extended slightly and the antero-ventral surface of the head lies practically horizontal. A depression U-shaped in section near the anterior extremity is the invagina-

TEXT-FIG. 1.



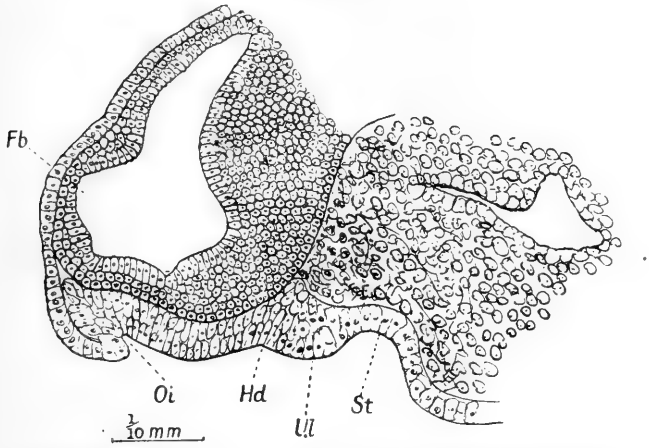
Petromyzon planeri. Embryo just prior to hatching, rudiment of hypophysis and stomodaeum.

AdO, adhesive organ. *A.O.*, accessory organ. *D.W.*, dorsal wall of fore-gut. *F.b.*, fore-brain. *H.c.*, hypophysial cavity. *H.d.*, hypophysial depression. *H.r.*, hypophysial rudiment. *H.s.*, hypophysial strand. *I.*, infundibulum. *L.l.*, lateral lobes of 'Uebergangsteil'. *N.*, notochord. *N.c.*, cartilage of nasal capsule. *O.ch.*, optic chiasma. *O.i.*, olfactory invagination. *O.n.*, olfactory nerve. *P.A.*, pars anterior. *P.E.*, pineal eye. *P.I.*, pars intermedia. *P.N.*, pars nervosa. *S.i.h.*, solid ingrowth of the hypophysis. *St.*, stomodaeum. *T.c.*, trabecula cranii. *U.*, 'Uebergangsteil'. *U.l.*, upper lip.

tion of the olfactory organ. Farther back is the larger similarly U-shaped invagination of the stomodaeum; but between them is a slight dent on the surface corresponding to a region where ectoderm cells are pushing in beneath the brain. This is the

rudiment of the hypophysis. The region of ectoderm between it and the stomodaeum is no longer flat but convex, being the beginning of the expansion of the upper lip. Is the dent the beginning of a true invagination or is it due to the expansion of the upper lip ? The latter I believe to be the true interpretation. It bears no resemblance to the normal appearance of Rathke's pocket or any invagination, as may be seen by com-

Text-fig. 2.



Petromyzon planeri. Embryo just hatched, beginning of hypophysis depression.

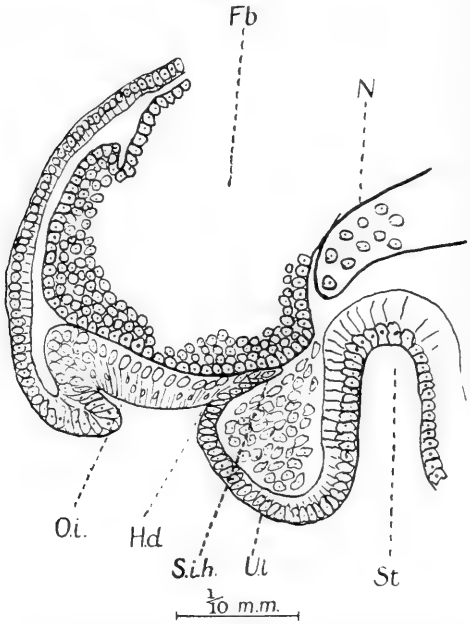
paring it with the stomodaeum or the olfactory organ. An invagination presents one concave curve; the hypophysis depression is made up of two convex curves meeting at an apex. There is a solid inpushing of cells at this stage although the depression is a mere dimple, whereas in those cases where the hypophysis arises by invagination it is sunk beneath the surface by means of that invagination which must be almost as deep (see Text-fig. 23 *a*).

The upper lip continues to increase in size, and the hypophysis depression comes to assume the form of a cleft which deepens and the upper and lower sides of which become more and more parallel as the upper lip enlarges. At the same time the sides of the upper lip grow up with the sides of the anterior

surface of the head so as to leave the hypophysis depression as a tube which becomes deeper by the lengthening of its walls. The beginning of this process is shown in Text-fig. 3, and it is more accentuated in Text-figs. 4 and 5.

The apex of the V, i.e. the limit of the depression, does not move farther back relatively to the rest of the head. It never

TEXT-FIG. 3.



Petromyzon planeri. Embryo fourteen days old, beginning of expansion of the upper lip.

quite reaches the optic chiasma. Meanwhile the solid ingrowth has extended until it almost reaches the tip of the notochord. To verify this I have resorted to measurements, though I do not set much store by them owing to the difficulty of measuring with accuracy and of selecting standard measuring lines since no points can be assumed to be fixed.

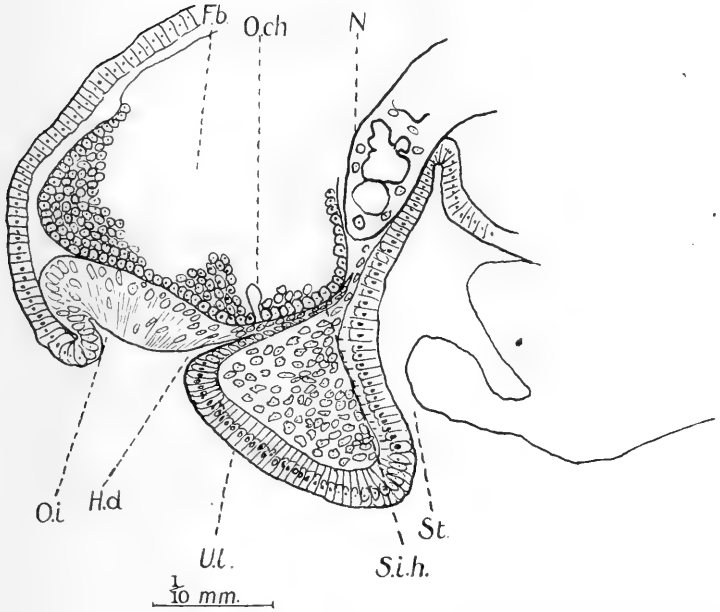
A. Distance from apex of depression to notochord.

B. Distance from apex of depression to extremity of upper lip.

Embryo represented

In Figures :	*A.*	*B.*
3	30	15
4	30	25
5	30	60
6	50	120
7	80	140
8	280	440

TEXT-FIG. 4.



Petromyzon planeri. Embryo twenty-two days old.

Since the measurements are all to the same scale the units are unimportant.

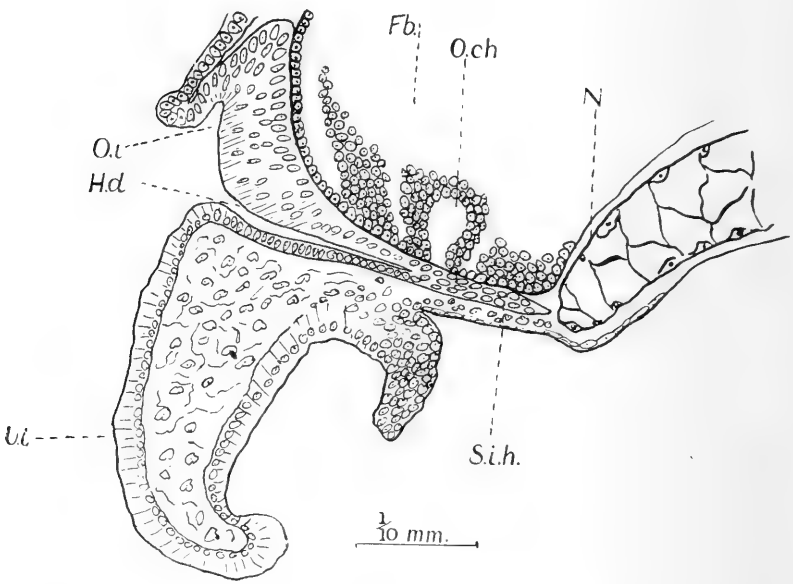
The fact that the distance between the apex of the depression and the notochord does not decrease, while that between the apex and the extremity of the upper lip increases rapidly, supports my contention that the hypophysis depression is not an invagination but is brought about by the relative growth

of the upper lip. The relations of the apex to the optic chiasma confirm this.

The solid ingrowth of the hypophysis extending by itself from the region of the optic chiasma to the notochord does so not by the deepening of the hypophysis depression but by its own lengthening.

It is usually stated that the monorhinal condition of the

TEXT-FIG. 5.



Petromyzon planeri. Embryo thirty-two days old.

lamprey is due to the connexion of the hypophysis with the olfactory organs which thus communicate with the exterior by means of a single (hypophysial) aperture. It appears to me to be easier to regard the agent as the upper lip in conjunction with the sides of the anterior surface of the head, which by their expansion cause both organs to be situated in the same secondary depression.

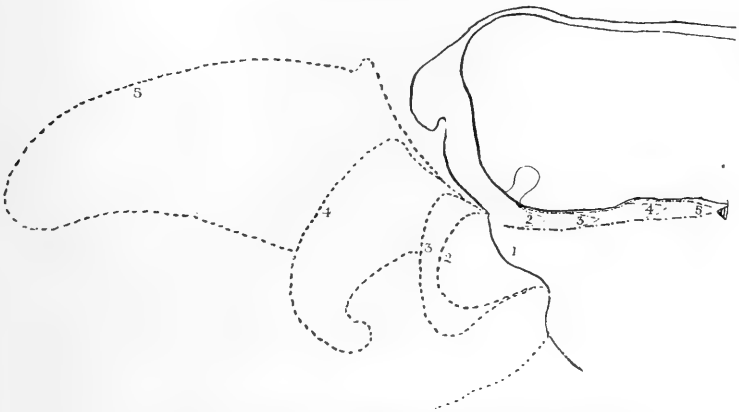
The hypophysis of Petromyzon then belongs to the class of

those which develop by a solid inpushing. A diagrammatic representation of the growth of the upper lip and formation of the hypophysis depression is given in Text-fig. 6.

The Formation of the Hypophysial Cavity.

The solid ingrowth lies between the dorsal wall of the gut and the floor of the brain, separated from them by well-marked membranes. In the stage represented in Text-fig. 7 no glandular

TEXT-FIG. 6.

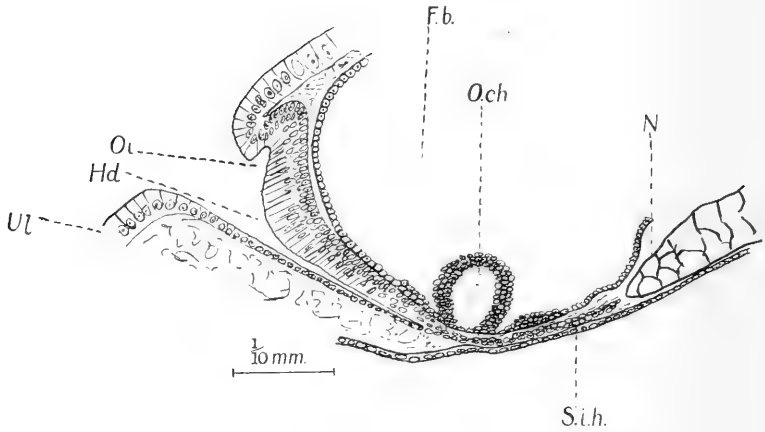


Petromyzon planeri. Diagram showing the expansion of the upper lip and formation of the hypophysis depression.

differentiation has as yet occurred. The first indication of the histological differentiation which will produce the future pituitary body is to be found in the floor of the brain. Just dorsal to the posterior portion of the hypophysis a thickening of the brain-floor occurs composed of neuroglia (Text-fig. 8), thus foreshadowing the pars nervosa. There is no trace of hypophysial cavity.

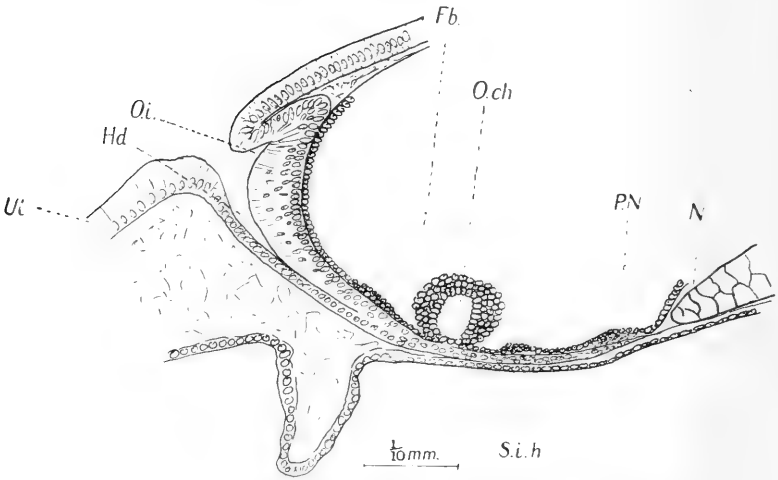
In a later stage (35 mm., Text-fig. 9, and under higher magnification in Text-fig. 10) the hypophysis is beginning to undergo its histological differentiation (changes which will result in the formation of the glandular elements which it contributes to the pituitary body). Beneath the optic chiasma the strand of tissue

TEXT-FIG 7.



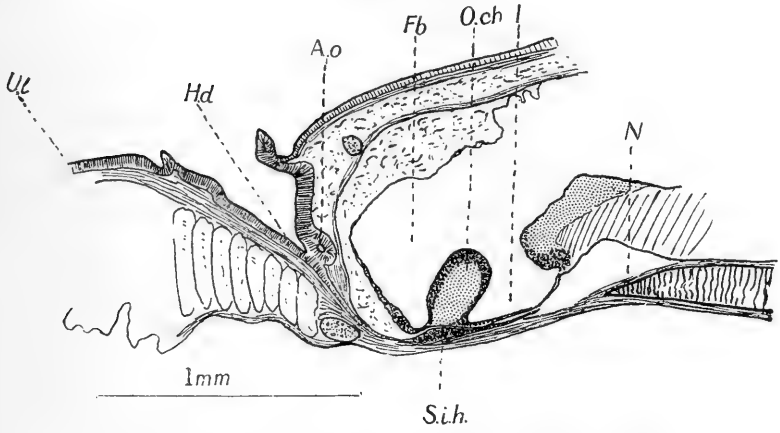
Petromyzon planeri. Embryo 10 mm. long.

TEXT-FIG. 8.



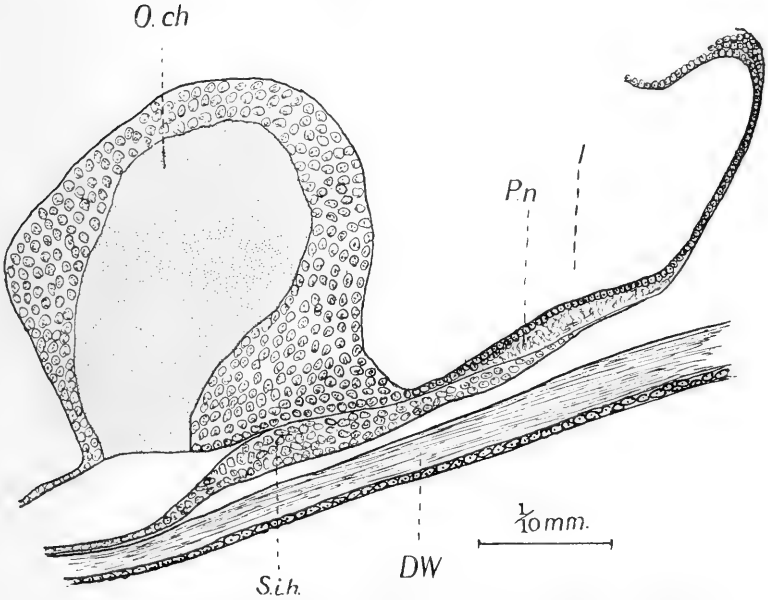
Petromyzon planeri. Embryo 15 mm. long. Appearance of neuroglia thickening in the wall of the infundibulum.

TEXT-FIG. 9.



Petromyzon planeri. Embryo 35 mm. long.

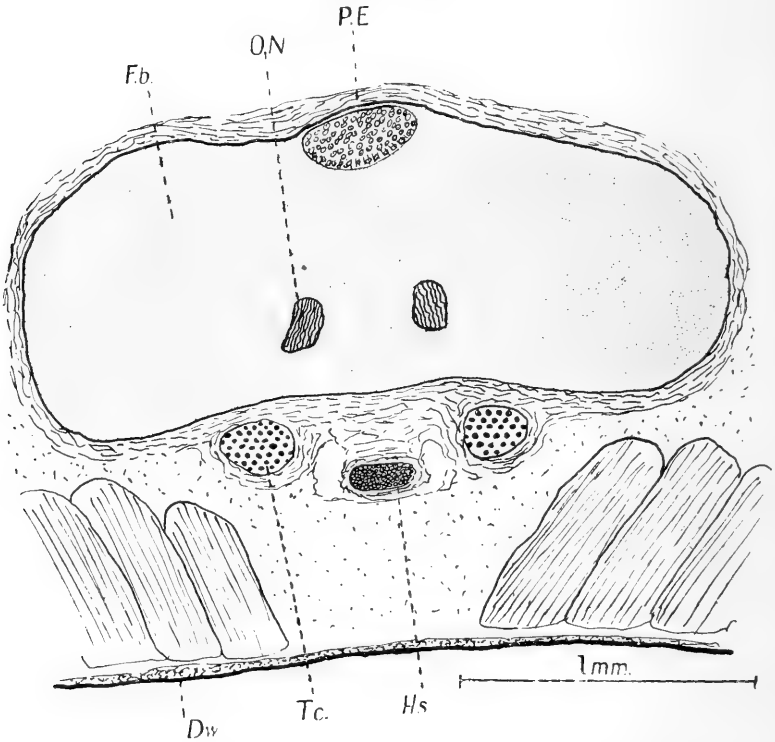
TEXT-FIG. 10.



Petromyzon planeri. The same embryo highly magnified. Differentiation of the glandular elements. No trace of a hypophysial cavity.

thickens and its cells become ovoid and glandular and less closely packed. Dorsally this thickening is for the greater part of its length closely adpressed to the floor of the infundibulum. The layer of neuroglia which separates the cells lining the infundibular cavity from the outer membrane of the brain-

Text-fig. 11.

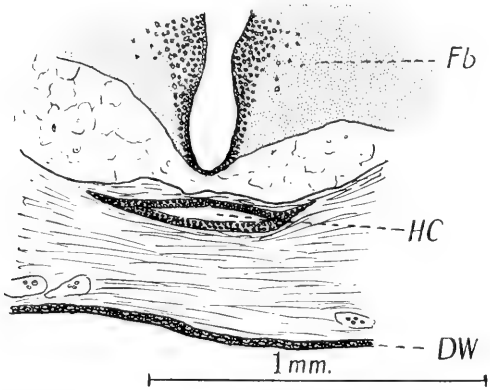


Petromyzon fluviatilis. Ammocoete. Transverse section just posterior to the apex of the external depression showing absence of hypophysial cavity.

wall has also thickened. All this time the apex of the hypophysis depression has not moved, and the differentiation of the glandular hypophysis takes place from a solid strand of cells, there being still no true hypophysial cavity.

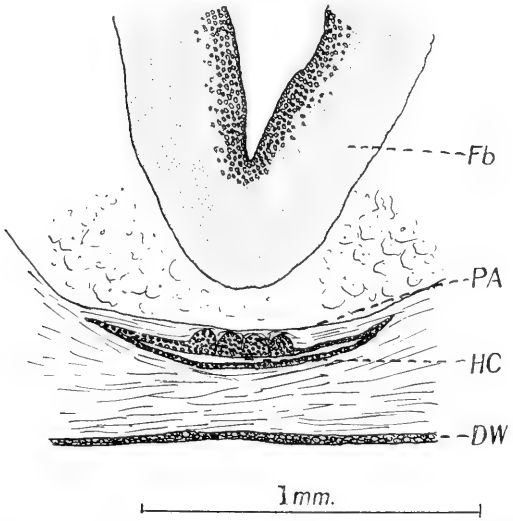
In the Ammocoete a cavity appears in the region just anterior to the gland. It has no connexion with the external depression

TEXT-FIG. 12.



Petromyzon fluviatilis. Ammocoete. Transverse section slightly farther back and anterior to the gland showing the hypophysial cavity in the middle of the hypophysis.

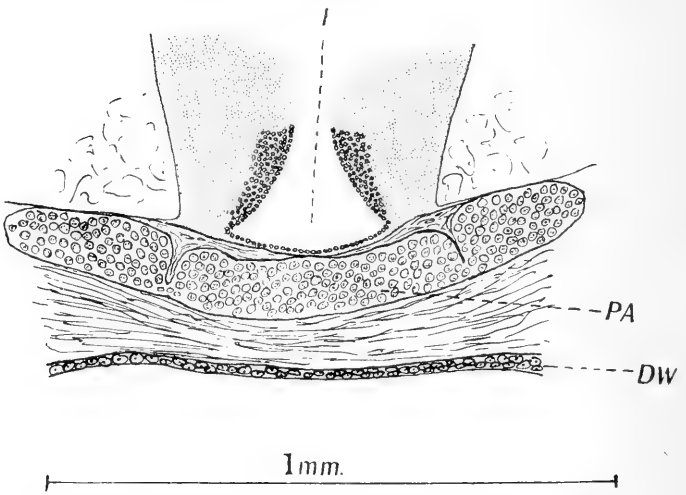
TEXT-FIG. 13.



Petromyzon fluviatilis. Ammocoete. Transverse section still farther back showing the relation of the gland to the hypophysial cavity.

(Text-fig. 11) and is hollowed out in the middle of the strand (Text-fig. 12), its walls being two or more cells thick. More posteriorly the cavity extends for some distance ventrally to the glandular body, the latter forming its dorsal wall (Text-fig. 13). It extends farther back laterally than it does in the median plane. Transverse sections posterior to this region show no cavity at all (Text-figs. 14 and 15). The relations of the

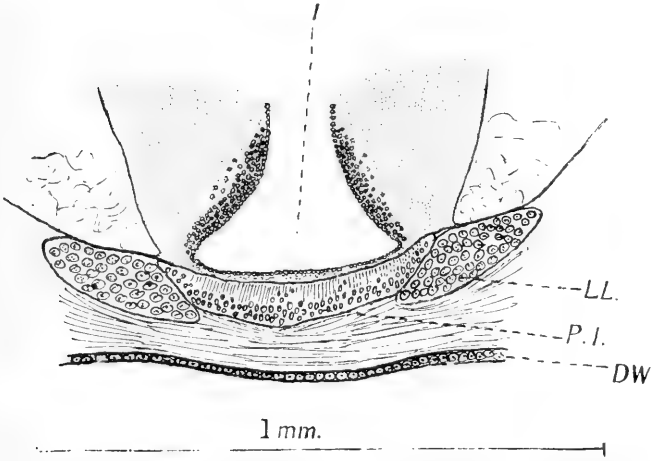
TEXT-FIG. 14.



Petromyzon fluviatilis. Ammocoete. Transverse section behind the limit of extension of the hypophysial cavity showing the lateral lobes of the ' Uebergangsteil '.

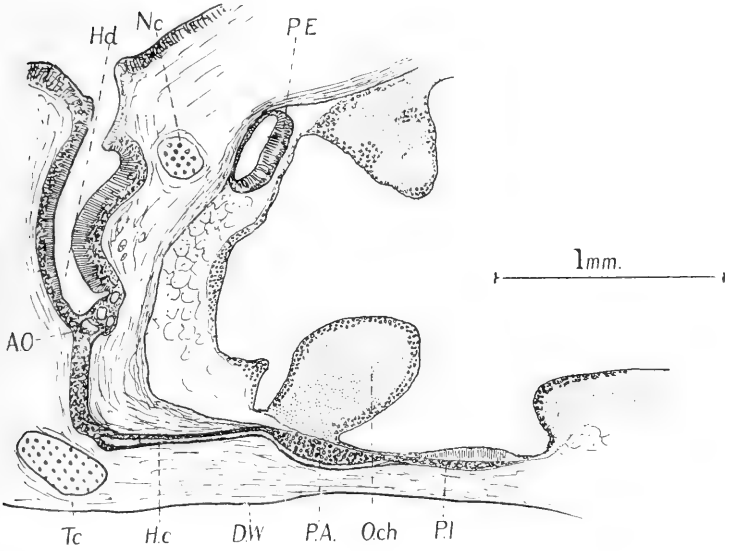
hypophysial cavity are shown in Text-fig. 16, which is a sagittal section of an Ammocoete. It arises in the middle of the hypophysis tissue without communication with the hypophysis depression. The glandular tissue forms its dorsal wall, and at this time the pars anterior and pars intermedia, though distinguishable, are in contact, being continuations the one of the other in a straight line. The hypophysial cavity does not separate them. Text-fig. 17 is a high-power view of this stage. The distinction between the parts of the pituitary body

TEXT-FIG. 15.



Petromyzon fluviatilis. Ammocoete. Transverse section showing the lapping of the lateral lobes back round the pars intermedia.

TEXT-FIG. 16.

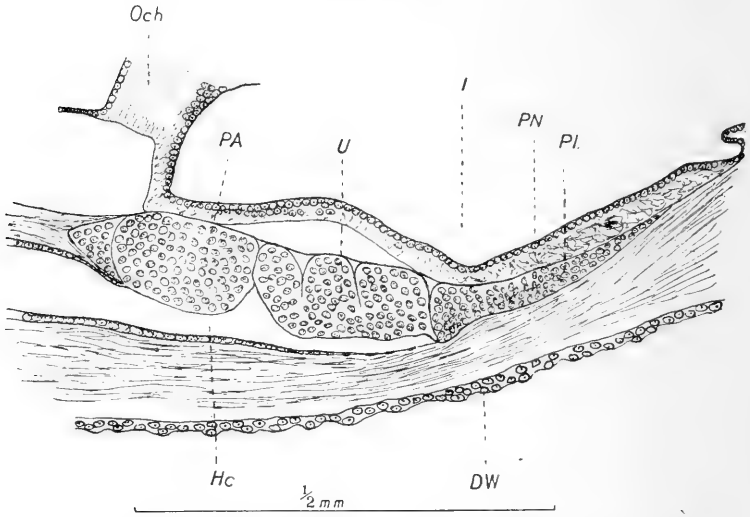


Petromyzon fluviatilis. Ammocoete. Longitudinal section.

is plain. Text-figs. 11 to 18 are of Petromyzon fluviatilis, the conditions in planeri are similar.

At a later stage the hypophysial cavity acquires more definite walls, and dorsally the wall separates itself from the glands (Text-fig. 18). The pars intermedia is the first to lose connexion, a band of connective tissue passing between it and the cavity

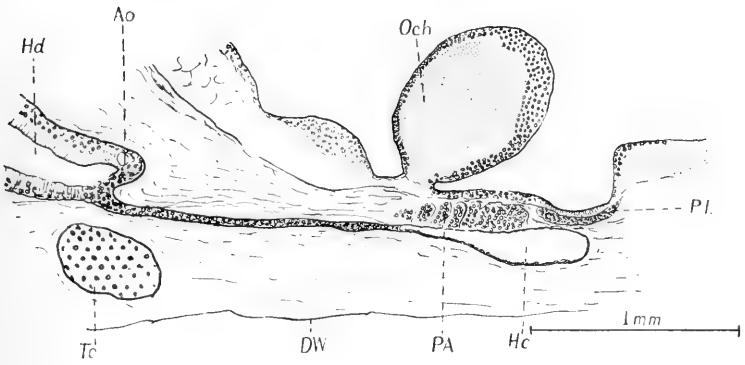
Text-fig. 17.



Petromyzon fluviatilis. Ammocoete larva more highly magnified, showing the relations of the hypophysial cavity, the pars anterior, pars intermedia, pars nervosa, and 'Uebergangsteil'.

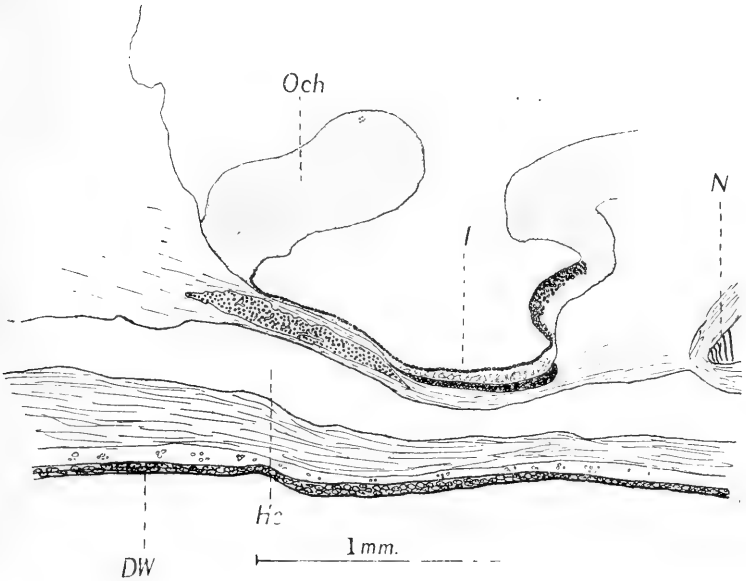
and also between it and the pars anterior separating it from that also. The pars intermedia, practically devoid of blood-vessels, is now in intimate connexion with the pars nervosa of the infundibulum, and its condition is now similar to that which it presents in the adult. The pars anterior is highly vascular. The cavity becomes more spacious and extends ventrally and posteriorly. It acquires a connexion with the exterior through the hypophysis depression by a split which occurs along the hypophysis strand. In the adult (Text-figs. 19, 20, and 21) the separation of the glands from the hypophysial cavity has gone further and they are now firmly encapsuled

TEXT-FIG. 18.



Petromyzon fluviatilis. Ammocoete. Further extension of the hypophysial cavity and separation from the glands.

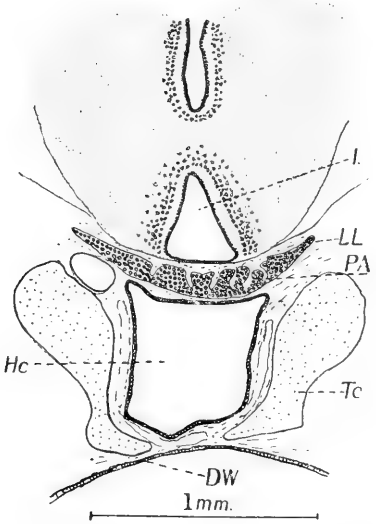
TEXT-FIG. 19.



Petromyzon planeri. Adult. Longitudinal section showing the separation of the glands from the hypophysial cavity and the great extension of the latter.

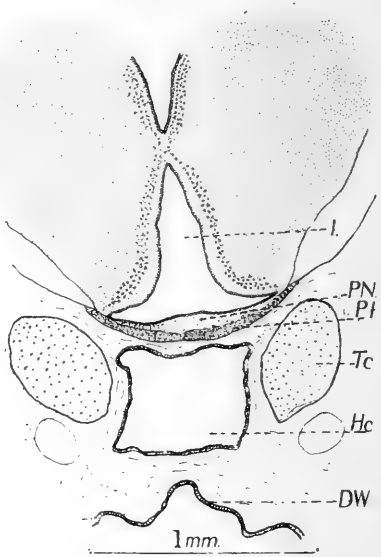
in connective tissue. The hypophysial cavity has reached huge dimensions, and is now the well-known sac extending back between brain and gut and communicating with the exterior by the single ' nasal ' aperture.

TEXT-FIG. 20.



Petromyzon planeri. Adult. Transverse section through the pars anterior.

TEXT-FIG. 21.



Petromyzon planeri. Adult. Transverse section through the pars intermedia.

Discussion.

The hypophysial cavity of Petromyzon is remarkable for several reasons. Briefly its characteristics may be considered. It develops in the middle of the hypophysis tissue which was a solid inpushing. This is paralleled by many other vertebrates. It remains in connexion with the exterior throughout adult life. This condition is only found elsewhere in Polypterus and Calamoichthys. In these a persisting connexion between the hypophysial cavity and the stomodaeum is referred to by Sedgwick and Wiedersheim, and it would be of great interest to know its development.

The connexion with the exterior may be regarded as a delayed appearance of Rathke's pocket. The adaptive nature of this connexion in Myxine where the hypophysial cavity also communicates with the gut suggests that the hypophysial cavity is secondarily modified, as does also the size of the hypophysial cavity in these forms. The fact that it has lost all connexions with the glandular portions also lends support to this view. For whatever the primitive function of the hypophysis may have been, it was an ectodermal organ sunk beneath the skin and the hypophysial cavity must be supposed to have served to keep the organ in communication with the exterior. In vertebrates above Cyclostomes where a hypophysial cavity exists it is in association with the glandular portions of the organ, though functionless since the latter have adopted the method of internal secretion. In Cyclostomes the cavity is separated from the glands in the adult by a good thickness of connective tissue, although in earlier stages it was in contact with them and they even formed its dorsal wall. It is this fact which enables one to believe that the hypophysial cavity of Petromyzon is homologous with that of other forms, a homology which would be difficult to establish from the adult. But even here Petromyzon is peculiar. In vertebrates typically the hypophysial cavity separates the pars anterior from the pars intermedia. In Petromyzon, as we have seen, the glands differentiate before the appearance of a cavity and the pars anterior and pars intermedia lie in a straight line with the cavity (when it does form) beneath them. Starting from this condition, i.e. the hypophysis arising from the front and growing backwards, and the hypophysial cavity horizontal with the glands on the brain side (dorsal), the pars nervosa of the pituitary very slightly developed and not projecting ventrally; the conditions obtaining in higher vertebrates can be derived when the following changes are taken into account.

(i) The hypophysis grows up from beneath and meets the brain at right angles. The consequence of this is that that portion of the roof of the primitive hypophysial cavity in connexion with the pars anterior which was dorsal and

horizontal in Petromyzon is rotated through 90° and lies anterior and vertical.

(ii) The down-growth of the infundibulum in connexion with the specialization of the pars nervosa forces the pars intermedia down also so that it lies posterior to the hypophysial cavity. In this manner the hypophysial cavity comes to lie between

TEXT-FIG. 22.

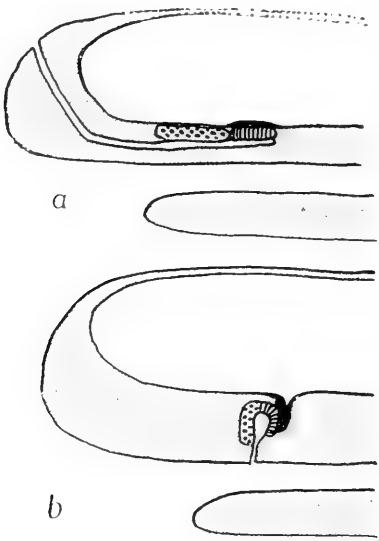


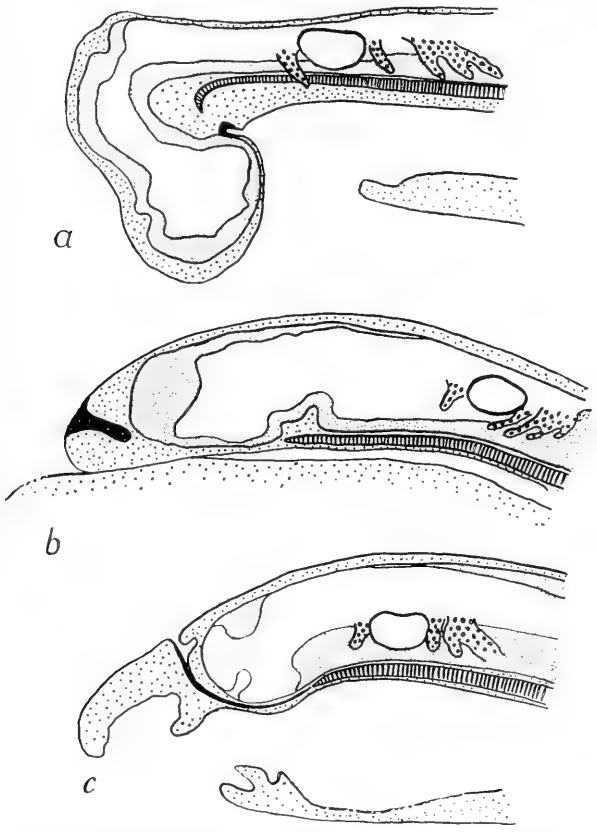
Diagram illustrating the difference in relations between the glands and the hypophysial cavity in (*a*) Petromyzon and (*b*) higher vertebrates. Pars anterior dotted, pars intermedia lined, pars nervosa black.

the pars anterior and the pars intermedia. This is shown diagrammatically in Text-fig. 22; and it is on such lines that I would explain the difference between the relations of these organs in Cyclostomes and higher forms.

Swale Vincent (1922) gives a figure of the pars intermedia on both sides of the cleft, i.e. his pars anterior is separated from the cleft by a strip of tissue labelled pars intermedia. If the cleft should represent the true hypophysial cavity, this

is incompatible with the theory which I am putting forward here. The strip of tissue in question is probably a portion of the pars anterior the cells of which are slightly modified since

TEXT-FIG. 23.



Diagrammatic comparison between (*a*) Squalus, (*b*) Amia, and (*c*) Petromyzon showing the position of the hypophysis and infundibulum.

here they act as an epithelium and line a cavity. Swale Vincent does not describe this structure in the text, so that not much importance must be placed upon his figure. The

evidence is in favour of the cleft representing the true hypophysial cavity, and to quote Biedl (1913) ' the anterior and posterior lobes are separated by a cleft more or less broad. This represents the vestige of the embryonic hypophysial cavity. The posterior wall of this cavity is directly opposed to the posterior lobe and forms its anterior limit in the shape of a strip . . . known as the pars intermedia.'

The identification of the hypophysis with Kölliker's pit (and therefore the neuropore) of Amphioxus (Willey 1894) is open to the objection that in higher forms hypophysis and neuropore are found in one and the same animal without any connexion.

With regard to the Tunicates the researches of Julin and van Beneden led to these observers believing that the hypophysis is represented by the subneural gland. But the later observations of Willey, Seeliger, and others show that the neural gland is derived not from external ectoderm but from the nervous system. The gland may have some connexion with the neuropore, but later it is in communication with the buccal cavity and a shallow ectodermal invagination (or several) meets the tube growing out of the gland. It is possible that the shallow ectodermal invaginations alone may represent the hypophysis (Stendell 1914*b*). Dr. Hogben kindly permits me to make use of the fact that he found that extracts from the subneural gland of ascidians had no properties such as are present in extracts of the posterior lobe of the pituitary of all classes of Gnathostomes.

Dohrn's (1883) view that the hypophysial cavity of Petromyzon represents a pair of gill clefts is open to the objection that whereas gill clefts are formed by outgrowth of endoderm, the hypophysis is ectodermal.

Haller (1896) believes that the hypophysis of Cyclostomes is secondarily modified, but he describes a lumen in the pars intermedia the existence of which has never been confirmed.

Woerdeman (1914) agrees that the glandular elements are differentiated from a solid strand of tissue in the absence of any cavity, and also that the greatest part (' Grösstenteils ') of the hypophysial depression is due to the overgrowth of the

upper lip. In the hypophysis of Gnathostomes he divides the hypophysial cavity into three parts; the most posterior he terms Rathke's pocket and separated from it by a constriction he distinguishes the remainder of the cavity as 'Mittelraum' and 'Vorraum'. Applying these homologies to Petromyzon he gets the external hypophysis depression to correspond with the 'Vorraum', the glandular portion of the hypophysis with Rathke's pocket, and the upper lip with that region situated just behind Rathke's pocket in Gnathostomes. The consequence of this is that the olfactory organs and mouths of Cyclostomes and Gnathostomes are not homologous. Such conclusions are unacceptable. The recognition of the secondary nature of monorhiny, the relations of the olfactory organs to the olfactory nerves and brain and those of the trigeminal and facial nerves to the mouth are against his view. Besides, it does not appear that the acceptance of his divisions of the hypophysial cavity facilitates their interpretation.

We are left then with the view that the hypophysial cavity of Petromyzon is homologous with that of other forms but differs from them by secondary modifications.

The Primitive Position of the Hypophysis.

The most striking feature about the hypophysis of Petromyzon at first sight is the fact that it has nothing to do with the mouth, but communicates with the exterior on the dorsal side of the head. The latter feature is, as previously stated, due to the great expansion of the upper lip with the front of the head, so that when a stage before the upper lip has developed (Text-fig. 2) is considered, the hypophysis faces ventrally. But even then it has no connexion with the stomodaeum. The limits of the stomodaeum are not easy to define since they are not marked by any structural peculiarity; but they may be taken as being the points where the concave curve of the invagination changes and becomes convex. The fact that in the majority of vertebrates, viz. Selachians and Amniotes, the hypophysis develops from within the stomodaeum has

led to the view that this is its primitive position (Stendell 1914*a*).

Recently, however, attention has been drawn to those cases where the hypophysis arises outside the stomodaeum and just anterior to it. In this connexion the works of Götte (1915) and Atwell (1919) for Amphibia, of Reighard and Mast (1908) and the writer for Amia, of Wells (unpublished) for Clupeus may be mentioned. In these cases the hypophysis has no connexion with either mouth or nose, and this position Scott (1883) believes to be primitive. Where the hypophysis arises within the stomodaeum, i.e. in Selachians and Amniotes, there is a great development of the fore-brain and cranial flexure; this rotation of the anterior part of the head causes the ventral elements of the head to lie relatively farther back and accentuates the stomodaeal invagination. This I believe to be the cause of the hypophysis being situated in the stomodaeum in these forms, but the difference between the two types is more apparent than real. In addition there is the fact that the hypophysis is ectodermal tissue which must get into contact with the infundibulum. Where the fore-brain is large and the cranial flexure pronounced this can most conveniently be done through the stomodaeum. But primitively the fore-brain cannot have been large and there was less cranial flexure. This condition is preserved in the Teleosts and Amphibia, and here the typical position for the hypophysis to arise is outside and dorsal to the stomodaeum. Text-fig. 23 is a diagrammatic comparison between Squalus, Amia, and Petromyzon showing the modifications brought about by the fore-brain and the cranial flexure.

Not only are the ventral elements of the head of Squalus pushed backwards, but the dorsal elements are pulled forward owing to the outer side of the curvature of the cranial flexure being dorsal. So the dorsal nerve-roots lead backwards to their respective gill arches whereas in Amia they incline forwards. The anterior position of the heart in Amia is partly due to the embryo being flattened out on the yolk.

Support is lent to the view that this is the primitive position

of the hypophysis by Goodrich's (1917) suggestion that the hypophysis is represented in Amphioxus by the deep groove and depression known as the pre-oral pit in the larva and wheel organ in the adult. This, as its name implies, is anterior to the mouth.

There remains the question as to whether an invagination (Rathke's pocket) or a solid ingrowth is the more primitive method of formation of the hypophysis. It is dangerous in a point of this kind to attempt to induce phylogeny from ontogeny, for the mode of development obeys embryonic conditions. I should suggest that the hypophysis of the primitive vertebrate was an invagination as is the pre-oral pit of Amphioxus, but that when the combination with the infundibulum forming the pituitary body was evolved, the mode of development became influenced by the distance which the ectodermal tissue has to travel.

So in Petromyzon or Amia or Amphibia the ingrowth is solid, in Selachians and Amniotes it tends to be hollow.

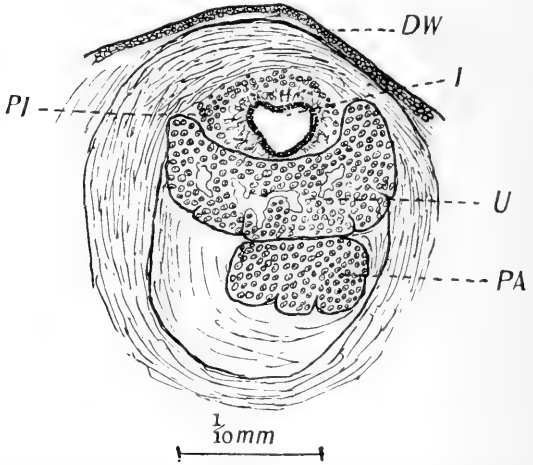
The Pars Tuberalis.

To the description of the main glandular elements of the pituitary body of Petromyzon I have little to add. The pars anterior is made up of two portions, an anterior lobe composed of chromophil cells, and a posterior of chromophobe. This latter portion is termed by Stendell (1914*a*) the ' Uebergangsteil ', by Gentes ' the middle lobe '. Stendell (1913) regards it as morphologically part of the pars anterior (' Hauptlappen '); Sterzi as part of the pars intermedia. It is seen in the ammocoete of Petromyzon fluviatilis in longitudinal section in Text-fig. 17, where it occupies the region between the pars anterior and the pars intermedia. The outer sides of this ' Uebergangsteil ' lap back round the pars intermedia on each side, as seen in transverse section in Text-fig. 15 and in horizontal in Text-fig. 24. Text-fig. 25 is a reconstructed view of the whole organ from the ventral surface. This fact was also observed by Woerdeman (1914).

Recently attention has been paid to the pars tuberalis as

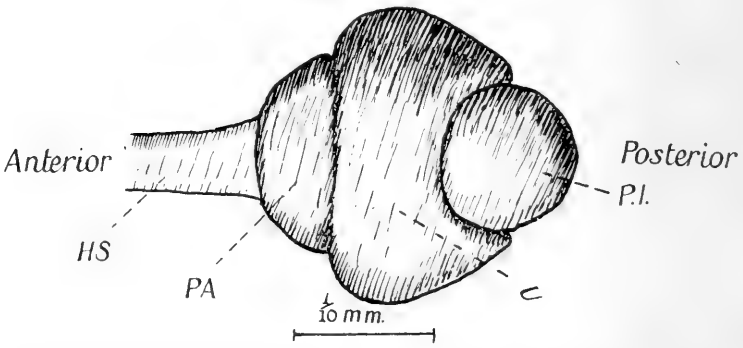
a distinct element of the pituitary body, notably, by Tilney (1913), Woerdeman (1914), Baumgartner (1916), and Atwell

TEXT-FIG. 24.



Petromyzon fluviatilis. Ammocoete. Horizontal section showing the lapping of the lateral lobes round the pars intermedia.

TEXT-FIG. 25.



Petromyzon fluviatilis. Reconstruction of the pituitary body of an ammocoete seen from the ventral surface after removal of the ventral wall of the hypophysial cavity.

(1919). Woerdeman considers the possibility that its homologue is to be found in the accessory organ (Text-figs. 8 and 9)

situated in the nasal capsule described by Scott (1888). This organ has been studied and is being described by me elsewhere. It develops in close connexion with the olfactory organ and has no histological or topographical similarities with the pars tuberalis of other forms.

In the description of its origin in other forms the pars tuberalis arises as lateral processes (lobuli laterales) which develop acini composed of chromophobe cells, and which are closely adpressed to the brain. According to Tilney it arises in con nexion with the pars intermedia (his pars infundibularis). If the pars tuberalis is represented at all in Petromyzon, I suggest that it is the ' Uebergangsteil '. Its cells are chromophobe (Herring, 1910), it has lateral extensions which are closely adpressed to the brain-wall, and is the only part of the organ not otherwise accounted for. From its position between the pars anterior and the pars intermedia it can hardly be said to arise in connexion with the one rather than with the other, though Sterzi considers it to be more closely related to the pars intermdia.

II. Amia calva.

Introduction.

The hypophysis of Amia calva is described by Dean (1896) as developing late. He regarded it as ectodermal, though he admitted that its ventral limit could not be distinguished from the endodermal roof of the fore-gut.

Subsequently Prather (1900) investigated the question and concluded that the hypophysis of Amia was of endodermal origin and derived from the roof of the fore-gut.

Later still, Reighard and Mast (1908), as a result of their researches, believe that Prather was misled in his conclusions by artifacts in his preparations due to shrinking, and they claim that the hypophysis of Amia arises from the ectoderm close to the neuropore, dorsal to and separate from the stomodaeum.

Smith (1914) regards the hypophysis as of ectodermal origin, but thinks that endoderm cells are contributed to it.

Stendell (1914*a*) makes no mention of Amia, and remarks that the Ganoids are in need of much further study in this respect. In view of the diversity of opinion on this matter and the fact that the confirmation of either of these two theories must lead to important conclusions with regard to the hypophysis, I determined to examine my preparations of embryonic and larval stages of Amia.

The material consisted of sets of serial sections, sagittal, horizontal, and transverse; and I may say at once that the remarks made by Reighard and Mast with regard to the care necessary in making the preparations are well founded. By shrinking of the egg-membrane the contained embryo is often compressed and the limits of its organs are sometimes difficult to make out with certainty. Thionin was found to be an efficient stain although it unfortunately fades. For bringing out the basement membranes Lichtgrün or acid fuchsin were found to be useful as counter-stains after safranin and methylene blue.

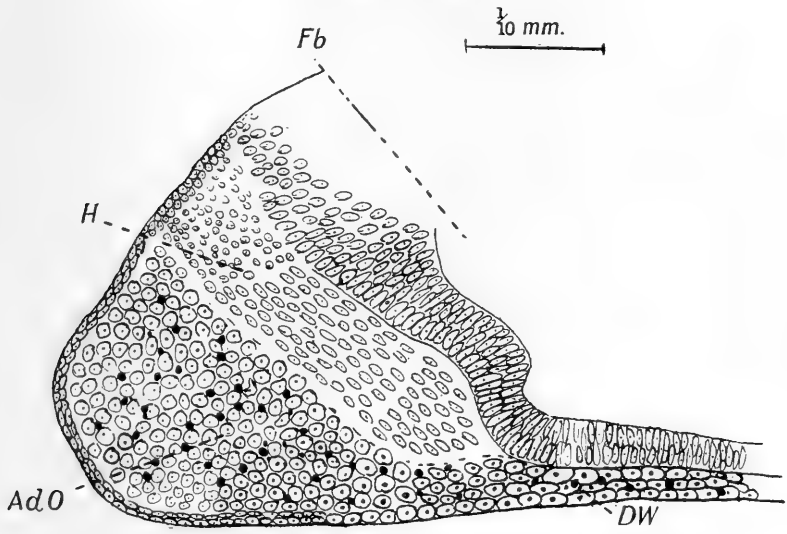
The Origin of the Hypophysis.

The earliest stage in which the hypophysis was visible is shown in sagittal section in Text-fig. 26. The brain is in contact with the antero-dorsal ectoderm in the region where the neuropore has closed. The antero-ventral region of the head is occupied by a mass of large endoderm cells containing abundant yolk and destined to form the adhesive organ. Between the latter and the brain is a tract of smaller cells almost devoid of yolk-granules, in contact with the ectoderm antero-dorsally, and postero-ventrally tapering into a point where the brain comes into contact with the endodermal roof of the fore-gut. There can be no doubt that these cells originate from the ectoderm.

The next stage is shown in small scale in Text-fig. 27 and under higher magnification in Text-fig. 28. The tract of ectodermal cells described in the previous figure is more compressed and denser. Anteriorly it shows traces of previous connexion with the inner layer of superficial ectoderm of the front of the head.

The line of demarcation between it and the underlying endoderm is not easy to see, but with an oil-immersion objective traces of the original basement membrane of the endoderm can be observed. More striking is the difference in yolk content, for whereas the hypophysis (since such I believe these ectoderm cells to be) is practically devoid of it, the endoderm cells both of the roof of the fore-gut and of the adhesive organ contain

TEXT-FIG. 26.



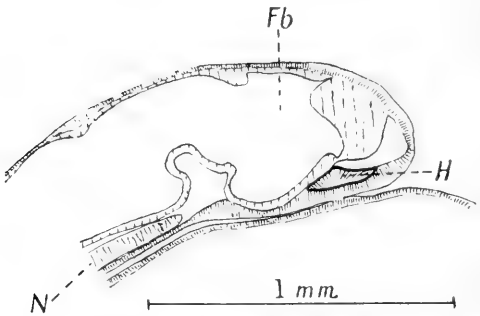
Amia calva. Young embryo (coiled round the yolk) showing the rudiments of the adhesive organ and of the hypophysis.

numerous large granules. At the same time the brain appears to be pressing down on the hypophysis and squeezing it against the endodermic roof of the fore-gut.

In the next stage (Text-figs. 29 and 30) this pressure has increased, for not only is the hypophysis less thick but it has impressed its convex dorsal and ventral surfaces into the corresponding concavities of the brain and gut-roof. The floor of the brain which in previous stages was bent dorsally in the region of the recessus opticus here continues flat for a further distance forwards. It looks as if the brain and the

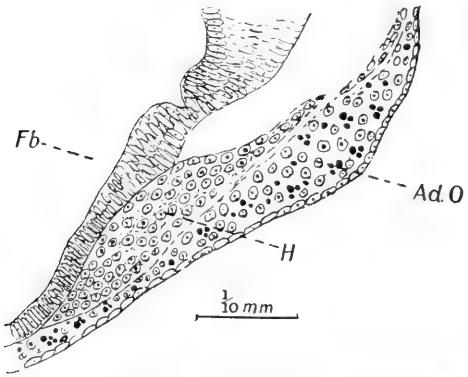
front of the head generally were growing forward and the hypophysis caught tight between the brain and the gut-roof were prevented from joining in this forward movement and

TEXT-FIG. 27.



Amia calva. Slightly later stage than the preceding, the hypophysis is beginning to lose connexion with its point of origin.

TEXT-FIG. 28.



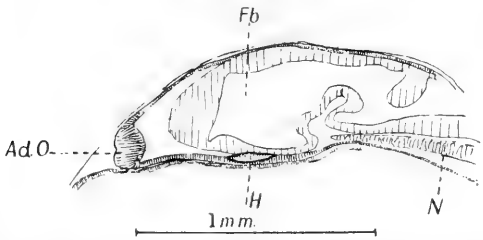
Amia calva. The same as Text-fig. 27, higher magnification.

so comes to lie relatively farther back. The demarcation between hypophysis and gut-roof is again hard to pick out, but the restriction of large yolk-granules to the latter is plain.

As development proceeds the brain and the front of the head generally seems to have grown and extended anteriorly. But

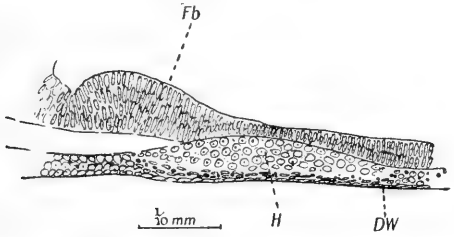
the distance between the hypophysis and the anterior extremity of the notochord is much reduced. This may mean that the tip of the notochord has grown forward, or that the hypophysis itself has moved back. The tip of the notochord appears to be stationary and to bear fairly constant relations to the limits of the hind-brain, so that the movement must be sought for

TEXT-FIG. 29.



Amia calva, 6 mm. long. Hypophysis completely separated from its point of origin and wedged between the brain and the dorsal wall of the gut.

TEXT-FIG. 30.

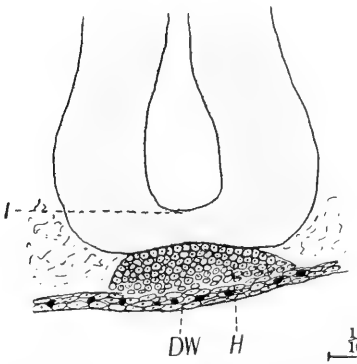


Amia calva. The same as Text-fig. 29, higher magnification.

in the hypophysis itself. Such movement could hardly be due to active migration since the hypophysis is firmly held between brain-floor and gut-roof, but is rather to be explained as the passive result of the movement of the latter. This movement may be due to the ' recoil ' resulting from the forward growth of the anterior of the fore-brain as suggested by Reighard and Mast, or to shrinkage of the gut-roof in the region posterior to the hypophysis, which would have the effect of drawing back

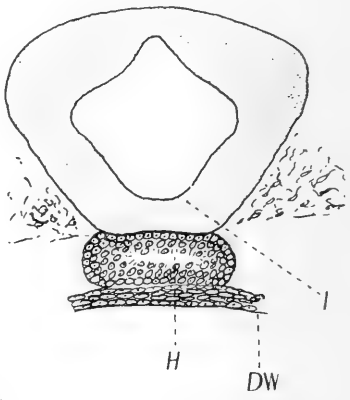
the structures anterior to it. In transverse section the hypophysis about this stage appears as in Text-fig. 31. At first sight it looks as if the hypophysis must have been derived in situ from the underlying endoderm cells. But careful observation with high powers reveals the differences that have been met with before between the cells of the hypophysis and the endoderm cells, viz. absence of yolk and different orientation. In many cases the limiting membrane of the gut-roof

TEXT-FIG. 31.



Amia calva. The same as Text-fig. 29. Transverse section.

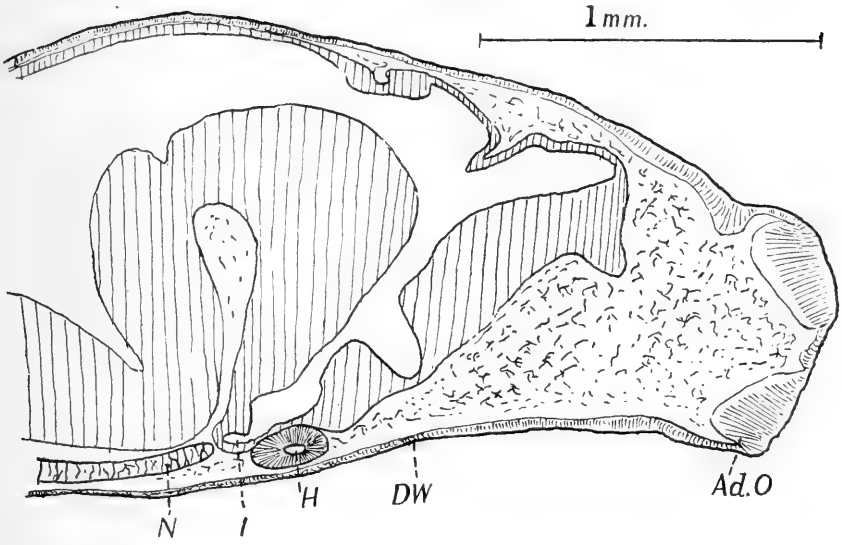
TEXT-FIG. 32.



Amia calva, 8 mm. long. Transverse section. Beginning of the hypophysial cavity.

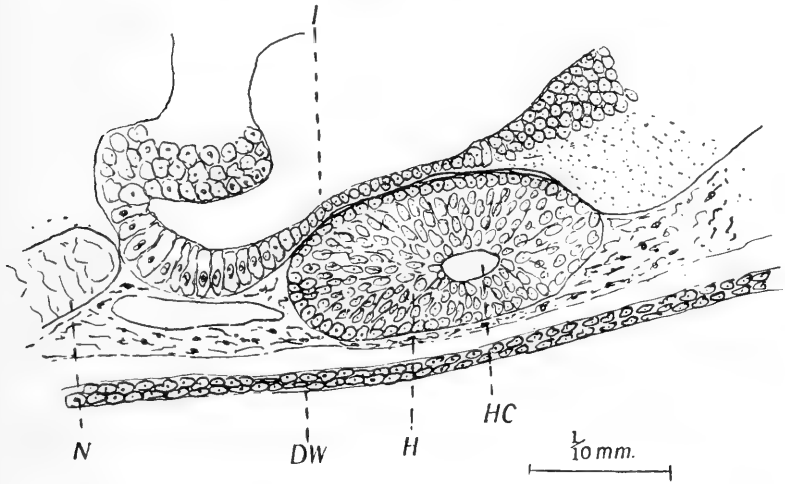
appears to be continuous with that of the hypophysis, and this fact puzzled me for some time, but I believe it to be without significance and due to the close apposition of the hypophysis to the gut-roof. In some sections the real discontinuity of the membrane can be seen. The hypophysis is still in contact with the gut-roof, and at this stage the beginning of the hypophysial cavity can be observed (Text-fig. 32). Mesenchyme begins to make its way between the floor of the brain and the gut-roof, and in the next stage (Text-figs. 33 and 34), where the hypophysis has separated off the endoderm, it is enclosed by a layer of mesenchyme.

TEXT-FIG. 33.



Amia calva. 11 mm. long. The hypophysis with a hypophysial cavity separated from the dorsal wall of the gut by connective tissue.

TEXT-FIG. 34.



Amia calva. The same as Text-fig. 33, higher magnification.

The hypophysial cavity is now distinct with the cells arranged radially around it. There is as yet no distinction between the cells on opposite sides of the cavity, the differentiation into pars anterior and pars intermedia has not yet appeared. On the side of the brain there is no pars nervosa, though the infundibular cavity and the recessus saccularis are already very distinct.

Summary.

1. The hypophysis of Petromyzon arises as a solid ingrowth.

2. The depression in which the hypophysis and olfactory organs come to lie is formed by the great expansion of the upper lip in conjunction with the sides of the anterior surface of the head.

3. The beginning of the histological differentiation of the glandular elements takes place from a solid strand before the appearance of any cavity.

4. The hypophysial cavity arises late as a split in the thickness of the hypophysis, and afterwards extends in both directions communicating forwards with the external depression.

5. The homologue of the pars tuberalis is probably to be found in the 'Uebergangsteil' of Stendell, a chromophobe portion situated between the pars anterior and pars intermedia.

6. The hypophysis of Amia is derived from the ectoderm, thus agreeing with all other known forms.

7. It arises outside the stomodaeum on the anterior surface of the head.

8. It is a solid ingrowth which loses all connexion with its point of origin, and within which the hypophysial cavity makes its appearance at a later stage.

9. Outside and in front of the stomodaeum is probably the primitive position of origin of the hypophysis, without connexion with either mouth or nose.

10. In Selachians and Amniotes where the fore-brain is early very large and the cranial flexure pronounced, the hypophysis arises further posteriorly and so is included in the hollow of the stomodaeum.

11. Although probably primitively hollow, the rudiment of the hypophysis is often solid. Such diversity is brought about by embryonic developmental conditions, perhaps the distance separating the rudiment from the infundibulum.

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The Relation of the form of a Sponge to its Currents.

By

G. P. Bidder, Sc.D.[1]

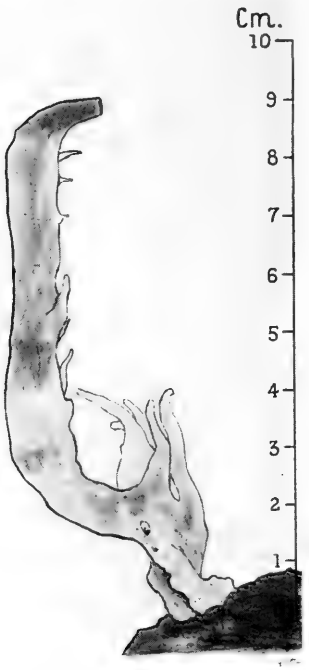
With 12 Text-figures.

All zoologists know that from the large holes, which we call oscula, on a sponge, an outgoing current may be detected in life. During several months in Naples I investigated this current, using litmus and carmine solutions, and carmine and indigo in suspension. I worked with two calcareous species of sponges, having oscula at the end of tubular prolongations, which reach the size and shape of a child's thumb in the case of Leucaltis, and of a child's finger in the case of Leucandra aspera (Text-fig. 1). The solutions were either placed on the surface of the sponge, to be sucked in by its currents (Text-fig. 2), or dropped by a pipette through an incision into the cloaca—the cavity of the tubular prolongation. In the latter case the time taken for the colour to be thrown out at the osculum, though liable to many corrections, afforded on the whole the most trustworthy determinations of oscular velocity : the cloaca being wider than the osculum, the observed

[1] This paper was read before the British Association at Hull, September 1922. A preliminary note was published in 'Proc. Camb. Phil. Soc.', 1888, vol. vi, p. 5. (See also 'Quart. Journ. Micr. Sci.', vol. 38, p. 28; 'Proc. Roy. Soc.', vol. 64, p. 61 ; and I. B. J. Sollas, 'Camb. Nat. Hist.', vol. i, p. 235.) The experiments were made in the Naples Zoological Station in 1887, 1888, and 1889, where I occupied the Cambridge University table, and in 1890, 1891, and 1892 at a table allowed me by the great kindness of the late Professor Anton Dohrn. For a long time I proposed to myself to make a further series of experiments to clear up doubtful points, but recognizing that I shall not now do so, I have reconsidered all the experiments this year (1922) and recalculated all results and formulae.

cloacal velocity would be multiplied by 4, 5, or 6, as the case might be, to obtain the oscular velocity. A pretty method was arrived at accidentally (Text-fig. 3), when I found the coloured jet marked by dark beads or nodes, caused by my pulse shaking

TEXT-FIG. 1.



Leucandra aspera var. gigantea (A. 11).[1]

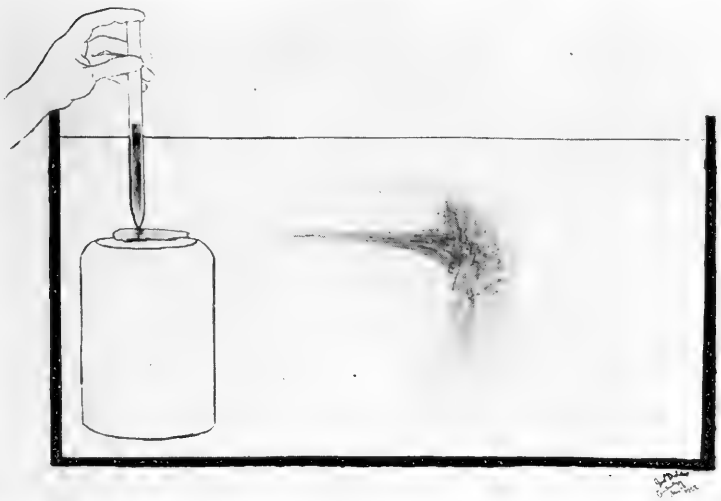
the pipette; the length between any two nodes, divided by three-quarters of a second, gives the core-velocity at that part of the jet.

The fastest oscular velocity recorded directly for the

[1] This sponge came from the Porto Militare, and I regret that I have drawn it erect instead of pendent. Vosmaer states that this metamp of L. aspera is found only on the keels of boats (cf. p. 314).

L. aspera of Text-fig. 1 was 7 cm. a second, and this was the basis adopted in the calculations of this paper. I have now changed them, in consequence of the conclusion that the actual mean oscular velocity when the sponge was in the sea was 8·5 cm. a second (Appendix, Note 1).[1] From Parker's experi-

TEXT-FIG. 2.



ments on the pressure in Stylotella, a siliceous sponge, its velocity is considerably higher than that of Leucandra; we shall see later that this could be conjectured from its structure.[2]

In quite still water such a current from Leucandra goes

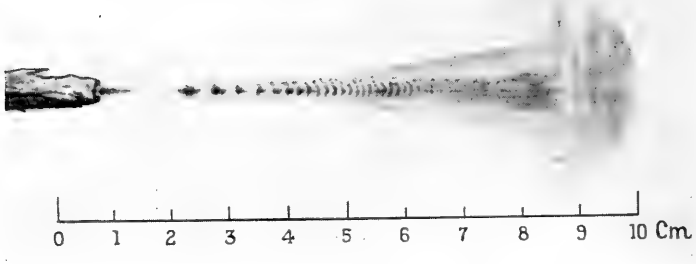
[1] This was the conclusion from observations of length of jet and velocity; I cannot put it forward as an exact physical measurement, but as a final judgement after considering upwards of 1,000 unsatisfactory and imperfect observations, of the nature described in the text. Note 6 (Appendix) indicates that the figure 8·5 was a lucky judgement, and is probably close to the true velocity.

[2] The velocity in Stylotella will be less than Parker calculates, as he does not allow for the friction in the canals of the sponge.

10 or even 20 inches before coming to rest.[1] Speaking exactly, it does not really come to rest at this distance, but reaches a velocity not higher than that of the slow return-current, slightly indicated in Text-figs. 2 and 3, which is necessarily established to fill up the region from which this water has been removed.

Text-fig. 4 is a diagram of the currents which must exist around a bath-sponge in still, deep water. The swift vertical jets from the oscula on the top surface carry the used and

Text-fig. 3.



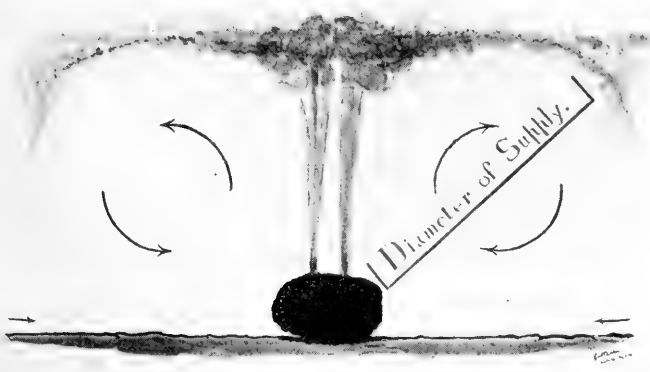
fouled water to a stratum some feet above the sponge; slow currents, in the plane at right angles to the jets, creep in from all directions along the sea-bottom to feed the intaking pores, which cover the general surface of the sponge. If the water be absolutely still, there is established between these afferent and efferent currents a re-entrant vortex, whose section is a circle in any radial plane through the osculum.

The diameter of that circle I call the Diameter of Supply; and the angle between the directions of the intake and outflow currents, which in sessile sponges (Text-fig. 4) is a right-angle, and in pedunculated sponges (Text-figs. 9 and 10) is 110° to 120° or more, I call the Angle of Supply.

[1] See Note 5, (12).

On these two factors depends the life of the sponge, or of any other fixed or stationary organism in still water. The outgoing current carries with it water which has been filtered of food, in which carbonic acid has been substituted for oxygen, into which the poisonous products of metabolism have been excreted. In still, or nearly still water, the angle of supply and the diameter of the circle of supply measure the chance that some convection current or drift will carry away that water,

TEXT-FIG. 4.



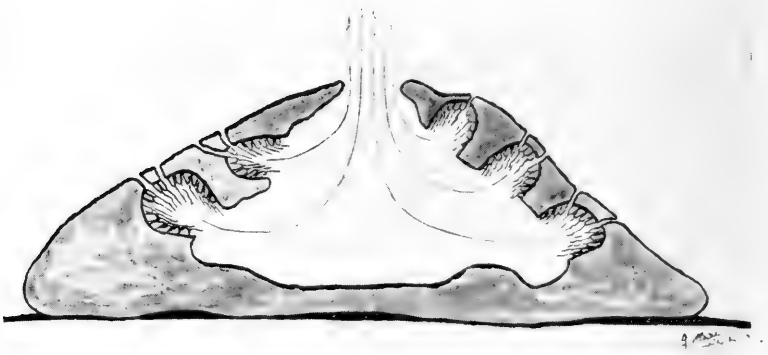
useless for life, before the slow eddy of return brings it down to the plane of the ingoing current. According to the distance to which it is so carried is the percentage of clean, unused water which enters the organism, and according to this percentage is the chance of life of the organism; and in a slow tidal channel it is clear that the distance to which the foul water will be carried by the tide before it is drawn back to the plane of the ingoing currents, depends directly upon the length of the oscular jet.

The length of this jet was shown by the experiments to vary as the initial velocity—a result to be expected by elementary theory, though a full theory would be difficult. With jets of the same initial velocity, but from oscula of different size,

the distance carried appeared to be proportional to the diameter of the osculum, although, in consequence of the oscula used having small range in size, this result was not so certain. The rough formula indicated by the experiments is that, using centimetres and seconds, the length of the jet approximates numerically to twelve times the product of its initial velocity and the diameter of the osculum.

That among jets of equal velocity the distance carried should

Text-fig. 5.



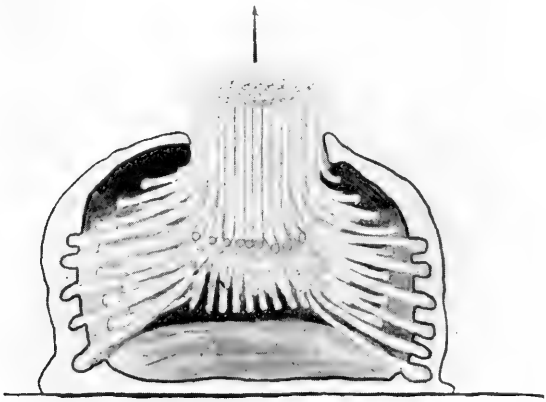
Rhagon.

be proportional to the oscular diameter, might also be expected by elementary theory; since per centimetre of length the weight of water increases as the square of the diameter, while the surface exposed to friction increases only as the diameter. Consequently the ratio of the moving weight to the resisting surface increases as the diameter, and with equal velocity a jet 4 mm. wide may be expected to go twice as far as a jet 2 mm. wide.

This consideration enables us to understand the advantage gained by the union of many unicellular flagellates to make one thimble-shaped Olynthus, or by the union of many Olynthi opening into a central cloacal cavity to form a Rhagon (Text-fig. 5) or a Sycon. Suppose, in Text-fig. 6, we unite a hundred

Olynthi, each ejecting an efferent stream 1 mm. wide, and raise up their colonial wall to enclose a common efferent aperture 1 cm. wide, thus forming a hypothetical Rhagon with an efferent aperture having its area equal to the aggregate oscular area of the 100 Olynthi. We can visualize the stream from each Olynthus as a thread, and the stream from the Rhagon as a cord of a hundred threads issuing side by side. We see at once

Text-fig. 6.



Efferent streams. Hypothetical Rhagon with wide aperture.

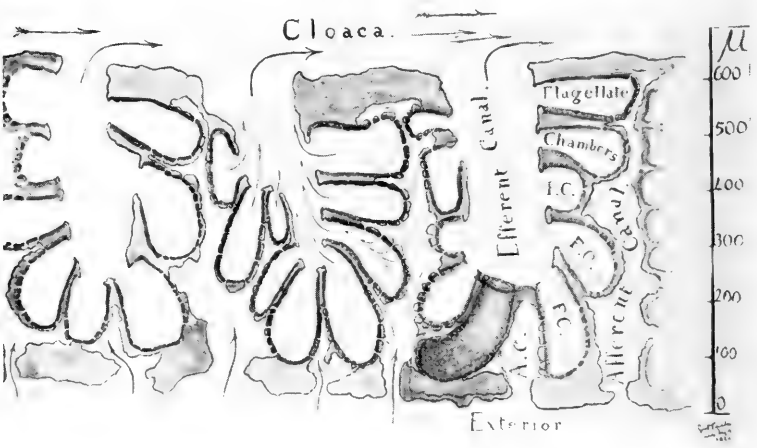
that most of the threads lie altogether inside the cord, with their surfaces entirely protected by other moving threads from friction against still water. The mass and initial velocity of the Rhagon's jet is that of its 100 constituent jets; but for each centimetre of length the external surface of the Rhagon's jet is only one-tenth of the total surface of the constituent jets, consequently the friction to which it is exposed is one-tenth of their friction, and the combined jet will travel about ten times as far as each of the constituent jets would go separately.

Coalescence is therefore advantageous in increasing the diameter of supply; and, as in all living things and in the

works of engineers, absolute magnitudes are determined by the relations of the consequences of increase in length to the consequences respectively of increases in area and in volume.

But sponges are not mere coalesced Protista: they have varied cellular differentiation and at least two organs. The first is the perforated membrane of tissue which is formed by the flagellate cells, or on which they stand (Text-fig. 8), thereby ensuring to them absolute separation of the water which leaves

Text-fig. 7.



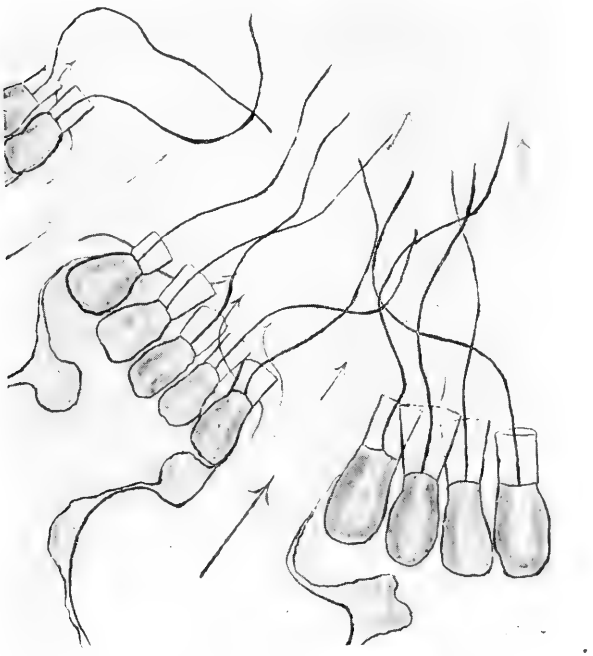
Wall of Leucandra aspera.

them from the water which supplies them; this organ is possessed by no choanoflagellate, and characterizes the sponges. The second is the canal system, which we may better call the hydraulic organ, in which the form of every part is wonderfully modified for the advantage of the whole aggregate. In the canal system all agree that progressive changes have occurred again and again in similar order along many lines of descent in sponges. We can show these changes to have as one of their necessary consequences large increase in oscular velocity, with consequent increase in the diameter of supply, and that therefore each successive change has left the sponge a more self-

sufficient food-catching machine than its ancestors, so as eventually to make a fixed organism which is independent of chance currents from waves and tides.

Watching under the microscope a flagellum with such a rapid period of vibration that the eye only sees a mist

TEXT-FIG. 8.



Wall of flagellate chamber, with two afferent pores.

terminated by the two extreme positions, we are apt to think, and to say, that the flagellum is moving rapidly ; and much theory has been written about the mechanics of the collar-cells and choanoflagellates, on the assumption that food-particles are thrown through the water at express-train speed by this rapidly moving flagellum, as a savage hurls a projectile with a throwing-stick, or as civilized man drives a ball with a club.

Really, far from express-train speed, no part of the most rapidly moving flagellum ever attains the rapidity of motion of a snail. We forget, as we look through a microscope magnifying 1,000 diameters, that though distance is magnified, time is not magnified, and therefore any velocity is $\frac{1}{1000}$ what it appears to be. I have estimated the vibrations of the flagella in healthy and lively collar-cells at twenty to the second.[1] The flagellum is 30 μ long; therefore if it vibrates through an angle of 60°, its tip travels 30 μ each half-vibration and 60 μ each complete vibration, making twenty vibrations 1·2 mm. per second; which is 14 ft. an hour. This is the speed of only the extreme tip of the flagellum, the base being motionless; so that some 7 ft. an hour is the mean speed of the middle point of this invisibly rapid flagellum.

I confess that I have often adduced this calculation to show why there are no flagella in the cloaca of Leucandra, where the mean velocity is $1\frac{1}{4}$ centimetres a second, or twenty times the speed of the flagellum. But, really, viscous flow at a mean velocity of 1 cm. means an axial velocity of 2 cm. diminishing gradually to zero on the walls. The bareness of the cloaca we must attribute to the fact that the sponge flagella do not, and cannot, act like oars, an action which requires direction of movement and nervous co-ordination. All observers agree that the movements of sponge-flagella are neither co-ordinated, synchronous, nor in parallel planes.[2] A collar-cell flagellate surface is comparable mechanically to a seine-net with a number of fishes fixed by their gills in the meshes. Their movements cannot establish a current along the face of the net: that would involve their tails all striking strongly in the same direction and weakly in its opposite. But if the net be fixed, the uncoordinated movement of their tails will draw water through the net from the side on which are their noses. Sponge collar-cells are similarly capable of

[1] 'Quart. Journ. Micr. Sci.', vol. 38, p. 17.

[2] Permanent sections of Oscarella show the pinacocytal flagella of the afferent canals looking as if they work as oars, and far enough apart to do so without collision; say, one to the area of 30 collar-cells.

drawing water through the perforated membrane on which they stand ; from its position the walls of the cloaca cannot be so perforated, so it bears no collar-cells. It is true that sponge-flagella can accelerate a current down which they lie, but for this their position is least useful when they stand on a wall parallel to the current, and most useful on a wall at right angles to it. To this latter position they become more and more limited in the progressive development of the canal system, in which flagellate tubes become progressively shortened until only the perforated hemispherical end of the chamber is left.

The remarkable achievement of the perfected hydraulic organ in sponges is that from this waving of hairs $\frac{1}{100000}$ of an inch in thickness at a mean speed of 7 feet an hour, there is produced an oscular jet with an axial velocity of over half a foot a second (280 times the speed of the flagellum), which in Leucandra throws to the distance of 9 inches five gallons a day or a ton in six weeks (Appendix, Note 3).

It is, of course, clear that when we combine many slow streams into one narrow channel the velocity is increased. I computed in several sponges the aggregate cross-section of the stream through the body in various parts of its course. In the Leucandra of Text-figs. 1 and 7, 10 cm. long, there were about $2\frac{1}{4}$ million flagellate chambers, with a total transverse area of $52\frac{1}{2}$ sq. cm., or 1,700 times the area of the osculum, which is 0·031 sq. cm.; so that the mean velocity at the osculum is 1,700 times as high as in the chambers.

At $8\frac{1}{2}$ cm. a second, a quarter of a cubic centimetre (0·26 c.c.) will issue from the osculum each second, and to replace it a quarter of a cubic centimetre must have passed through the $2\frac{1}{4}$ million chambers, that is $\frac{1}{8650}$ of a cubic millimetre through each, or 116,000 cubic μ; which through a chamber 54 μ diameter (transverse area 2,300 sq. μ) implies a rate of flow of 50 μ a second, or $\frac{1}{1700}$ of that at the osculum, as above.

The flagellate chamber (Text-fig. 7) is a blind, thimble-shaped tube, the water entering through perforations in the walls. The total area of the wall-surface is eight times the cross-

section of the tube, so that the water entering has this transverse area of channel as it passes the flagella (Text-fig. 8), and therefore the velocity of only 6 μ a second.[1] Below the flagella half the channel is occupied by the necks of the collars, so that between them the water moves at the rate of 12 μ a second, and a particle of food takes a second to travel the length of a cell.

Slow as we thought the movement of the flagella, at $\frac{1}{2}$ mm. a second, the water on which they act is stationary by comparison, and they can get on their full work. And the remarkable anomaly in sponges, that through considerable evolution their motor-cells remain their ingestive cells, ceases to be surprising when we realize that both functions are alike localized at the position where the current is slowest.

The one second during which a food-particle is passing the collar-cell is not such a short time for its capture as would at first appear. It allows of a good many events in the cell's life: we know of twenty double vibrations of the flagellum, with the metabolic cycles which they imply. The biological magnitude of an interval of time is measured by the number of events which can happen in it; and since every event requires the motion of something from one position to another, therefore where the distances between positions are smaller, events can happen more rapidly. Every motion is produced by an acceleration—such as gravity, or the stress of contracting protoplasm—and with a given acceleration the time required to move over a certain distance from rest is as the square root of that distance: a stone takes a second to fall 16 ft., but to fall a quarter that distance takes half a second. Therefore in a biological world whose linear dimensions are $\frac{1}{1000}$ those of our own, there will be some thirty times as many events in a second as in our own, since thirty-two times thirty-two is a thousand; and I suggest that in the biological time of the flagellate cell the one second during which a particle of food is passing would compare with half a minute in our external life. (We must put the adjective 'external', because our psychical events which happen 'with the speed of thought', are events

[1] Note 6 gives reason for supposing double these velocities in full health.

in a cellular or intracellular world where distances may be even smaller than those about a flagellate cell. Hence, always, their rapidity has been noted as of a different order to that of common external events.)

There is also a purely physical point deserving attention in the conditions of the world under an immersion lens. When we watch flagella working under a high power, the water seems to have lost its fluidity: a particle moved with apparent swiftness by a flagellum loses its motion at once. The general appearance is as if the flagella were labouring in thick gum, or treacle; and to understand microscopic physics it is a serviceable short-cut to think of the water as treacle. The energy of a projectile to overcome the resistance of the medium through which it is thrown is as its mass multiplied by the square of its velocity; loss of energy from the resistance is as its surface multiplied by the velocity and by the distance traversed. We magnify its apparent mass as the cube of the magnification, and the square of its apparent velocity as the square of the magnification; so that the apparent energy of the projectile is magnified as the fifth power; but the energy lost, measured by surface multiplied by velocity and distance traversed, is only magnified as the fourth power. Consequently, with 1,000 diameters, the water offers a thousand times the retarding effect which we expect, on the projectile which we think we see; and the ratio is even higher with the small projectiles which concern us.[1] With velocities among which 25 ft. an hour is the swiftest, at distances among which $\frac{1}{1000}$ of an inch is very great, the viscosity of water is the predominant phenomenon; and this world at which we are looking is a world of pushing, not of throwing.

When the flagellum pushes in with its stroke a minute droplet of water into the flagellate chamber (Text-fig. 8), it creates

[1] Sir J. J. Thomson kindly informed me that, according to Stokes's law, the resistance of water to the movement of a minute sphere is proportional to the diameter, not to the square of the diameter. This would make the apparent retardation under the microscope a million instead of a thousand times the expected retardation.

a pressure there which forces an equal amount of water out into the efferent canal (Text-fig. 7), and so the pressure created in the chamber is transmitted to the efferent canal, and thence, with a loss by friction, to the cloaca. The chamber is distended by this flagellar pressure, as the elastic bag of a squeeze-pump is distended, and the stretching of the chamber-walls is resisted by the surface tension and elasticity of the tissue, as the stretching of a soap bubble is resisted by the surface tension of soapy water in air. The text-books have long pointed out that, as the canal system is specialized, the diameter of the chambers become smaller, and they change from cylinders to hemispheres and spheres. This change, therefore, directly increases the possible pressure in the chambers, and therefore the diameter of supply. There is twice the pressure in a soap bubble 1 in. in diameter that there is in a soap bubble 2 in. in diameter; and similarly, reduction in the size of flagellate chambers proportionally increases the pressure which their tension can balance. In Leucandra I calculate from computation of the oscular current [1] and the friction in the canals that the pressure is between $\frac{3}{5}$ mm. and $1\frac{1}{5}$ mm. of water in the cylindrical flagellate chambers 54 μ wide. The same tension would support double the pressure in spherical chambers of the same diameter, and four times the pressure in spherical chambers of half the diameter; so that from my results the pressure in a sponge with spherical chambers 27 μ in diameter would be $2\frac{1}{2}$ to 5 mm. of water, and in 35 μ chambers would be 2 to 4 mm. In spherical chambers of 35 μ, by direct experiment, Parker found the pressure of $3\frac{1}{2}$ to 4 mm. of water in Stylotella. We may therefore conclude with some safety that the pressure in Leucandra is close to 1 mm. of water, and that the healthy tension of the chamber-wall tissue is nearly alike in this and in Stylotella (for the latter 0·00034 gm. weight per centimetre, or less than $\frac{1}{80}$ of the surface tension of petroleum in water). In the smaller spherical chambers of Stylotella this tension can support three times the pressure of the large cylinders of Leucandra, and so

[1] See Appendix, Note 6.

we may expect nearly twice the oscular velocity, and consequently nearly twice the diameter of supply, with this admittedly more specialized type of canal system.

This pressure of 1 mm. water is transmitted, with a loss from friction, through the efferent canals and cloaca to the open osculum, where the potential energy of the compressed water is converted into the kinetic energy of the swift oscular jet.

We are familiar with such conversion of the potential energy of compressed air in an air-gun into the kinetic energy of the escaping bullet, and of the potential energy of the compressed water at the bottom of a cistern, converted into the kinetic energy of the jet from a garden-hose. We know well, with the garden-hose, that when the water will only go 3 ft. from the open hose, it will throw a jet 30 ft. from a fine tube-nozzle. This is because with the open pipe, delivering perhaps 10 gallons a minute, there is a flow of 5 ft. a second through the 1 in. hose-pipe, with great loss of energy in every foot of the pipe from friction. Putting on a nozzle which will only deliver 1 gallon a minute, the velocity within the hose is lowered to 6 in. a second, $\frac{19}{20}$ of the loss by friction is avoided, and the potential energy of the cistern is transmitted almost undiminished through the hose, in any part of which there is nearly the full pressure of the cistern. If we stop the nozzle with the finger, we have the full pressure of the cistern throughout the hose, and can feel it on the finger. This is not perceptibly diminished by allowing a fine thread of water to escape, and its velocity of issue approximates to the full theoretic velocity due to the cistern head, but it does not travel far owing to the smallness of its mass compared with the surface of friction it exposes to the air. As the jet is allowed to increase in volume, so does the velocity increase in the hose-pipe, and the consequent successive loss of energy in each yard of its length ; and we can feel the pressure on the finger noticeably diminish and can see that the velocity of the jet consequently decreases, though with its greater volume it travels farther.

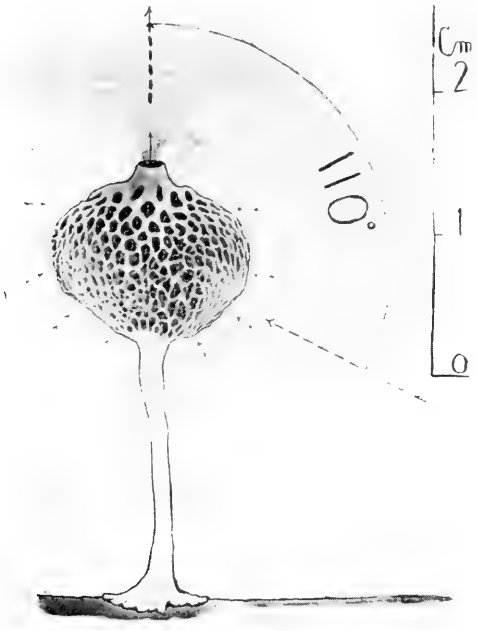
So with the sponge. The narrow osculum, 2 mm. diameter, in

Leucandra is comparable to the tube-nozzle on the hose. It means that pressure is transmitted from the flagella through the water of the canals, the water moving so slowly that loss in friction still leaves enough energy at the osculum to make a strong stream. As with the hose-pipe, were we to close the osculum more, there would be less energy lost, and the velocity at the osculum would rise, with a pin-point hole, from $8\frac{1}{2}$ cm. to 13 cm. a second, but the tiny jet could barely travel $1\frac{1}{2}$ cm. instead of 24 cm., while the quantity would not be a hundredth of that necessary for nourishment. With the osculum half its existing diameter, the velocity would be increased to 11 cm. instead of $8\frac{1}{2}$ cm., but the length of the jet reduced to three-quarters and the quantity of water to only one-third what we now find them. On the other hand, were the same Leucandra shaped like a cornucopia, with the widest part of its cloaca as osculum, the quantity of water passing would be increased by one-sixth of the existing quantity, but the velocity would be only $1\frac{1}{2}$ cm. a second, and the jet consequently less than half the present length. For a given pressure, acting through a given length of channel of fixed width, there is an optimum value for the size of the osculum (as we have all found with the garden-hose) above and below which it will not carry so far; with measurements of the flagellar pressure, and of the number and dimensions of the canals, an equation can be made to determine this optimum value. I have calculated it for the Leucandra of Text-fig. 1 (Note 5), but my computations of the canals and currents are not close enough to say more than that the theoretically best diameter for the osculum of this specimen is 2·6 mm. $\pm\frac{1}{4}$ mm.; the preserved diameter being 2 mm. Rough computations for other specimens, and for a Sycon, confirmed the conclusion that the osculum is always at any rate near to the optimum size for producing the greatest diameter of supply; and that this is the explanation of the small and definite oscula which we have all noted as characteristic of the majority of sponges with high canal system.

The secret of the repeated development of this common type of sponge is the reduction of internal velocities, so that

a larger proportion of the energy produced by the flagella is transmitted to the osculum in the untaxed form of pressure. The cloaca must be much wider than the osculum for the cloacal current to be slow; hence the bottle-shaped cloaca with a small orifice, which led our forefathers to compare to the stomach and mouth of animals the pressure-chamber

Text-fig. 9.



Clathrina blanca. Angle of supply 110°.

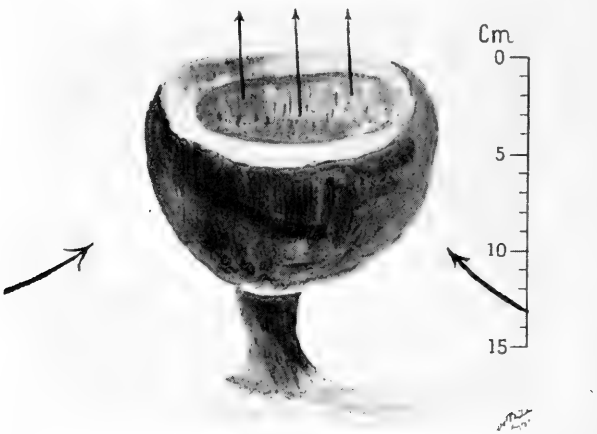
and vent of the hydraulic organ of sponges, evolved with the advantage, not of the retention and digestion of food, but of the forcible removal of excreta.

I shall not here discuss the sluggish currents of Clathrina and Leucosolenia; nor the evolution of subdermal spaces, and their possible development as muscular pumps with the collars acting as valves (as in the strange reversed canal

system described by Vosmaer in Spirastrella).[1] But modifications concerned with the angle of supply demand brief notice in regard to our main thesis.

Stalks of greater or less length (Text-fig. 9) increase the angle of supply in many sponges, and when this is the case in sponges of any size, the osculum opens out, as admixture of incoming and outgoing streams becomes less probable, and oscular velocity therefore less important. This gives rise to the

Text-fig. 10.



Calyx lieberkuhnii (modified from O. Schmidt).

well-known Neptune's-cup form (Text-fig. 10), found in many groups, and the expanded lip intervenes between the two streams.

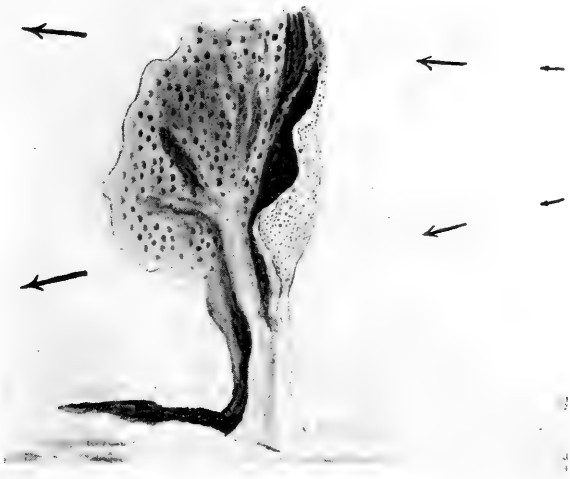
If this cup becomes set on one side (Text-fig. 11), the efferent stream passes away forwards, while the intake is at the back of the cup, and the angle of supply approaches to 180°. In still water, with the oscular jet horizontal, the length of the jet becomes infinite, whatever its velocity; because with the angle of supply 180° there is no back eddy, and the friction on the surrounding water serves by degrees to set it in motion

[1] Siboga-Expeditie, 'The Genus Spirastrella', p. 49.

TEXT-FIG. 11.

Phakellia ventilabrum (combined from Johnston and Bowerbank).

TEXT-FIG. 12.



Phakellia conulosa (reconstructed from Dendy).

in the direction of the jet. So, since maintenance of oscular velocity is now of no advantage, the osculum opens out completely to allow the maximum quantity of flow regardless of oscular energy. The cloaca becomes a flat surface (Text-fig. 12), and the whole sponge a disk or fan with one intake face and one outflow face. If such a sponge be in really still water the surrounding water will by degrees be all set into slow motion, in the direction from intake to outflow, and the condition of life approaches that of the deep-sea sponges and polypes.

It is a puzzling fact at first that in most of the Hexactinellida we can detect no hydraulic evolution nor hydraulic efficiency; puzzling until we remember that in the great depths where they live, an unchanging current sweeps slowly from the poles to the equator. They have but to spread a net across it, and, whatever their mechanical inefficiency, they have incoming and outgoing streams 180° apart; the flagella have only to work the water through the many meshes formed by the feet of the collar-cells. The cavity of the Hexactinellida is no pressure-chamber: it is even perforated to let the onflowing water sweep out that which is befouled. Food is brought to them, waste is taken away. For them in their eternal abyss, with its time-like stream, there is no hurry, there is no return. Such an organism becomes a mere living screen between the used half of the universe and the unused half—a moment of active metabolism between the unknown future and the exhausted past.

APPENDIX.

Note 1. The Velocity of a Jet of Measured Length.

The empiric formula, in centimetres and seconds,

$$L = (12 \pm 2)\ V.B. \times \{1 - 0{\cdot}023\ (20 - t)\}$$

where L is the length of the oscular jet,
V its mean velocity at the osculum,
B the diameter of the osculum,
t the temperature in degrees Centigrade,

may be of use to naturalists who are able to observe in a large tank the length of the jet from a mollusc or other fixed organism, and the width of the aperture. It is thus possible, for jets not exceeding 180 cm., to obtain a value for the approximate issuing velocity, and therefore of the quantity of water filtered by the animal and of the effective work performed by its cilia. The correction for temperature is theoretical.

Thus the figure of $8{\cdot}5 \pm 1{\cdot}5$ cm. per second, which I have finally adopted as the mean oscular velocity, at 18° C., of Leucandra aspera gigantea (Text-fig. 1), healthy and in natural conditions, is deduced from the oscular streams of 30 cm. and even 45 cm. long, measured from recently gathered sponges. These indicate a mean oscular velocity of 8 cm. per second, and from the reduction which this undergoes under the best aquarium conditions, it may be concluded that the velocity of any gathered sponge is less than in the sea.[1]

The formula was deduced from measurements of velocity on sponges some time in the tanks whose streams had shortened to 15 cm., 10 cm., 5 cm., or even 1 cm. ; such languid velocities being more easy to measure. The constant (12 ± 2) has been adopted as a compromise between a group of experiments by different methods which agree on the value $L/VB = 10{\cdot}5 \pm 1$ and several experiments which group themselves about $L/VB = 14$.[2] The distance to which the cloud drifts will obviously be affected by the position of the jet with reference to the walls of the tank : with accurate physical experiment and definition of the conditions a closer result could be obtained ; but for aquarium observations it is probable that the conditions cannot be sufficiently identical to reduce greatly the probable error of the ratio L/VB.

[1] In cold water, flagella have a lower metabolism with which to drive a more viscous fluid, and the energy of the oscular current is less. When increase of temperature first becomes injurious, diminishing vitality is compensated by diminishing viscosity ; with still higher temperatures the change in viscosity is less and the injury greater (cf. 'Linn. Soc. J.', 34, p. 317).

[2] Experiments in June and July, temperature unfortunately not observed.

Note 2. Regulation of the Size of the Osculum.

In two examples of Leucandra aspera (one with an iris-like oscular sphincter) I cut off the oscular end for preservation, and observed that the aperture contracted to one-third of its normal diameter. Mechanical stimulation failed to induce further contraction.

There was more than once evidence that the oscular aperture contracted when the current through it grew feebler, though the contraction was not sufficient to keep from diminution either the oscular velocity or the length of the jet. In a Leucaltis, observed over 24 days, the osculum contracted to half its original diameter, while the length of the jet diminished from 18 cm. to 1 cm. I give this series of measurements, which shows also that when the current recovered (probably with a lower temperature) the osculum widened again.

July	7,	7,	8,	9,	9,	11,	11,	11,	20,	21,	22,	23,	29,	29,	30,	30,	30,	31
Diameter of osculum	·38	·35	·30	·25	·18	·32	·28	·25	·20	·205	·20	·20	·17	·16	·18	·18	·18	—
Length of jet	15+	9	7	6·5	2	13	8	7½	6·5	6·7	6·2	4·4	·9	·5	1·6	1·0	·5	·7

Parker found in Stylotella (' Journ. Exp. Zool.', 8, p. 784) that the oscula close when the external water is still; this may be called ' Parker's reaction '. In Leucandra and Leucaltis it is shown by the sphincter of the osculum being inhibited from contraction by the movement of water over its internal surface. If the reaction is such that there is neither contraction nor relaxation in response to the velocity characteristic of the species (Note 5 (12)) the optimum osculum will be maintained at all stages of growth.

Note 3. Delivery of Water and Secretion of Lime.

In the Leucandra aspera of Text-fig. 1, taking the mean oscular velocity at 8·5 cm. per second (see Note 1), the area of the osculum being 0·031 sq. cm., the delivery per second was 0·26 c.cm., or 16 c.cm. per minute; that is ·9 litre an hour, 90 litres in 4 days, and a ton in 45 days (see Note 6).

This sponge was gathered on May 20, and from Vosmaer ('Mitth. Z. S. Neapel', v, pp. 486, 487) was probably only a month old, during which time its volume and the volume of its delivery must be supposed to have increased by some 40 per cent. every day in geometrical progression. Then the total amount of water passed during its whole life would be the equivalent of $3\frac{1}{2}$ days of its final delivery, and it would have extracted food from a total weight of 80 kg. of water.

By weighing the preserved sponge alternately in alcohol and water I found the total volume 0·31 c.cm., weight 0·59 gm., ∴ sp. gr. 1·9. Allowing 2·5 for the sp. gr. of the spicules and 1·4 for that of the dry protoplasm, this gives

	Total Volume.	*Dry Weight.*
	c.cm.	*gm.*
Spicules of Leucandra aspera (Text-fig. 1)	·15	·38
Protoplasm	·16	·21
	·31	·59

Therefore from 80 kg. of water the sponge abstracted a total weight of 0·38 gm. of carbonate of lime, or 0·005 gm. per kg., or about one-third of the amount of carbonate of lime which can be dissolved in pure water free from carbonic acid, and about one-twelfth of the total lime in an average sample of sea-water. If we suppose the sponge's life to have been longer, or the osculum to have been more dilated (Note 6), the percentage proportion of lime extracted is proportionately less.

Note 4. Fan-shaped Sponges.

There is a possibility that these, like the Hexactinellids, are found always in a permanent current, on which they depend for subsistence.

After this paper was read at the British Association, Sir W. Herdman kindly informed me that in the deeps off Scotland (where the sponges were found from the figures of which Text-fig. 11 was made) there are many places where the current sets only one way. And Professor Stanley Gardiner added that in 30 fathoms off the Seychelles (where the sponge

was found of Text-fig. 12) there is a constant current down the slope away from the shore. The hypothesis is possible that the fan-shaped form only occurs in response to the stimulus of a constant current (compare Note 2) across which its plane is extended; but that if the current turns tidally from all points of the compass, the fan grows up across each direction in turn, so that an open cup is formed.

The advantage of the fan in still water is shown in the text, but in a current turning tidally the efferent stream will be driven back on the sponge for half the day. In such a position the vertical oscular stream is the common form, because this forms an equal angle with the supply from whatever point it comes. Oyster-shaped sponges, with oscula on the edges, are possibly from a channel where the tide runs alternately from two opposite points; they may be called ' pectinate '.

Sponges living always in the surf, or long flexible sponges, such as Chalina oculata, which point downstream from their stalks, have of course no need to do more than to lift the outflowing water sufficiently from their surface for the current down which they lie to bear it free of their more apical parts.

The conditions discussed in this paper affect sponges which are left long in tide-pools, and sponges which inhabit depths where there is inappreciable wave-motion, and where currents are feeble.

Note 5. Calculations of Pressure and Optimum Size of Osculum.

(Mathematical basis of the paper.)

The loss of energy in a tube from resistance due to viscosity in unit of time is

$$8\pi\mu v^2 b,$$

where v = velocity,
b = length of tube,
μ = index of viscosity.

Therefore if E be the loss of energy per second in N similar tubes,

$$E = 8\pi\mu \cdot Nu^2 b.*$$

Let q be the quantity of water passing per second through the N tubes in parallel, the loss of energy per second is

$$\epsilon = 8\pi\mu \,.\, Nb\frac{q^2}{a^2},$$

where a is the aggregate area of the cross-sections of the tubes.

In the sponge the whole of the water passes in succession through

(1) Afferent canals;
(2) Flagellate chambers;
(3) Efferent canals;
(4) The cloaca.

For the whole system, therefore, the loss of energy due to resistance is the sum of the losses in these four systems, which may be represented

$$\sum \epsilon = 8\pi\mu\left(\sum \frac{N\,.\,b}{a^2}\right)q^2.$$

Let
$$8\pi\mu\left(\sum \frac{N\,.\,b}{a^2}\right) = F. \tag{1}$$

Then the energy reaching the osculum per second is

$$E = Pq - Fq^2, \tag{2}$$

where P is the pressure maintained by the action of the flagella.

But if v be the velocity at the osculum, the energy of the jet per second is

$$E = \rho \,.\, q\frac{v^2}{2}, \tag{3}$$

where ρ is the density of the water; and if x be the diameter of the osculum

$$q = \frac{\pi}{4}x^2 \,.\, v.$$

* It was the late Professor Sir G. G. Stokes, in 1888, who supplied me with this formula, and a clearly written exposition of its meaning, which could be understood by the ignorant. I cannot allow my use of it to appear in print without a tribute to his kindness to a then young man, unknown to him, with no recommendation but a somewhat shameless request for assistance.

Therefore from (2) and (3)

$$\rho \frac{v^2}{2} = P - Fq, \tag{4}$$

$$= P - \frac{\pi}{4} Fx^2 v.$$

$$\therefore \quad v^2 + \frac{\pi}{2} \cdot \frac{F}{\rho} \cdot x^2 v = 2 \frac{P}{\rho}. \tag{5}$$

If l be the length of the oscular jet, I find by experiment (p. 298 and Note 1) that

$$l = Cvx. \tag{6}$$

* Substitute $\frac{l}{Cx} = v$ in (5), therefore

$$\frac{l^2}{c^2 x^2} + \frac{\pi}{2} \cdot \frac{F}{\rho} \cdot \frac{x^2 l}{Cx} = \frac{2P}{\rho}, \tag{7}$$

$$\therefore \quad \frac{2}{C^2} + \frac{\pi}{2} \cdot \frac{F}{\rho} \cdot \frac{x^3 l}{C} = \frac{2Px^2}{\rho}. \tag{8}$$

Now l has a maximum or minimum when $\frac{dl}{dx} = 0$. Differentiating (8),

$$\frac{2l}{C^2} \cdot \frac{dl}{dx} + \frac{\pi}{2} \cdot \frac{F}{\rho C} \left(3x^2 l + x^3 \frac{dl}{dx} \right) = \frac{4Px}{\rho}.$$

Therefore when $\frac{dl}{dx} = 0$, $\frac{\pi}{2} \cdot \frac{F}{\rho C} \cdot 3l = \frac{4P}{\rho x}$.

$$\frac{l}{C} = \frac{8P}{3\pi Fx}.$$

Substitute this value in (7), therefore l has a maximum or minimum when

$$\left(\frac{8P}{3\pi F} \right)^2 \cdot \frac{1}{x^4} + \frac{4P}{3\rho} = \frac{2P}{\rho},$$

* I have to thank Mr. G. I. Taylor, F.R.S., who has very kindly read the first proof-sheets of this paper, for giving me this simple demonstration of (9), from (5) and (6), to replace my own very clumsy differentiation.—June 8, 1923.

or $$\left(\frac{8P}{3\pi F}\right)^2 \cdot \frac{1}{x^4} = \frac{P}{\rho}\left(2 - \frac{4}{3}\right) = \frac{2}{3}\ \frac{P}{\rho}\cdot$$

$\therefore$ when $$x^4 = \frac{64P^2}{9\pi^2F^2} \cdot \frac{3\rho}{2P} = \frac{32P}{3\pi^2F^2}\cdot \qquad (9)$$

(Note that the value of C is not involved in this equation.)

$$\therefore \quad x^4 = 1{\cdot}081\rho\frac{P}{F^2}, \text{ or taking } \rho = 1{\cdot}025,\ x^4 = 1{\cdot}11\frac{P}{F^2}\cdot$$

The negative and imaginary roots do not concern us, for x is necessarily rational and positive; and since P and F are finite and positive, this equation gives a finite and rational value for x, and therefore, from (5), l is also rational, positive and finite.

But there is no jet from an aperture of infinite radius, because the velocity is zero, and there can be no jet from a closed aperture;

therefore when $x = \infty$, $l = 0$; and when $x = 0$, $l = 0$;

therefore the value of x given by (9) corresponds to a greater value of l than that when $x = 0$, or when $x = \infty$; and as it is the only positive and finite value for x for which $\frac{dl}{dx} = 0$, therefore the corresponding value of l is the only positive maximum of l, and the length of the oscular jet has its greatest value when the diameter of the osculum has the value X, where

$$X = \sqrt[4]{1{\cdot}11\frac{P}{F^2}} = 1{\cdot}03\sqrt{\frac{\sqrt{P}}{F}}\cdot \qquad (10)$$

If a second sponge precisely similar to A. 11 were to have the oscular end of its cloaca united with the oscular end of the cloaca of A. 11, to make a twin sponge with a single osculum, we should have twice the number of afferent and efferent canals, &c., and two cloacae; so that in the computation of F, N and a would both be doubled, with the result that, comparing F_2 for the twin sponge with F of the original sponge, by (1)

$$F_2 = 8\pi\mu\left(\sum\frac{2Nb}{4a^2}\right) = \frac{F}{2}\cdot$$

With twice the number of flagellate chambers, q_2 will be approximately equal to $2q$, therefore $F_2 q_2 = Fq$. The pressure in the flagellate chamber depends solely on the structure and vigour of the flagellate cells, velocity there being so slow that kinetic energy is always negligible, therefore $P_2 = P$, whatever the number of chambers.

But, from (4), $$P = \frac{\rho v^2}{2} + Fq. \qquad (11)$$

$$P_2 = \frac{\rho (v_2)^2}{2} + F_2 q_2.$$

Therefore $v^2 = v$, and the velocity from the osculum is the same in sponges of similar canal systems, irrespective of size; that is, of the number of similar units which are grouped to expel water by one osculum; and for Leucandra aspera gigantea in health, from Note 1, $$L = 12 \times 8{\cdot}5\, B = 100\, B. \qquad (12)$$

Now by (10) $$X = 1{\cdot}03 \sqrt{\frac{\sqrt{P}}{F}}.$$

Therefore for the twin sponge

$$X_2 = 1{\cdot}03 \sqrt{\frac{\sqrt{P_2}}{F_2}} = 1.03 \sqrt{\frac{2\sqrt{P}}{F}} = X\sqrt{2}.$$

Similarly, if m similar units, for each of which the optimum oscular diameter is X, be united to one osculum, and X_m be the optimum diameter of this, then

$$X_m = X\sqrt{m}.$$

With similar sponges the external volume may be taken as the approximate measure of the number of similar units aggregated into one individual; or, more conveniently, the product of length, breadth, and thickness may be taken as the measure. Calling this product M, its value for A. 11 is 4·1 c.c., and we shall find in Note 6 that for A. 11 $X = {\cdot}25 \pm {\cdot}03$.

Therefore, for Leucandra aspera gigantea of similar canal-system, the optimum diameter of the osculum in centimetres is numerically

$$X_m = {\cdot}25 \sqrt{\frac{M}{4{\cdot}1}} = {\cdot}12 \sqrt{M},$$

and generally for any one species and metamp

$$X_m = \alpha \sqrt{M},$$

the area of the osculum varying as the volume of the sponge.

For a sponge like the bath-sponge, with N oscula, the sum of whose diameters is $\sum X$, approximately,

$$\sum X = N\alpha \sqrt{\frac{M}{N}} = \alpha \sqrt{NM}.$$

NOTE 6. ARITHMETICAL TESTS, DATA, AND CONCLUSIONS.

From camera lucida drawings of the canals and their apertures in the Leucandra aspera of Figs. 1 and 7 ('A. 11' of my records) F is computed in the table below to be 180 ± 30, the relative velocities being confirmed by the times taken by litmus to pass through the walls of the sponge and through its cloaca (p. 293). Using this value in (11), with $\rho = 1{\cdot}025$, $v = 8{\cdot}5 \pm 1{\cdot}5$, we find for A. 11 the equation

$$P = 37{\cdot}0 \pm 14 + (1200 \pm 420)x^2, \qquad (13)$$

so that, if $x = {\cdot}20$, the diameter of the osculum measured in spirit, then

$$P = 85 \pm 30 = {\cdot}9 \text{ mm.} \pm 3 \text{ mm. of water};$$

and by equation (10), the optimum diameter of the osculum

$$X = {\cdot}235 \pm {\cdot}025.$$

Note 2 shows the need of a probable correction in the value of x. The Leucandra 'A. 11' was 4 hrs. under experiment before being preserved, and its velocity had sunk to less than half its original value. If we may reason from the observations on Leucaltis we should expect the diameter of the osculum to have been reduced by 30 per cent., and therefore that for a velocity of 8·5 cm. it had been ·28 cm. wide, instead of the ·20 cm. measured after preservation.

With $x = {\cdot}28$,

$$P = 131 \pm 45 = 1{\cdot}33 \text{ mm.} \pm {\cdot}46 \text{ mm. of water},$$

$$X = {\cdot}26 \pm {\cdot}03.$$

The conditions under which the diameter of the osculum is equal to the theoretically best diameter are found by substituting the value of X in (10) for x in (11), giving the relations

$$\left.\begin{aligned} P &= 1{\cdot}55\, v^2 \\ x^2 &= 1{\cdot}33\, \frac{v}{F} \end{aligned}\right\}. \qquad (14)$$

So that, with $F = 180$,

$$\text{if} \quad v = 8{\cdot}5\,;\ x = X = {\cdot}251,\ P = 112\,;$$
$$v = 9{\cdot}0\,;\ x = X = {\cdot}258,\ P = 126\,;$$

the latter being acceptable values. On the other hand, we may ascertain what error is indicated in F, if we assume that in healthy life x was ·28 cm. as suggested above, and that P (cf. p. 306) was exactly proportional to the pressure of 370 ± 25 found by Parker in the 35μ spherical chambers of Stylotella:

The chambers of Leucandra being cylindrical and 54μ in diameter,

$$P = (370 \pm 25) \times \tfrac{1}{2} \times \tfrac{35}{34} = 120 \pm 8.$$

By (14) $$v = \sqrt{\frac{P}{1{\cdot}55}} = \sqrt{\frac{120 \pm 8}{1{\cdot}55}} = 8{\cdot}84 \pm {\cdot}3\,;$$

by assumption $x = {\cdot}28$;

by (14) $$F = \frac{4}{3} \cdot \frac{v}{x^2} = \frac{4}{3} \times \frac{8{\cdot}84}{{\cdot}0784} = 150.$$

The diminution of viscosity with warmth would reduce F from 180 at 15° C. to 150 at 22° C. This is no unlikely temperature for the Porto Militare in the summer, so that the investigation shows the data in harmony with each other and with the theories of the paper.

The arithmetical coincidence suggests that the probable errors of the observations are overstated. The errors could not be calculated statistically, and I could not estimate them at smaller figures. I much regret having been unable to claim greater exactitude.

TABLE OF DATA FOR CALCULATION OF VELOCITIES AND RESISTANCE (L. aspera. 'A. 11').

	Number.	Mean length.	Aggregate transverse area.	$\therefore F = \cdot 28 \frac{N}{a^2}$†	Velocity. (1)	(2
Afferent canals . .	81000	·06	4·2	99	·06	·13
Subchoanal space . .	—	—	200	—	·0013	·0026
Flagellate chambers .	2,250000	$\cdot 019 \times \frac{1}{3}$*	52·5	1	·005	·010
Efferent canals . .	5200	·08	2·5	47	·10	·21
Cloaca . .	1	$10 \times \frac{1}{2}$*	·21	33	1·3	2·5
Osculum . .	1	—	(1) ·031 (2) ·062	—	8·5	8·5
Whole sponge . .				180		

Therefore, for A. 11, $F = (180 \pm 30) \times \{1 - \cdot 024\,(t - 15)\}$, where $t°$ is the temperature Centigrade.

* The factors $\frac{1}{3}$ and $\frac{1}{2}$ allow for the water entering by holes along the walls.

† At 15° C. From equation (1).

(1) Calculated for oscular diameter ·20, area ·031, delivery ·26.

(2) Calculated for oscular diameter ·28, area ·062, delivery ·53.

On the Development of the Hypobranchial, Branchial, and Laryngeal Muscles of Ceratodus. With a Note on the Development of the Quadrate and Epihyal.

By

F. H. Edgeworth, M.D.

With 39 Text-figures.

As is well known, Wiedersheim stated that laryngeal muscles exist in Lepidosiren and Protopterus, but are absent in Ceratodus. He admitted, however, that the specimen investigated was badly preserved.

In a recently published paper (1920) on the laryngeal muscles of Amphibia, I suggested that, possibly, they might be found in better specimens. Owing to the skill and perseverance of Dr. Bancroft I have come into possession of some well-preserved heads, and also of a series of embryos up to the stage of 30 mm. in length for purposes of investigation. The material also enabled me to examine the development of the hypobranchial and pharyngeal muscles, which, together with other structures, have been the subject of an elaborate memoir by Greil. In the description given by Semon of the development of Ceratodus the embryos were depicted in a series of stages numbered from 1 to 48, the last mentioned and oldest stage described being an embryo of 17·8 mm. Greil's description is based on Semon's stages, and also extended to stage 48. The embryos described in this paper had been fixed in formalin, and their lengths are given in millimetres. Their relation to Semon's stages will be found in an appendix.

A tabular statement of the synonyms of the names employed has also been appended.

Occipital Myotomes and Nerves. Fürbringer (1897) stated that, in the adult stage of Ceratodus, there are two or three occipital and two occipito-spinal nerves.

x_v	y_{vd}	z_{vd}	a_{vd}	b_{vd}	
	y_v	z_{vd}	a_{vd}	b_{vd}	
	y_v	z_v	a_{vd}	b_{vd}	
	y_v	z_v	a_{vd}	b_{vd}	c_{vd} c coming out between skull and vertebra.

The Plexus cervicalis is formed from x, y, z, or y, z. Sewertzoff (1902) stated that in a 15·7 mm. embryo there are five occipital myotomes in front of the first vertebral arch, the first two being in process of reduction. There are ventral spinal roots corresponding to the fourth and fifth. He identified the fourth myotome with x of Fürbringer, so that the first five are u, v, w, x, y.

Greil (1918) stated that no vertebral arches are present in a 15·7 mm. embryo, and that they are developed in a 17·8 mm. embryo. Five occipital myotomes are present in front of the first vertebral arch in the latter stage. The first two have no corresponding nerve-roots, the third has a (variable) ventral root, the fourth a ventral root, and the fifth a ventral and a (variable) dorsal root. The Nervus hypobranchialis (which = the Plexus cervicalis of Fürbringer) is formed from the variable third, and the fourth and fifth nerve-roots. He identified these five myotomes with v, w, x, y, z of Fürbringer's terminology.

On comparison of these statements it would appear that (1) Sewertzoff's 15·7 mm. embryo was a little more advanced in development than Greil's 17·8 mm. embryo—vide infra. The embryos I have examined at these stages agree with those of Greil, so that, in all probability, Sewertzoff's embryo was somewhat shrunken. (2) Sewertzoff regarded the fourth myotome—counting from before backward—and Greil the

third myotome as myotome x of Fürbringer's classification. The explanation of the difference of opinion is that Sewertzoff's embryo was one in which the variable nerve x was absent. The variation certainly occurs, e.g. in an embryo of 20 mm. Nerves x, y, z were present; in one of 26 mm. (vide Text-fig. 26) there were only nerves y and z. I therefore follow Greil's nomenclature. Atrophy of myotomes takes place from before backwards, as stated by Sewertzoff. Thus in a 20 mm. embryo only a few fibres of myotome v persisted, in one of 24 mm. myotome v has altogether disappeared and also the ventral part of myotome w.

Coraco-hyoideus and Genio-coracoideus. Greil stated that the Coraco-hyoideus is developed from downgrowths of the third to the sixth myotomes, i.e. myotomes x, y, z, a. These downgrowths separate from the myotomes above, fuse together, and form the Coraco-hyoideus, which extends from the shoulder-girdle to the hyoid bar. The primordium of the Genio-coracoideus separates from the ventral edge of the third myotome (i.e. foremost) constituent of the Coraco-hyoideus in a 13·9 mm. embryo, and fuses with its fellow, forming a median muscle which elongates forwards to the jaw and backwards. The posterior end forks right and left, and in a 17·8 mm. embryo—the latest stage investigated—reaches the antero-posterior level of the first branchial arch. This method of development of the anterior constituent of the hypobranchial spinal muscles is not a usual one, and the initial stages were not depicted. The first figures given, i.e. Nos. 424–9, show the Genio-coracoideus already developed as a median muscle, partly in front of and partly underlying the anterior ends of the Coraco-hyoidei.

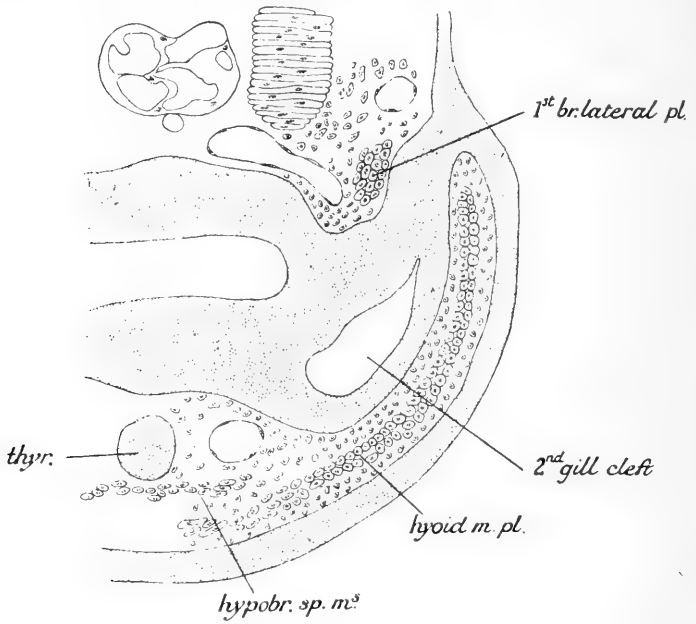
I find that in a 9·5 mm. embryo (Text-figs. 1–5) the pericardium extends forwards to the hyoid segment, and its anterior end is ventral to the hinder edge of the thyroid outgrowth. The primordium of the hypobranchial spinal muscles lies laterally to the pericardium, and, as this lessens in size, approximates to its fellow. In front of the pericardium the two columns come together and lie beneath the thyroid. The anterior part

of the primordium consists solely of yolk-laden cells, the posterior part of muscle-cells.

In a 10·5 mm. embryo (Text-figs. 6–9) the pericardium has

TEXT-FIGS. 1–5.

Embryo 9·5 mm., transverse sections; Text-fig. 1 is the most anterior.

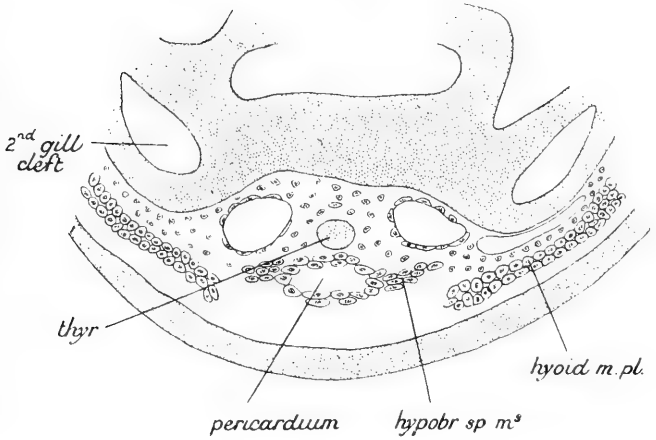


ABBREVIATIONS TO TEXT-FIGURES.

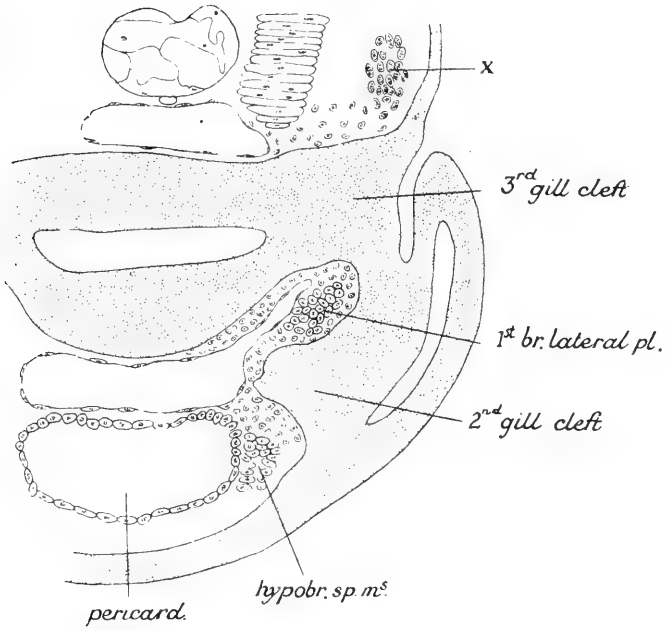
aud. cap., auditory capsule. *ceratobr.*, ceratobranchial. *coraco-hy.*, Coraco-hyoideus. *const. br.*, Constrictor branchialis. *epi-br.*, epibranchial. *genio-cor.*, Genio-coracoideus. *g. petros.* ix, ganglion petrosum ix. *hypobr. sp. ms.*, primordium of hypobranchial spinal muscles. *lateral pl.*, lateral plate. *lev.*, Levator arcus branchialis. *m. pl.*, muscle-plate. *Mx.*, myotome x. *Nx.*, nerve x. *operc.*, opercular fold. *parachord. c.*, parachordal cartilage. *pericard.*, pericardium. *peric. perit. duct*, pericardio-peritoneal duct. *thyr.*, thyroid body. *1st vert. arch*, 1st vertebral arch. Roman numerals, cranial nerves.

retreated a little, and its anterior end is 56 μ behind the thyroid. The primordium of the hypobranchial muscles has separated into anterior and posterior parts—the Genio-coracoideus and

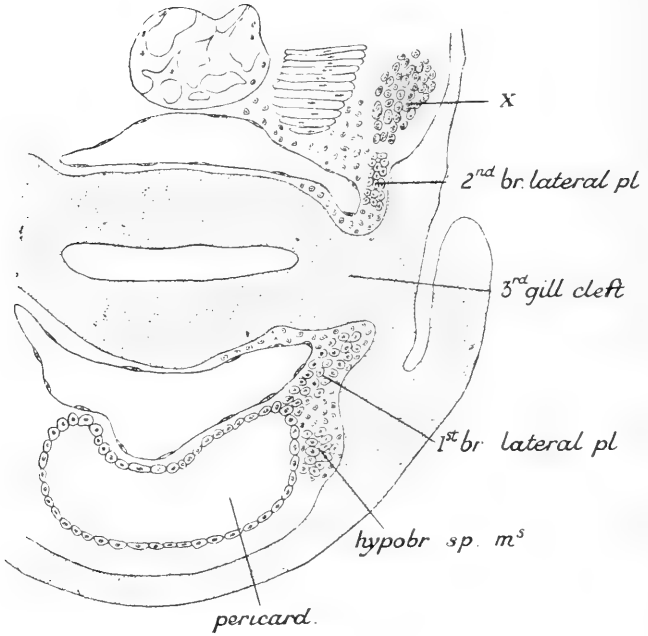
TEXT-FIG. 2.



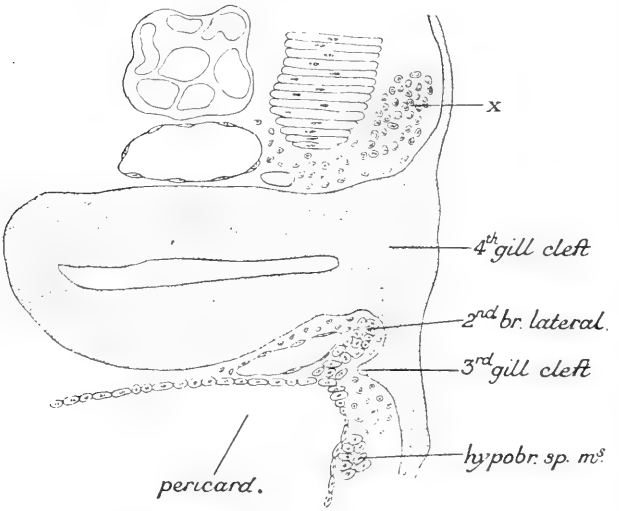
TEXT-FIG. 3.



TEXT-FIG. 4.



TEXT-FIG. 5.



the Coraco-hyoideus. The Coraco-hyoideus lies laterally to the pericardium, and its anterior end is 56 μ behind the thyroid. The Genio-coracoidei form a λ-shaped structure. The anterior median part is ventral to the thyroid: it extends behind this for 104 μ, diverging into two lateral ends which lie beneath the anterior ends of the Coraco-hyoidei. The Genio-coracoidei consist of yolk-laden cells, the Coraco-hyoidei of muscle-cells. In an 11 mm. embryo the anterior end of the Genio-coracoidei extends a little farther forwards—in front of the thyroid, and in a 12 mm. embryo reaches Meckel's cartilages. Its cells become transformed into muscle-cells in a 13 mm. embryo.

The Genio-coracoidei extend slowly backward, diverging into right and left halves. These reach the level of the third branchial arch in a 20 mm. embryo, and become attached to the lateral edges of the median cartilage—called 'sternum' by Greil—which forms the ventral constituent of the cartilaginous shoulder-girdle, in the 28 mm. embryo.

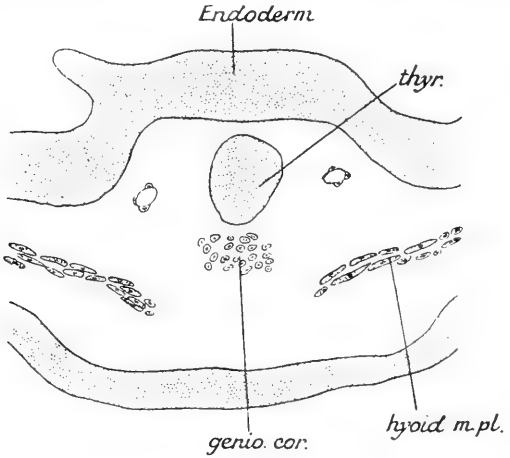
The above may be summarized in the statement that the primordium of the hypobranchial spinal muscles extends forwards, laterally to the pericardium, and in front of this joins its fellow. It then separates into Genio-coracoideus and Coraco-hyoideus. The Genio-coracoidei, in contact with each other from the first, form a median structure which extends forwards to the jaw and backwards to the shoulder-girdle.

Fürbringer stated that the Genio-coracoideus of the adult form extends from the mandible to the shoulder-girdle (coracoid and clavicula) and has one tendinous inscription on its inner, i. e. dorsal surface. This, he said, gives rise to the idea that it originally consisted of two myomeres, but this structure may be secondary. This latter supposition is confirmed by the fact that in a 28 mm. embryo (in which the posterior end of the muscle has reached the 'sternum') there are no inscriptions in the muscle. The same explanation applies to the three inscriptions depicted in the muscle by Maurer.

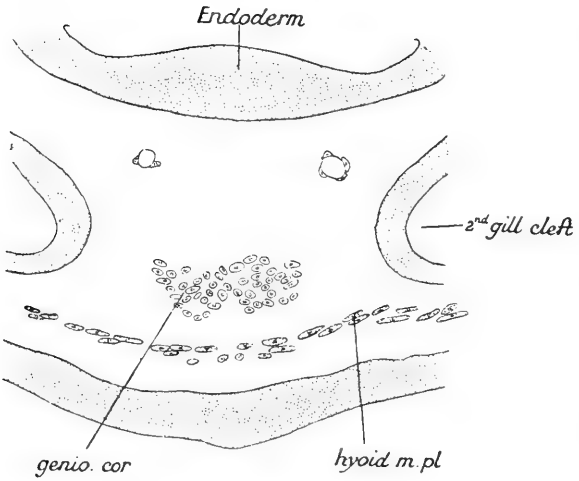
Innervation.—Fürbringer stated that the Coraco-hyoideus and Genio-coracoideus are innervated by the Plexus cervicalis, i. e. Nervi spinales x, y, z, or y, z.

TEXT-FIGS. 6–9.

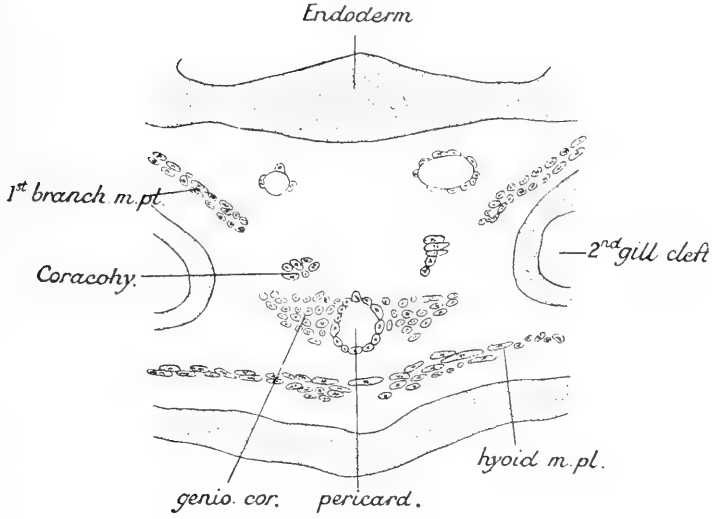
Embryo 10·5 mm., transverse sections; Text-fig. 6 is the most anterior.



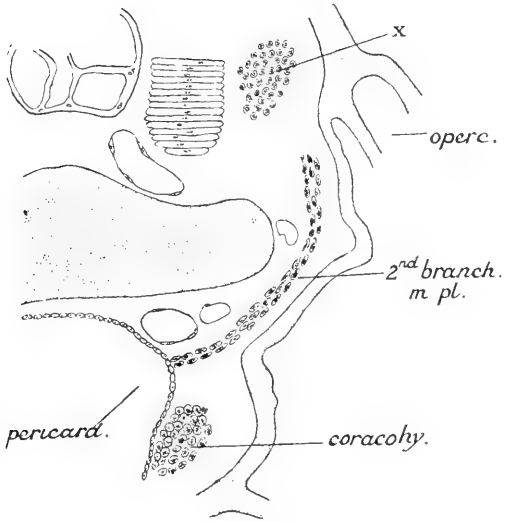
TEXT-FIG. 7.



TEXT-FIG. 8.



TEXT-FIG. 9.



Greil stated that the Coraco-hyoideus is innervated by the N. hypobranchialis (derived from Ni. occips. x, y, z, or y, z) and by the R. hypobranchialis of N. occipito-spin. a, whilst the Genio-coracoideus is innervated by the R. hypohyoideus of N. posttrematicus ix. He did not refer to Fürbringer's statement.

I find in a 27 mm. embryo that an anterior branch of the N. hypobranchialis enters the posterior end of the Genio-coracoideus, and have not found any branch of the IXth nerve entering the muscle.

The Genio-coracoideus and Coraco-hyoideus of Ceratodus are homologous with the Genio-thoracicus and Coraco-hyoideus of Protopterus and Lepidosiren. Agar has shown that the latter is developed from myotomes x, y, z ; but did not describe the development of the Genio-thoracicus.

The Genio-coracoideus of Ceratodus resembles the Genio-coracoideus s. Coraco-mandibularis of Selachii and the Genio-branchialis s. Branchio-mandibularis of Ganoids in that it is formed from the anterior constituent of the hypobranchial spinal muscles and subsequently grows backwards overlapping the posterior constituent—Coraco-hyoideus.

On the Source of the Branchial Muscles.—Greil stated that in a 5·9 mm. embryo the mesoderm lateral to the branchial region of the alimentary canal—between this and the ectoderm—is continuous with the epithelium of the pericardium, and is to be regarded as ' lateral-plate '. In 6·6 to 9·8 mm. embryos these lateral plates degenerate into connective tissue, and their place is taken by downgrowths from the first and second myotomes. The cells of these downgrowths are distinguishable from those of the lateral plates by the shape of their nuclei and the later absorption of their yolk-granules. Processes from the first myotomes penetrate the first three branchial arches, whilst one from the second myotome forks over the sixth gill-cleft into the fourth and fifth arches. In a 10·2 mm. embryo these downgrowths separate from the myotomes above and become the source of the branchial musculature.

I find that in an embryo of 9·5 mm. (Text-figs. 1–5) the cell-

columns in the first and second branchial segments, which consist of yolk-laden cells, are not continuous with the myotome above, but are continuous with the pericardial wall below. I interpret them as lateral plates. In an embryo of 10·5 mm. (Text-figs. 6–9) the pericardium has retreated a little, and its anterior end is 56 μ behind the thyroid, just in front of the lower end of the first branchial segment. In this segment is the first branchial muscle-plate, the lower end of which is detached from the pericardial wall. In the second branchial segment is the second branchial muscle-plate, the lower end of which is continuous with the pericardial wall. The difference between the two segments is owing to the slight retardation in development from before backwards—from segment to segment. I use the term 'muscle-plate' to denote those cells of the lateral plate which are obviously muscle-cells and the primordia of the branchial muscles. Though still containing yolk-granules they are distinguishable from the other cells of the lateral plate.

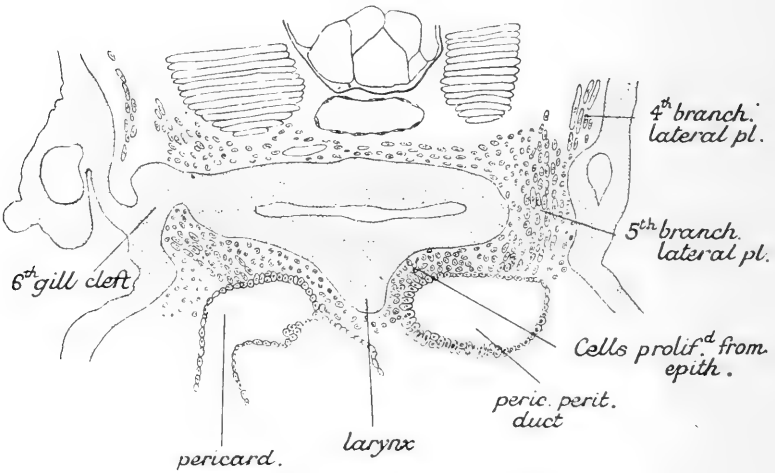
In explanation of the figures it should be added that in the 9·5 mm. embryos the branchial arches slope downwards and slightly backwards, in the 10·5 and 12 mm. embryos they are vertical, in the 16 mm. embryo they slope downwards and forwards.

I thus fail to find any continuity between the myotome above and the cell-columns in the first and second branchial segments in a 9·5 mm. embryo, i.e. at a stage when, according to Greil, such a continuity exists. The same is true of a 9 mm. embryo. Further, in a 10·5 mm. embryo what is obviously the second branchial muscle-plate is continuous with the pericardial wall. The difference in length of these embryos are so slight that it is improbable that—as is demanded by Greil's theory—what is 'lateral-plate' in the 9·5 mm. embryo is replaced by downgrowth from myotome in the 10·5 mm. embryo. Again, Greil's theory fails to explain why a muscle-plate derived from myotome downgrowth should ever be continuous with the pericardial wall. I also fail to find any differences in the shape of the cell-nuclei between the upper and lower parts of the cell-columns of these segments in the 9·5 mm. embryo, as is stated by Greil.

I am therefore of opinion that the evidences presented by these embryos are sufficient to warrant a rejection of Greil's statement that the branchial muscles are derived from downgrowths of the myotomes above, and to show that they are derived from the lateral plates—as is usual in Vertebrates.

What is said above in relation to the first and second branchial segments applies also to the third, fourth, and fifth.

Text-fig. 10.



Embryo 11 mm., transverse section.

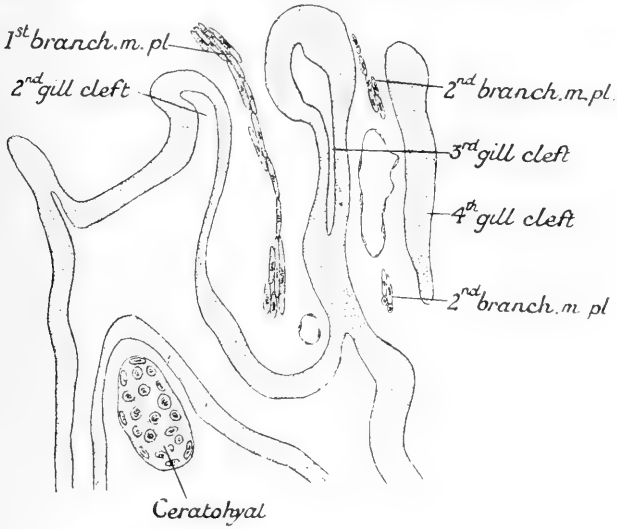
Constrictores branchiales and Levatores arcuum branchialium.—Both these muscles are developed in the first four branchial arches, but a Levator only in the fifth. Their anatomy in the adult stage was first described by Jaquet (1897), and subsequently—with more accuracy—by K. Fürbringer (1904). Greil stated that the Levators in the first four arches are developed from the upper part of the mesoblast (i.e. myotome downgrowth) in the arches, whilst the Constrictors are developed from cells given off from the muscles at their upper and lower ends. His words are ' welcher jedoch keinen Rest des primären den ganzen Bogen durch-

ziehenden axialen Mesoderms bildet, sondern durch Züge spindeliger Zellen, welche von der Dorsal- und Ventralseite (Levatores und Interbranchiales) stammend, vorwachsen, geschlossen wird'. This occurs in the description of the 17·8 mm. stage (p. 1355). No figures were given illustrating this derivation of the Constrictors.

In 10·5 and 12 mm. embryos, and as is additionally shown in

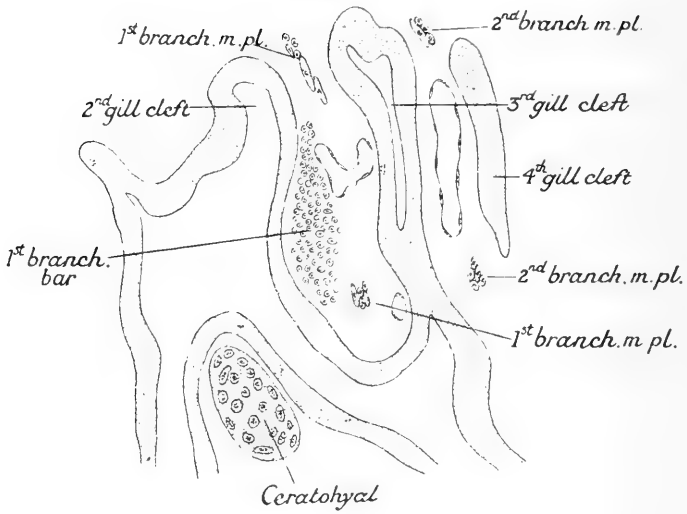
TEXT-FIGS. 11–13.

Embryo 12 mm., sagittal sections; Text-fig. 11 is the most external.

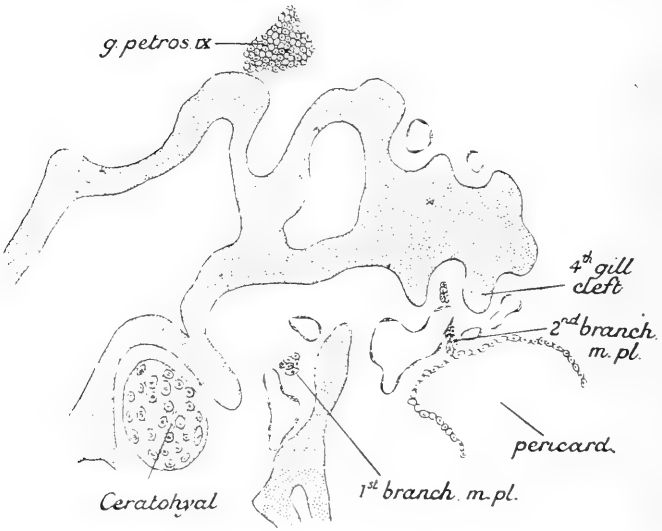


Text-figs. 11–13 taken from sagittal sections of a 12 mm. embryo, the first and second branchial muscle-plates form vertical strips through the whole extent of the arches. The ventral end of the first branchial muscle-plate is detached from the pericardial wall, and has grown a little forwards—towards, but not yet reaching, the Ceratohyal. The ventral end of the second branchial muscle-plate is continuous with the pericardial wall (Text-fig. 14). These muscle-plates, slightly convex outwards, pass down external to the primordia of the branchial bars.

TEXT-FIG. 12.

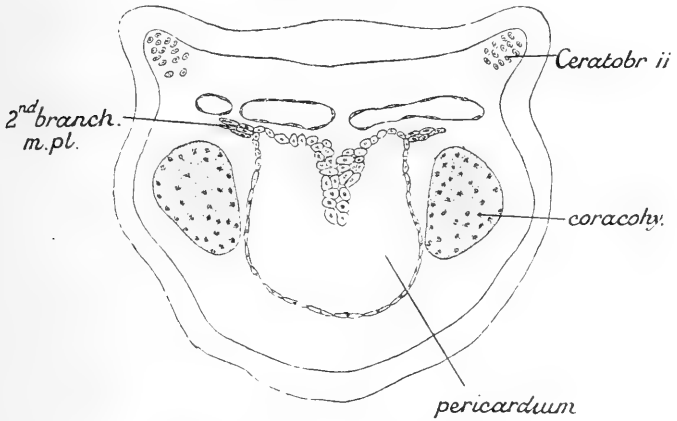


TEXT-FIG. 13.



In an embryo of 13·5 mm. (Text-fig. 15) the ventral end of the first branchial muscle-plate has separated and grown forwards to the Ceratohyal, forming the Branchio-hyoideus. The rest of the muscle-plate persists. The lower end of the second branchial muscle-plate has become detached from the pericardial wall and grown inward, forming the Transversus ventralis ii, and is detached from the vertical strip above.

Text-fig. 14.



Embryo 12 mm., transverse section.

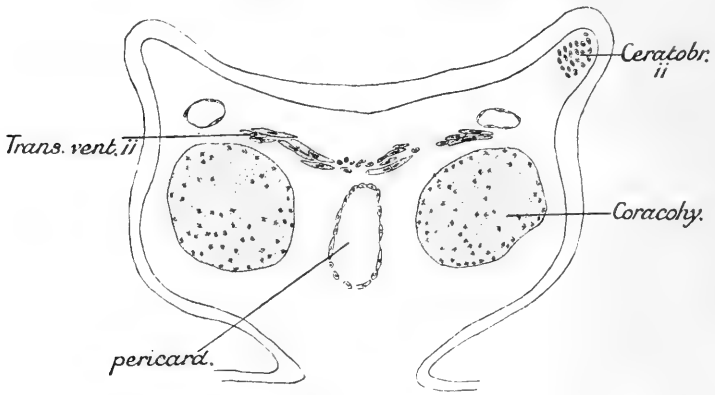
The development in the third and fourth arches is similar to that in the second.

In an embryo of 16 mm. (Text-figs. 18–20) the first four branchial bars have separated into Epi- and Cerato-branchial elements and have chondrified, the process being most complete in the first. The upper end of the first branchial muscle-plate has separated into an inner and outer portion—the inner is the Levator and is inserted into the Epi- and Cerato-branchial i, the outer is the upper end of the Constrictor branchialis, which is continued down through the arch to its lower end. In the second branchial arch separation into these two constituents is not complete, and in the third and fourth branchial arches has barely begun, owing to the progressive retardation in development from before backwards. In a 17·5 mm. embryo

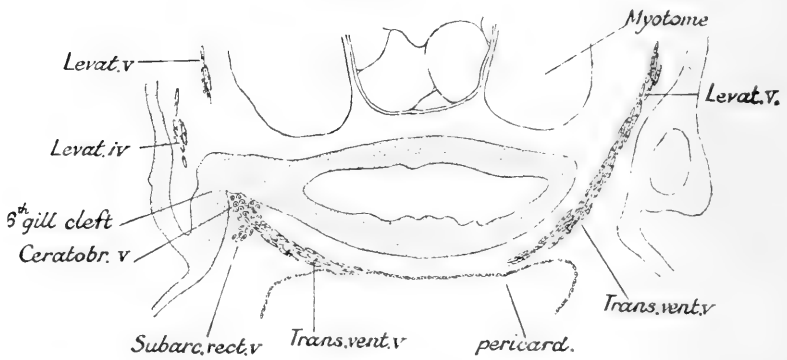
the separation has occurred in these also. In a 27 mm. embryo the lower ends of the Constrictors have grown forwards. Each is attached to the lower end of the Ceratobranchial of the

TEXT-FIGS. 15–17.

Embryo 13·5 mm., transverse sections; Text-fig. 15 is the most anterior; Text-fig. 17 is 32 μ behind Text-fig. 16.



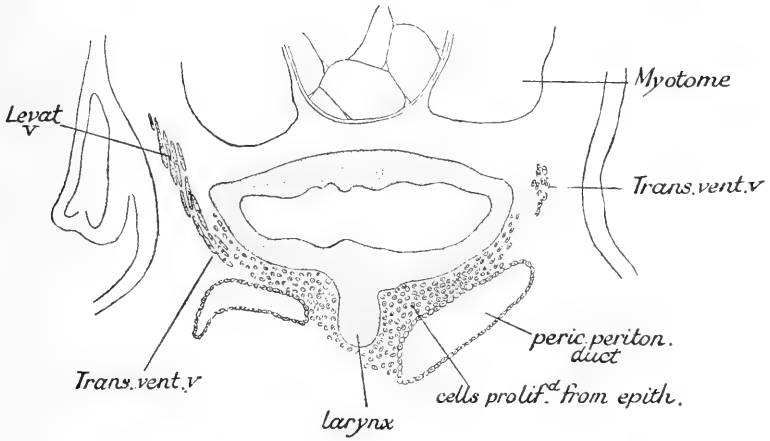
TEXT-FIG. 16.



next anterior arch—the condition described in the adult by K. Fürbringer.

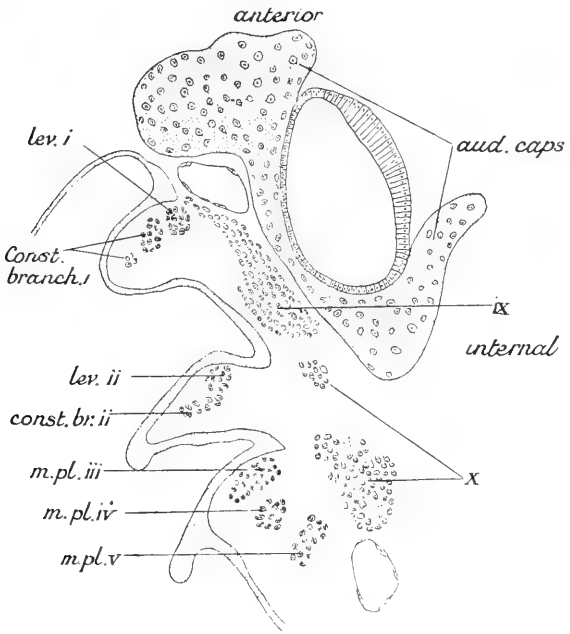
The above-recorded observations show that the Constrictores branchiales are the direct descendants of the branchial muscle-

TEXT-FIG. 17.



TEXT-FIGS. 18–20.

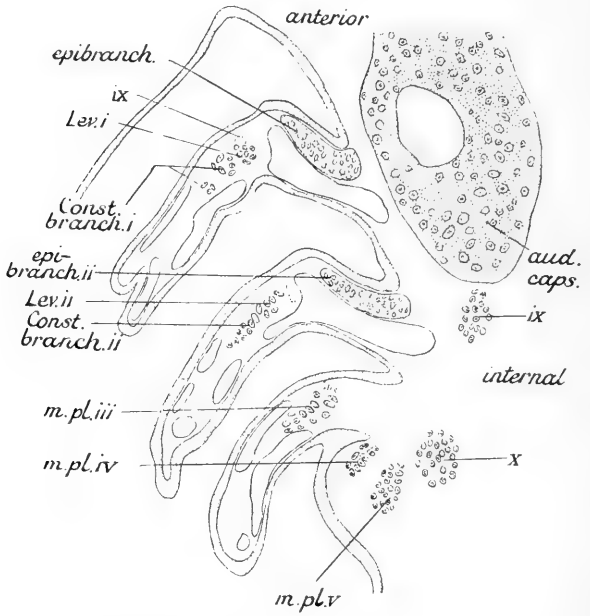
Embryo 16 mm., horizontal sections; Text-fig. 18 is the most dorsal.



plate, and that the Levatores arcuum are separated from their upper ends.

In the case of the fifth branchial arch Greil stated that the mesoblast (i. e. myotome downgrowth) forms the Dorso-pharyngeus. I find that the development is similar to, but not identical with, that of the more anterior arches. In the

Text-fig. 19.

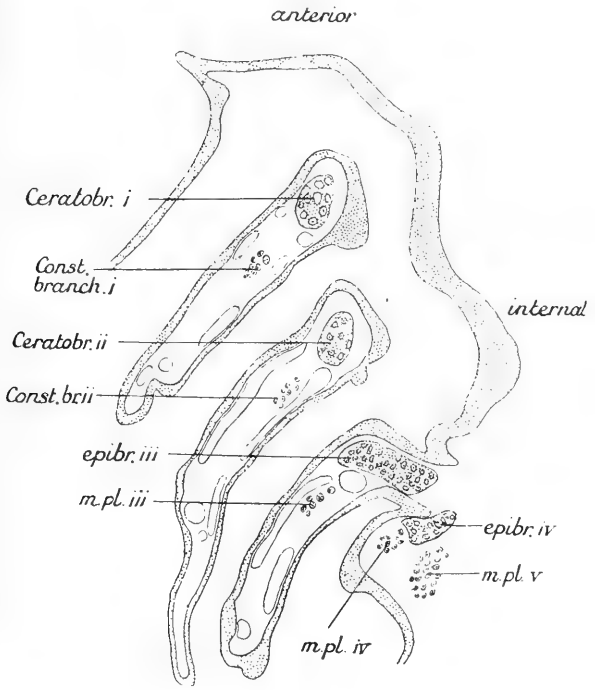


11 mm. embryo (Text-fig. 10) there is a lateral plate, the ventral end of which is continuous with the pericardial wall. In the 13·5 mm. embryo (Text-figs. 16, 17) the muscle-plate is formed; and its ventral end has separated from the pericardial wall and grown inwards forming the Transversus ventralis v. In the 16 mm. embryo some few fibres of the muscle-plate are attached to Ceratobranchial v., but the majority are continuous with the Transversus as in the 13·5 embryo. The condition is a more primitive one than in other arches. The upper part might be called a Levator or a Constrictor branchialis. The

Cucullaris is separated from it in a 14 mm. embryo—as was described by Greil.

It is not known whether the Constrictores branchiales and Levatores arcuum are present in Protopterus and Lepidosiren. Pinkus (1895) did not mention any individual branchial muscle

TEXT-FIG. 20.



in his description of the cranial nerves of Protopterus. The Constrictores are homologous with those of Selachii, the Levatores with those of Ganoids and Amphibia.

Subarcuales recti and Cleido-branchialis.—The Subarcualis rectus i. s. Branchio-hyoideus was first described by Fürbringer (1897), who stated that it is a muscle passing from the Ceratobranchial i. to the Ceratohyal. It was also described in the following year by Jaquet. Greil stated that it is developed

from the ventral end of the mesoblast (i.e. myotome downgrowth) in the first branchial arch. As stated above, I find it to be developed from the ventral end of the first branchial muscle-plate.

Behind this muscle are two others, also longitudinal in direction—the Subarcualis rectus v. and the Cleido-branchialis. The latter was first described by Fürbringer under the name Coraco-branchiales, of which he said five are present, passing from the shoulder-girdle to the five Ceratobranchials. The fifth is the broadest and some of its fibres are attached to the skull. The others are slender. Greil described the development of these muscles as follows. The Subarcualis rectus v. is developed in a 17·8 mm. embryo by forward growth from the mesoblast (i.e. myotome downgrowth) of the fifth branchial arch. Its anterior end becomes attached to the ventral ends of the branchial bars. The Cleido-branchialis is derived from a process of the mesoblast of the fifth branchial arch which grows forwards, separating into three or four pointed extremities (' Zipfel ') which reach the ventral ends of the branchial bars. It forms an aberrant band of muscle which grows in the same direction, but is separate from the Subarcualis rectus v. and gains a secondary relation to the shoulder-girdle.

I find that these two muscles are developed from a single primordium which appears in a 13·5 mm. embryo (Text-fig. 16) as a slight forward growth from the junction of Levator v., and Transversus ventralis v. This primordium extends forwards, reaching the antero-posterior level of the third branchial arch in a 14 mm. embryo and that of the second branchial arch in a 16 mm. embryo (Text-fig. 28). In the last-mentioned stage its hinder part has increased in vertical depth and its postero-inferior angle is attached to the Cleithrum. In a 28 mm. embryo (Text-fig. 29) the primordium has fully separated into the muscles it forms, viz. the Subarcualis rectus v. passing from the fifth to the first Ceratobranchial, and the Cleido-branchialis. This latter muscle is posteriorly attached to the ventral surface of the Cleithrum and separates into fasciculi which, passing dorsal to the Subarcualis rectus v.,

are inserted into the ventral ends of all five Cerato-branchialia. The fasciculus inserted into the first is confluent with the anterior part of Subarcualis rectus v.

Innervation.—According to Fürbringer and Greil, Subarcualis rectus i. is innervated by the IXth. I can confirm this. According to Fürbringer the Cleido-branchialis is innervated by the Plexus cervicalis; according to Greil both the Subarcualis rectus v. and the Cleido-branchialis are innervated by the N. ultimus vagi (Quartus Vagi), i. e. the nerve to the fifth branchial arch. I can confirm Greil's statement.

The Subarcualis rectus i. s. Branchio-hyoideus is homologous with the similar muscle in Lepidosiren, Protopterus, and Amphibians. Its innervation is not known in these Dipnoans. In Amphibia, as in Ceratodus, it is innervated by the IXth.

The morphological nature of the hinder longitudinal muscles is uncertain. Fürbringer held that the Cleido-branchialis is homologous with the Coraco-branchiales of Selachii. Greil did not express any opinion other than that quoted above.

The muscles in Ceratodus are developed from a single primordium which grows forward from the fifth branchial arch. The posterior end of the muscle gains a secondary relation to the Cleithrum, and subsequently an almost complete separation into two muscles takes place. The shoulder-girdle is situated far forwards and has an oblique position—from dorso-posterior to ventro-anterior—its lower part underlying the branchial region. If this represents the phylogenetic development of the muscles, as is probable, the original form was probably a Subarcualis rectus v., passing from the fifth to the first Ceratobranchial, and the Cleido-branchialis is a secondary muscle. The developmental evidence thus leads to rejection of Fürbringer's theory.

It is not known whether there is a Subarcualis rectus v. in Protopterus and Lepidosiren, but Fürbringer described a homologue of the Cleido-branchialis in these Dipnoans and stated that the innervation is, as in Ceratodus, from the Plexus cervicalis.

The Subarcualis rectus v. is probably derived from a Sub-

arcualis rectus v. passing from the fifth to the fourth Ceratobranchial, and resembling the Subarcualis rectus iv a of Urodela in its forward extension to the first Ceratobranchial. I do not know of any homologue of the Cleido-branchialis in other groups.

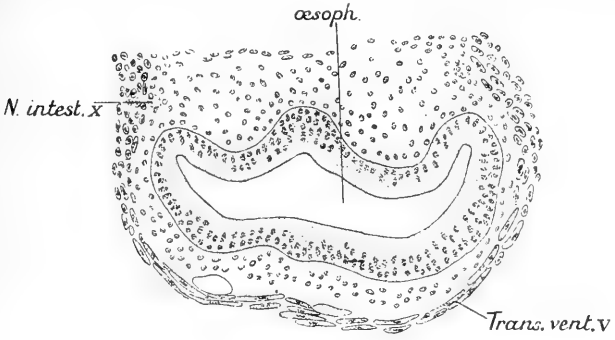
Transversi ventrales ii, iii, iv, v are present—Greil stated that they are developed from the ventral ends of the mesoderm, i. e. myotome downgrowth, in the second, third, fourth, and fifth branchial arches by inward growth. I find that they are developed by inward growth from the ventral ends of the branchial muscle-plates (vide supra). I would add that Transversus ventralis iv. is not always developed, possibly owing to the relatively great size of Transversus ventralis v. Greil held that the Subarcualis rectus i. s. Branchio-hyoideus, developed in the first branchial arch, is serially homologous with the Transversi ventrales of the hinder arches. But it is difficult to think that a longitudinal muscle in one arch is serially homologous with a transverse one in another when neither changes its direction during development. In larvae of Ichthyophis and Siphonops a Subarcualis rectus i. and a Transversus ventralis i. are both developed in the first branchial arch. In Ceratodus, too, a Subarcualis rectus and a Transversus ventralis are developed in the fifth branchial arch. Transversi ventrales iv. and v. occur in Lepidosiren and Protopterus. The Subarcuales recti and Transversi ventrales are homologous or serially homologous with those of Ganoids and Amphibia.

Transversus ventralis v. and Sphincter oesophagi et laryngis.—The Transversus ventralis v., as stated above, is developed in a 13·5 mm. embryo, as an inward growth from the ventral end of the fifth branchial muscle-plate (Text-figs. 16, 17). It extends back behind the posterior edge of the sixth gill-clefts to a greater degree laterally than in the median line, the distances being 96 μ and 40 μ, i. e. the posterior edge of the muscle is concave from side to side. The muscle in subsequent stages spreads backwards below the anterior part of the oesophagus (Text-figs. 21–4). This is concurrent with a backward shifting of the larynx (vide infra),

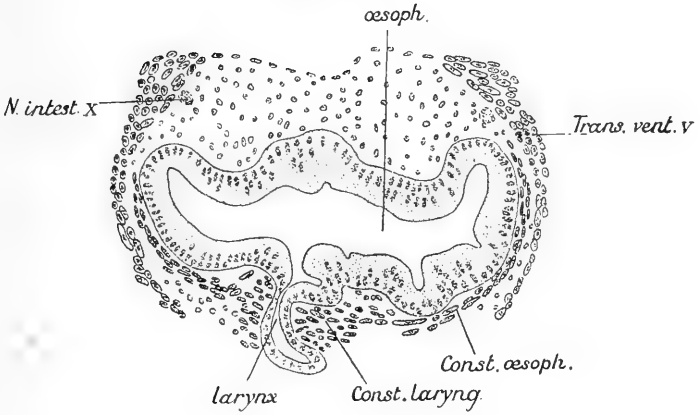
so that in a 30 mm. embryo—the latest embryonic stage investigated—the larynx is not covered by the muscle.

TEXT-FIGS. 21–4.

Embryo 20 mm., transverse sections; Text-fig. 21 is the most anterior.



TEXT-FIG. 22.

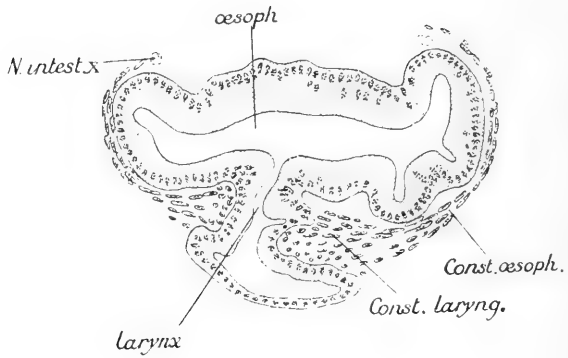


Greil represents the Transversus ventralis v. in his figure of a model of a 17·8 mm. embryo (Taf. lxiv, fig. 3) with a markedly convex posterior edge. But I have not found this in embryos of 14, 15, 16, 17·5, 20, 24, 28, and 30 mm. It is concave from the first and remains so during the extension

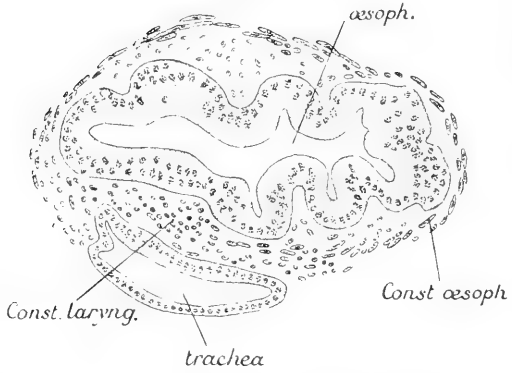
backwards of the muscle, i.e. the posterior extension takes place as fast laterally as in the mid-line.

The posterior part of the Transversus ventralis v. forms the ventral constituent of the Sphincter oesophagi et laryngis, but

Text-fig. 23.



Text-fig. 24.

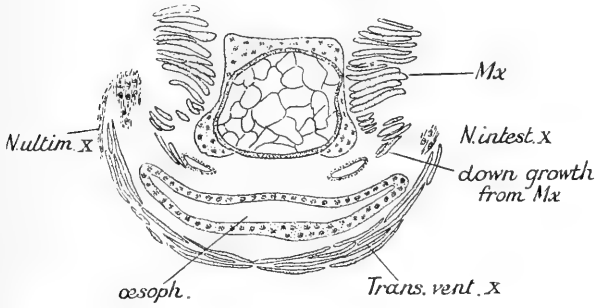


no distinction can be made between the muscles. They form a continuous structure. The edges of the ventral constituent slightly lap round the lateral edges of the oesophagus in a 16 mm. embryo (Text-figs. 21, 22).

In a 24 mm. embryo (Text-fig. 25) a downgrowth takes place on each side from myotome x—downwards, backwards, and inwards, towards the upper part of the oesophagus, a little in

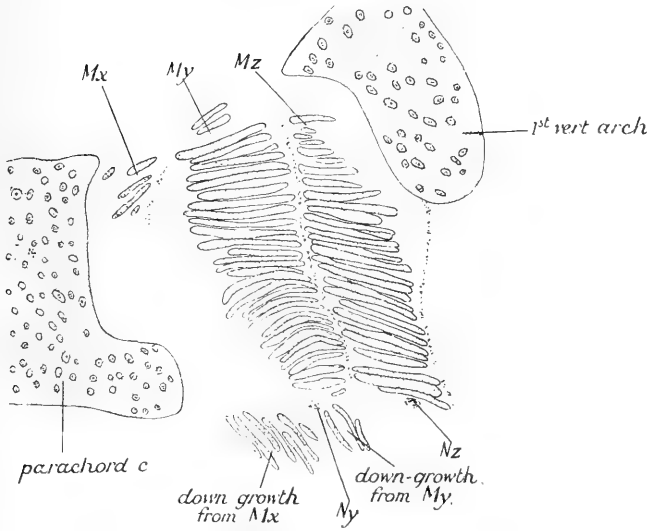
front of the larynx. In one of 26 mm. (Text-fig. 26) this down-growth has separated from its myotome, and there is a second

TEXT-FIG. 25.



Embryo 24 mm., transverse section.

TEXT-FIG. 26.



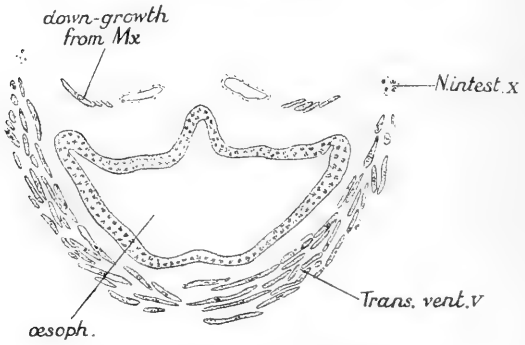
Embryo 26 mm., sagittal section.

downgrowth from myotome y. In an embryo of 28 mm. (Text-fig. 27) this, too, has separated from its myotome, and

the two downgrowths form together a muscle-plate dorsal to the oesophagus. The Sphincter oesophagi et laryngis is thus developed from two constituents—a ventral derived from a backward extension of Transversus ventralis v. and a dorsal derived from the downgrowths of myotomes x and y.

The only changes which take place between this condition and the adult form (as determined by transverse sections) is that the Sphincter is completed by medial and lateral spread of its dorsal constituents, and it extends a little farther backwards in the mid-ventral line so as to underlie the larynx.

Text-fig. 27.



Embryo 28 mm., transverse section.

The Transversus ventralis v. is said by Greil to be innervated by the N. ultimus vagi, i.e. the nerve of the fifth branchial arch. My observations confirm this, and I can add that no additional branch of the vagus is developed in later stages for its hinder part, i.e. the ventral part of the Sphincter oesophagi et laryngis. This is in harmony with its development. The dorsal part of the Sphincter—developed from myotomes x and y—is innervated by branches of the Ni. occips. x and y, or by the latter only when there is no N. occip. x.

Wiedersheim (1904) described the adult condition of the Sphincter oesophagi et laryngis in all three Dipnoi,[1] and

[1] He gave figures of Protopterus and Lepidosiren, but not of Ceratodus.

Göppert (1904), subsequently, in Protopterus. These writers employed the term ' Constrictor pharyngis ' on the theory that it represents the musculature of atrophied hinder branchial segments. But the theory receives no support from the developmental phenomena in Lepidosiren, as described by Agar (1907), nor could I see any evidence in its favour in Ceratodus. I have therefore employed the term ' Sphincter oesophagi et laryngis '.

Agar stated that the ventral portion of the Sphincter oesophagi et laryngis of Lepidosiren is derived from cells budded off from the inner walls of the pericardio-peritoneal ducts, and its dorsal portions from downgrowths of the occipital myotome y (' possibly, but not probably, the downgrowth extends to x and z also '). These two constituents coalesce and form a complete sphincter muscle.

As, however, the ventral part of the Sphincter is continuous anteriorly with Transversus ventralis v. in all three adult Dipnoi, and this is not excluded by the figures given by Agar of the first stage of its development in Lepidosiren, it is possible that this part is, as in Ceradotus, due to a backward extension of Transversus ventralis v.

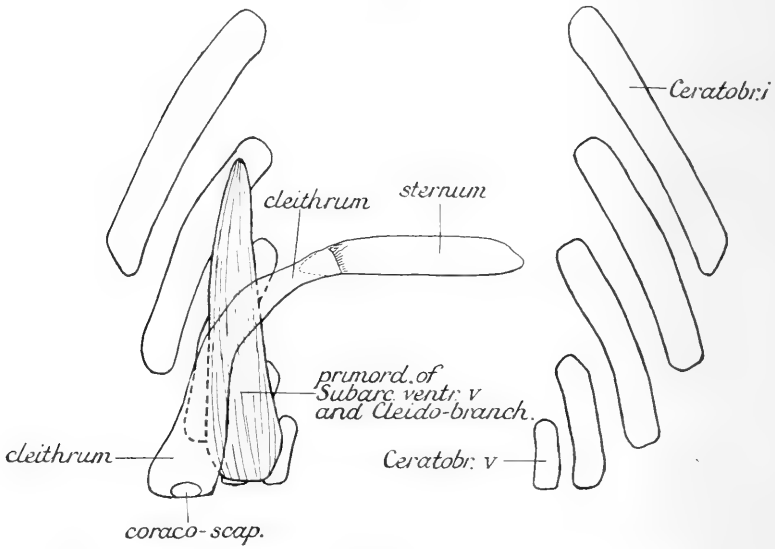
The ventral part of the sphincter of Lepidosiren is said by Agar to be innervated by the N. muscularis vagi. This is apparently the nerve of the fifth branchial arch, and the innervation would thus agree with that of Ceratodus. But the description given by Pinkus of the innervation of the muscles in the branchial region in Protopterus is very vague, and exact information is needed. The dorsal portion of the Sphincter of Lepidosiren is innervated, in accordance with its derivation, by Nervus occip. y (Agar) ; Lepidosiren and Ceratodus are similar in this respect.

Larynx.—Neumeyer (1904) stated that the larynx is developed in a 10·9 mm. embryo immediately behind the branchial region, Kellicott (1905) that it developed in a 11·6 mm. embryo in the ventral wall of the pharynx near the commencement of the oesophagus, Greil (1913) that it developed in a 11·6 mm. embryo at the level of the fifth branchial segment,

i. e. behind the sixth gill-clefts. No observer made mention of any subsequent backward shifting of the larynx.

I find that there is a slight trace of the laryngeal outgrowth in a 10·5 mm. embryo, but it is quite clear in a 11 mm. embryo (Text-fig. 10) as a median ventral outgrowth of the pharyngeal and oesophageal epithelium, extending from the anterior border of the fifth to 8 μ behind the posterior border of the sixth gill-cleft.

TEXT-FIG. 28.



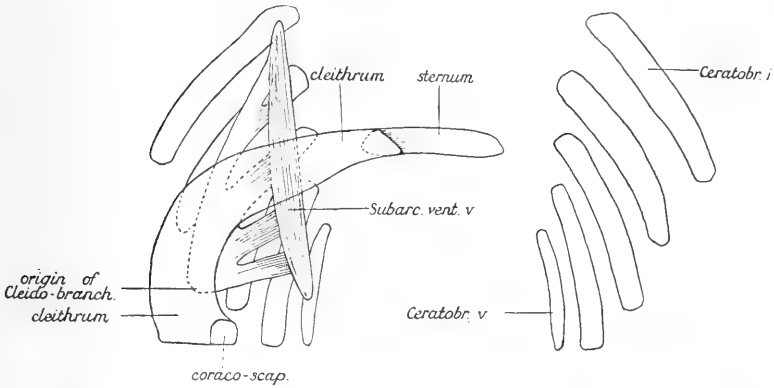
Embryo 16 mm. Model of Ceratobranchialia and Subarcualis rectus v.[1]

In a 12 mm. embryo the continuity of the laryngeal epithelium with the pharyngeal and oesophageal epithelium extends from 16 μ in front of the anterior border of the sixth gill-cleft to 72 μ behind it. In a 13·5 mm. embryo the continuity extends from 72 μ behind the sixth gill-cleft to 152 μ behind it. In a 20 mm. embryo it extends from 480 μ behind the sixth gill-cleft to 600 μ behind it, and the oesophagus opens into the stomach 140 μ

[1] In Text-figs. 28 and 29 only the front parts of the Cleithrum and Coraco-scapulare are represented. The epibranchialia i–iv are not shown.

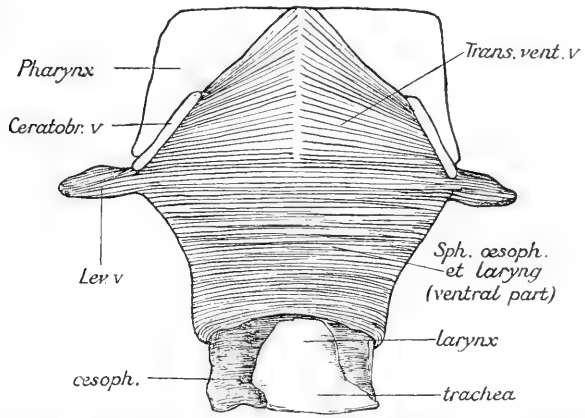
behind the posterior border of the larynx. The pre-laryngeal region of the oesophagus at this stage is thus 460 μ, the laryngeal region 120 μ, and the post-laryngeal region 140 μ in length. The above can be summed up in the statement that the larynx

TEXT-FIG. 29.



Embryo 27 mm. Model of Ceratobranchialia, Subarcualis rectus v, Cleido-branchialis, and shoulder-girdle.

TEXT-FIG. 30.



Embryo 20 mm. Model showing Transversus ventralis v. and Sphincter oesophagi et laryngis (ventral part); the laryngeal muscles are not represented.[1]

[1] Text-figs. 28, 29, and 30 were drawn by Miss Cross.

is developed as a median ventral outgrowth of the endoderm in the last (fifth) branchial segment and anterior end of the oesophagus, and subsequently shifts backward relative to the sixth gill-cleft so as to be situated entirely in the oesophageal region. During this period the larynx increases in size, though to a far less extent. Thus in the 12 mm. embryo the epithelium of the larynx is continuous with the endoderm above over a length of 86 μ from front and back ; in the 20 mm. embryo over a length of 120 μ. The trachea begins to be formed in the 13·5 mm. embryo as a posterior outgrowth of the larynx.

The larynx of Ceratodus is thus developed in the same position as in Menopoma, but unlike that of Menopoma it subsequently shifts backwards. The backward extension of Transversus ventralis v. is in relation to this. There is no similar extension of Transversus ventralis iv. in Menopoma.

Oesophageal and Laryngeal Muscles.—The only statement that Greil made in regard to these muscles is that in a 13·9 mm. embryo the free mesoderm cells increase in numbers round the oesophagus. These cells are chiefly of paraxial origin. They form a mantle round the gut and become for the most part smooth muscle-cells.

I find that a Constrictor oesophagi and a Constrictor laryngis are present in the adult, and that they are developed from cells which are budded off from the epithelium of the pericardio-peritoneal ducts in an 11 mm. embryo (Text-fig. 10). These cells increase in numbers and spread round the larynx and oesophagus, and in a 16 mm. embryo form the above-mentioned muscles, which are better developed in a 20 mm. embryo from which the figures are drawn (Text-figs. 22–4). The Constrictor oesophagi extends from the level of the anterior edge of the larynx to the posterior end of the oesophagus. In the laryngeal region its fibres are interrupted ventrally and pass towards the epithelium of the larynx through the fibres of the Constrictor laryngis. Behind the larynx the fibres encircle the oesophagus. The anterior part of the Constrictor oesophagi thus acts additionally as a dilatator of the larynx, but this part is continuous with the posterior part, and remains so up to the adult state. A separate Dilatator laryngis is not formed.

The Constrictor laryngis consists of short muscle-cells encircling the larynx in a horizontal plane. In transverse sections they are most obvious as such just in front and behind the larynx. The Constrictor laryngis gradually increases in size, and in the adult forms a muscle of many fasciculi which are penetrated by the dilatator fibres of the Constrictor oesophagi.

Innervation.—In a 27 mm. embryo the N. intestinalis x. passes back dorso-lateral to the oesophagus just within the upper edge of the ventral constituent of the Sphincter oesophagi et laryngis. At the anterior edge of the Constrictor oesophagi it gives off a ventral branch which passes to the larynx, the Constrictor oesophagi and Constrictor laryngis.

The oesophageal and laryngeal muscles of Ceratodus thus resemble those of Menopoma in that they are differentiated from cells which are proliferated from the splanchnic layer of the coelomic epithelium.

Pinkus described in Protopterus a fine twig from the N. intestinalis x. to the mucous membrane of the pharynx and larynx. This is the homologue of the laryngeal branch of the N. intestinalis x. in Ceratodus.

The evidence hitherto available suggests the probability that the Dilatator and Constrictor laryngis, and the Dilatator laryngis (and also the Constrictor oesophagi if microscopical examination shows it to be present) of Lepidosiren will be found to be developed from the cells which were shown by Agar to be proliferated from the pericardio-peritoneal ducts.

But, however this may be, comparison of the adult anatomy of Ceratodus, Protopterus, and Lepidosiren shows that the laryngeal muscles of Ceratodus—consisting as they do of a Constrictor laryngis and the (unseparated) fibres of the Constrictor oesophagi which act as a dilatator—are the most primitive existing in vertebrates.

I have the pleasure of thanking Dr. Bancroft for the embryonic and adult stages of Ceratodus, and the Bristol University Colston Society for defraying the expenses incurred in the investigation.

Relationship of Semon's Stages to Lengths of Embryo.

Stage 35 = 5·9 mm.	Stage 43 = 10·8 mm.
,, 36 = 6·4 ,,	,, 44 = 10·9 ,,
,, 37 = 6·6 ,,	,, 45 = 11·6 ,,
,, 38 = 8·3 ,,	,, 45¼ = 12·0 ,,
,, 39 = 9·0 ,,	,, 46 = 13·9 ,,
,, 40 = 9·8 ,,	,, 47 = 15·7 ,,
,, 41 = 10·2 ,,	,, 48 = 17·8 ,,
,, 42 = 10·3 ,,	

Synonyms.

Ceratodus.	
Genio-coracoideus.	Genio-coracoid, Humphry.
	Coraco-mandibularis, Jaquet, Greil.
	Coraco-mandibularis s. Coraco-cleido-mandibularis, Fürbringer.
	Unnamed, Maurer.
Coraco-hyoideus.	Coraco-hyoid, Humphry.
	Coraco-hyoideus, Jaquet.
	Coraco-hyoideus s. Coraco-cleido-hyoideus, Fürbringer.
	Cleido-hyoideus, Greil.
	Unnamed, Maurer.
Protopterus.	
Genio-thoracicus.	Genio-hyoideus, Owen.
	Deep layer of superficial stratum of ventral muscle, or Genio-hyoid, Humphry.
	Rectus inferior corporis (anterior part), Jaquet.
	Coraco-mandibularis s. thoracico-mandibularis, Fürbringer.
	Unnamed, Maurer.
Coraco-hyoideus.	Retractor ossis hyoidei + Coraco-hyoideus, Owen.
	Cervicalis profundus s. Coraco- or Ventro-hyoid, Humphry.
	Thoraco-hyoideus, Jaquet.
	Coraco-hyoideus s. Coraco-cleido-thoracico-hyoideus, Fürbringer.
	Coraco-hyoid, Agar.
	Unnamed, Maurer.

Lepidosiren.		
Genio-thoracicus.	Gerade untere Stammmuskel (anterior part of), Hyrtl.	
	Unnamed, Maurer.	
Coraco-hyoideus.	Retractor ossis hyoidei and Coraco-hyoideus, Hyrtl.	
	Coraco-hyoid, Agar.	
Ceratodus.		
Levatores arcuum branchialium.	Levatores arcuum branchialium, K. Fürbringer.	
	Cranio-branchiales, Jaquet.	
	Levatores arcuum branchialium (first four) and dorsal part of Dorso-pharyngeus (fifth), Greil.	
Constrictores branchiales.	Branchiales, Jaquet.	
	Interbranchiales, K. Fürbringer.	
	Branchiales septales, Greil.	
Cucullaris.	Scapulo-branchialis, Jaquet.	
	Levator scapulae, Greil (1907).	
	Dorso-clavicularis s. Trapezius s. Clavicularis, Greil (1913).	
Subarcularis rectus i. s. Branchio-hyoideus.	Cerato-hyoideus internus, Fürbringer.	
	Grand abducteur du premier arc branchial, Jaquet.	
	Cerato-hyoideus, Greil.	
Subarcualis rectus v.	Dorso-branchialis, Dorso-cerato-branchialis, Greil.	
Coraco-branchialis.	Coraco-branchialis, Fürbringer, Jaquet.	
	Coraco-branchialis, Cleido-branchialis, Coraco-cleido-branchialis, Greil.	
Transversi ventrales ii and iii.	Muscle chiasmique, Jaquet.	
Transversus ventralis ii.	Interbranchialis anterior.	Greil.
Transversus ventralis iii.	Interbranchialis posterior.	
Transversus ventralis iv.	Interbranchialis iv.	
Transversus ventralis v.	Interbranchialis v. s. ventral part of Dorso-pharyngeus.	
Sphincter oesophagi et laryngis.	Ceratodus.	Constrictor pharyngis, Wiedersheim.
	Protopterus.	Constrictor pharyngis et laryngis, Wiedersheim.
		Constrictor pharyngis, Göppert.
	Lepidosiren.	Constrictor isthmi faucium, Hyrtl.

Sphincter oesophagi et laryngis.	Lepidosiren. Constrictor pharyngis et laryngis, Wiedersheim. Constrictor pharyngis, Agar.
Constrictor oesophagi.	Ceratodus, present.
	Protopterus, ?
	Lepidosiren, ?
Dilatator laryngis.	Ceratodus. Not a separate muscle.
	Protopterus. Dilatator, Wiedersheim. Pharyngo-laryngeus, Göppert.
	Lepidosiren. Dilatator, Wiedersheim.
Constrictor laryngis.	Ceratodus, present.
	Protopterus. Sphincter laryngeus, Göppert.
	Lepidosiren, ?

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NOTE.

On the development of the Quadrate and Epihyal.

The Hyomandibula of Ceratodus was first described by Huxley (1876), who stated that it is a small four-sided cartilage with a short conical process at its antero-ventral angle. This process is embedded in the dorsal and posterior part of the hyosuspensorial ligament.

The cartilage was subsequently described by v. Wijhe (1882), who identified it with a Hyomandibula, or possibly an Interhyal.

Ridewood (1894) stated that the hyosuspensorial ligament passes from the top of the Ceratohyal to a protuberance of

[1] The only copy of this in the United Kingdom that I know of is in the Library of the Royal College of Surgeons, Lincoln's Inn Fields.

the Quadrate. The Hyomandibula was always found dorsal to the ligament. It is a rhombic cartilage applied at its anterior edge to the ' cranial cartilage ' (apparently the otic process), and with a ventral process which is attached to the upper surface of the suspensorial tubercle. The ventral process may be a separate cartilage. In one specimen he found a small accessory cartilage embedded in the ligament. Sewertzoff (1902) stated that the Quadrate is formed independently of the chrondrocranium and fuses with it by three processes —a ' palatobasal ' uniting it with the trabecula, an ' ascending ' uniting it with the alisphenoidal wall, an ' otic ' uniting it with the external wall of the auditory capsule. He did not describe any pterygoid process. A Hyomandibula is present, lying just dorsal to the ligament connecting the top of the ceratohyal with the Quadrate.

K. Fürbringer (1904), in the 17·8 mm. stage, described a cartilage which lies behind the Quadrate, and, internally, is continuous with the anterior part of the auditory capsule. At an earlier stage the cartilage is separate from the cranium. This cartilage he identified as probably that described by Huxley. It did not exactly correspond in position with the Hyomandibula of Sewertzoff.

Krawetz (1911) stated that two cartilages are developed behind the Quadrate, one, the ' Hyomandibula ' which lies in a cell-column (' Strang '), passing from the Quadrate to the auditory capsule, and a second, the Symplecticum, in close association with the ligament passing from the ceratohyal to the otic process of the Quadrate. These two cartilages are in connexion by a connective tissue, ' Strang '. The first-named of these cartilages is that represented by K. Fürbringer in his figures of the embryonic stage, the latter that represented by Sewertzoff. These two cartilages with the connecting ligament represent the upper part of the hyoid bar which is ' zerfallen ' into two parts.

The statement of Krawetz that the upper part of the hyoid bar is ' zerfallen ' into two parts appears to be an inference only, as he does not state that a single cartilage or structure

had been seen in one stage and two cartilages in a succeeding one; but the opinion he expressed is confirmed by the observations described below.

Greil (1913), who did not refer to the observations of Sewertzoff, K. Fürbringer, or Krawetz, stated that the Quadrate has a 'processus anterior (trabecularis)' which springs from the trabecula and anterior portion of the parachordal, and a Processus oticus connecting it with the otic capsule. It has also (in a 11·5 mm. embryo) an inconstant, rudimentary, and transitory pterygoid process at the point where the Processus anterior springs from the trabecula.

In his figs. 421–3 (pp. 1154–6) he depicted a dorsal process of the Quadrate, naming it 'Proc. asc. Qu.' This, however, is evidently the otic process, which passes upwards and backwards to the otic capsule. On p. 1390 and in fig. 537, in his description of a 17·8 mm. embryo, he described and depicted a 'Knorpelspange' which 'stellt eine secundäre Verbindung des Sphenolateralknorpels mit der Pars anterior des Palatoquadratus her'. This process is external to the N. ophthalmicus profundus (v.), and is evidently the ascending process of Sewertzoff.

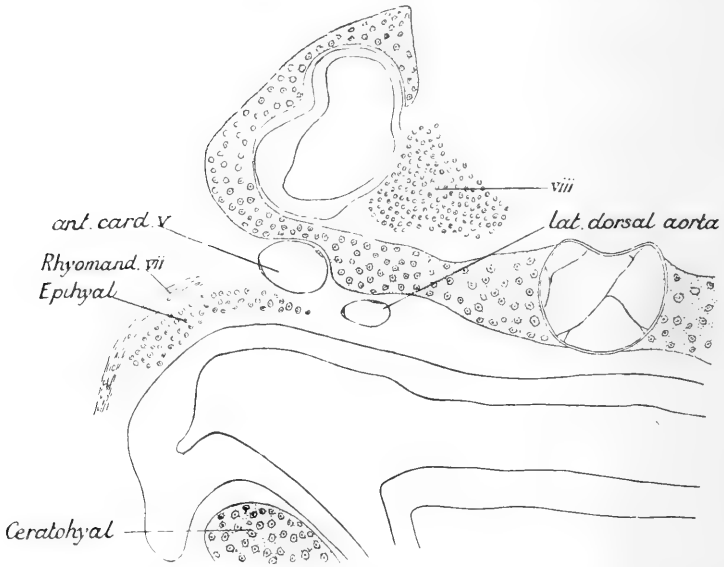
In a 17·8 mm. embryo, Greil described two or three egg-shaped or oblong pieces of cartilage adjoining the dorsal edge of the Ceratohyal and over which the N. hyomandibularis vii. (which passes out of the skull above the vena capitis lateralis and close under the otic process of the Quadrate) passes outwards. In the figs. 543 and 544 he depicted two small cartilages, the inner of which is ventro-external to the vena capitis lateralis. These cartilages he called Epihyalia s. Hyomandibularia. He also stated that a small cell-column passes from the dorsal corner of the ceratohyal to the lateral surface of the otic capsule and probably forms the primordium of small cartilaginous collections.

I find that the primordia of the Quadrate and Meckel's cartilage are first visible in a 10·5 mm. embryo as a continuous U-shaped column of cells on the inner side of the continuous primordium of the masticatory muscles and Intermandibularis.

It is not yet chondrified and not continuous with the trabecula or parachordal. In an 11 mm. embryo the primordium has separated into Quadrate and Meckel's cartilage, which are chondrified. The Quadrate is continuous with the junction of the trabecula and parachordal by a cartilaginous basal process, and also has ascending and otic processes which are continuous

TEXT-FIGS. 31, 32.

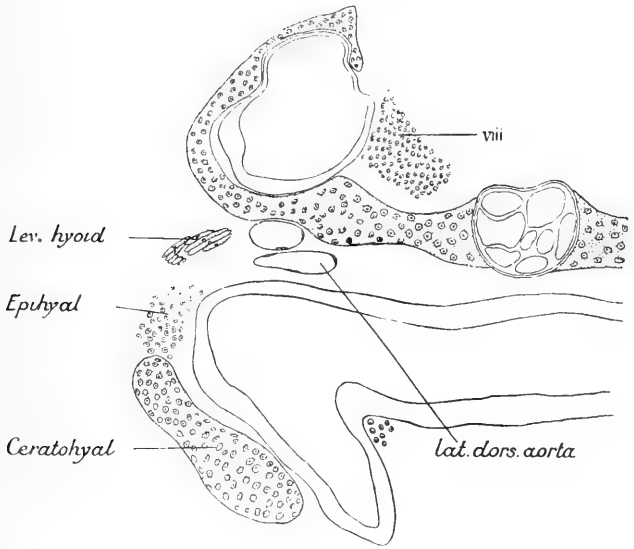
Embryo 13 mm., transverse sections; Text-fig. 32 is 42 μ behind Text-fig. 31.



with the trabecular wall and otic capsule. The upper ends of these processes are not chondrified until the 13 mm. stage. I have not seen any pterygoid process of the Quadrate in any one of several embryos of 11 and 12 mm. in length. The structures are depicted in Text-figs. 33 and 34 from a 13·5 mm. embryo. The observations thus confirm those of Sewertzoff. In a 13 mm. embryo (Text-figs. 31, 32) the Ceratohyal passes upwards and backwards in the hyoid segment. From the top of the Ceratohyal a curved tract of cells, the Epihyal, passes

upwards and slightly forwards, and then inwards below the auditory capsule, but is not yet attached to this. The Epihyal has separated in a 13·5 mm. embryo into three parts (Text-figs. 35–7). The upper end—the primordium of the oto-quadrate cartilage—is slightly chondrified and connected by a narrow neck to the floor of the junction of the auditory

Text-fig. 32.

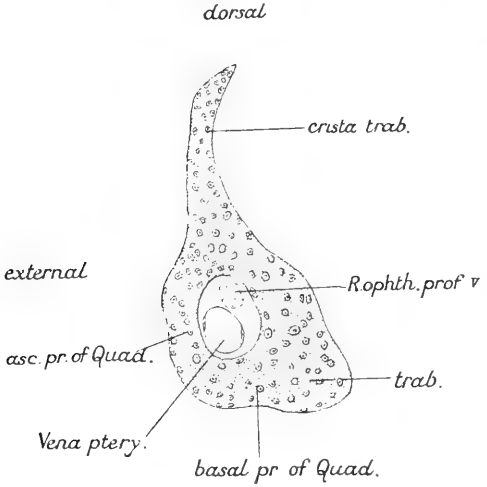


capsule with the parachordal. The middle portion—the Interhyal—is an aggregate of cells between the outer end of the oto-quadrate cartilage and the hyosuspensorial ligament. It is separated by a little gap from the former, but abuts against the latter. The lower end, consisting of cells in a slightly fibrillated matrix, is the primordium of the hyosuspensorial ligament. It does not yet extend forwards to the Quadrate, nor until the 17·5 mm. stage.

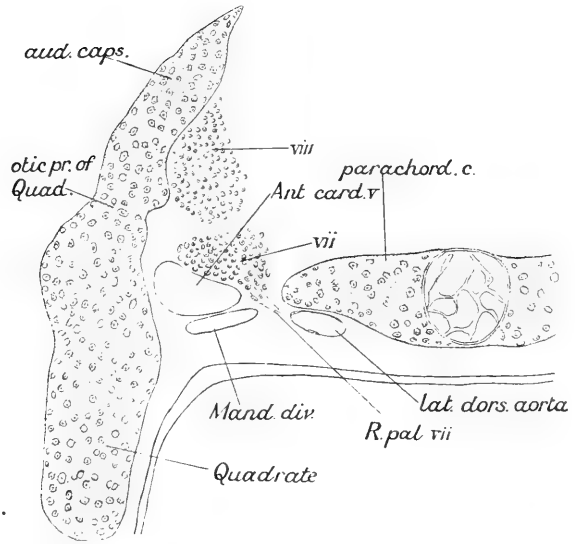
The outer end of the oto-quadrate cartilage joins the inner surface of the otic process of the Quadrate in a 17·5 mm. embryo. The Interhyal chondrifies in a 16·5 mm. embryo and subsequently enlarges (Text-fig. 38). In a 17·5 mm.

TEXT-FIGS. 33–7.

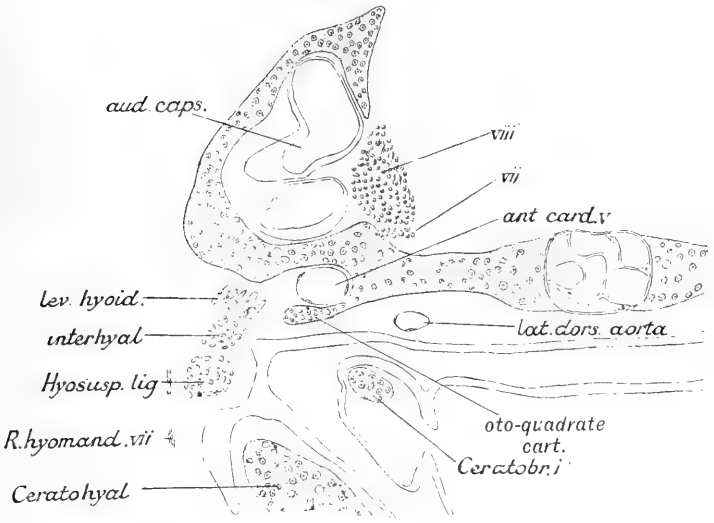
Embryo 13·5 mm., transverse sections; Text-fig. 33 is the most anterior.



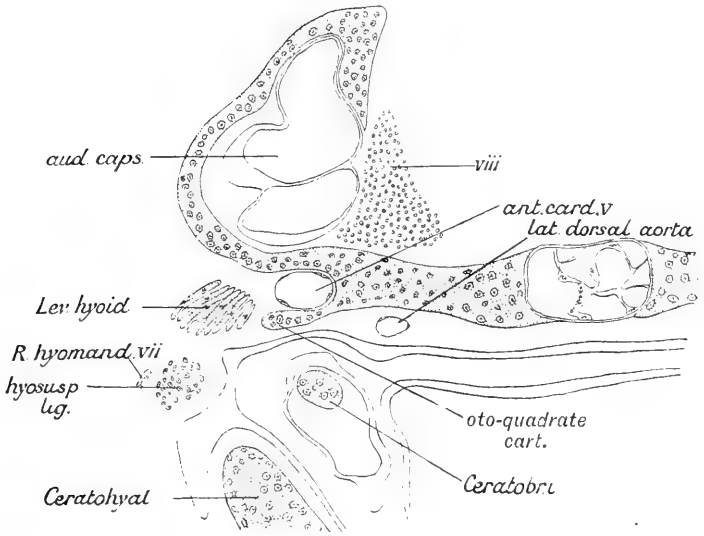
TEXT-FIG. 34.



TEXT-FIG. 35.

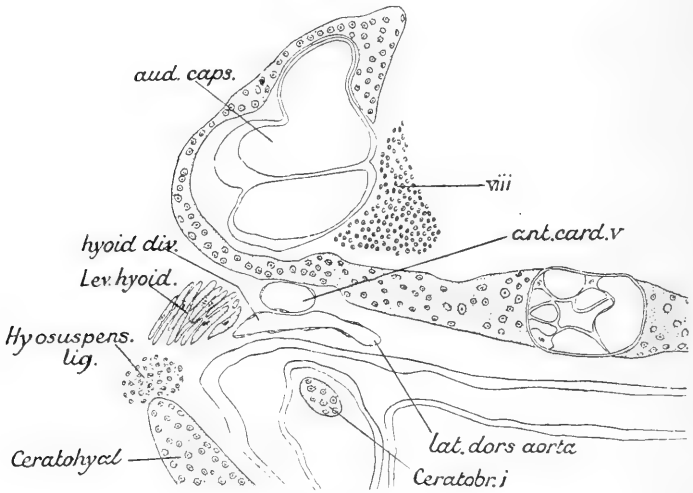


TEXT-FIG. 36.

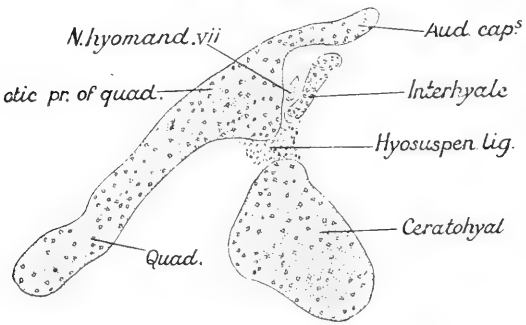


embryo the suspensorial tubercle has developed on the back of the otic process of the Quadrate and the anterior end of the hyosuspensorial ligament joins it. These structures are shown

Text-fig. 37.



Text-fig. 38.

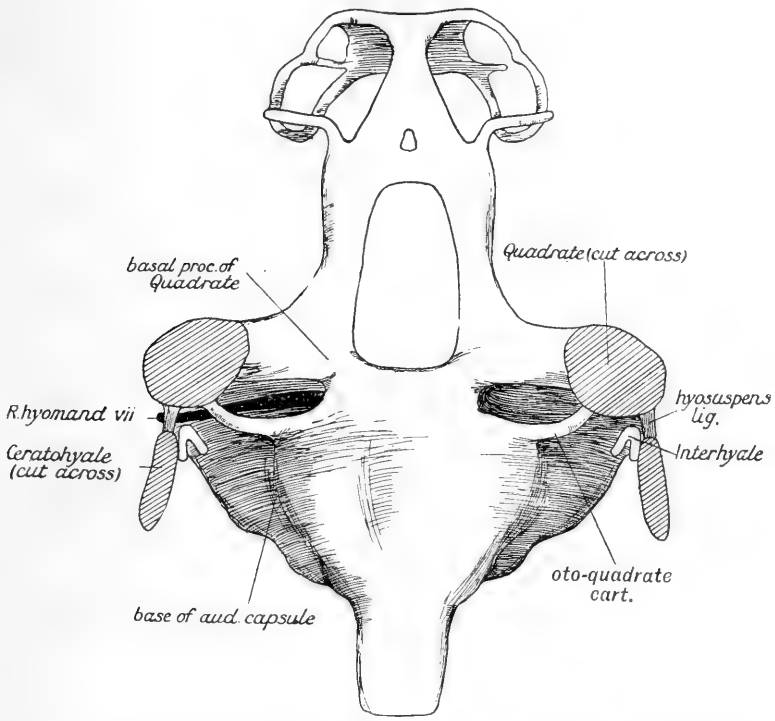


Embryo 26 mm., sagittal section.

in Text-fig. 39 made from a model of a 27 mm. embryo. The lateral end of the oto-quadrate cartilage turns forwards and joins the Quadrate. The Interhyal is a <-shaped structure, apex forwards, between the lateral edge of the auditory capsule

and the hyosuspensorial ligament, and immediately behind the Quadrate. The ligament passes from the upper end of the Ceratohyal to the Quadrate. The R. hyomandibularis vii. passes outwards dorso-anterior to the oto-quadrate cartilage,

TEXT-FIG. 39.



Base of model chondrocranium of a 27 mm. embryo. The Quadrate and Ceratohyal have been cut across. The R. hyomandibularis vii. is represented only on the right side.

dorsal to its lateral end, then between the Interhyal and the Quadrate, and downwards external to the hyosuspensorial ligament.

The oto-quadrate cartilage is the structure called 'Hyomandibula' by K. Fürbringer and Krawetz and probably the inner of the two 'Epihyalia' depicted by Greil.

The Interhyal is the cartilage called 'Hyomandibula' by Huxley, Ridewood, v. Wijhe, and Sewertzoff, 'Symplecticum' by Krawetz, and the outer of the two 'Epihyalia' of Greil. The development, however, shows that no one of these names is quite suitable, and the second suggestion of v. Wijhe is adopted.

The hyosuspensorial ligament was so called by Huxley and succeeding authors.

Comparison of the upper end of the hyoid bar with that of the first four branchial bars suggests that the tract of cells which separates into the above-mentioned structures is serially homologous with the Epibranchialia, and it is accordingly called Epihal.

The above can be summarized as follows. A precartilaginous tract, the Epihyal, extends from the upper end of the Ceratohyal upwards and inwards beneath, though not at first attached to, the auditory capsule. This Epihyal separates into three portions—from above downwards the oto-quadrate cartilage, the Interhyal, and the hyosuspensorial ligament. The first-named gains secondary attachments, its inner end to the base of the chondrocranium and its outer end, subsequently, to the Quadrate. The Interhyal chondrifies later than the oto-quadrate cartilage. The hyosuspensorial ligament slowly extends forwards to the Quadrate.

Additional Literature.

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On Golgi's Internal Apparatus in spontaneously absorbing Tumour Cells.[1]

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With Plates 19 and 20.

Introductory.

The results obtained by the study of the Golgi apparatus of growing tumours of the mouse, rat, and guinea-pig were set forth in a previous paper (**7**) in which also the literature of the subject was summarized and discussed. The apparatus was found to be constantly present in the healthy cells of all tumours examined, in most of which it appeared with certain characteristic features. These were maintained through the successive regrafting of the same growths even when the general histological picture of some of them had somewhat changed and the tumour cells and their apparatus had become hypertrophic or had undergone partial degeneration.

It is now proposed to describe the behaviour of the apparatus during the spontaneous absorption of some of the same tumours, a point which could not be properly dealt with in the previous work. Such an investigation has not, as yet, been carried out, though it is important because it gives the opportunity for studying the modifications of the apparatus in cells of either epithelial or connective-tissue origin, undergoing regressive changes after a period of active proliferation, a phenomenon obtained with difficulty under different experimental conditions.

As shown in another paper (**6**), spontaneously absorbing

[1] Part of the expenditure incurred for carrying out the present research was defrayed by a grant from the Royal Society. The material was obtained through the kindness of Dr. J. A. Murray, Director of the Laboratories of the Imperial Cancer Research Fund.

tumours of small laboratory animals are at first invaded by elements of the lymphocytic type and then by connective-tissue cells, the process generally ending in the complete disappearance of the tumour cells and the production of a scar structurally similar to that seen during the healing of certain inflammatory lesions of the subcutaneous connective tissue. When studying the apparatus of absorbing tumour cells, that of some of the elements taking part in the production of the scar was observed and will be also briefly described.

Material and Methods.

The following malignant new growths in various phases of spontaneous absorption were examined:

Mouse.

Jensen. Alveolar carcinoma.
Twort. Alveolar carcinoma.
27. Papillary cystic adeno-carcinoma.
91. Haemorrhagic and cystic alveolar carcinoma.
113. Slightly haemorrhagic alveolar carcinoma.
155. Fissure forming adeno-carcinoma.
206. Alveolar carcinoma.
630. Squamous cell carcinoma.
37 S. Spindle cell sarcoma.

Rat.

Rat 9. Adeno-carcinoma with dense hyaline stroma.

All tumours were investigated by the cobalt nitrate method with the precautions suggested in the previous work. A certain number of tumours were likewise investigated by the modification of Veratti's potassium antimoniate method previously described. But this time the results obtained were not so satisfactory as when investigating the apparatus of growing tumours. This was probably due to the fact that absorbing new growths often contain only a small number of healthy cells and a great deal of detritus which becomes so intensely impregnated by the potassium antimoniate method as to render

extremely difficult the interpretation of the histological pictures. It is necessary to add here that a description of Veratti's original method was published by Barinetti in a paper which had been previously overlooked (**1**).

No conclusive results were reached by the investigation of the Rat 9 carcinoma either by the cobalt nitrate or by the potassium antimoniate methods in spite of repeated attempts made in different stages of the absorption process. Successful impregnations were obtained only when most of the tumour cells were in a healthy condition, and it is therefore proposed not to include this tumour in the following description. However, the fact was worth mentioning because it has a parallel in a previous observation regarding a transplantable liposarcoma of the guinea-pig. In that case satisfactory results were obtained by Golgi's arsenious acid method, but not by others. The two observations taken together seem to indicate that biological conditions, through which tissues may be passing, have sometimes a decisive influence on the reaction to which the silver impregnation is due. Ernst's (**10**) recent investigations on certain phenomena of adsorption are in favour of this supposition.

The Apparatus of Spontaneously Absorbing Carcinomata and Sarcomata of the Mouse.

Jensen.—As previously described, the apparatus of the healthy cells of this tumour has a perinuclear or juxta-nuclear position, and generally consists of minor parts ring- or loop-like in shape. This is also seen in the small group of unaltered cells shown on one side of Pl. 19, fig. 1, which was drawn from a zone of transition between a surviving nodule and an absorption area. In most of the degenerating tumour cells the apparatus is recognized because of its characteristic aspect, the ring- and loop-like shapes being scattered in the cytoplasm instead of being collected in one juxta-nuclear formation. Only in a relatively small number of cells an irregular fragmentation of the apparatus is observed, though this phenomenon becomes more and more apparent and lastly predominates

when and where the absorption process is most advanced. In such places, however, the degeneration of the tumour cells has gone so far that they are hardly recognizable.

In transitional zones like that shown in Pl. 19, fig. 1, multinucleated protoplasmic masses are now and then observed, apparently due to the conglutination of a variable number of degenerating tumour cells. Next to almost each of the nuclei included in these pseudo-giant cells, typical remnants of the apparatus are frequently seen with arrangements similar to that described in connexion with a polymorphous cell sarcoma of the mouse (see Pl. xxi, fig. 29, of the previous work, 7). A fusion of the apparatus of the single cells into a common and centrally situated formation, as observed under different conditions, was not met with in specimens from the Jensen carcinoma.

When the absorption process is nearing its end and the connective-tissue proliferation leading to the formation of the scar is very much advanced, the number of the carcinomatous cells is very small, and these are recognized with difficulty, particularly by the method used in this investigation. Nevertheless elements are now and then found which can be safely considered as surviving tumour cells. Their identification is chiefly based on the size and aspect of their nuclei and apparatus. As shown by Pl. 19, fig. 2, this has a much more robust appearance than that of the other cells, most of which are large wandering cells and fibroblasts mixed with a few leucocytes. In some of the tumour cells the apparatus still possesses the characteristic aspect to which reference has already been made; in others it is uncommonly large and looks like a somewhat granular and disintegrating juxta-nuclear structure. The apparatus of the connective-tissue cells is much smaller, irregular in shape and structure, and often stretched in various directions.

Twort.—The healthy cells of this tumour are provided with an apparatus in the main smaller and more distinctly reticular than that of Jensen's carcinoma. The changes observed during absorption are similar to those above described,

though a simple disintegration of the apparatus into a structureless material was the prevalent feature of many specimens. A transformation of the apparatus into a large juxta-nuclear formation was sometimes observed (Pl. 19, fig. 3, *tu.*), but not the fusion of various elements into pseudo-giant cells as in the case of the Jensen tumour.

In advanced stages of absorption of the Twort carcinoma accumulations of large macrophages were found. Most of them contained only formless débris of argentophile material, but some possessed a small and irregularly shaped apparatus (Pl. 19, fig. 3, *m.*) situated close to the nucleus and on the whole similar to that of the connective-tissue elements above mentioned.

Tumour 27.—The apparatus of the healthy cells of this adeno-carcinoma generally consists of short rods collected in a bunch on that side of the nucleus which is turned towards an existing or virtual glandular lumen. Only in some groups of cells it appears formed of reticular portions irregular in size, shape, and distribution. In absorbing tumours of the same strain the typical rod-like aspect survives only in a rather small number of cells. Most of them either show the picture occasionally observed in growing tumours or convey the impression that the rods forming the apparatus have swollen into elliptical or roundish shapes within which a minute light space can be detected. It has been impossible to decide whether these spaces are really empty or contain a material which does not take the silver. Pl. 19, fig. 4, is a good instance of this condition which, in the specimens investigated, extended to wide areas, easily distinguished from the unaltered tumour portions by the pale colour of the nuclei. These showed in addition a strong tendency to fuse into agglomerations in which the boundaries between cell and cell could hardly be made out even by very high magnifications.

These observations were at first found a little surprising. Other absorbing tumours of the same strain were therefore carefully investigated, but with results which did not essentially differ from those already obtained. The number

of cells provided with an apparatus of the characteristic type described varied considerably from tumour to tumour and from place to place of the same tumour ; but whenever the absorption process had manifested itself, the apparatus showed in varying degrees the changes shown in Pl. 19, fig. 4.

It is interesting to note that the same fact was even more clearly observed in dividing tumour cells. Mitotically dividing cells frequently occur in spontaneously absorbing tumours even when these are much reduced in size and nearing complete disappearance. One of such cells is shown in a part of Pl. 19, fig. 4, and another in the centre of Pl. 20, fig. 5. In both cases the fragments of the apparatus (dictyosomes) to be subdivided between the daughter cells no longer have the aspect generally observed in growing tumours of small solid clumps of argentophile material, but appear as irregularly rounded or elliptical shapes with a light central portion, as in most of the absorbing cells at rest. Pl. 20, fig. 5, was drawn from a specimen of a tumour in a very advanced phase of absorption, and the apparatus of some cells show signs of the approaching complete disintegration.

Tumours 91 and 206.—The apparatus of the healthy cells of these two growths has a fine reticular structure, more delicate in carcinoma 206 than in carcinoma 91. During absorption the characteristic aspect of the apparatus is still recognized for a time in the cells of both of them. In the case of tumour 91, however, the apparatus is soon reduced to a granular material with loss of the previous reticular arrangement. This is well shown in Pl. 20, fig. 6, which was drawn from a place where the various phases of this degenerative process could be seen one next to the other. In other areas of the same absorbing tumour, appearances almost identical with that exhibited by the central portion of Pl. xxii, fig. 32, of the previous work were sometimes noticed. In such instances the similarity between the disintegrative phenomena affecting the apparatus of both the tumours now considered was rather striking, though in carcinoma 206 the gradual fragmentation of the previous fine network was plainer. As shown by Pl. 20, fig. 7, a reticula

appearance still survived in spite of the breaking up of the whole structure into small portions and threads, only now and then united by thinner filaments.

Tumours 113 and 155.—The apparatus of the healthy cells of these two carcinomata is very small and therefore less suitable for observations of the kind considered in the present paper. In the cells of areas undergoing absorption the apparatus occurred, in both tumours, in the form of short rods or small clumps closely arranged, one next to the other, on one pole of the nuclei, but no structural details could be made out in the intensely impregnated fragments. In more advanced phases of absorption these fragments were still smaller and frequently indistinguishable from formless débris.

In conditions of this sort elements provided with a small and well impregnated apparatus were often seen, but accurate observations, particularly of serial sections, led to the conclusion that such elements were connective-tissue cells similar to those shown in part of Pl. xxii, fig. 32, of the previous work and in Pl. 19, fig. 2, of the present one.

Tumour 630.—The regressive changes exhibited during absorption by this squamous cell carcinoma are identical with those previously observed in keratinizing areas of the same tumour and need no further description. When absorption is very advanced the tumours consist of small nodules of a more or less completely keratinized material surrounded by a shell of proliferated connective tissue in which a considerable number of multinucleated giant cells are found. As shown by Pl. 20, fig. 8, some of them are provided with remnants of a centrally situated and reticular apparatus, some only contain a finely granular material, the granules being sometimes arranged in such a way as to convey the impression that they also have arisen from the fragmentation of a perhaps formerly reticular apparatus. The presence of many giant cells in the above-mentioned situation is probably due to the fact that in the absorption phase in which they were noticed, nothing survived of the old tumour but a hard substance in many respects similar to a foreign body. The fragmentation of their apparatus may

be attributed to the process of involution through which also the proliferated connective-tissue cells presumably pass before disappearing with the remains of the tumour.

Together with the giant cells, either in the same situations or between the keratinized nodules, elements of the large wandering cell type were frequently observed. They were provided with a small either reticular or compact apparatus similar to that of elements of the same kind previously mentioned and described also by Verson (**17**), though in different pathological conditions.

37 S.—In the absorption areas of this sarcoma of the mouse the apparatus undergoes a simple process of granular disintegration, frequently manifesting itself at a period in which the outward aspect and structure of the tumour cells is otherwise almost unaltered. Apart from the size of the cells, the phenomenon is like that observed in the foreign body giant cells described in connexion with Pl. 20, fig. 8. As easily deduced from a comparison between Pl. 20, figs. 8 and 9, in both cases the apparatus soon becomes transformed into an accumulation of small argentophile granules, the arrangement of which now and then reminds one of a pre-existing reticular structure. This observation is not without interest, because the cells exhibiting the changes described have in common a connective-tissue origin. Pl. 20, fig. 9, was drawn from the peripheral portion of a nodule, the central cells of which still appeared in a healthy condition and possessed an apparatus identical with that shown in Pl. xvi, fig. 19, of the previous work. Only a few of the peripherally situated cells were provided with an intact apparatus, though remains of it could be recognized even in elements showing many signs of degeneration, such as loss of a great part of the nuclear chromatin, liquefaction, and fusion of the cell-bodies.

General Considerations.

One of the principal facts resulting from the present investigation is the relative resistance of the apparatus to altered biological conditions even when these lead to a complete

degeneration of the cells concerned. The moment, in which the disintegration of the apparatus begins, appears to vary within wide limits and for causes which are beyond our present means of observation. However, changes of the apparatus are on the whole noticed when the external aspect and structure of the cells seem otherwise unaltered. After this initial phase a period follows during which portions and fragments of the apparatus continue to be recognized until the extreme degree of cell degeneration is reached. This is in agreement with previous observations on growing transplantable tumours (**7**) and with the results obtained by other authors in different fields of investigation. It corroborates the suggestion resulting from Bowen's recent work (**4, 5**) that the apparatus plays perhaps an important rôle in the economy of the cell, though we must confess with him our ignorance as to the exact nature of this rôle.

A second point deserving brief discussion is the different behaviour of the apparatus during the absorption of different tumours. In some of them, as in certain connective-tissue cells, it soon becomes transformed into a granular material which, though persisting during cell degeneration, no longer possesses any definite structure; in others this terminal disintegration is reached through stages during which it either swells into peculiar shapes or breaks into fragments and pieces which are for a time endowed with certain distinctive features. In certain cases it even passes through a sort of hypertrophic condition which, in the material examined, could not be more closely studied. These phenomena are probably influenced, if not determined, by the different structure of the apparatus in the healthy cells of the tumours investigated, and confirm the opinion previously expressed as to the existence of a well-defined relation between the apparent structural type and the mode of being of the apparatus in the living cells.

The disintegration of the apparatus during the spontaneous absorption of certain tumours has a parallel in the observations made by other authors and myself under different pathological or physiological conditions. For instance, the breaking up of

the apparatus in certain growths (Jensen's carcinoma, tumours 206 and 91) has a striking similarity with Penfield's retispersion (**15, 16**) and with alterations of the same kind observed in degenerating nerve-cells by Battistessa (**2, 3**), Marcora (**14**), Da Fano (**8**), and others. Some of the changes exhibited by the apparatus in tumour 27 have an almost surprising resemblance with the modifications exhibited by the apparatus in germ-cells as described by Gatenby (**11**) and his collaborators Woodger (**12**) and Ludford (**13**), and more recently by Bowen (**5**). The same applies to the changes noticed by Da Fano (**9**) during the involution of the mammary gland at the end of lactation. The phenomena described by the above-mentioned authors and in the present paper were noticed in tissues so different that no detailed comparison is possible. However, they were worth brief mention because they seem to indicate that the fragmentation of the apparatus, whether under the influence of physiological or pathological stimuli, is up to a point determined by the as yet obscure part taken by the apparatus in various cell activities.

Summary.

In continuation of previous work the behaviour of Golgi's apparatus during spontaneous absorption of transplantable tumours of the mouse is described. In some of them the apparatus soon becomes transformed into a granular almost structureless material ; in others this terminal phase is reached through stages which are probably determined by the characteristic structure exhibited by the apparatus in the healthy cells of the corresponding tumours. The modifications of the apparatus during such stages have a striking resemblance with the changes observed in different tissues under various physiological conditions. The connective-tissue cells, which invade the tumours as they are absorbed, are provided with a small, irregular, and sometimes reticular apparatus which is identical with that described by other authors in similar elements though in different pathological processes. In some of these elements

the apparatus undergoes changes not altogether different from those observed in absorbing tumour cells. In general, the fragmentation of the apparatus begins when other cell constituents are still apparently unaltered, but its fragments seem to possess a great power of resistance to degenerative conditions.

London,
March 1923.

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DESCRIPTION OF PLATES 19 and 20.

All figures have been drawn by means of the camera lucida at the magnification indicated for each of them, and are from spontaneously absorbing tumours treated by the cobalt nitrate method for the demonstration of Golgi's internal apparatus. They are fully described in the text.

Reference Letters.

d.tu., degenerating tumour cell; *f.*, fibroblast; *g.c.*, giant cell; *ps.g.c.*, pseudo-giant cell; *k.*, area of keratinization; *m.*, macrophage; *plc.*, polymorphonuclear leucocyte; *tu.*, tumour cell; *w.c.*, wandering cell.

Fig. 1.—Jensen's carcinoma (387 A). Moderately progressed absorption. (×1160.)

Fig. 2.—Jensen's carcinoma (399 B). Advanced absorption; surviving tumour elements amongst proliferated connective-tissue cells. (×1620.)

Fig. 3.—Twort's carcinoma (123 A) at the end of the absorption process. One surviving tumour cell; group of macrophages. (×1620.)

Fig. 4.—Adeno-carcinoma 27 (122 C) in initial phase of absorption. (×1000.)

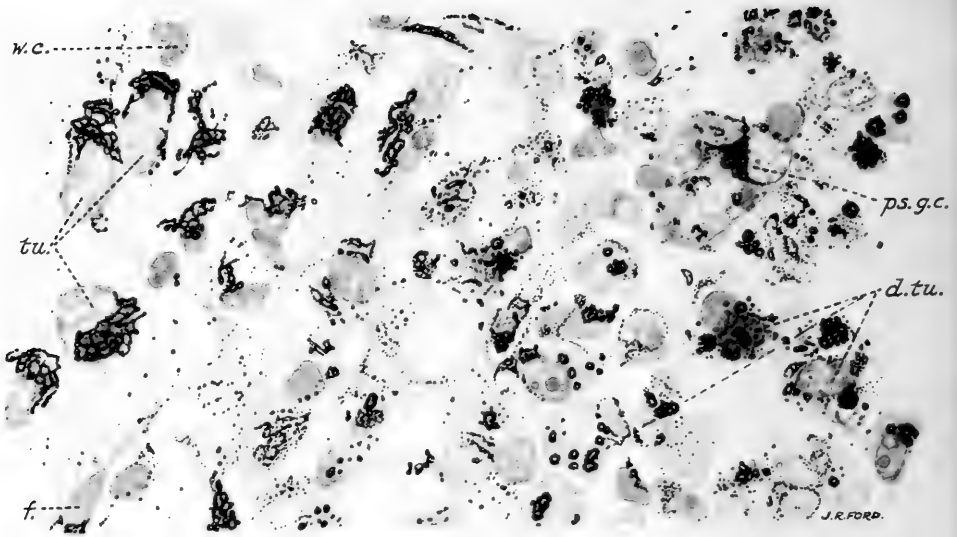
Fig. 5.—Adeno-carcinoma 27 (136 C). Group of surviving cells when the tumour was nearing complete absorption. (×1620.)

Fig. 6.—Alveolar carcinoma 91 (153 B). Internal apparatus in different stages of fragmentation during spontaneous absorption. (×1620.)

Fig. 7.—Alveolar carcinoma 206 (331 A). Breaking up of reticular apparatus during absorption. (×1620.)

Fig. 8.—From connective-tissue capsule enveloping keratinized remains of squamous cell carcinoma 630 (126 A). Connective-tissue cells and foreign body giant cells with fragmented apparatus. (×800.)

Fig. 9.—From the peripheral layers of an absorbing nodule of sarcoma 37 S (140 A). (×1240.)



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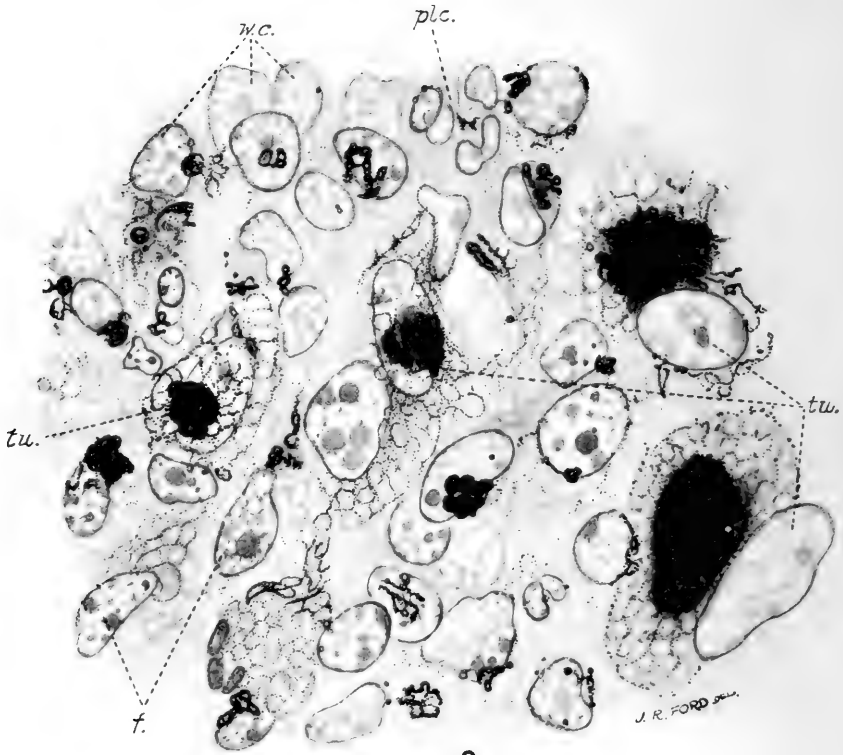

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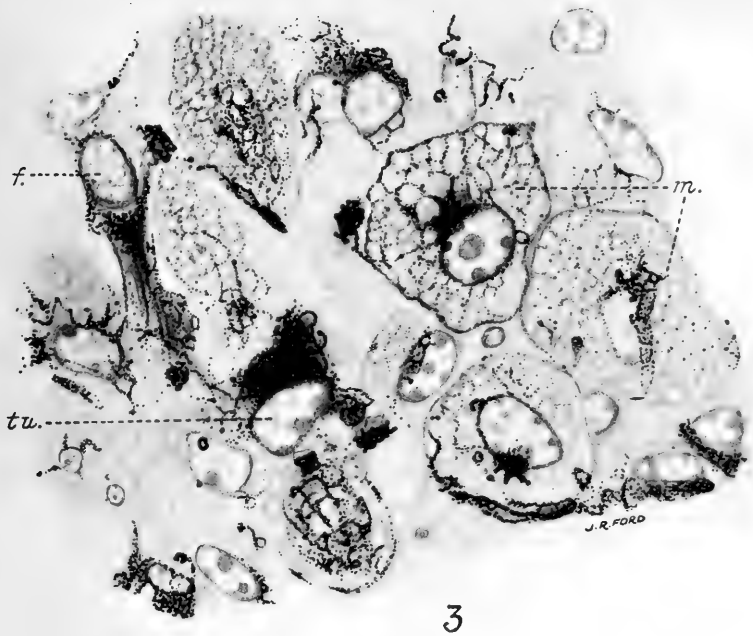

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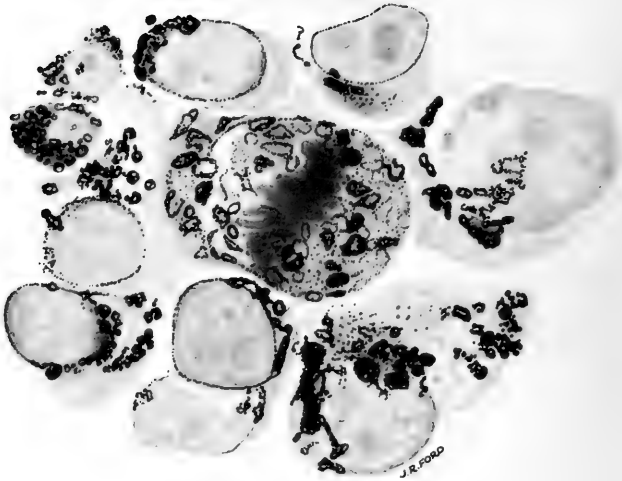

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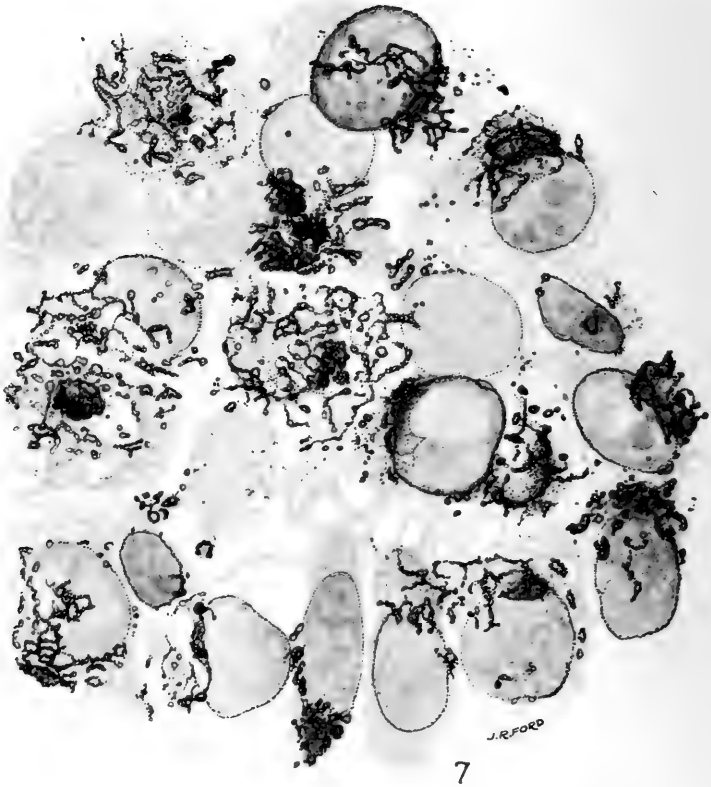

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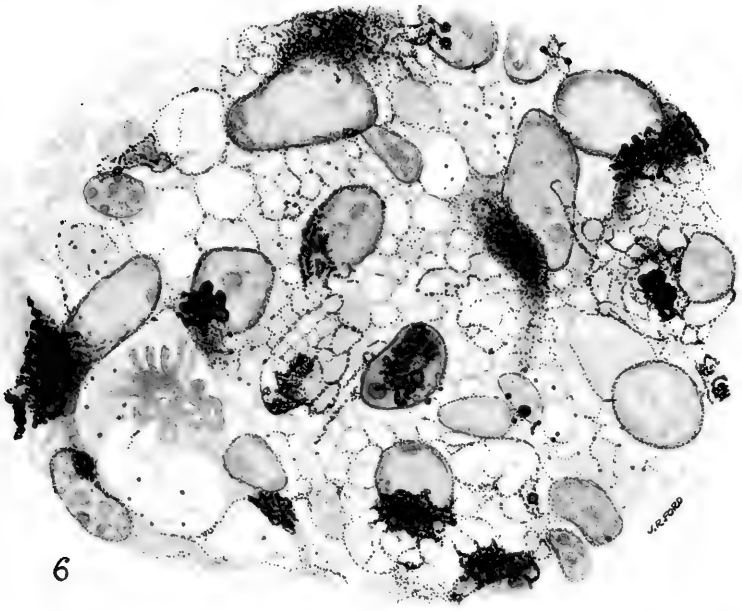

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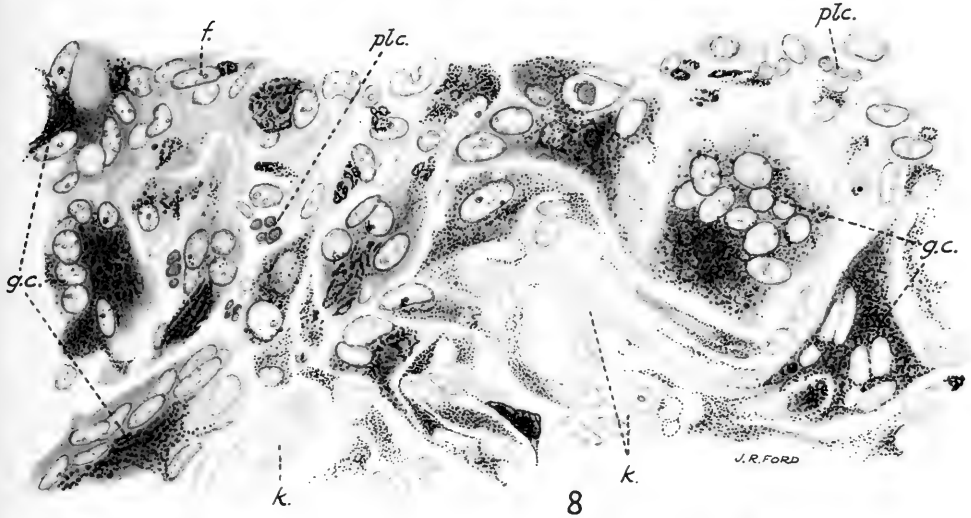

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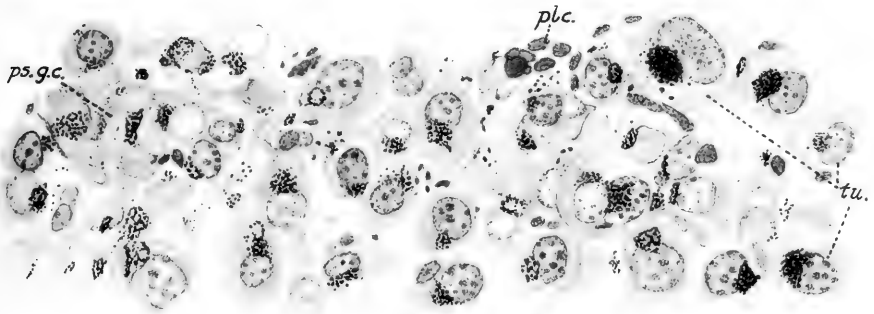

9

The Golgi Bodies of a Coccidian.

By

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With Plate 21.

For many months we have been collecting material of the protozoan fauna of the gut of Lithobius forficatus. Our object was to study the Golgi apparatus, if such were present, and to examine also the behaviour of the mitochondria. We chose the form Adelea because we believed that its life-history was well known, and that there would be no special difficulty in identifying the stages. In this we have been disappointed—we found that Siedlecki and other workers have left the matter in a confused state : we have been obliged to spend many months trying to piece together the various parts of the life-history : at present we are not sure of any of the stages except those described in this paper.

Mr. Dobell (in literis) has answered some of our questions as well as he was able considering that his own paper on the subject of Adelea was written many years ago at the beginning of his investigations on the Protozoa. In our first material nothing except Adelea seemed to be present ; but subsequently we found to our regret that the Coccidian (Eimeria schubergi?) stages were present side by side with those of Adelea. Minchin, in his ' Introduction to the Study of the Protozoa ', has brought out the fact that not only has there been a confusion as to the stages in the life-history of many Coccidia, but that in the case of Adelea, Eimeria, and Barroussia, there has been even a confusion of species described under the one name. That this confusion is not difficult to bring about can best be understood by trying to identify the various species and stages ; we ourselves have been much puzzled in the endeavour

to identify Siedlecki's stages of macro- and micro-schizonts. We have no wish to enter into a purely protozoological field, but later we shall have to come to some decision ; at present we do not find any clear evidence for the macro- and micro-schizonts of Siedlecki, but wish to leave the matter open. We feel sure, however, that the stages we definitely identify in the present paper are schizonts and gametocytes. No sporogony stages are described.

Several special methods, which show the Golgi apparatus of the metazoan cell, have been used by us. In our material they demonstrated a hitherto undescribed structure in Adelea.

Our reasons for identifying this intra-cellular structure as the Golgi apparatus are as follows :

1. Its staining and fixing reactions are identical with those of the Golgi bodies of the metazoa.

2. It occupies an excentric juxta-nuclear position and spreads out in the cell cytoplasm, as does the Golgi apparatus in many metazoan cells, e.g. the egg, and the nerve-cell.

3. It consists mostly of the very characteristically shaped crescents and beads, known, in the case of the metazoan cell, as dictyosomes.

4. As in the metazoan cell, these protozoan dictyosomes can be found dividing by themselves in the ground cytoplasm.

An account of the details of our technique and of our reasons for making the statement in paragraph 1, above, will be given in a later paper.

Schizogony.

The merozoite and sporozoite of certain Coccidia are said to be much alike : small nuclear differences have been described : in Eimeria schubergi the nucleus of the merozoite is said to have a distinct karyosome, absent in that of the sporozoite. On this basis the stage drawn in Pl. 21, fig. 5, would be a merozoite—it has a karyosome ; at the present stage we prefer to call the latter a nucleolus (*n*). Such small crescentic coccidians occur commonly in areas of the Lithobius gut, where the asexual multiplication occurs. They are found

among the cells of the gut, intra- and inter-cellular in position, and often in the lumen.

In such stages one can impregnate a ring-like structure, just near the nucleus, in exactly the same position, and of the same general appearance as the Golgi bodies of many metazoan cells. In Pl. 21, fig. 2, is drawn freehand at a very high magnification, a number of these structures we now identify as the Golgi apparatus ; the latter consists, as in sponge, coelenterate, and many other cells, of dictyosomes (or Golgi crescents and bent rods) lying upon the surface of a thickened protoplasmic zone or centrosphere. Whether or not a centrosome exists in Adelea, there is certainly a darker (denser) zone associated with the sickle-shaped dictyosomes.

As the merozoite (sporozoite) grows and becomes definitely a trophozoite, the Golgi apparatus develops into an important part of the cell : in Pl. 21, fig. 11, G, is the Golgi apparatus of a growing coccidian ; the apparatus is seen to consist of a number of bent rod-shaped structures, and it is produced by the growth and fragmentation of the original Golgi fragment of the younger cell ; the nucleolus, hither excentric, becomes more centrally disposed in the nucleus.

In Pl. 21, figs. 12 and 13, the Golgi apparatus is seen to have fragmented partly and the separate dictyosomes are irregularly scattered, though a main aggregation is found at G, in a juxtanuclear position.

Such cells proceed to division ; in Pl. 21, fig. 14, is a stage with four nuclei (two shown), and the Golgi bodies had been gathered into four rough groups, one around each nucleus (N). The Golgi bodies at this stage were spherical, crescentic, or granular ; the next stage is shown in Pl. 21, figs. 16 and 17, where each one of the many nuclei had near it, its own quota of Golgi beads, crescents, and granules. In Pl. 21, fig. 16, the apparatus consisted of closely associated crescents, in fig. 17, of much coarser rings and crescents. Such stages are followed by ones showing the formation of cell-walls, and separation of the merozoites and their subsequent scattering and growth again.

There were from 20 to 30 nuclei in the last stages of division :

no centrosomes could be identified. Among our material one finds all stages from cells like that in Pl. 21, figs. 16, 17, to corps en barillet stages, one of which is depicted in Pl. 21, fig. 18. This is a group of agametes or daughter individuals produced by division of a trophozoite, and now ready to break away into its component parts. Each merozoite has a nucleus with an excentric nucleolus (*n*) which is always turned away from the Golgi apparatus (G): no exception to this rule has been found to occur. The Golgi apparatus is either formed of several little crescents together making a little granule, or it is elongate.

In Pl. 21, fig. 20, is a large trophozoite with a completely scattered Golgi apparatus (G), which may be seen to lie among other granules (Y) whose nature we do not care to examine in this paper. Whether this is an individual of Eimeria or Adelea we are not sure. Many trophozoites of the Lithobius parasites have completely scattered Golgi bodies.

Occasionally one finds schizonts in which some body like the Golgi apparatus (Pl. 21, fig. 15, GX) lies in the centre of the cell, and appears to be taking no part in dictyokinesis. This is rare.

♀ AND ♂ GAMETOCYTES.

In Pl. 21, figs. 19 and 21, are two cells which we can positively identify as ♀ gametocytes—the ♂ gametocyte rests upon them. The Golgi apparatus of the ♀ gametocyte is much like that of the trophozoites already described in Pl. 21, figs. 11, 12, and 13: in some cases it is much more scattered. In the ♂ gametocyte we never found at this association stage a juxta-nuclear and discrete apparatus as in the merozoites in Pl. 21, fig. 18. Even in preparations where the Golgi apparatus of the ♀ gametocyte was beautifully marked as clear black rings and crescents on a yellowish or grey background, the ♂ gametocyte was found only to contain a few black granules (GX) generally stuck in its periphery and of doubtful nature. While we cannot positively identify a Golgi apparatus in the ♂ gametocyte at this stage, the small granules which are present, and which might represent

the Golgi bodies, are much fewer and smaller than those found in the ♀ gametocyte.

Examination of a large number of cells which we believe to be ♂ gametocytes before association has enabled us, we think, to throw some light on this matter: in Pl. 21, figs. 1, 3, 4, 6, and 9, are what we believe to be ♂ gametocytes. In them the apparatus is rarely juxta-nuclear, but has fragmented, and its elements seem to have passed to the periphery and are struck beneath, or on, the cell-wall. We have found many ♂ gametocytes in association, with no signs of any Golgi bodies, except a number of these peripheral black granules. We believe that in the ♂ gametocyte the Golgi apparatus is mainly extruded or absorbed. We doubt very much if it takes any part in fertilization. In Pl. 21, fig. 9, is a ♂ gametocyte prepared in the same manner as the cell in Pl. 21, fig. 20. In Pl. 21, figs. 7 and 8, are two cells, which may be intermediates between ♂ and ♀ gametocytes—such intermediates often occur in the metazoa: in Pl. 21, fig. 7, there is certainly an apparatus at G, the nature of the granules at GX is more doubtful, but in both figures there are elements which seem to be passing to the periphery.

The ♂ gametocyte often stains so darkly that it is difficult to make out much of its structure. In Pl. 21, fig. 10, is an example of a ♂ gametocyte containing a large granule which did not appear to be taking any part in the activity of the cell in which it lay: there were no clear dictyosomes.

Discussion.

In this paper we have described what we consider to be the Golgi apparatus of the protozoan Adelea. This Golgi apparatus is dissolved away by the same fluids, preserved by the same reagents, and stained by the same methods as the Golgi apparatus of the metazoan cell.

During growth, the coccidian Golgi apparatus, like that of the metazoan egg, spreads out through the ground cytoplasm in the form of curved banana-shaped rods or dictyosomes. So far as we could observe, the Golgi apparatus of Adelea

takes no part in the formation of the yolky bodies which are to be found in both trophizoites and gametocyte.

In the asexual multiplication phase where cell-division takes place, the Golgi apparatus behaves as is usual in metazoan cells: it becomes sorted out into subequal groups around each dividing nucleus, and each ultimate daughter nucleus has gathered near it a part of the original apparatus. We have therefore established that a true dictyokinesis takes place in the protozoan Adelea.

The Golgi apparatus is found in every schizont and merozoite we have examined, and strangely enough it always lies oriented in a special manner with reference to the excentric nucleolus (karyosome) of the oval nucleus: the Golgi bodies lie always away from the nucleolus. The exact significance of this we do not know: it means possibly that this nucleolus does not contain the body which during division is responsible for shepherding the Golgi elements into groups around the syncytial nuclei (Pl. 21, figs. 16, 17). Whether a true centrosome, either intra- or extra-nuclear is present, we are unable to say: previous workers are mainly against the view that an extra-nuclear centrosome occurs in Adelea. Suffice to say at present that within, or near, the coccidian nucleus is some body with the power of attracting the dictyosomes, as occurs in the case of the metazoan centrosome.

The interesting period of conjugation of the gametocytes and of fertilization provided us with no facts worth recording at length. We found not a jot or tittle of evidence for the view that the Golgi apparatus of the male takes part in the process of fertilization; this period is undoubtedly the most difficult to study, and we are not at present satisfied with our material of these stages.

So far as the senior writer is concerned, this examination of the Golgi bodies of a coccidian has been disappointing from the point of view of the phylogenetic origin of the Golgi apparatus; we have found a typically metazoan Golgi apparatus, which acts just like that in the metazoan egg, during oogenesis, and which, as in most cases of metazoan fertilization,

takes no part in the process. We have an apparatus in the coccidian which acts normally at dictyokinesis. It is evident that in this protozoon we have the typical apparatus already formed and established.

The search for a primitive apparatus must be carried out among forms other than the Sporozoa, as Hirschler's and our work amply shows.

The Golgi bodies probably arose in connexion with the terminal bead of the flagellum of some primitive flagellate. The outer layer of the bead might have been differentiated to form a lipoid store-house or elaborator of the energy-yielding materials necessary for the nutrition of the locomotor organ. From its primitive position in the metazoan cell, always associated at some time with the centrosome-centrosphere complex, we cannot but believe that in the early history of the cell the Golgi apparatus and the centrosome were evolved side by side, or thea pparatus from the centrosphere, in some way. This speculation can only be tested when further evidence is produced. An important field, altogether neglected hitherto, is opened up to protozoologists. The latter more than any of their fellow biologists are interested in the architecture of the organisms which they study, and they should attack the problem with the special methods explained elsewhere (**5**).

Summary.

1. There is a true Golgi apparatus in the Coccidia (Pl. 21, figs. 11, 12, 16, 17, 18, 21).

2. It consists of separate dictyosomes or crescentic rods, with the power of fission as in metazoa (Pl. 21, figs. 2, 12, 17).

3. During growth the excentric Golgi apparatus (Pl. 21, fig. 5) becomes larger and tends to spread out in the cell (Pl. 21, figs. 12, 13).

4. During division of the schizont the Golgi elements are attracted into subequal groups of dictyosomes and granules around each nucleus, as happens in most metazoan cell-visions.

5. No centrosome was identified—the Golgi elements are probably attracted by some other body in the nucleus.

6. Each daughter schizont receives a part of the Golgi apparatus of the mother cell.

7. The peculiar nucleolus (or karyosome) of the merozoite (corps en barillet stage) always lies at one end of the nucleus. The Golgi apparatus always takes up its position outside at the other end (Pl. 21, fig. 18).

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3. Hirschler.—"Über Plasmastrukturen in den Tunicaten-, Spongien-, und Protozoenzellen", 'Anat. Anz.', Bd. xlvii, 1914.
4. Siedlecki.—"Étude cytologique et cycle évolutif de Adelea ovata", 'Annal. Instit. Pasteur', 13, 1899.
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6. King and Gatenby.—'Nature', March 1923.

DESCRIPTION OF PLATE 21.

Lettering.

G, Golgi apparatus. GX, bodies possibly either Golgi apparatus modified or derived from the Golgi apparatus. N, nucleus. *n*, nucleolus (karyosome). Y, yolk-like bodies.

Figs. 1, 3, 4, and 10.—Male gametocytes not containing a normal Golgi apparatus. The granules GX, impregnate like the Golgi apparatus, but resemble them neither in position nor morphology.

Fig. 2.—The Golgi apparatus of a number of merozoites drawn free-hand.

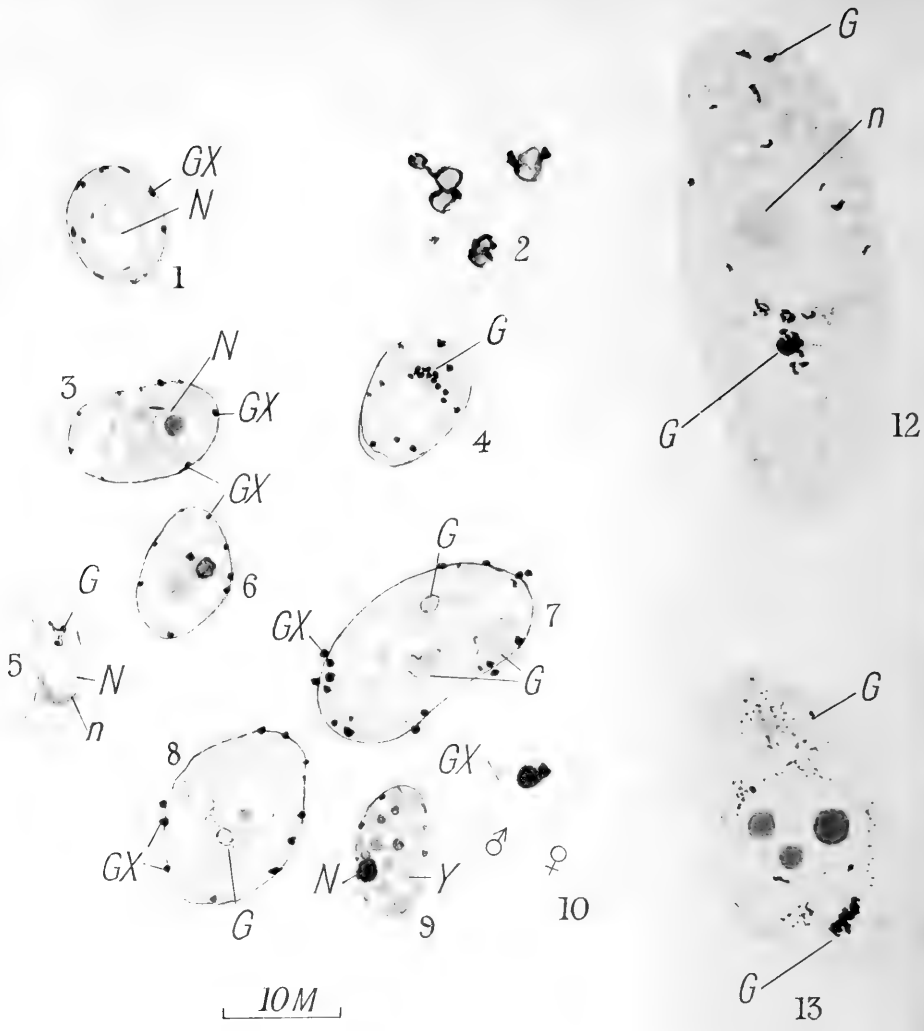
Fig. 5.—Young trophozoite just after separation of merozoites.

Fig. 6.—Male gametocyte containing an excentric juxta-nuclear Golgi apparatus, and also numbers of the granules GX, one near the Golgi apparatus.

Figs. 7 and 8.—Cells too large to be normal male gametocytes, but showing what seems to be the degeneration of the Golgi apparatus.

Fig. 9.—Shows yolky bodies in male gametocyte.

Figs. 11, 12, 13.—Trophozoite (schizont) showing Golgi apparatus.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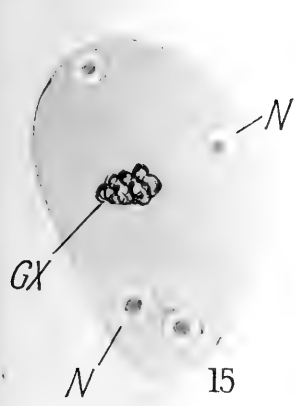

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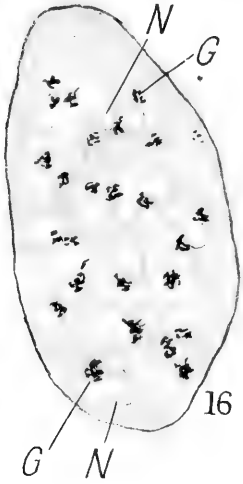

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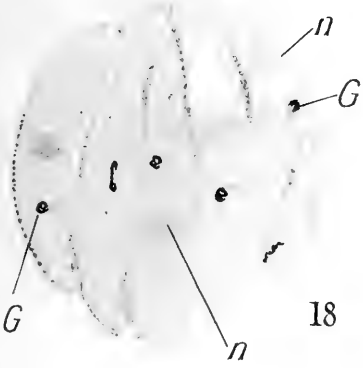

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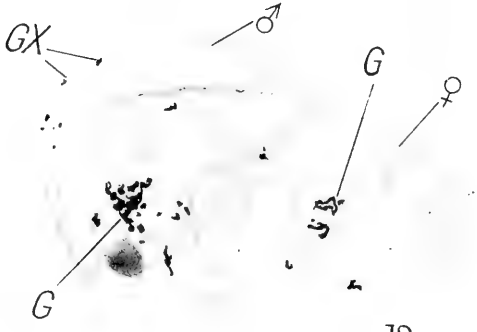

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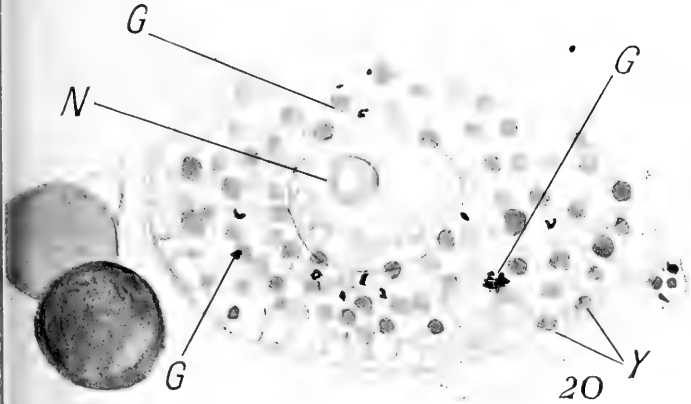

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Figs. 14, 16, 17.—Stages in schizogony showing dictyokinesis of Golgi elements. In fig. 17 the nuclei are not shown, all the dark rings being Golgi bodies.

Fig. 15.—Four-nuclear stage of schizogony showing the Golgi apparatus (GX) apparently abnormally situated in the centre of the cell and taking no part in division.

Fig. 18.—'Corps en barillet' stage showing the merozoites, each with a pale nucleus in which at one end lies the nucleolus, *n* (karyosome); at the opposite pole, outside the nuclear membrane, is the Golgi apparatus.

Figs. 19, 21.—Association of gametocytes; in the ♂ the granules GX were the only bodies which impregnated by Golgi-apparatus methods.

Fig. 20.—Coccidian trophozoite with scattered Golgi elements, and much 'yolk', Y; the cytocyst lies around the cell.

Some Observations upon Spirostomum ambiguum (Ehrenberg).

By

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With Plates 22 and 23 and 9 Text-figures.

Contents.

1. Historical.

The genus Spirostomum was first mentioned by Ehrenberg, but no definition nor description was given. Its systematic position and the question of the number and identity of the species contained in it was a subject for discussion for many years. Later, Dujardin (**7**) gave a very satisfactory description of the genus in the following words :

'Corps cylindrique très-allongé et très-flexible, souvent tordu sur lui-même, couvert de cils disposés suivant les stries obliques ou en hélice de la surface ; avec une bouches située latéralement au delà du milieu, à l'extrémité d'une rangée de cils plus forts.'

He recognized, however, only Spirostomum ambiguum as a true species.

It is to Dr. Stein (**28**) that we are indebted for a comprehensive and beautifully illustrated description of the genus, together with a detailed account of the vicissitudes of nomenclature through which it had passed since its discovery by Ehrenberg. Stein recognizes two species of Spirostomum, S. ambiguum (Ehrenberg) and S. teres (Claparède et Lachmann) (**5**). Previously Perty (**24**) had included another form in the genus, and to it he gave the name S. semivirescens. His observations, which were founded on a single specimen, are regarded by Stein as being of too superficial a nature to justify the creation of a new species. He believed it to be merely a variety of Spirostomum ambiguum.

Pénard (**23**) recognizes S. ambiguum and S. teres as true species, and to these adds S. filum. This latter, although described by Ehrenberg as Uroleptus filum, was placed tentatively in the genus Spirostomum by Bütschli (**3**) and Claparède et Lachmann. Stein does not classify it among his species. None of the above workers, with the exception of Ehrenberg, had seen it personally, but has relied upon Ehrenberg's figures for their data. Pénard has actually seen it, and feels quite certain that it justifies the position he has given it as a third species of the genus Spirostomum.

2. Material and Methods.

The Spirostoma from which the cultures were started were obtained from ponds in North Cheshire, principally in the neighbourhood of Ringway and Styal near Manchester. They were most numerous in rather deep ponds, with muddy bottoms covered with decaying vegetable matter, and with Lemna covering the surface. A good supply was obtained during the drought of the summer of 1921 whilst the ponds were low and the water fairly concentrated, but all through the autumn and winter, though a large number of ponds were visited, including those visited in the summer, very few specimens were obtained. In May and June they became plentiful again and obviously were multiplying rapidly, since many dividing forms were collected.

Fixation.—The fixation of Spirostomum ambiguum is very unsatisfactory because the animal possesses very highly developed powers of contraction. Attempts to narcotize them with chloroform, ether, carbon-dioxide, or by the action of Epsom salts proved unsuccessful, though the narcotics were used in minute quantities and in very dilute solutions.

Bouin's solution and hot or cold Schaudinn's solution are both good fixatives for the nuclear structures. For whole mounts hot Schaudinn's solution gives the best results, since the contraction of the cell is less with this fixative. When whole mounts were required, the animals, together with as small a quantity of culture fluid as was possible, were placed on a slide smeared with egg albumen. The hot Schaudinn's solution was dropped rapidly on the animals whilst they were extended. Contraction of the whole body invariably took place, but by this method there was no shrinkage of the endoplasm from the ectoplasm. This contraction of the body was not really very disastrous, since the main outline of the meganucleus and its relative position were easily studied in the living animal and fixed preparations were required for detailed study.

It was found best, whenever possible, to starve the material

for a few hours prior to fixation. This had the effect of removing all the undigested food which otherwise would have obscured the details of nuclear structure.

Animals destined to be sectioned were fixed in bulk in a watch-glass with warm Schaudinn's solution or with Bouin's solution. The fixative was washed away and the animals were removed to a narrow tube where they were treated with the different percentages of alcohol. When in 50 per cent. alcohol they were lightly tinged with borax carmine to facilitate their orientation in the paraffin wax. They were cleared with xylol. After they had been cleared they were transferred to a watch-glass containing xylol in which paraffin wax was gradually dissolved. By means of a warm, fine pipette they were transferred to pure wax, contained in a clean porcelain dish, and left in the embedding oven for about two minutes. The animals, together with some of the wax, were dropped upon a glass slide which previously had been smeared with egg albumen. They were orientated with a warm needle before the wax solidified. The solidified wax was then shaved into small blocks.

Staining.—Borax carmine, alum carmine, paracarmine, Delafield's haematoxylin, aqueous iron haematoxylin, and Dobell's alcoholic iron haematoxylin all proved to be useful stains. Aqueous iron haematoxylin gave the best results for sections, but the alcoholic modification of the stain was generally used for whole mounts, since in aqueous solution the animals often became detached from the slide.

Methyl green in 1 per cent. acetic was used for fixing and staining animals not needed as permanent preparations.

The method generally used for clearing whole mounts was to soak the dehydrated preparations in clove oil for about twenty minutes and to wash away the oil with xylol before mounting in Canada balsam. Hairs were used to support the coverslips because there is a tendency for the unsupported coverslips to crush so large an animal as Spirostomum.

Observations on Living Specimens.—For isolation of an animal for repeated observations it was found best

to use a hollowed slide, which, when it was not under observation, was kept in a moist chamber. Although hanging drops were used at first, they were abandoned later, when it was found that when the animal moved to the edge of the drop, which it normally did, it rapidly disintegrated there.

To facilitate observations on living Spirostoma, Caragheen extract was used. This slows down their movements considerably; but, since their shape becomes somewhat distorted with the density of the medium and disintegration often follows, it is not advisable to use it when keeping the animals under observation for a long period. It was very useful, however, for the study of ciliary structures.

Feeding Methods.—For following the course of ingested material, finely powdered carmine or Indian ink in culture solution were both used. A dilute solution of milk in culture solution was also used, as was also finely powdered yolk of egg.

3. Observations on the Morphology of Spirostomum ambiguum.

As a description of the general morphology and movements of Spirostomum ambiguum Dr. Stein's (**28**) excellent account has not been improved upon. It will be sufficient here, before passing on to a detailed account of the various structures, to mention that Spirostomum ambiguum is a large, elongated ciliate belonging to the order Heterotricha. The peristomial groove, which terminates in the mouth, is lateral in position, but the distance of the mouth from the anterior end of the body may vary considerably in different individuals. The peristomial membranellae extend from the extreme anterior end to the mouth, around which they curve in a spiral manner. The meganucleus is long and moniliform. The numerous small micronuclei are situated close to the meganucleus.

Contractile Vacuole.—Spirostomum ambiguum is bounded externally by a relatively thin layer of ectoplasm, on the outer side of which is the thin cuticle. The ectoplasm

has none of the coarsely vacuolated structure characteristic of the endoplasm.

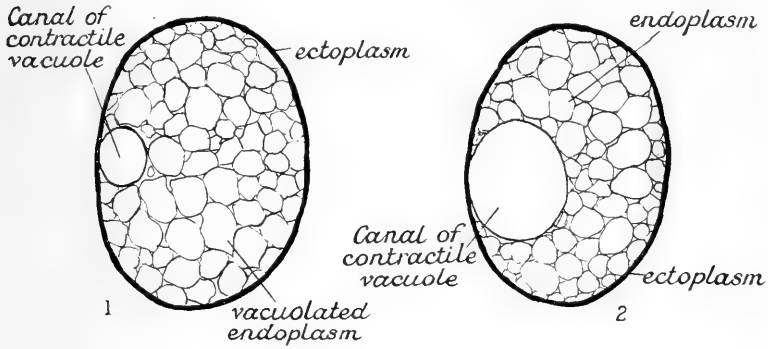
The contractile vacuole lies at the posterior end of the animal. There is a long feeding canal stretching from the anterior end of the animal to the contractile vacuole into which it opens. When distended with fluid the contractile vacuole fills almost completely the posterior end of the animal, and only a very narrow band of endoplasm lies along one side of it, down which the food passes to the median cytopyge (see p. 408). The relative size of the vacuole to that of the whole body, and also its shape, varies with the variety of Spirostomum ambiguum, and this point will be dealt with later.

Transverse sections of Spirostomum ambiguum show that the outline of the contractile vacuole and its canal is perfectly definite. The vacuole and its canal lie immediately below the ectoplasm (see Text-figs. 1 and 2). When full the canal protrudes far into the endoplasm, by which it is almost completely surrounded.

When contraction of the vacuole is about to take place the liquid passes down the feeding canal, which normally closes behind it, and into the vacuole proper, which becomes very much distended. Normally the voiding of the contents to the exterior immediately follows this, but in some cases, particularly in partially narcotized animals, and also often in animals kept for a long time in hollow slides with a small quantity of liquid, evacuation does not immediately take place. The animal continues to swim about with a large, closed vacuole at the posterior end. When the vacuole is about to be emptied an opening is formed at the posterior end at the base of a slight depression; the body-walls surrounding the vacuole contract from before backwards, the liquid is forced out of the opening and the vacuole disappears. This complete contraction of the contractile vacuole gives the posterior end of the animal a compressed appearance. The new vacuole and feeding canal are formed in exactly the same position as was occupied by the preceding one.

Endoplasm and Nuclei.—The endoplasm consists of large vacuoles separated by narrow meshes of fairly fluid protoplasm. In the endoplasm lies the long moniliform meganucleus. In the living animal its form can be followed quite easily, since its denser structure and greater powers of refraction readily distinguish it from the rest of the protoplasm. Normally it consists of a single unbranched chain, extending in a fairly straight or slightly zigzag manner from the anterior end to the contractile vacuole. The lobes, which vary con-

Text-figs. 1 and 2.



siderably in size, are joined together by commissures which may be either almost as wide as the lobes themselves or very narrow. The lobes vary also in number. The least number I have ever seen in any member of the large variety was ten and the greatest number was fifty.

In some animals, the nucleus, although it was normal in length, had an unconstricted, vermiform shape and in places was slightly coiled. In all other respects the individuals seemed quite normal and the position of the mouth (see part on Fission) did not point to any very recent or immediately approaching fission. I found, however, by isolating these individuals and keeping them under observation for a number of hours which varied with the individual, that lobation eventually did take place and that it was in this case delayed for a much longer

period after fission than usual. A similar phenomenon was observed by Johnson (**13**) during his study of the Stentors.

Stein (**28**) describes cases where the meganucleus was only a quarter of the body length, was not lobated, and lay in the anterior end of the body. These were, I feel certain, also stages in fission.

The meganucleus is surrounded by a nuclear membrane which adheres firmly to the nucleoplasm. It is best shown in individuals which have been fixed and stained in methyl green and acetic, especially if the cytoplasm has been teased out prior to fixation.

In preparations well fixed and stained with iron haematoxylin the internal structure of the meganucleus is plainly visible. It consists of numerous granules, which stain deeply with iron haematoxylin, embedded in a fairly homogeneous matrix.

Greenwood (**9**) terms these deeply staining granules macrosomes, and describes in addition to these other minute granules which do not stain deeply with haematoxylin but do so with borax carmine. These latter she calls microsomes. In my preparations with borax carmine the nucleus seems to have a finely granular appearance, but the large granules (i. e. Greenwood's macrosomes) do not combine with this stain.

In preparations stained with iron haematoxylin the macrosomes are seen to be present in both the commissures and lobes of the nucleus ; but if the commissures are very narrow they are confined to the lobes alone. The macrosomes vary in size from minute dots barely visible at a high magnification to masses up to 10 μ or more in breadth (Pl. 22, figs. 1, 2, and 3). Often these granules are surrounded by lightly staining areas. Since these are not always present it is possible that they are due to the fixative and are not to be interpreted as part of the normal nuclear structure. The macrosomes vary greatly in shape ; they are generally round, but may be oval, pear-shaped, or even roughly oblong. The medium-sized granules often show a single vacuole in the centre (Pl. 22, fig. 1, *vac.*), whilst invariably within the large masses one or more vacuoles

are present (Pl. 22, fig. 2, *vac.*). Occasionally as many as five vacuoles have been seen in one large macrosome, each vacuole being separated from those adjacent to it by strands of the darkly staining substance of which the macrosome is composed.

The presence of small non-vacuolated granules, and medium or large-sized ones containing one or more vacuoles, in the same lobe of the nucleus is quite common. Sometimes all the granules present in the nucleus are without vacuoles, whilst in others they are all large with many vacuoles.

Collin (**6**) describes similar macrosomes and microsomes in the nuclei of Acinetaria. The microsomes he believes to be true chromatin grains, whilst the macrosomes he regards as true nucleoli.

Owing to the fact that I have not had time or opportunity as yet to study these structures in detail in Spirostomum, I do not propose to offer any speculation as to their nature. I should, however, like to add that since there exist all degrees of vacuolation and non-vacuolation, and since whenever large masses with numerous vacuoles are present the actual number of masses is small, it seems to me very probable that the large vacuolated masses (i.e. Greenwood's macrosomes) are formed by a flowing together of a number of the smaller macrosomes and a subsequent vacuolation from several centres.

Animals having large multivacuolated macrosomes in their meganuclei do not seem to be otherwise abnormal, and show no signs of degeneration in the cytoplasmic structures. Since the degree of vacuolation seems to be independent also of the degree of growth after fission it seems quite probable, as Greenwood suggests, that it is due to diet or to some temporary condition of the culture medium.

The Micronuclei.—The micronuclei of Spirostomum ambiguum are minute in size and difficult to find. They completely escaped the notice of Stein (**28**). Maupas (**17**) was the first to discover their existence. They lie close to, but are not attached to, the meganucleus (Pl. 22, fig. 1, *M.N.*). In structure they consist of a central endosome, presumably

composed of chromatin, since it stains darkly with the various haematoxylin and carmine stains and with methyl green. This endosome seems to be homogeneous and is surrounded by a pale area or halo, around which there appears to be a definite membrane.

In his summary of our knowledge of the multinucleate ciliates Calkins (**4**) says that ' Balbiani, in his earlier work at least, held that the number of micronuclei is always the same as the macronuclei, or in beaded forms, as many as there are segments of the macronucleus '. He goes on to say that Maupas (**18**), Gruber, Bütschli, and others disproved this view. They found that the numbers were the same in some ; in other cases, of which Stentor is an example, the micronuclei outnumber the segments of the macronucleus ; whilst in other forms, including Spirostomum ambiguum, the segments of the macronucleus outnumber the micronuclei. I can fully endorse the statement that the micronuclei do not correspond in number to the lobes of the meganucleus, for I have seen individuals in which a number of lobes had no micronuclei near to them, whilst others have two, or in some cases three, four, or five to each lobe. The micronuclei are found opposite to the commissures as well as opposite to the lobes.

In the majority of individuals which I examined, however, the number of lobes of the meganucleus is greater than the number of micronuclei. In a number of cases there have been nearly twice as many lobes of meganucleus as micronuclei present. On the other hand, quite an appreciable number of individuals have been observed in which the micronuclei were approximately equal in number to the lobes of the meganucleus or slightly exceeded them. In one case where the number of lobes in the meganucleus was only ten, twenty-six micronuclei were present.

From these observations it seems clear that, subsequent to fission, in the change from a vermiform to moniliform type of nucleus, there is no correlation between the number of constrictions appearing in the meganucleus and the number of micronuclei present in the daughter Spirostomum.

Abnormalities in the Form of the Meganucleus. —A number of individuals from different cultures have been found in which the meganucleus was abnormal. These observations include individuals of both the major and minor varieties (see below).

One member of the major variety was found whose meganucleus had a short branch, consisting of two lobes and a commissure, given off from one of the commissures. This was the only case of a branched meganucleus met with.

A fairly common abnormality was the division of the meganucleus into two pieces; in one case three pieces of meganucleus were present. Such conditions might be brought about by the snapping of a delicate commissure. Another method was revealed, however, whilst watching a normal individual divide. During the contraction of the meganucleus towards the anterior end of the animal, the posterior end of the meganucleus was seen to come apart from the rest and to follow as a separate small oval fragment in the wake of the rest. Elongation subsequently took place, and, when the constriction of the cell occurred, the anterior daughter contained a whole daughter meganucleus, whilst the posterior daughter contained its share of the major fragment and the separated minor fragment. Each of these latter gave rise to a piece of moniliform meganucleus.

The most interesting cases of abnormalities, however, were found in a five months' old culture of the minor variety of Spirostomum ambiguum. The cilia of all were normal, but the protoplasm seemed denser and more granular than usual, although it showed no signs of the vacuolation usually associated with degenerate forms. The meganucleus had lost its moniliform appearance and lay collected in masses in the endoplasm. In some cases it took the form of three or four rounded masses separated from one another by quite distinct gaps. In one case the meganucleus was represented by a big sphere in the anterior end separated by a wide space from the remainder, which took the form of four lobes of the normal moniliform type.

In three cases the meganucleus was broken up into three or more rounded spheres lying in the endoplasm, the hindermost of which had passed down the body and lay as a small refractive ball at the extreme posterior end. Stained preparations of two of these animals showed that their meganucleus was composed of darkly staining granules packed more closely together than they normally are but not vacuolated. Each sphere of meganucleus was surrounded by micronuclei. The remaining individual was isolated on a well-slide, supplied with a little of the original culture medium and put into a moist chamber. After about twenty-four hours it was again observed. The spheres of the meganucleus were in practically the same position in the anterior part, but the posterior sphere had disappeared. Whether it had been absorbed or had passed out of the body I cannot say ; but its position in relation to the cytopyge on the previous day seemed to suggest that the latter fate had befallen it. The animal was kept two more days without any important changes taking place. At the end of that time it died.

The culture in which these cases were found was an old leaf one. The animals in it were very few and no case of division was observed while it was under observation. From the lack of food vacuoles in the animals it was obvious that the culture was in an impoverished state, and these abnormalities were no doubt due to starvation.

4. Methods of Cultivation.

The first attempts to form cultures of Spirostomum ambiguum were made with hay infusions. A similar solution to Woodruff's standard hay infusion (**31**) was made, the formula being 10 grammes of chopped hay in 1 litre of tap-water, and raised to the boiling-point for a few minutes. A culture basin containing a quantity of this fluid was inoculated with a few Spirostoma. In a few hours it was found that all the animals had died. Experiments were then made with 75 per cent., 50 per cent., and 25 per cent. dilutions of the fluid with

tap-water. In the stronger solutions the Spirostoma died almost immediately, whilst although those in the weakest solutions lingered for a few days, they showed no signs of multiplying and ultimately locomotion was suspended and disintegration followed.

During his work upon Spirostomum teres, Maupas (**19**) fed the material upon a solution of flour in water, which he added to their own pond water. In order to find out whether á similar medium would suit Spirostomum ambiguum, 0·150 grm. of flour was added to 100 c.c. of tap-water and boiled for ten minutes, these being the amounts used successfully by Calkins (**4**) for Uroleptus mobilis. Varying volumes of this solution were added to culture dishes and to test-tubes which contained Spirostoma together with pond-water and a little débris. No marked success followed this experiment. In two cases the test-tube cultures lived for some days but no division was seen. Mixtures of flour, hay, and pond-water in various proportions, and solutions of Lemco and Vitmar were all in turn tried without any success.

Pond-water, together with the slimy, decaying leaves from the bottom of ponds, was boiled for about ten minutes in order to free it from any organisms which might be present. The boiled leaves were placed into test-tubes with about 10 c.c. of the water in which they had been boiled. The tubes were filled up with pond-water boiled to free it from any organisms. At first approximately 2 c.c. of Woodruff's standard hay infusion was added. This hay infusion was cooled and then allowed to stand open to the air for twenty-four hours before it was added to the cultures. By this means the hay infusion was inoculated with a plentiful supply of bacteria. That it is necessary to use newly made infusions is shown by the work of Peters (**25**), who showed that the maximum development of bacteria in a hay infusion is reached in approximately the first three days. Further, Hargitt and Fray (**10**) have isolated from old hay infusions many kinds of bacteria which are toxic to Paramoecium. It is not improbable therefore that these old infusions contain bacteria which are toxic to other ciliates as

well. Later, the addition of hay infusion was found to be unnecessary.

The tubes were allowed to stand for not less than four days at a constant temperature of 20° C. or for a longer period at a lower temperature in order that bacteria might multiply and decomposition of the leaves and débris set in. Each tube was then inoculated with a few Spirostoma.

In order to discover whether any one particular kind of leaf is more suitable for the cultivation of these animals than another, cultures were made of oak leaves, beech leaves, rushes, and leaves of potomageton respectively. It was found, however, that no individual leaf gave such good results as did a mixture of several different kinds.

At first no multiplication of the Spirostoma took place, although in the majority of the tubes the animals remained alive. After a week had elapsed a smell of decay, in which the odour of sulphuretted hydrogen could be detected easily, issued from the tubes. The cultures darkened in colour, and in a number of cases microscopical investigation revealed the presence of Beggiatoa and numbers of minute green flagellates. The Spirostoma then began to increase rapidly until, about a month after the making of the cultures, they were present in large numbers.

A comparison of these cultures with the ponds in which Spirostomum ambiguum is numerous seems to show that by this method conditions approximately similar to those of their natural environment are obtained artificially. The odour of sulphuretted hydrogen, so noticeable when collecting in the type of pond in which Spirostomum ambiguum is numerous, evidently arises from the decaying vegetable matter. Lauterborn (**14**) believes its presence to be characteristic of the environment necessary to what he calls a ' sapropelic ' fauna, and Spirostomum ambiguum figures in his list of such sapropelic organisms.

There seems to be a direct relation between the presence of sulphuretted hydrogen and a thriving condition of the Spirostoma. When the amount of sulphuretted hydrogen is

very great the Spirostoma die. Whether the Spirostoma are directly dependent upon the sulphuretted hydrogen or upon some product of protein decomposition during which sulphuretted hydrogen is liberated, or whether the kind of bacteria upon which they flourish best is dependent upon it, I am at present unable to determine. The last supposition, however, seems the most likely.

While these experiments were being made excellent cultures of Amoeba proteus were being obtained by Sister Monica Taylor's wheat method (**29**). Since large ciliates were often plentiful in such cultures it seemed probable that Spirostomum ambiguum might be grown in a similar medium. To test this assumption, two wheat grains, boiled to stop germination, were put into a test-tube, which was then filled up with aquarium water, previously boiled to free it from any living organisms. The tubes were allowed to stand in the incubator at a temperature of 20° C. from four to five days to favour the development of a thick bacterial growth. Spirostoma were then added. Excellent results were obtained from this method, thicker cultures being obtained than from the former cultures. In addition to the Spirostoma, Chilomonas, green flagellates, and pink bacteria often developed in these cultures. The wheat cultures are not only easier to prepare, but have the additional advantage that the bacterial food-supply of the ciliata, and therefore the cultures themselves, lasts longer than it does in the leaf-extract medium.

It is interesting to note that long, narrow test-tubes seem to be necessary for the success of these cultures. All attempts to cultivate Spirostomum in either leaf or wheat extract in shallow, wide dishes were unsuccessful. Spirostomum evidently thrives best in deep water where the surface area, and therefore the amount of dissolved oxygen, is small. That it was not the shallowness of the medium which killed the cultures was shown by attempting to grow Spirostoma in wide, deep jars, when the result, or rather the lack of result, was the same as in the case of the wide, shallow dishes.

5. Food Cycle.

In Spirostomum ambiguum from a healthy culture numbers of large, round food-balls can be observed throughout the endoplasm. These food-balls may be formed of very small green flagellates, clumped closely together to give them a morula-like appearance ; others are brownish in colour and are composed of compact masses of bacteria. Some large, pink-coloured balls are present owing to the animal having fed upon pink bacteria present in the culture. On one or two occasions I have seen Chilomonads in the endoplasm, enclosed in a large fluid vacuole. That these Chilomonads were not yet dead was shown by their undulating movements. It is not usual, however, for Spirostomum ambiguum to ingest anything so large.

In some cultures the food-bodies were entirely bacterial, in others bacteria and flagellates mixed together formed the food-balls, whilst in others the food-balls were composed entirely of green flagellates. The animals were in a flourishing state in all three cases.

Individual Spirostoma were examined for the presence of fluid vacuoles surrounding the food-balls. In the case of bacterial food-balls lying anterior to, or slightly posterior to, the mouth, distinct fluid vacuoles could be seen encircling them. These vacuoles were absent from balls close to the posterior end. Vacuoles similarly encircled balls of bacteria mixed with flagellates. In the case of flagellate balls, only a very thin film of fluid could be detected, or in many cases the balls seemed to be embedded in the coarsely vacuolated endoplasm, without the intervention of a vacuole, whatever their position in the animal might be.

It was soon realized that a definite circulation of the food took place in the endoplasm. An attempt to trace the cycle was made by placing individuals in tap-water for a sufficient length of time to allow all the food to pass out of the body, and then isolating them in well-slides containing some culture solution rich in green flagellates. Unfortunately all the

animals treated in this way refused to feed. After many unsuccessful attempts a few animals were persuaded to feed from such a culture solution in small test-tubes.

The food is wafted down the long peristomial groove to the cytostome by the peristomial membranellae. At the base of the cytostome the food is gathered into a sphere, which varies considerably in size from a ball only just visible under the $\frac{2}{3}''$ objective, to one-half the width of the animal's body. The food-ball then passes forward towards the anterior end of the body. Its movement is, comparatively speaking, rapid. On reaching the anterior end of the body it moves to the posterior end in a course parallel to its former one. After regaining a position approximately level with the cytostome its progress becomes much slower, and with many halts it passes down the side of the contractile vacuole, along the narrow strip of endoplasm found in this region, to the cytopyge. This cytopyge appears at the base of a slight depression situated in the middle of the posterior end. The undigested material is evacuated slowly, one sphere at a time.

Since the movement of the food-balls at the posterior end of the animal is so slow, there is often an accumulation of these spheres in the neighbourhood of the contractile vacuole.

The course followed by flagellate or bacterial food-bodies is shown in Text-fig. 4.

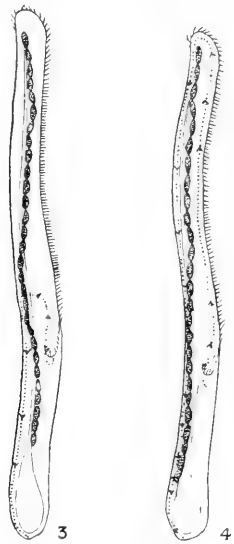
In order to discover whether the course taken by food-balls was similar in the case of substances of no food value, animals were taken straight out of the culture and put directly into a suspension of finely powdered carmine in culture solution. The particles of carmine were wafted to the cytostome. The granules of carmine were collected at the base of the cytostome exactly as were the bacteria in the cases cited above, but the carmine grains were packed much more loosely together. Like the bacterial balls, the carmine balls moved from the base of the cytostome forward, but instead of passing right to the anterior end they passed inwards, as shown in Text-fig. 3, and then continued backwards down the opposite side of the body to

the mouth. They were evacuated in the same manner as were the remains of the nutritious particles. Smaller clumps of carmine move more rapidly than larger clumps.

In cases where the animals were full of balls of nutritious material before feeding, it could be seen that the carmine balls moved backwards more rapidly than did nutritious balls.

A similar experiment in which Indian ink was used instead of carmine gave identical results.

TEXT-FIGS. 3 AND 4.



The above observations differ from those of Lund (**16**) on Bursaria, in that in Spirostomum the ingested material follows a definite course through the body. When the material ingested is of a nutritious nature, the balls travel to the anterior end and then move backwards following a course parallel to the meganucleus. In her work upon the food vacuoles of Carchesium, Greenwood (**8**) states that the food vacuoles travel round the meganucleus. In Spirostomum ambiguum, and in Paramoecium also, according to Metalnikov (**20**), substances of no nutritive value take a much shorter course,

move much more rapidly, and are therefore expelled in a much shorter time than nutritious ones.

A suspension of hard-boiled yolk of egg in water was fed to some Spirostoma. The particles ingested were observed to follow the course taken by carmine granules and Indian ink. A similar course was followed by globules of raw milk. From this it seems apparent that raw milk and yolk-granules are not nutritious to Spirostomum ambiguum, the animal evidently being unable to digest them.

6. Varieties of Spirostomum ambiguum.

During the observations made upon a number of different cultures of Spirostomum ambiguum it soon became evident that two distinct varieties were present. That these were not stages in a developmental cycle was proved by making pure cultures of each.

Stein (**28**) describes a number of varieties of which his Pl. ii, fig. 10, and Pl. iii, fig. 3, show two chief types. These two main varieties, corresponding to the ones present in the cultures, are also recognized by Roux (**26**), and are termed by him Spirostomum ambiguum major and Spirostomum ambiguum minor. These two varieties differ from one another in a number of important details, the most striking of which is size.

The major variety is usually much broader in proportion to its length than the latter. The average length of the ordinary members of the major variety, when fixed with warm Schaudinn's solution, is 800–900 μ, whilst that of ordinary members of the minor variety, when treated in the same way, is 400–500 μ. The posterior end of the minor variety is truncated, whilst that of the major variety is rounded. The protoplasm of the major variety is yellowish in colour, whilst that of the minor variety is greyish white and the endoplasm is less coarsely vacuolated and more granular in the latter than in the former. In the major variety the peristomial membranellae extend from the extreme anterior end to some point posterior to the middle of the animal's body. In some individuals of

the major variety the mouth may be situated almost at the extreme posterior end, about level with the middle of the contractile vacuole. This variation in the position of the mouth depends, as will be seen later, upon the degree of growth to which the animal has attained since the last fission. In the minor variety the peristomial membranellae extend from the extreme anterior end backwards to the mouth, which usually is in the anterior third of the body length.

The shape of the contractile vacuole also differs in each form. In Spirostomum ambiguum major it is a pear-shaped vessel, almost as broad as the animal, but never occupying more than an eighth of the animal's length. In the minor variety it is much larger, generally filling, when fully expanded, the posterior quarter or even third of the animal. In some cases it has been observed to fill half the total length. The shape of the contractile vacuole in the minor variety tends towards an oblong.

The time between contractions of the vacuoles in the two varieties varies. Thus, in the major variety the average time obtained from repeated observations on a large number of animals from four different cultures was every eight and a half minutes. The maximum period between contractions which was ever observed was ten minutes and the minimum seven minutes. In the minor variety the average was sixteen minutes. On two occasions thirty and thirty-four minutes respectively elapsed between contraction of the vacuoles of individuals of this variety. Since these two figures were so different from the rest they were not included in the average.

The meganucleus in both varieties is similar in form and structure, but I have never seen large, multi-vacuolated macrosomes in the meganucleus of the small variety. Since the contractile vacuole is very large, the length of the meganucleus in proportion to the body length in the small variety is less than in the large variety.

The micronuclei in the small variety are similar to those of the majority variety except that they are smaller and often rather disc-shaped.

The chief differences between the two varieties, therefore, can be summarized as difference in size, in colour of protoplasm, in position of mouth and length of peristomial area, and finally in the shape and relative size of the contractile vacuole and in its periods of contraction.

7. Reproduction.

A. Observations on the Growth and Reproduction of Spirostomum ambiguum during Cultivation.

In many cultures of the large variety of Spirostomum ambiguum (see p. 404) made by both the culture methods described above, it was observed that the size of the individuals in different cultures varied enormously. Observations made upon animals grown in a rich wheat or leaf culture medium and kept at a constant temperature of 16° C. in the incubator, showed that they were larger than those grown in a similar culture at a higher temperature. Even when the culture was not very rich in food and was kept at a temperature of about 16° C. the individuals were very large. That these differences in size were not due to differences of race in the Spirostoma was proved by reversing the conditions, when the animals altered in size correspondingly.

Further, it was observed that individuals in the cultures which were kept at 20° C. divided more rapidly than did those in cultures kept at 16° C. or lower. This was confirmed by starting two cultures similar in every way and containing the same number of Spirostoma but keeping one at 20° C. and the other at 16° C. The former increased more rapidly than the latter.

This is probably the whole reason for the variations in size found in Spirostoma ambiguum major in the various cultures. When the cultural conditions favour rapidly repeated divisions the individuals become smaller than the normal size of the species. At each division their size is halved and the intervals between successive divisions are so short that they

are unable to reach normal size again before the next division occurs. After this division they are therefore still smaller than half the normal, and this decrease in size is progressive, producing a culture full of small individuals.

On the other hand, in other cultures the stimulus to division was evidently weak or in abeyance, although assimilation and growth were in no way impaired. The Spirostoma divided, therefore, infrequently, and grew to far beyond the average size, sometimes attaining as much as two and a half times the size of the ordinary individuals.

In starving cultures also, or in tap-water, they become very small. The cytoplasm seems to decrease more rapidly than the nucleus, since in such animals the nucleus is much coiled together.

A curious phenomenon, observed in most cultures on certain occasions, was the agglomeration of many Spirostoma into balls and strings. In an undisturbed condition of such a culture these clumps were suspended in the fluid, but they sank to the bottom if they were agitated. This condition was very marked at the time of conjugation in nearly all the cultures which contained conjugants. Lebedew (**15**) describes a similar massing together of individuals of Trachelocerca in the material from which he obtained his conjugants. He believed that the animals congregated around food-bodies. I have seldom been able to find any food forming the nucleus of the balls occurring in my cultures. Calkins (**4**) states that in Uroleptus mobilis epidemics of conjugation 'are invariably preceded by a characteristic massing or agglomeration of individuals'. By transferring these masses to other dishes containing fresh culture medium, he invariably obtained epidemics of conjugation.

In many cultures Spirostoma were observed adhering to the sides of the tubes, evidently by the mucous secretion described in this animal by Jennings (**12**). It is possible gently to draw them away a little from the solid to which they adhere without severing the mucus. I am of the opinion that the suspended agglomerations are formed in a similar way, by

the adhesion of many animals by a mucous secretion, and that this condition is particularly prevalent at times of conjugation. Possibly by its means the future conjugants first adhere one to another before any protoplasmic connexion is established.

Some attempts were made to induce conjugation in the large variety of Spirostomum by experimental means.

Maupas (**19**) produced conjugation in S. teres by subjecting them to alternations of a rich diet and a period of semi-starvation. A similar process was tried with Spirostomum ambiguum but without any result.

In his work upon the conditions for conjugation in Paramoecium, Hopkins (**11**) has produced conjugation by subjecting the animals to a preliminary period of semi-starvation for about two weeks and then adding food and a small percentage of various solutions of inorganic salts. The salt solutions used were :

0·00002 N solution of ferric chloride,
0·00025 N solution of potassium chloride,
0·001 N solution of sodium chloride,
0·0001–0·0004 N solution of calcium nitrate.

Somewhat similar experiments have been done by Zweibaum (**32**). He, too, has been able to produce conjugation, and has found that ferric chloride gives the best results. I was unable, however, to induce conjugation in Spirostomum by any of these methods. Ferric chloride, in the quantities used by Hopkins, was an excellent stimulant to division, and cultures treated in this way gave rise to great numbers of very small Spirostoma, which, some weeks later, presumably when the effects of the salt had been lost, returned gradually to a normal size.

Conjugation was first observed in the large variety of Spirostomum ambiguum on May 4, 1922. It occurred in a wheat culture which had been kept at a constant temperature of 20° C. since March 13. On May 7 conjugating pairs were observed in two other wheat cultures, both of which had also been kept at the above constant temperature. One culture dated from January 4 and the other from the end of

March. All three cultures were quite normal, no experiments having been performed upon them to induce conjugation. There was no appearance of a true epidemic such as Mulsow (**21**) found in Stentor coeruleus and Stentor polymorphus in May 1911, when he obtained some 2,000–3,000 pairs of conjugants. A few pairs were observed each morning in each culture for about a fortnight, and after that the cultures again became normal.

For about a fortnight previous to conjugation being observed, microscopic observation of a few individuals taken at random from these cultures had shown that the protoplasm had become dark in colour and somewhat granular in appearance. But the protoplasm of all conjugants observed was of the normal light colour. Whether the protoplasm darkens in colour previous to conjugation and then grows light again when this takes place, or whether individuals destined to conjugate remain light, in which case we must suppose that all such individuals had escaped my observation, I cannot say with certainty. From the fact that in some leaf cultures (see below) the protoplasm of all individuals observed was dark, and that later almost all individuals in the culture conjugated, the former suggestion seems to be the more probable.

Since all these cultures had been kept at a constant temperature, it was impossible that the sudden rise in room temperatures, which took place about the above dates, could have stimulated the Spirostoma to conjugate.

On May 24 a number of conjugating individuals were observed in one wheat culture and in two leaf cultures which had been kept always at the ordinary laboratory temperature, then about 22° C. The period of conjugation lasted for about ten days. In two of the cultures only a few pairs were found to be conjugating, but in one leaf culture on an average five or six pairs were removed each morning. This gave a fairly high percentage of conjugating individuals. That the cultures were in a good condition was shown by the fact that division took place frequently in the non-conjugating organisms.

In his paper upon the conjugation of the Stentors Mulsow (**21**)

concludes that it was unfavourable conditions in their environment which caused them to conjugate, since, when the conjugants were removed, all non-conjugants left in the cultures died within a few days. That this did not apply to my cultures of Spirostoma was shown by the fact that the non-conjugants continued to live quite normally when left in the undisturbed cultures after the period of conjugation had passed. The fact that conjugation took place in both wheat and leaf cultures was interesting, since it indicated that the raw material from which the culture medium was made was not the factor inducing conjugation. It is probable, however, that the physical and chemical constitution of an extract of leaves is not so widely different from that of an extract of wheat that it would affect the behaviour of the organisms in this respect. This point also seems worthy of more detailed investigation, since Baitsell (**1**) found that in pedigreed cultures of Stylonichia pustulata conjugation occurred on two occasions in animals kept in a beef medium, whereas it never occurred in those forms kept in hay infusion though they were identical in age to the former. From this he concludes that conjugation is induced by external conditions affecting the organism and that it bears no relation, in this form at least, to a particular period of a ' life-cycle '.

Since all the cultures in which conjugation had so far taken place had been stocked from the descendants of Spirostoma obtained on one occasion from a pond near Styal (near Manchester) in July 1921, it was at first thought possible that the length of time during which the animals had been cultivated might be a factor influencing conjugation. It is a well-known theory that Protozoa multiply for a long period without conjugation, after which the rate of multiplication decreases and a period of depression ensues in which the animals degenerate and die unless they are stimulated to renewed division by conjugation. This theory was first suggested by Maupas (**19**).

From June 14 to June 22, however, conjugation was observed in a leaf culture of Spirostomum ambiguum which had

been collected from a pond at Hale in Cheshire only a fortnight before. This new culture had been kept in the incubator and had divided repeatedly. Moreover, the individuals which were not conjugating were dividing actively during the period of conjugation. The proportion of conjugants was greater in this culture than in any other. The culture was started from about ten individuals, and, since approximately forty pairs of conjugants were removed during the third week, the conjugants must have been capable of repeated division immediately prior to conjugation, in which case a senile condition was impossible. That conjugation took place in a culture where the division rate was high was found by Baitsell (**1**) in Stylonichia pustulata, but, whereas this culture of Spirostomum ambiguum continued to flourish after the period of conjugation had passed, the non-conjugants in the culture of Stylonichia pustulata became degenerate and died out.

In one of two of the wheat cultures in which conjugation was observed, numerous Colpidia and Paramoecia were present in addition to Spirostomum ambiguum. Conjugation was never observed among any members of the two former species. Whatever the conditions might be inducing conjugation in Spirostomum ambiguum, they did not have the same effect on the Paramoecia and Colpidia.

My experiments and observations have not, therefore, up to the present time, thrown any new light upon the factors causing conjugation. They seem to indicate that the seasonal factor is an important one, in Spirostomum ambiguum at any rate, and I must hope that further and more detailed work will enable me to follow out such hints as I have so far gained. An important part of such work would be the study of this organism in its natural surroundings in its native ponds and ditches.

B. Fission.

It is to Stein (**28**) that we are indebted for the first description and figures of fission in Spirostomum ambiguum. He observed the phenomenon in four cases, three of which were

in the small variety and one in the large variety. As he relied upon freshly collected material this was not strange, for even in healthy cultures individuals undergoing division often are not numerous. This is, no doubt, due to the fact that a period of two to three days elapses between divisions even in an animal subjected, as far as it is possible for us to tell, to excellent conditions. Divisions takes place during the night as well as in the day time ; for in the morning usually there are present in the cultures a number of animals in the last stages of division, or some which have recently divided.

As the process of division differs very little in the two varieties, it is unnecessary to give a description of each ; it will be sufficient to describe it as it occurs in the major variety and to note any deviation which occurs in the minor variety.

In order to follow the entire process of division it was found best to isolate individuals which showed the first signs of coming fission and to observe them through the whole process. Stained preparations were made at different stages.

The time taken to complete the phenomenon varies in different individuals, but the average time is from seven to eight and a half hours ; even then, the two daughters, though separated, have not attained the normal form. This is especially true of the form of the meganucleus. Simpson (**27**) gives one to two hours as the time required for division in Spirostomum ambiguum ; but this I take to mean the actual division of the animal's body into two parts irrespective of nuclear changes.

In the major variety the mouth, in animals about to undergo division, lies midway between the anterior and posterior ends. Large numbers of animals belonging to the major variety have been observed undergoing division, and in every case the mouth of the parent was at the middle of the body length. In his single observation on fission in this variety Stein describes the old peristome as extending through the anterior two-thirds of the body length, and the new peristome of the future daughter as developing in the posterior third of the body. He further states that after half an hour's observation the beginning of

the division of the body showed between the old and new peristomes. In such cases division would be unequal; one daughter would be two-thirds the length of the other. On no occasion have I seen any such inequality in size between the two daughters.

In the individuals of the small variety about to undergo fission the old peristome lies in the anterior third and the new peristome forms in the middle to posterior third of the body.

Animals about to divide are always much longer than the average-sized individual of the same culture. This length is reached by a gradual process of growth, principally in the region behind the mouth.

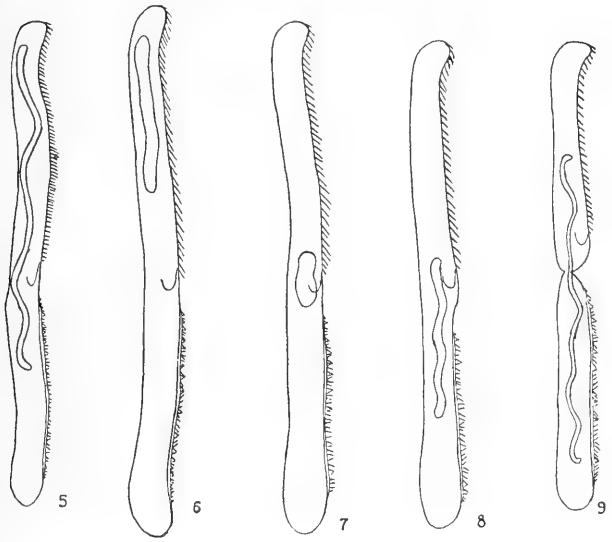
The first indication of coming fission is cytoplasmic, not nuclear. It consists of the formation of the peristomial membranellae of the new cytostome. In the major variety the anterior end of the new membranellae is almost level with the posterior curve of the old. The first indication of the formation of these daughter membranellae is a slight ridge in the posterior half of the body, running parallel with the rows of the body cilia. This ridge gradually becomes more pronounced, and along it the new membranellae are formed. At first these are very small but rapidly grow larger. The immature membranellae are much shorter in proportion to their width than are the mature ones. This gives them a somewhat leaf-like appearance. The movements of these developing membranellae, almost until the separation of the two daughters, are very irregular, many of the individual membranellae moving in different directions to their neighbours, which gives the whole a ragged appearance. This lack of co-ordination in movement is very noticeable when compared with the steady, undulating motion of the mature membranellae.

The formation of the membranellae is usually well advanced before the nucleus shows any intimation of approaching fission. The times given above were taken from the beginning of the nuclear changes. The somewhat zigzag form of the meganucleus becomes straightened, the lobation is gradually lost, and its shape becomes vermiform. Stained preparations of this stage

show that the granular structure of the nucleus is quite normal. The micronuclei, too, are normal (Text-fig. 5).

The meganucleus next begins to contract and incidentally to thicken. In some individuals contraction begins before the lobation is completely lost, but in the majority of cases the former is the normal method. In almost all individuals the anterior part of the meganucleus remains in its normal

TEXT-FIGS. 5, 6, 7, 8, AND 9.



position, and the posterior and middle parts contract towards it in such a way that the almost completely contracted meganucleus lies in the anterior end of the body (Text-fig. 6). Its shape is still vermiform but much thickened and less than a quarter of the length of the body.

All the sections which have been studied of animals at this stage show darkly stained granules irregularly dispersed over a mesh-work. These meshes do not combine with stains so strongly as do the granules. Preparations of stages intermediate between the expanded and the anteriorly contracted stages of the meganucleus show that the micronuclei move

forwards with the meganucleus, but that they become much swollen and stain very lightly (Pl. 22, fig. 4, *M.N.*). At this stage, when the meganucleus lies in the anterior end, the micronuclei are almost three times their normal size. They are surrounded by a distinct halo. Some are close to the meganucleus, but others are a slight distance away from it.

The meganucleus moves backwards until it comes to lie in the middle of the animal's length, at the same time contracting still further (Text-fig. 7). The movement backwards to the middle of the body is fairly rapid. Here the meganucleus remains for a relatively much longer period. In appearance it is a roughly oval, dense structure. Its form is not rigid but slowly changes, showing protuberances at the side which gradually disappear and reappear at other places.

In many sections of animals with the meganucleus fully contracted in the centre of the body it has not been possible to find any micronuclei. In one or two specimens large, pale-staining micronuclei have been seen; these evidently have not yet undergone division. In other cases small, intensely staining micronuclei have been found near the meganucleus. It seems quite probable that these are the products of division, and I hope to say more about them in a further paper. In all further stages of division in Spirostomum ambiguum the micronuclei are small and stain normally.

From the fact that, when the micronuclei of conjugants are about to divide, they become swollen and similar in appearance to the ones described above, it is to be concluded that these micronuclei divide some time during the migration backwards of the anteriorly contracted meganucleus, or soon after it takes up its central position. Unfortunately I have never actually seen any division taking place in any preparations, and conclude therefore that the division must take place very rapidly. A similar difficulty was experienced by Johnson (**13**) whilst working upon the division of Stentor.

During the time when the meganucleus is fully contracted in the centre of the body, a slight dilation becomes visible in the feeding canal of the contractile vacuole, on a level

with the anterior end of the meganucleus. This is the beginning of the daughter contractile vacuole.

A gradual elongation of the meganucleus next takes place (Text-fig. 8). A curious phenomenon in the elongation of the meganucleus, observable also in its contraction, is that this process does not take place equally fast at each end of the mass. Elongation takes place more rapidly in the posterior part than in the anterior, so that while the posterior half of the animal has quite a long developing meganucleus only a short part projects into the anterior half. The elongating meganucleus does not expand in a straight line but is often coiled in its course. Both anterior and posterior ends have the shape of a crook, which often persists even after the separation of the two daughters.

During the elongation of the meganucleus a slight constriction of the body can be observed a little posterior to the anterior cytostome. This gradually becomes more pronounced and marks the point of the future separation of the two daughters.

Coincident with the development of this constriction is the gradual enlargement of the dilation in the feeding canal of the contractile vacuole. For some time it continues to empty with the contents of the original vacuole, but a considerable length of time before the separation of the two daughters it becomes disconnected from the posterior part of the canal and contracts independently of the posterior vacuole. This separation of the anterior contractile vacuole from the posterior one seems to take place as soon as the constriction of the cytoplasm is sufficiently deep to allow the excretion of the fluid through a pore in the median line of the constriction.

Although the meganucleus in the anterior half seems to grow more rapidly immediately prior to the separation of the two daughters than that in the posterior part, the length of meganucleus in each daughter at the time of separation is still a trifle unequal (Text-fig. 9). This inequality seems to be adjusted later. The meganucleus divides into the two

daughter meganuclei just before the cytological separation of the two daughters takes place.

The newly separated daughters can be distinguished from ordinary individuals by the fact that they are shorter; and also the meganucleus is vermiform and not moniliform. Further, the cytosome is always at the posterior end, and the peristomial membranellae therefore extend practically the whole of the animal's length. Since growth takes place much more rapidly behind the cytostome than in front of it, the cytostome appears to move forwards gradually, so that in animals about to undergo fission it is central in position.

Lobation of the meganucleus seems to take place at varying times after the separation of the daughters. There seems to be no correlation at all between the number of micronuclei present and the number of lobes formed in the meganucleus. It seems probable, as Collin suggested for the nuclei of Acinetaria, that lobation is governed by the varying tensions of the nuclear membrane.

C. Conjugation in the Major Variety of Spirostomum ambiguum.

Stein (**28**) observed conjugation in Spirostomum ambiguum on July 28, 1857. Balbiani also observed it in this species. Conjugation, as stated in Part 7, was seen by the present worker in a number of individuals of the major variety of this species during May and June 1922.

It was noticed that the conjugants were considerably smaller than the ordinary individuals. Stein describes a similar condition in his specimens. It was also noticed that the pairs of conjugants in any one culture showed less variation in size than non-conjugants of the same culture. The greater amount of variation in size in the non-conjugants was probably due largely to the fact that all stages in growth between newly separated daughters and individuals about to divide were present, whereas, from the fact that the cytostome was always central in position with regard to the body-length, it was

apparent that the conjugants were all at the final stage of growth when division should commence. No information throwing light on the means by which the smallness in size in the conjugants is arrived at, was obtained. In his biometrical study of conjugation in Paramoecium caudatum Dr. Raymond Pearl (**22**) found ' that conjugant individuals when compared with non-conjugants were shorter and narrower and less variable both in length and breadth '. He also showed that there was a high degree of correlation between the lengths of the two members of conjugant pairs. He proved, by numerous careful measurements, that such a high degree of homogamic correlation was not due to the random pairing of individuals in a ' homogeneous population of low variability '. Miss Watters (**30**) has obtained similar results with regard to the relationship in size between conjugants and non-conjugants in Blepharisma undulans. Such rough observations as have been made during the present study of conjugation seem to indicate that similar relationships exist between the conjugants themselves and between the conjugants and the non-conjugants in cultures of Spirostomum ambiguum.

Both conjugants are, as was noticed by Stein (**28**), attached along the peristomial groove. This makes the taking in of food during conjugation impossible. From the fact that conjugants, even in the earliest stages of conjugation, are rarely found containing ingested food, it would appear that ingestion ceases some time prior to conjugation.

The peristomial membranellae are not absorbed during conjugation. Generally the anterior end of one individual of the pair is attached to a point a little posterior to the anterior end of the peristomial groove of the other. The attachment ends at the cytostome. Since the cytostome is central in position, it follows that conjugating individuals are attached for half the body-length. Stein (**28**) in his figure of a pair of conjugants depicts them as being attached to one another from the extreme anterior end of each. Quite a number of pairs attached in this way have been met with during the present observations, but the method of attachment in the majority

has been in the manner described above. When they are attached in the manner described by Stein (**28**) the posterior ends are level, since the conjugants are almost without exception equal in size; when they are attached in the more common manner, however, the posterior end of one individual projects beyond that of the other. Observations of individuals just beginning to conjugate showed that the anterior ends were the first to become attached. Sections show that the conjugants are joined by a thin sheet of ectoplasm and that the endoplasm of the individuals does not mingle.

The contractile vacuole seems to function in a normal manner during conjugation, though the average time of its contraction was not studied.

One of the difficulties encountered in studying conjugating pairs is that newly attached individuals often become separated in drawing them up a pipette. Such severed conjugants have never been observed to become reattached but sooner or later die. Maupas (**19**) experienced similar difficulties whilst working upon conjugation in S. teres. Whilst working upon Paramoecium, however, Calkins found that conjugants, if severed before there had been any exchange of nuclear material, would live and divide in a normal manner. Similar experiments upon artificially severed conjugants were carried out by Baitsell (**1**), but without exception the severed conjugants all degenerated and died within the twenty-four hours following the operation.

The time taken from the attachment of a pair of conjugants to the time of their separation varies between sixty and seventy-two hours. It is very difficult to be absolutely certain of the duration of conjugation in a pair, since it is practically impossible to remove for observation a pair which are only just becoming attached. They invariably become separated during the removal from the culture to the depression slide.

In order to secure permanent preparations of as many stages of conjugation as possible, the conjugants were removed from the culture and placed into test-tubes, which contained some of the culture solution, and fixed at different intervals. This

unfortunately entailed a rather high death-rate, but it seemed unavoidable. Further, the supply of material was meagre and the technical difficulties considerable.

For an hour or two subsequent to the attachment there was no change in the meganuclei or micronuclei of either conjugant.

The first big change to take place was the breaking up of the meganucleus into isolated segments by the snapping of its commissures (Pls. 22 and 23, figs. 7 and 13). The greater number of the segments of the meganucleus migrated towards the anterior end of the conjugant's body and came to lie in the area opposite to the line of attachment. A few of the posterior segments invariably remained in the neighbourhood of the contractile vacuole and never migrated forwards. The fact that the meganucleus became fragmented during conjugation was noticed and figured by Stein (**28**). He did not describe it as actually fragmenting, the pair of conjugants upon which he worked evidently having passed this stage when he first observed them.

In some preparations made before fragmentation of the meganucleus and in almost all the ones made afterwards, vacuoles were observed in the substance of the meganucleus. These vacuoles varied in size, sometimes attaining to half the size of the lobes of the meganucleus. Sometimes only one vacuole was present in each lobe, but in other cases three or four were present. The vacuoles often projected, causing the nuclear membrane to bulge outwards. In stained preparations these vacuoles were colourless, but lightly staining spheres could be seen in the centre of some of them (Pl. 22, fig. 5, *vac.* and *p.*). I am unable to offer any explanation of the nature of these spheres. They were present at all stages from the fragmentation of the meganucleus to the early stage of the exconjugant. They were evidently a product of the degeneration taking place in the meganucleus, and might result from the coagulation by the fixative of some fluid in the vacuole. This suggestion unfortunately is not very plausible, since it demands either that the spheres should be present in all the vacuoles, which was not the case, or else that the contents

of different vacuoles varies, which seems a very improbable hypothesis.

These vacuoles, present in the meganucleus during conjugation, are not to be associated in any way with the vacuoles inside the larger ' macrosomes ' of a normal meganucleus. The former were found in the interstices of the granular structure and represent a vacuolation of the nuclear sap ; whereas the latter appeared within the substance of the ' macrosomes '. Although small macrosomes were always present in the meganucleus of conjugants, no large nor vacuolated ones were ever seen in the degenerating meganuclei of conjugants. It is almost unnecessary to remark that this does not mean that the presence of large vacuolated macrosomes can never be coincident with conjugation, but merely that, in the comparatively small number of conjugants that have been studied, they have not been present. However, if the vacuolated-macrosome condition is to be regarded as being caused by an unknown factor in the culture, it may be that this same factor is not suitable for inducing conjugation.

No further visible changes took place in the meganucleus until after the separation of the conjugants.

In newly separated exconjugants the fragments of meganucleus were present and stained as intensely as in conjugants. These fragments contained vacuoles, as did those in the conjugants. In preparations made at a slightly later stage the vacuoles had become more pronounced and now bulged outwards greatly. In some specimens they appeared to have burst, for circular cavities could be seen in the fragments of meganucleus.

Absorption of the old meganucleus took place during the first few days subsequent to separation. Gradually the fragments took up the stain less intensely, and often a clear space could be seen in the cytoplasm surrounding them. Not all the fragments were absorbed at the same time. Complete absorption of the meganucleus did not take place until the rudiments of the new meganucleus had attained to a considerable size (Pl. 22, fig. 6, *L.* and *A. A.*).

The first stages in the formation of the rudiments of the meganucleus have not been seen. Preparation of the earliest stages obtained showed two or more thin discs about the middle of the exconjugant. Since these discs were denser than the surrounding cytoplasm, they could be seen in living exconjugants. The ground substance of these disc-shaped rudiments of the meganucleus stained very feebly, scarcely more intensely than did the surrounding cytoplasm. They contained a number of deeply staining granules. In the earlier stages these granules were distributed through the interior of the disc, but later they appeared to migrate to its periphery. Some of these granules showed one or more vacuoles inside them and seemed to be identical to the macrocomes of the meganucleus of normal individuals of Spirostomum ambiguum (Pl. 22, fig. 8, *M.V.*).

The normal number of these meganuclear discs present in the exconjugants was two. Occasionally four, and in one preparation six, were seen. Whether these large numbers arose by division of an original two, or whether the normal two were formed first, and later nuclei, which normally remain as micronuclei, became converted into them, is not clear.

Although I succeeded in keeping exconjugants alive in test-tubes until seventeen days after the separation of the conjugants, there were no signs of constrictions appearing in the rudiments of the meganucleus to form the moniliform meganucleus. The few individuals that remained alive so long died after seventeen days, and the cessation of conjugation left me without any material with which to make another attempt.

Owing to the small quantity of material at my disposal, the smallness in size of the micronuclei, their great number, and the difficulties of staining them whilst undergoing division, my observations upon them are very fragmentary, a fault which I hope to rectify in an additional paper.

The first change in the micronuclei during conjugation occurred some time after the fusion of the conjugants and before the severing of the commissures of the meganuclei. This change consisted of the gradual swelling up of the majority

of the micronuclei whilst still in their old position close to the meganucleus. Their staining powers decreased as their size increased.

The increase in bulk of the chromatin sphere of the micronucleus did not take place at the expense of the surrounding halo, since this too increased in size and appeared as a wide, clear area surrounding the swollen micronuclei (Pl. 23, fig. 13). It does not seem probable, therefore, that the increase in size of the micronuclei was due to the absorption of fluid from the surrounding halo, unless the latter obtains fresh liquid from the surrounding cytoplasm. As they increase in size the micronuclei move a little distance away from the meganuclei.

In a large number of the animals studied a few of the micronuclei, particularly those situated towards the posterior end of the conjugant, appeared to be unaffected by this change, and they retained their minute size. Such micronuclei were often near to isolated fragments of meganucleus at the posterior end, even after the majority of the micronuclei were well advanced in division. Their fate was not known, but since they were never present in the exconjugant immediately after separation, one may presume that they were subsequently absorbed.

When the majority of the isolated segments of the meganucleus migrated towards the anterior end of the conjugants (see p. 425), the greater number of the swollen micronuclei performed a similar migration and came to lie in the cytoplasm between the scattered lobes of the meganucleus. A few of the swollen micronuclei remained amongst the lobes of the meganucleus at the posterior end of the body. These underwent the same changes as did those at the anterior end.

In their new position the micronuclei continued to swell. Since the amount of chromatin did not increase, but was merely distributed through a greater bulk, the micronuclei became very pale and difficult to study. When they had attained to their greatest size the swollen spheres began to stain unevenly as though the chromatin was becoming aggregated at certain points (Pl. 23, fig. 12). It then became apparent

that the chromatin was gathering at one side of the halo (Pl. 23, fig. 16), the rest of the micronucleus being almost colourless. At the opposite side of the micronucleus to the aggregation of chromatin a projection appeared, lengthening until it touched the membrane at the edge of the halo. Threads formed between the chromatin aggregation, which now became broken up into definite granules, and the apex of the projection (Pl. 23, fig. 11). This I took to be the beginning of the formation of a spindle ; but, in his work upon Stentor, Mulsow (**21**) states that such a condition may be preparatory to the formation of the spindle or a stage in degeneration prior to absorption of the micronuclei, both stages being remarkably alike.

Thus it is not possible to state with certainty that all micronuclei in this stage gave rise subsequently to spindles. No stages in the formation of the second pole were discovered.

The formation of the spindle took place inside the micronuclear membrane. The structure took up the entire space and no halo was to be seen. Many of the spindles were almost globular in shape (Pl. 23, figs. 10 and 15) ; a few, however, were more diamond-shaped (Pl. 23, fig. 9). In the globular form the apices were flattened, but in the others they were quite pointed. Whether this difference in shape was accidental or was the expression of different stages in the division process is not certain, but the latter interpretation seems to me more probable.

No centrioles were ever seen. The spindle-fibres were usually quite obvious. They appeared to fuse and form groups when approaching the apex of the spindle. In the diamond-shaped spindle the fibres appeared to converge at one point, but in the globular spindles the groups of fibres did not all come together at the pole (Pl. 23, figs. 10 and 15).

Spindles were present at the same time in both conjugants, but division did not take place simultaneously in all the micronuclei of the conjugant, since, besides spindles at the equatorial plate stage, micronuclei at the swollen stage prior to division (see Pl. 23, fig. 12) were present, as were also micronuclei which were not so swollen or pale. Some of these

very swollen micronuclei possibly were about to degenerate and not to divide. No stages demonstrating the formation and crossing over of the gamete nuclei nor the degeneration of the surplus ones have been obtained.

In a preparation of an exconjugant, made soon after its separation, a spindle was seen at the anterior end of the body. This spindle (see Pl. 23, fig. 14) was more elongated than those present in the conjugants. It was immediately surrounded by denser protoplasm than that of which the rest of the body was composed. It also was at the equatorial plate stage. A very careful investigation of the rest of the animal did not reveal the presence of other spindles nor of other micronuclei. This spindle was therefore regarded as that of the zygote nucleus, and it was concluded that the other micronuclei had all degenerated. Subsequent stages in division of the nuclei in the exconjugant were not seen.

No further stages of the division of the micronuclei were discovered in the exconjugants. In two exconjugants four, and in one preparation eight, micronuclei were discovered in the neighbourhood of the two rudiments of the meganucleus. In no preparations were micronuclei found attached to the edge of the meganuclear discs as Mulsow found in exconjugants of Stentor.

I should like to take this opportunity to thank the many friends who have so kindly given me helpful advice and suggestions. Particularly I should like to thank Professor Hickson for the stimulating interest he has taken in the work. My sincerest thanks are offered also to Dr. G. Lapage for the help and encouragement he has given me throughout; to Mr. Wadsworth for his advice particularly on many matters of technique; and to Dr. Clifford Dobell for the assistance he so kindly gave to a stranger. Without their help the omissions and mistakes in this paper would have been considerably greater.

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9. EXPLANATION OF PLATES 22 AND 23.

Fig. 1.—Part of the meganucleus of an ordinary individual. *L.*, a lobe of the meganucleus. *C.*, a commissure. *M.N.*, a micronucleus surrounded by a halo. *m.*, a non-vacuolated macrosome. *vac.*, a macrosome containing a small vacuole. ×1200.

Fig. 2.—Part of the meganucleus of a normal individual. *MV.*, large macrosome. *vac.*, vacuole inside the macrosome. ×1200.

Fig. 3.—The terminal lobe of a similar meganucleus. ×1200.

Fig. 4.—Part of the meganucleus of an ordinary individual undergoing fission, with the meganucleus contracted towards the anterior end of the body. *X.*, the anterior end of the meganucleus. *M.N.*, swollen micronuclei surrounded by halos. ×1200.

Fig. 5.—Isolated lobes of the fragmented meganucleus of a conjugant. *vac.*, vacuoles inside the lobes. *p.*, pale-staining spheres appearing inside some of these vacuoles. ×1200.

Fig. 6.—The nuclear apparatus of a young exconjugant. *L.*, the darkly staining fragments of the old meganucleus. *A.*, the newly forming meganuclei, as yet very pale. The presence of as many as five newly forming meganuclei in one exconjugant is unusual. ×1200.

Fig. 7.—A preparation of a pair of conjugants showing the meganucleus in a fragmented condition. ×120.

Fig. 8.—One single large sphere of the newly forming meganucleus present in an exconjugant. *MV.*, macrosome-like bodies. ×1200.

Fig. 9.—Pointed spindle of a micronucleus in a conjugant. ×1200.

Fig. 10.—Globular spindle of a micronucleus in a conjugant. ×1200.

Fig. 11.—Swollen micronucleus with unevenly distributed chromatin at one side and spindle-threads forming at the other. ×1200.

Fig. 12.—Part of a conjugant. *M.N.*, micronuclei very much swollen. The distribution of chromatin in the swollen micronuclei is very uneven. *M.x.*, a micronucleus which is less swollen. *L.*, isolated lobes of the old meganucleus. ×1200.

Fig. 13.—Part of a conjugant. *L.*, isolated lobes of the meganucleus. *M.N.*, micronuclei which have begun to increase in size but are still close to the lobes of the meganucleus. ×1200.

Fig. 14.—The spindle of the dividing zygote nucleus in an exconjugant. ×1200.

Fig. 15. *A.* and *B.*, division spindles of micronuclei in a conjugant. Spindle *A.* has flattened apices. ×1200.

Fig. 16.—*M.S.*, a micronucleus approaching the spindle stage.

On some remarkable new Forms of Caryophyllaeidae from the Anglo-Egyptian Sudan, and a Revision of the Families of the Cestodaria.

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With Plates 24 and 25 and 1 Text-figure.

Contents.

Introduction.

Up to the present time ten more or less well-defined species of the Cestodarian family of the Caryophyllaeidae have been described. These ten species have been named as follows: Caryophyllaeus laticeps (syn. mutabilis, Rudolphi, 1801), Pallas (1781); C. tuba, Wagener (1854); C. fennicus, G. Schneider (1902); C. syrdarjensis, Skrjabin (1913); C. armeniacus, Cholodkovsky (1916); Mono-

bothrium terebrans, Linton (1893); M. hexacotyle, Linton (1898); Glaridacris catostomi, Cooper (1920); Archigetes appendiculatus, Ratzel (1868), and A. brachyurus, Mrázek (1908). Setting aside for the present the question as to whether the first eight of these ten species are rightly to be referred to the three genera named, it is to be remarked that all these ten forms are very similar in their general organization. Apart from minor differences in connexion with the genitalia, more marked differences in the muscular development and shape of the 'head', and the possession of a 'caudal' appendage by Archigetes, all ten species agree in general body-form, in bearing the sexual apertures in the last quarter of the body-length and in therefore having the ovary situated very near to the posterior end, and in the distance between the median 'isthmus' of the ovary and the genital openings being at most (in C. tuba) one-ninth the length of the body (the length of the body being measured from the hind end of the ovary to the anterior extremity in not unduly contracted specimens) and usually much less; also in the presence of a very small group of vitellaria situated posterior to the ovary.

The four new species of Caryophyllaeidae described in the present communication all differ from the ten species above named in at least two of the features just stated, and in three of these new species in all of these features, and since all four species represent marked departures from the type of Caryophyllaeus which, in the form of C. laticeps (Pl. 24, fig. 10), has grown familiar to zoologists, they are of more than usual interest.

My material consisted of specimens, already stained and mounted in balsam, contained in the collection of slides which belonged to the late Dr. A. J. Chalmers when Director of the Wellcome Tropical Research Laboratories at Khartoum, and kindly presented to the Wellcome Bureau by his successor, Major R. G. Archibald. The greater part of this material, though sufficiently well preserved for all ordinary purposes, is yet not good enough for minute histological observations,

despite much restaining and section-cutting on my part, and I have therefore omitted descriptions of the finer tissue structures ; I have also omitted to supply the details of structure of such organs as the cirrus and cirrus-sac, the uterus wall, the ovary, testes, and vitellaria, not because it is impossible to do so, but because I do not think that the information thus to be gained is, at least at present, worth supplying,[1] in view of the major differences separating these four new species from all species hitherto described.

Wenyonia virilis, gen. et sp. nov. Woodland, 1923.

Of this species (Pl. 24, figs. 1, 2), the most remarkable in form of the four to be described in this paper and the type species of the new genus, I possess altogether some twenty mature specimens and five small immature specimens. This parasite was found in the Nile Siluroid Synodontis schall, Bloch-Schneider, 1801, common at Khartoum, presumably in the intestine. Two of my specimens are much larger than the remainder, one of the two measuring 52·5 mm. in length with a maximum breadth of 3 mm., and the other (unmeasured and now sectionized) being of about the same dimensions. The other mature specimens range from 11 mm. to 16 mm. in length, with maximum breadths of 1·5 mm. to 1·7 mm. Apart from the difference of size of body and differences in the lengths of the several regions of the body relative to the length of the body as a whole, the two large specimens are identical with the smaller specimens, and I have no reason to believe that the former belong to a distinct species or variety. In shape of body Wenyonia virilis is very constant and characteristic (Pl. 24, fig. 1). The Caryophyllaeid body is usually divided into the three regions : (1) the ' Kopf ' or anterior extremity, usually distinguished from the next region by expansion, form, or muscular differentiation, or all three ; (2) the ' Hals ',

[1] In most cases these inquiries would involve considerable additional section-cutting of somewhat brittle material which has been flattened and preserved in balsam for some years. Should this additional information become necessary in the future, the material is always at hand.

the short region intervening between the Kopf and the anterior limit of the testes and vitellaria; and (3) the 'Rumpf' or remainder of the body containing the genitalia; but the so-called neck region is very ill-defined in any species of Caryophyllaeus and ceases to exist when the head is contracted, so that in many cases the body is simply divisible on this system into the head and the trunk regions. Since in W. virilis a new and conspicuous post-ovarian region is developed (Pl. 24, fig. 2)—a region inconspicuous in all previously described Caryophyllaeidae—I propose to dispense with the old 'trunk' region and subdivide the body of W. virilis[1] into the four distinct regions which are obvious even to the naked eye: (1) the head (including the 'neck' when present); (2) the testicular region, extending from the most anterior testes to the genital openings and containing the testes and anterior vitellaria; (3) the dilated and therefore conspicuous uterine region, extending from the genital apertures to the hind end of the ovary and containing the uterus, ovary, shell-gland, ootype, and the middle portions of the two elongated vitellarian strands; and (4) the post-ovarian region (Pl. 24, fig. 2, POVR), composing the rest of the body and containing the posterior more or less scattered vitellaria. The broad ovoid uterine region is plainly visible with the naked eye and is the region of maximum breadth.

In a specimen measuring 16 mm. in length the head was 2 mm. long and 1·3 mm. broad at the base, the testicular region was 2·2 mm. long and 1 mm. broad, the uterine region 3·5 mm. long and 1·5 mm. in maximum breadth, and the post-ovarian vitellaria extended nearly to the end of the body, i.e. for nearly 8 mm. In my largest specimen (52·5 mm. long, Pl. 24, fig. 1, *a*) the head was 2·1 mm. long and 1·1 mm. broad at the base, the testicular region was 10 mm. long and 2 mm. in maximum breadth, the uterine region 7 mm. long and 2·5 mm. in maximum breadth, and the post-ovarian vitellaria only extended about half-way down the remainder of the body,

[1] This regional subdivision will, of course, apply to the body of all species of Caryophyllaeids.

i.e. for about 16 mm. Thus the ratios of the lengths of the four regions to the length of the body in these two large and small specimens respectively differed considerably, being $\frac{2\cdot1}{52\cdot5}$ and $\frac{2}{16}$ for the head, $\frac{10}{52\cdot5}$ and $\frac{2\cdot2}{16}$ for the testicular region, $\frac{7}{52\cdot5}$ and $\frac{3\cdot5}{16}$ for the uterine region, and $\frac{33\cdot4}{52\cdot5}$ and $\frac{8\cdot3}{16}$ for the post-ovarian region ; but it is noteworthy that the combined lengths of the testicular and uterine regions in each of the two specimens occupied in each case about the same fraction of the body length, i.e. $\frac{17}{52\cdot5}$ and $\frac{5\cdot7}{16}$, both of which are roughly equivalent to $\frac{1}{3}$. The head and the tail thus appear to be the variable regions, as might be expected (vide infra).

The head region is very variable in form. Pl. 24, fig. 2, represents what may be regarded as its normal semi-contracted shape, but it can be elongated so that its outline becomes indistinguishable from that of the succeeding testicular region (Pl. 24, fig. 3, *a*, *b*), or, on the other hand, contracted to form a short globular mass (Pl. 24, figs. 3, *d*, *e*, 4). When semi-contracted there is often a short space between the broad base of the ' head ' and the most anterior testes (Pl. 24, fig. 2), but in many cases this space is absent. The surface of the head is marked by deep longitudinal creases lined by thick cuticle (Pl. 24, figs. 2, 3, 5, 6). These longitudinal creases vary in number in different specimens—from 13 or 14 up to 25 or 26—and are perhaps inconstant in the same individual, being dependent upon the contraction of transverse musculature. A transverse section through the base of the head (Pl. 24, fig. 6) shows well-marked longitudinal muscle-fibres scattered throughout the parenchyma between the thick subcuticula and the central muscle-free medullary area, and conspicuous transverse fibres running from side to side : muscle-fibres running obliquely and dorso-ventrally are not conspicuous. The excretory system lies at the base of the subcuticula and not on the edge of the medullary area. A longitudinal section through the contracted head (Pl. 24, fig. 4) shows that the transverse band often visible round and marking the base of the head (as shown in Pl. 24, figs. 2, 3, *a*) is due to a clustering of nuclei probably belonging to muscle-fibres. The two main longitudinal nerves

are also seen to unite just below the extreme tip of the head (Pl. 24, fig. 4). I could detect no 'faserzellenstränge', such as have been described by Will (**27**) in C. laticeps, and by Skrjabin (**23**) in C. syrdarjensis.

The testicular region is short relatively to the length of the body as compared with the same region in C. laticeps and other previously described species. It contains a central core of testes (Pl. 24, fig. 2, TES) in, and a thin strand of vitellaria (VIT) on each side of, the central medullary area. The finer branches of the vasa deferentia unite and eventually open into a median main vas deferens (Pl. 24, fig. 7, VDEF) which forms a stout cirrus surrounded by a large cirrus-sac (CIRS). The main vitelline duct lies on the inner side of each strand of vitellaria.

The cirrus opening (Pl. 24, fig. 2, CO) marks the boundary between the testicular and uterine regions. Immediately behind the male aperture lies another and somewhat smaller opening—the vagino-uterine aperture (Pl. 24, figs. 2, 7, VUO)—which, as the name implies, serves both as an entrance to the vagina and as an exit for the eggs from the uterus. An atrium or depression surrounding the two apertures is absent. The vagina (VAG) is a straight or slightly convoluted fairly wide tube which runs in the median line on the ventral side of the body direct from the vaginal aperture to the ovary. Opening into the vagina on its dorsal side and from the right, at a point immediately above the opening of the vagina to the exterior, is the narrowed anterior end of the uterus (Pl. 24, fig. 7, UOP). From the point where it thus opens into the vagina the uterus (UT) curves forward (thus rendering it impossible for the cirrus to enter it) and bends to the left in front of the male aperture, then turns posteriorly and crosses the vagina dorsally to the right, and thence pursues its course posteriorly as the dilated much convoluted canal shown in Pl. 24, figs. 2 and 8. In all of my mature specimens the uterus is full of shell-covered eggs. Posteriorly and immediately in front of the ovary, the vagina develops a slight but constant dilatation, the receptaculum seminis (Pl. 24, fig. 8, RCPS), then inclines to

the right, and just below the median isthmus of the ovary dilates into the ootype (OOTP), a large oval chamber which receives the openings of the oviduct (OPO), common vitelline duct (OVIT), and the large shell-gland (SHGL). From the hind end of the ootype arises the uterus (UT) as a slender convoluted duct which, lying dorsal to and to the left side of the median isthmus of the ovary, passes forward and, immediately anterior to the isthmus, turns to the right and commences its zigzag course anteriorly, attaining its wide lumen at a short distance in front of the ovary. The vagina and uterus thus form a complete ' circuit ' with a common opening to the exterior anteriorly, the proximal part or vagina serving for the inlet of the spermatozoa and the distal part or uterus serving for the exit of the fertilized eggs. In no other type of Cestode do these two ducts have a common external opening. The ovary or germarium is bipartite and of the form shown in Pl. 24, fig. 2. The two halves are united across the middle line by a transversely elongated receptacle—the isthmus (Pl. 24, figs. 2, 8, IOV)—into each end of which open a number of fine ducts (ODCTS) from one-half of the ovary. In all my mature specimens the isthmus is full of eggs. At the anterior end of the uterine region the mass of testes, divided into two posteriorly in the testicular region by the median vas deferens, ends as two diverging strands, one on each side of the anterior uterus. The marginal strands of vitellaria lie, one on each side, to the outside of the uterus. Pl. 24, fig. 12, represents a transverse section through the uterine region anteriorly, viewed from the hind aspect, in which the uterus (UT, the median space containing two eggs) is seen to be opening into the vagina (VAG, the left extension of the median space, both ducts having a common ventral opening (the vagino-uterine pore, VUO) to the exterior. This transverse section also shows the hind strands of the testicular mass (TES), the vitellaria (VIT) external to these, the two main longitudinal nerve-strands (N), the thick cuticle and subcuticula, the single outer well-defined zone of longitudinal muscle-fibres (LMUS), and the inconspicuous lateral dorso-ventral fibres.

The post-ovarian region—a region which is very short in

C. laticeps and other previously described Caryophyllaeids—is in W. virilis normally extremely long (Pl. 24, fig. 2, POVR) and very characteristic of the species. The only part of the genitalia it contains is an enormous posterior development and extension of the vitellaria (VIT), the two marginal strands of which these organs consist in the uterine and testicular regions here merging immediately behind the ovary into a more or less central core of scattered vesicles. The posterior extension of the vitellaria differs in different specimens. In my largest 52·5 mm. specimen and in a specimen only measuring from 11 to 12 mm. the vitellaria only extend about half-way down the post-ovarian region (Pl. 24, fig. 1, *a*), whereas in most other specimens (Pl. 24, fig. 2) the vitellaria extend nearly to the extreme end, but intermediate conditions doubtless exist, though I do not possess an example. At the extreme posterior end of this region there is developed a distinct muscular thick-walled vesicle (Pl. 24, fig. 2, TEXB), into which open the terminal excretory channels (EXCV).

I must mention finally that in two of my specimens the curious abbreviated condition of the post-ovarian region shown in Pl. 24, fig. 9, was found. This condition may be due merely to extreme local contraction, but since the concentration of the vitellaria hardly seems to be sufficiently great on this supposition, it is possible that in these two specimens the hind end of the animal had become detached during life and a new posterior extremity regenerated on the stump.

A brief description of other features of W. virilis remains to be supplied. The only portions of the nervous system my material allowed me to make out are the two main longitudinal marginal trunks (Pl. 24, fig. 4, N) already described as joining (JN) at the extreme anterior end of the head. I could not detect the other longitudinal nerves which have been described by Will (**27**) in C. laticeps, though possibly restaining of my material by special methods would display them.

The excretory system (Pl. 24, fig. 13) posteriorly was well observed in one of my specimens owing to accidental injection with some foreign substance. It consists at the extreme

posterior end of some four or five main longitudinal canals which unite into two or three to open into the excretory bladder (TEXB); more anteriorly these canals apparently branch into ten or more (Pl. 24, fig. 12, EXCV). All these main longitudinal trunks are connected by a network of finer vessels. In the head region (Pl. 24, fig. 6), especially at the base, a large number of peripheral canals are visible under the subcuticula, apparently belonging to a complicated dense network (cf. C. laticeps), and a few canals of about the same size are also visible in the centre of the medullary region, i.e. internal to the zone of longitudinal muscle-fibres. I could observe no distinction between 'ascending' and 'descending' vessels, and my material was not sufficiently well-preserved to allow me to detect flame-cells for certain.

The eggs (Pl. 24, fig. 11) contained in the uterus are ovoid and thin-shelled, and measured, when mounted in balsam, 37·6–41·3 microns in length and 20·1–25·6 microns in breadth. They are thus nearly half the size of the eggs of C. laticeps.

In addition to mature specimens of W. virilis I possess five immature specimens all about 5 mm. in length (Pl. 24, figs. 1, *d*, 14, 15). In the youngest of these (fig. 14) the only signs of developing organs are a strand of thickened tissue with expanded ends (VAGUT) representing the future vagina and uterus (this latter arises as a straight tube), the genital openings and the isthmus of the ovary, and cell-thickenings representing the future testes (TES). In an older stage (fig. 15) the uterus has become convoluted, the genital openings are distinct, the vas deferens is forming, and the rudiments of the vitellaria are apparent. It is noteworthy in these immature specimens that the post-ovarian region is evidently not a mere secondary outgrowth, as is shown by the anterior position of the rudiments of the hind ends of the vagina and uterus. It is also noteworthy that these very immature forms occur in the fish and not in a worm, and that they show no signs of a 'caudal' appendage comparable with that of the larva of C. laticeps. The form of the post-ovarian region in the next two species of Wenyonia to be described clearly

proves that this region cannot be homologized with a ' caudal ' appendage.

The definitions of the new genus Wenyonia—a genus named in honour of my friend Dr. C. M. Wenyon—and the species W. virilis will be stated below.

Wenyonia acuminata, sp. nov. Woodland, 1923.

Of this species (Pl. 24, figs. 16, 17) I possess four whole specimens, fragments of two others, and a number of longitudinal and transverse sections. The parasite is from the Nile Siluroid Synodontis membranaceus, Is. Geoffr., caught at Khartoum, and was presumably found in the intestine. Thus W. virilis and W. acuminata are found in two closely related species of Synodontis, and it also so happens that these two parasites are also more closely related to each other than to any other species of Caryophyllaeid, as will be evident from the ensuing descriptions. My four whole specimens measured 34·5 mm., 33·0 mm., 26·0 mm., and 17·5 mm. respectively, with corresponding maximum breadths of 1·5 mm., 1·3 mm., 1·2 mm., and 1·2 mm. In all the specimens the greater part of the body was almost uniform in breadth and not unlike that of certain Nematoda, while the head end tapered to a fine point (hence the name of the species) and the posterior extremity was ' stumpy ' and bluntly pointed. The maximum breadth of the body occurs, if anywhere, in the testicular region. The head in most of my specimens was not delimited from the rest of the body by any distinct base, though in two of the specimens (Pl. 24, fig. 16) a slight circular ridge or dark band appeared to mark a base, but this I am inclined to think was a transitory contraction, since in both of the specimens possessing a base the anterior testes and vitellaria both extended well anterior to this. In the other four whole specimens (of heads) and in my longitudinal sections the head is quite continuous in outline with the rest of the body (Pl. 24, fig. 17), and I shall consider of course that it ends posteriorly at the point where the anterior testes and vitellaria commence.

In my largest specimen (34·5 mm.) the head measured 3·5 mm. in length, the testicular region 11 mm., the uterine region 13·5 mm., and the post-ovarian region 6·5 mm. In the 26 mm. specimen the head was 4 mm. long, the testicular region measured about 10 mm. long, the uterine region 6·5 mm., and the post-ovarian region 5·5 mm. In the 17·5 mm. specimen the head was 2·5 mm. long, the testicular region 7·5 mm., the uterine region 5 mm., and the post-ovarian region 2·5 mm. There is thus some variation as regards the lengths occupied, relative to the entire body-length, by the several regions.

The head region appears to be practically constant in form and continuous with the testicular region. In transverse and longitudinal sections (Pl. 24, figs. 18, 19) it is seen that the subcuticula is not creased longitudinally, as in W. virilis, both the cuticle and subcuticula being thin. The longitudinal musculature (LMUS) also is not nearly so pronounced as in W. virilis, but, as in W. virilis, consists of a single outer zone. Transverse (TRMUS) and dorso-ventral (DVMS) muscle-fibres are also easily seen.

In the testicular region there is nothing calling for special comment, save that the testes end well in front of the cirrus pore—they do not extend behind as in W. virilis—and that the vitellaria do not extend so far forward as the testes. In some of my preparations the vitelline duct, which lies internal to the vitellaria on each side of the body, is very distinct.

In the uterine region it is seen (Pl. 24, figs. 17, 20) that the uterus is not nearly so voluminous as in W. virilis, and is much shorter, relative to the body-length. The ovary is more attenuated than in W. virilis, and its exact limits are not easy to ascertain; the vitelline strands are also very thin. The detailed conformation of the ducts in the regions of the genital openings and ovary are similar to that found in W. virilis.

The post-ovarian region is much shorter relative to the body-length (Pl. 24, fig. 17) than in W. virilis, and the vitellaria largely retain their marginal strand arrangement, otherwise it is similar to that in W. virilis.

The nervous system is similar to that in W. virilis, so far as the two main longitudinal trunks are concerned. The excretory system is also similar but is much less extensive, especially in the head region, when compared with that of W. virilis. As Pl. 24, figs. 19 and 20 show, there are about ten longitudinal excretory channels underlying the subcuticula and two in the medulla, both at the base of the head and in the uterine region.

The eggs (Pl. 24, fig. 21) are of the usual ovoid shape and measure (when mounted in balsam) 34·7–36·6 microns in length and 21·9–25·6 microns in breadth. They are distinguished from the smooth-surfaced eggs of W. virilis, C. laticeps, the two species yet to be described, and, so far as I know, all other species of Caryophyllaeidae, by the fact that under a magnification of a 1,000 diameters the shell is seen to be covered with minute spines (Pl. 24, fig. 21).

The specific characters of W. acuminata will be stated below.

Wenyonia minuta, sp. nov. Woodland, 1923.

Of this species (Pl. 24, figs. 22, 23) I possess one stained mounted specimen only. Fortunately the specimen is well stained, and I have been able to make out all the main features of its structure without restaining or sectionizing. This parasite was found in the Nile Siluroid Chrysichthys auratus, Günth., at Khartoum, presumably in the intestine. The total length of the body is only 3·5 mm., and the maximum breadth 0·8 mm. It is fully mature, the uterus being filled with eggs. The body is distinctly more flattened than in either of the two preceding species or in C. laticeps, the head is distinctly more of the C. laticeps type, and, as in C. laticeps, the post-ovarian region is short; on the other hand, as in the two preceding species, the genital apertures are situated in the anterior half of the body and the uterus is very long.

The head measured about 0·88 mm., the testicular region

about 0·7 mm., the uterine region nearly 1·5 mm., and the post-ovarian region about 0·46 mm.

Notable features concerning the genitalia are (1) the very long uterus, extending over twice the distance covered by the testis mass; (2) the position of the ootype (OOTP) on the left side and the initial coils of the uterus on the right side of the ovary; and (3) the opening of the uterus into the vagina from the left side (Pl. 24, fig. 23, is viewed from the ventral aspect), i.e. exactly the opposite of what occurs in the two preceding species;[1] (4) the breadth of the lateral rows of vitellaria; (5) the extension posteriorly of the testes to about the level of the genital openings; and (6) the apparent anterior extension of the ovary on each side to near the level of the genital apertures (though of this I am not quite certain, owing to the difficulty in my preparation of distinguishing the vitellaria from the ovarian follicles).

The two main nerve-trunks are visible anteriorly, and the excretory opening posteriorly.

The eggs are of the usual ovoid type and measure, when mounted in balsam, 32·9–40·2 microns in length and 20·1–25·6 microns in breadth.

The specific characters of W. minuta will be stated below.

Caryophyllaeus filiformis, sp. nov. Woodland, 1923.

Of this species (Pl. 25, fig. 24) I possess altogether thirty-one entire mounted specimens, and several in transverse and longitudinal sections. This parasite was found in the well-known Nile fish, Mormyrus caschive, L. (a Malacopterygian), at Khartoum, presumably in the intestine. Most of my specimens are mature, and these vary in length from 7·5 mm. to 24 mm. One or two smaller than this (circ. 5 mm.) do not appear to have eggs in the uterus. The usual maximum breadth is 1 mm., but occasionally the body may locally expand to as much as

[1] I at first concluded that I had viewed the specimen 'wrong way up', but re-examination proves that these statements are correct. I lay no stress on these differences, since it is more than possible that the arrangement of these ducts is variable in different individuals.

2 mm. The general shape of these parasites is indicated in Pl. 25, fig. 24, which in itself provides evidence that the body is very contractile and therefore variable in form and length. In the general topography (Pl. 25, fig. 25) of the genitalia (and in the structural details described below) these parasites are closely related to C. laticeps and other previously described species of Caryophyllaeus, since, as in these species, the genital openings are situated very near to the posterior end and the uterus is therefore relatively very short, and the testes extend over a great length of the body. One feature, however, in which C. filiformis markedly differs from C. laticeps and all other species of Caryophyllaeus is the total absence of vitellaria posterior to the ovary, and of a post-ovarian region, the ovary in most cases extending to the extreme end of the body.

In all specimens of C. filiformis the genital apertures are situated well within the last one-seventh of the body-length (from one-twelfth to one-seventh according to the length of the head). In six typical examples the lengths of the three regions were as follows: in a specimen measuring 21 mm. long, the head measured 10 mm. and the testicular region 9 mm.; in a specimen 23·5 mm. long, the head was 11·5 mm. and the testicular region 10 mm.; in a specimen measuring 21·5 mm. the head was 9 mm. and the testicular region 10·5 mm.; in a specimen measuring 20 mm. the head was 8 mm. and the testicular region 10 mm.; in a specimen measuring 11·5 mm. the head was 5·5 mm. and the testicular region 5 mm.; and in a specimen measuring 15·3 mm. the head was 5·5 mm. and the testicular region 8·3 mm. In five of the specimens (Pl. 25, figs. 26, *c*, *d*) the testes extended much more anteriorly, almost to the extreme front of the head—thus in a specimen measuring 22 mm. the head was only 1·5 mm. long, while the testicular region was 19 mm.—but this is not the normal condition, and intermediate conditions exist between this and the normal.

The head, as already stated, is extremely contractile and assumes the most various shapes (Pl. 25, fig. 26, *a*, *b*, *c*, *d*, *e*). When uncontracted it is fairly flat and ribbon-shaped (Pl. 25,

fig. 25), but it may also be pointed (Pl. 25, fig. 26, *c*, *d*) or excessively flattened, with a crenulated margin (Pl. 25, fig. 26, *e*); when contracted the head becomes transversely wrinkled and oval in transverse section (Pl. 25, fig. 27) and more robust (Pl. 25, fig. 26, *a*). In transverse section (Pl. 25, fig. 27) it is noteworthy that the longitudinal muscle-fibres are disposed into two distinct zones, an outer peripheral zone (SCLMUS) underlying the subcuticula, and an inner more powerful medullary zone (ILMUS), the two zones being widely separated. This condition of the longitudinal musculature (which is equally well-marked in the testicular region) is also found in C. laticeps, and it differs essentially from the single-zone condition of W. virilis and W. acuminata and probably W. minuta. Some transverse muscle-fibres lie immediately external to the medullary longitudinal musculature. In the anterior head region of some specimens I have observed longitudinal thickenings (Pl. 25, fig. 25) which may be the 'faserzellenstränge' of Will and Skrjabin. I have not examined them in detail.

The testicular region is also remarkable, not only on account of its length relative to the uterine region—a feature in which C. filiformis again resembles C. laticeps—but in the fact that the vitellaria throughout the greater part of this region practically form a ring round the testes, when viewed in transverse section (Pl. 25, fig. 28). In C. laticeps there is an approach to this annular arrangement, but, judging from Will's figures (**27**), it is not nearly so complete as in C. filiformis.

The uterine region (Pl. 25, fig. 29), as already stated, is very short, the uterus itself being short and but loosely coiled. The uterus opens anteriorly into the vagina from the left side (Pl. 25, fig. 25, is from the ventral aspect) and the ootype lies on the left side of the ovary, as in W. minuta. The male and female apertures are more separate from each other than is the case in the preceding species, a space of about 25 microns separating the hind border of the male aperture from the anterior border of the female, and this distance is fairly constant. In

C. laticeps, W. acuminata, and W. minuta the borders of the two apertures are contiguous, as is also usually the case in W. virilis. In all other respects the genitalia of C. filiformis appear to conform to the plan characteristic of Caryophyllaeus.

The two main nerve-trunks (Pl. 25, figs. 25, 27, 28, N) are visible anteriorly, and I have seen them (with difficulty) in my transverse sections.

The excretory channels (Pl. 25, figs. 27, 28, 29) are mostly situated between the inner and outer zones of longitudinal muscles, but a few also occur in the medulla. The vessels cut across in transverse sections form a part of a complicated network, and there appear to be no definite longitudinal vessels; posteriorly, however, some eight or ten longitudinal excretory channels open into the excretory bladder.

The eggs are of the usual ovoid smooth-shelled type and measure, when mounted in balsam, 62·2–69·5 microns in length and 29·2–32·9 microns in breadth, thus being exceptionally long.

The definition of this species, C. filiformis, will be supplied below.

The Genera and Species of the Caryophyllaeidae.

Skrjabin (**23**) has, with his description of Caryophyllaeus syrdarjensis, included a summary of the small differences which distinguish C. laticeps, C. tuba, C. fennicus, and C. syrdarjensis (all from Cyprinoid fishes) from each other, and C. armeniacus (Cholodkowsky, **2**) only differs from these principally in its large size (55 mm. in length), truncated head, and large eggs (80 × 45 microns). Linton (**10, 11**) has described two other species (also collected from Cyprinidae), which, on account of the form of the head, he refers to Diesing's (**6**) genus Monobothrium,[1] but, excepting

[1] The "Monobothrium serpentum n. sp." of v. Linstow ('Arch. f. Mikr. Anat.', Bd. lxii, 1903, p. 108), 1·4 mm. long, from a cyst in the mesentery of a snake, Patalung, cannot of course be referred to the Caryophyllaeidae. It is almost certainly a Dithyridium larva, several kinds of which have been described from snakes.

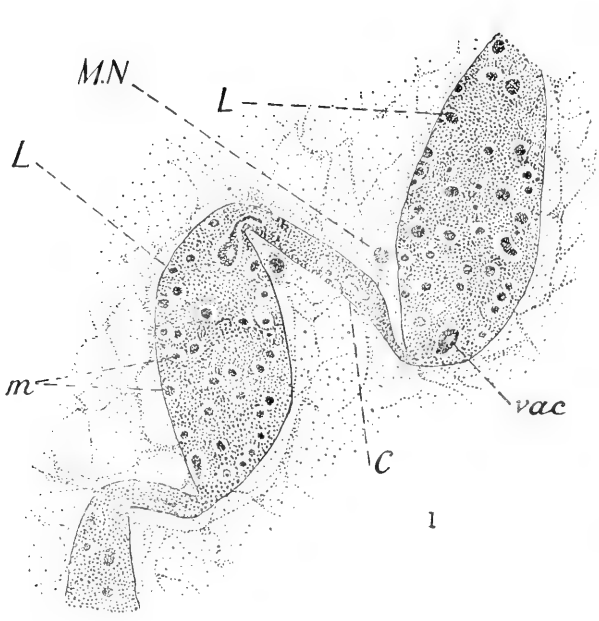
for the head, both these species also are essentially identical with the five species of Caryophyllaeus above named. Finally, Cooper (**5**) has described, under the name of 'Glaridacris catostomi', a species (from a Cyprinoid fish) which also 'closely resembles' the aforesaid five species of Caryphyllaeus and the two species described by Linton in general structure, and possesses a 'scolex' which is 'quite similar at least in outward appearance to that of Archigetes brachyurus, Mrázek'. Cooper appears to have been unacquainted with the work of Linton on 'Monobothrium', since he supposes that his 'Glaridacris' is 'the first member of the group [Caryophyllaeidae] to be described from America'. He bases his new genus 'Glaridacris' mainly on the characters of the 'scolex'. Now this 'scolex' of 'Glaridacris', 'when not strongly contracted, has somewhat the form of a truncated rectangular pyramid with the longer diameter in the transverse direction . . . the edges of the base and the apex protrude markedly, in this latter case forming a terminal disc comparable to that of many of the bothriocephalid Cestodes. The dorsal and ventral faces of the organ are each divided by two ridges converging towards the apex into three sucking grooves or loculi, of which the middle is best developed and most efficacious during life. It is also the last to become smoothed out with strong contraction of the whole scolex. The lateral loculi are, furthermore, not in the same plane with the medial one but inclined towards the corresponding ones of the opposite surface so that the edges of the scolex, especially just behind the terminal disc, are often not much thicker than the ridges between the loculi . . . the scolex of this form assumes a greater variety of shapes than that of any other tapeworm I have yet examined, in which respect it is comparable to the leaf-like anterior end of Caryophyllaeus.' From which description and from Cooper's figures, it is evident that the 'scolex' of 'Glaridacris', besides resembling that of Archigetes brachyurus, is extremely similar to the head of Linton's 'Monobothrium hexacotyle', which also has six loculi and a central papilla ('which may project forward as a sharp conical elevation or be

contracted to a low eminence') and is similarly 'versatile' in form. If, then, Linton is right in referring his species 'hexacotyle' and 'terebrans' (which latter has a head 'variable, subsagittate, wedge-shape or bluntly rounded') to the genus Monobothrium, it is evident that Cooper's species must likewise be referred to this genus, and 'Glaridacris' becomes a nomen nudum.

The genus Monobothrium was created by Diesing in 1863 (**6**) as one of three genera in his family of the Monobothria, i.e. forms with one 'bothrium'; the other two genera being Caryophyllaeus, Gmelin, and Diporus, Diesing. The genus Diporus can at once be eliminated as solely having reference to the 'Caryophyllaeus trisignatus' of Molin (**14**), a form [1] from the intestine of 'Gadus merlucius' which, in view of Molin's description and figures, cannot be referred with any degree of probability even to the Caryophyllaeidae and much less to any particular genus. The genus Monobothrium, according to Diesing's definition, only differs from Caryophyllaeus in that (*a*) the body is not 'depressum', that (*b*) the head is 'subcylindricum, bothrio, terminali subcirculari' instead of being 'dilatatum fimbriatum, bothrio terminali transverso bilabiato' (definition of Caryophyllaeus head), and that (*c*) the male and female genital apertures open into a common atrium ('apertura genitalis unica') instead of opening separately on the surface ('apertura genitalis feminea pene postposita contigua') as in Caryophyllaeus. Diesing describes two species of Monobothrium—M. tuba (the Caryophyllaeus tuba of Wagener (**15**) and Monticelli (**16**)) from the intestine of Tinca chrysitis, and M. punctulatum, the 'Caryophyllaeus punctulatus' of Molin (**14**) from the intestine of Conger vulgaris—another indeterminate organism described by this author which probably is not even a Caryophyllaeid.

The 'Monobothrium tuba' of Diesing was considered both by Wagener previously and by Monticelli subsequently

[1] Possibly a Tetrabothriid scolex, as Monticelli suggests.



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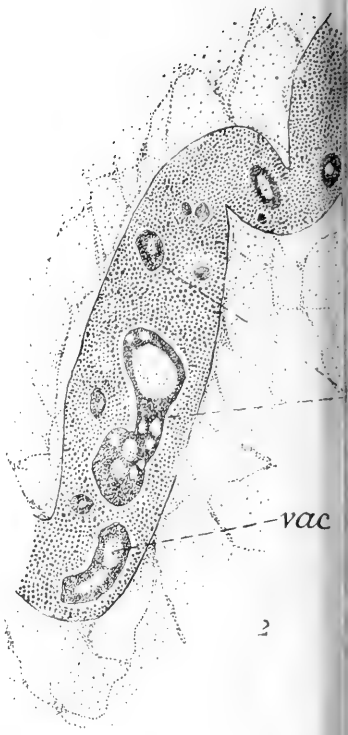

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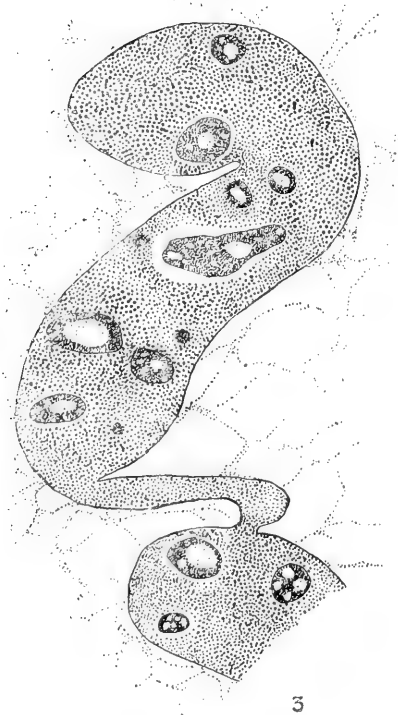

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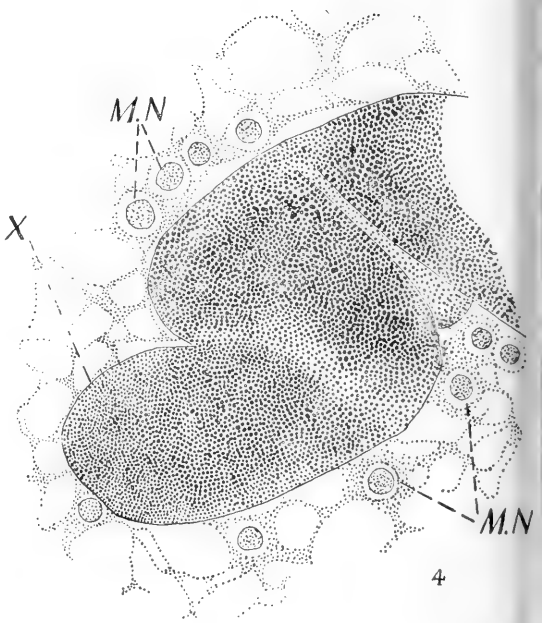

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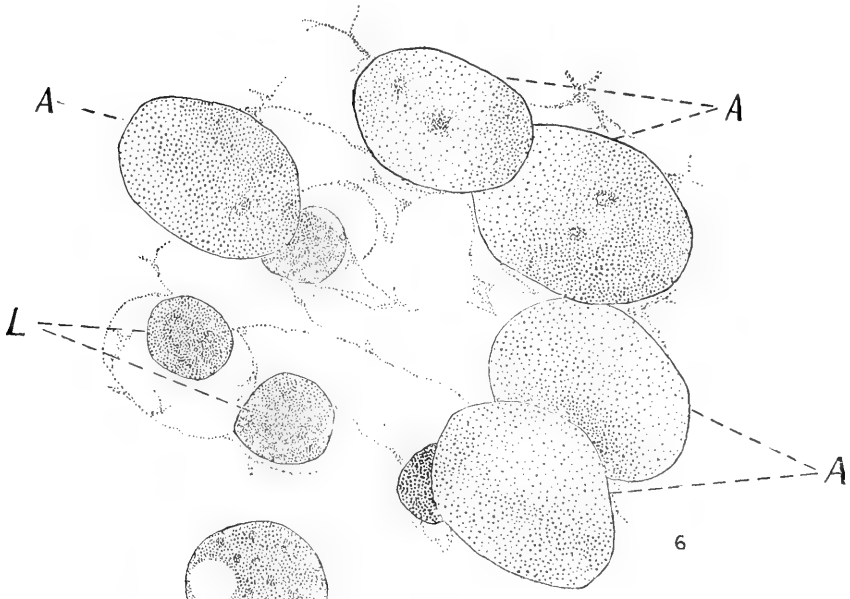

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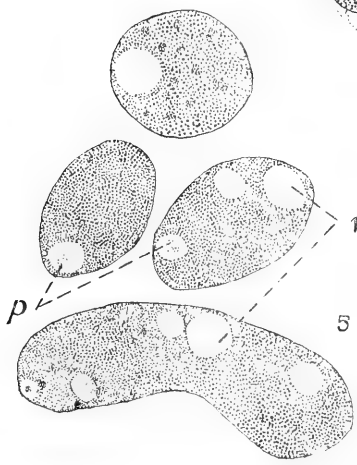

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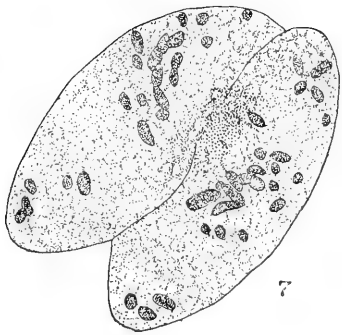

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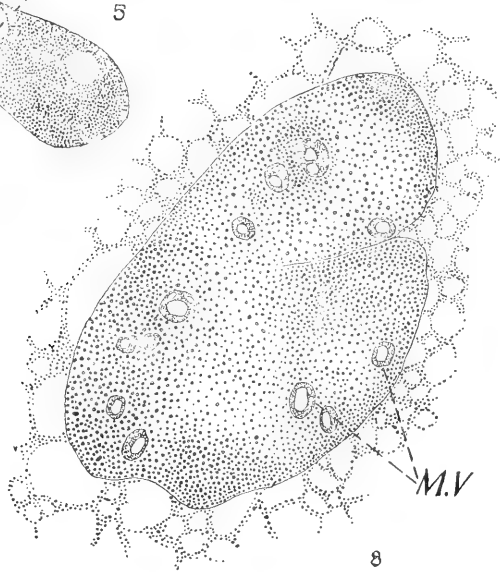

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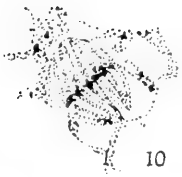

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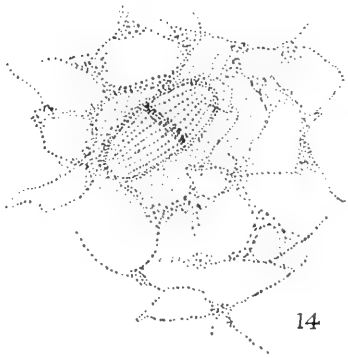

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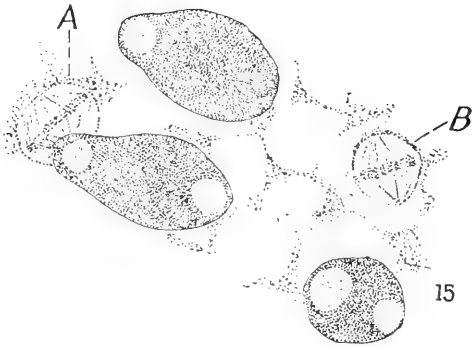

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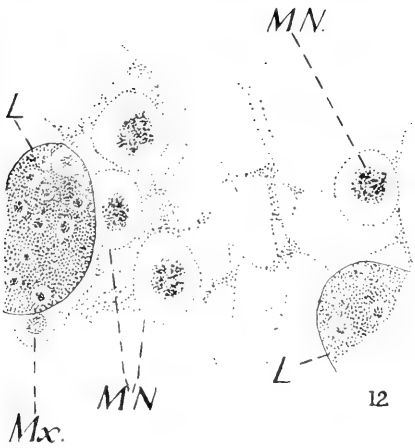

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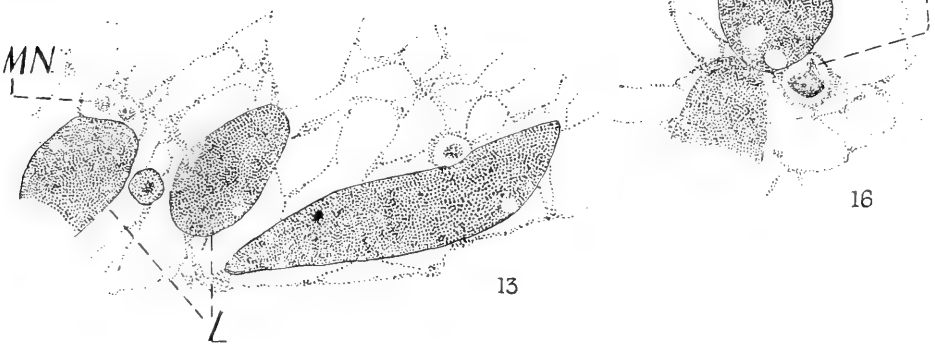

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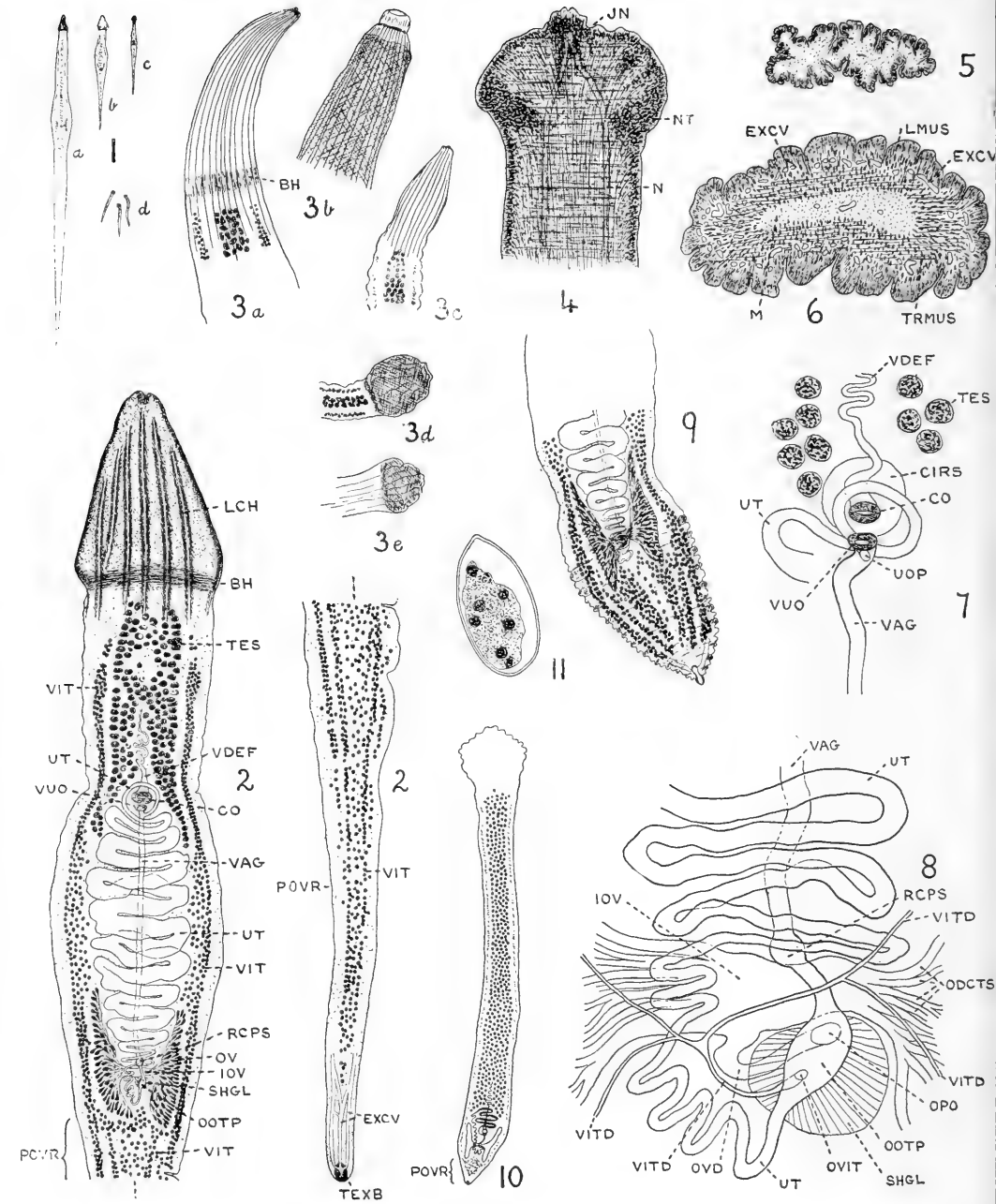
DESCRIPTION OF PLATES 24 AND 25.

Reference Letters.

BH, base of head; CIRS, cirrus sac; CO, cirrus opening; DVMS, dorso-ventral muscle-fibres; EXCV, excretory vessels; FZS, 'Faserzellenstränge'?; H, head region; ILMUS, internal layer of longitudinal muscle-fibres on edge of medulla; IOU, isthmus of ovary; JN, junction of the two main longitudinal nerve-trunks anteriorly; LCH, longitudinal creases or grooves in subcuticula of head; LD, local dilatation; LMUS, longitudinal muscle-fibres; M, medulla; N, main longitudinal nerve-trunk; NT, nuclear thickening at base of head; ODCTS, ducts from ovary opening into isthmus; OOTP, ootype; OPO, opening of oviduct into ootype: OV, ovary; OVD, oviduct; OVIT, opening of vitelline duct into ootype; POVR, post-ovarian region; RCPS, receptaculum seminis; SCLMUS, outer layer of longitudinal muscle-fibres underlying subcuticula; SHGL, shell-gland; TES, testes; TEXB, terminal excretory bladder; TRMUS, transverse muscle-fibres; UOP, opening of uterus into vagina; UT, uterus; VAG, vagina; VAGUT, rudiment of vagina and uterus; VDEF, vas deferens; VIT, vitellaria; VITD, vitelline duct; VUO, vagino-uterine opening.

N.B.—The magnifications given are those at which the figures were drawn. The 5 cm. scales provided on the two plates show, when compared with an actual 5 cm., the amount of reduction[1] which has occurred in reproducing the figures. All figures drawn by means of the camera lucida.

[1] Approximately $\frac{11}{25}$.



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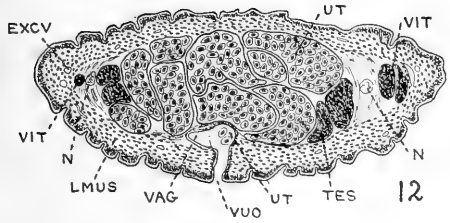
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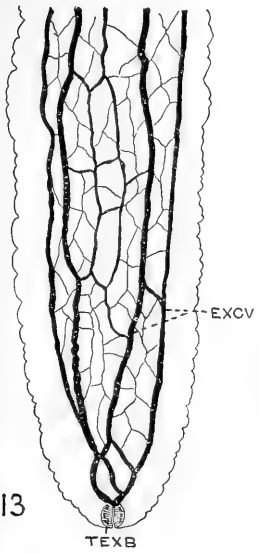
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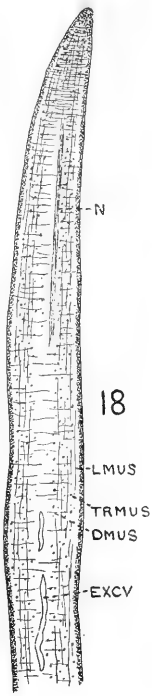
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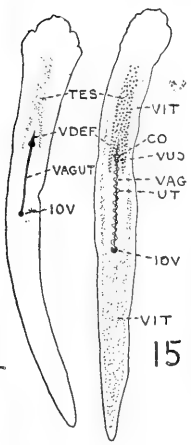
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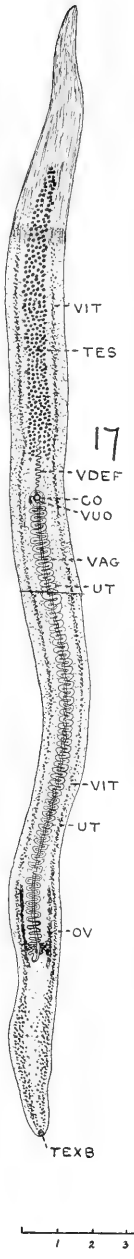
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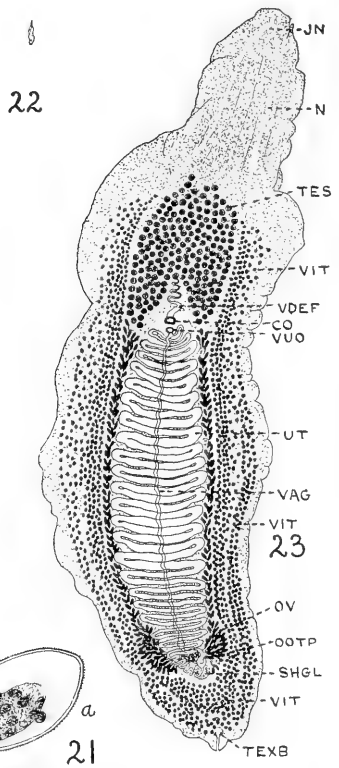
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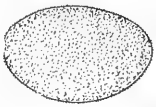

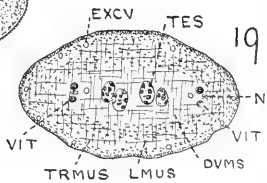
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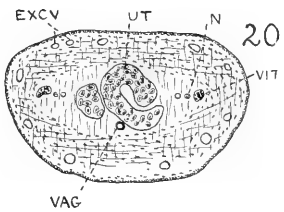
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Plate 24.

Wenyonia virilis.

Fig. 1, *a*, *b*, *c* (×2).—Adult specimens as mounted on slides and probably somewhat flattened.

Fig. 1, *d* (×2).—Three immature specimens.

Fig. 2 (×24).—Entire specimen magnified (the two halves of the figure should be continuous) and viewed from the dorsal aspect. The parts of the drawing round the sexual openings and the ovary have been made somewhat diagrammatic for the sake of clarity, and the uterus is in actuality full of eggs. The vitelline and other fine ducts are not shown.

Fig. 3, *a*, *b*, *c*, *d*, *e* (×24).—Figures showing the variations in form of the head region.

Fig. 4 (×35).—Horizontal section through a much contracted head.

Fig. 5 (×35).—Transverse section through the anterior region of the expanded head to show the longitudinal grooves (the thick cuticle and excretory channels are not indicated).

Fig. 6 (×78).—Transverse section through the posterior region of the head to show the longitudinal grooves, musculature and excretory network (thick cuticle not shown).

Fig. 7 (×112).—Ventral aspect of the genital openings and associated ducts.

Fig. 8 (×175).—Dorsal aspect of the ducts in the region of the ovary in outline.

Fig. 9 (×24).—The abbreviated (contracted ? regenerated ?) post-ovarian region in one of the two specimens showing this feature.

Caryophyllaeus laticeps.

Fig. 10 (×9).—C. laticeps from Tinca vulgaris, from the dorsal aspect (for comparison with Wenyonia spp.). The structures in the regions of the sexual apertures and ovary are represented somewhat diagrammatically, but the figure is otherwise correct. In most specimens of C. laticeps, however, the uterus does not extend anteriorly to the cirrus opening, at least to any extent.

Wenyonia virilis.

Fig. 11 (×1060).—Egg (contained in the uterus).

Fig. 12 (×78).—Transverse section through the uterine region, viewed from the front aspect, showing the vagino-uterine aperture.

Fig. 13 (×78).—The posterior excretory system artificially injected.

Figs. 14, 15 (×24).—Immature specimens showing developing genitalia.

Wenyonia acuminata.

Fig. 16 (×2).—Adult specimens (the dilated base of the head shown in two of the specimens is probably not a constant feature).

Fig. 17 (×9).—Entire specimen magnified and viewed from the dorsal aspect. The parts of the drawing round the sexual openings and the ovary have been made somewhat diagrammatic for the sake of clarity and the uterus is in actuality full of eggs. The vitelline and other fine ducts are not shown.

Fig. 18 (×55).—Longitudinal vertical section through head.

Fig. 19 (×55).—Transverse section through the anterior end of the testicular region.

Fig. 20 (×55).—Transverse section through the middle of the uterine region.

Fig. 21 (×1060).—Eggs in optical section and surface-view to show the minute spinelets on the shell.

Wenyonia minuta.

Fig. 22 (×2).—The adult specimen.

Fig. 23 (×60).—The entire specimen magnified and viewed from the ventral aspect. The parts of the drawing round the sexual opening and the ovary have been made somewhat diagrammatic for the sake of clarity and the uterus is in actuality full of eggs. The vitelline and other fine ducts are not shown. The anterior extension of the ovary to the level of the sexual openings is not certain, since the ovarian follicles and vitellaria are in this region almost indistinguishable in my preparation.

Plate 25.

Caryophyllaeus filiformis.

Fig. 24 (×2).—Adult specimens.

Fig. 25 (×55).—Entire specimen magnified and viewed in optical section from the ventral aspect (the three parts of the figure should be continuous). The parts of the drawing round the sexual openings, and the ovary have been made somewhat diagrammatic for the sake of clarity and the uterus is in actuality full of eggs. The vitellaria and other fine ducts are not shown. The vitellaria in actuality surround the testes in the testicular region—vide fig. 28.

Fig. 26, *a*, *b*, *c*, *d*, *e* (×24).—Drawings showing variation in form of the head and the variable extension of the testes and vitellaria anteriorly.

Fig. 27 (×78).—Transverse section through the head region.

Fig. 28 (×78).—Transverse section through the testicular region.

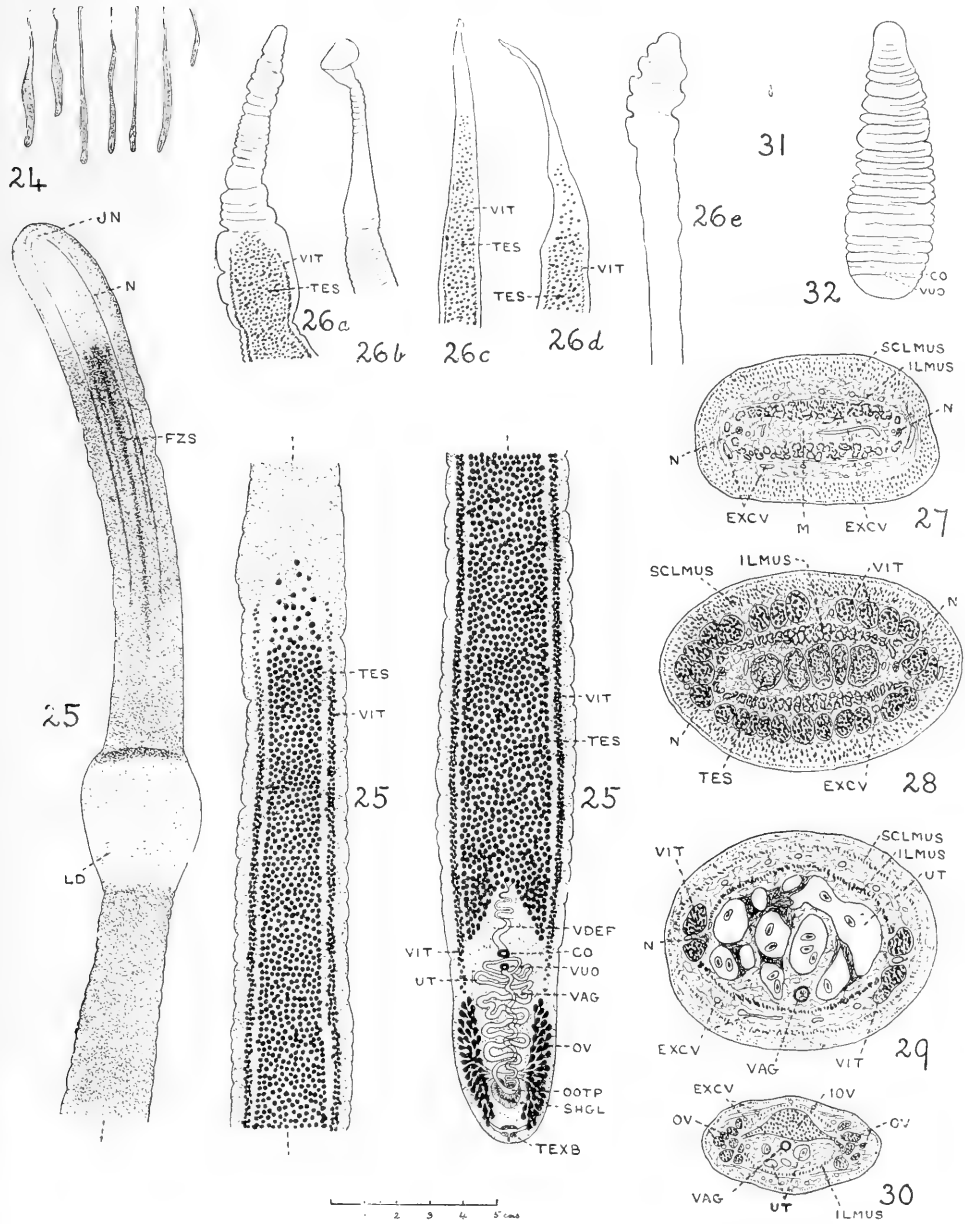
Fig. 29 (×78).—Transverse section through the anterior uterine region.

Fig. 30 (×78).—Transverse section through the median isthmus of the ovary. The vagina has at this level become dorsal to the uterus.

Immature Caryophyllaeid from Auchenoglanis occidentalis.

Fig. 31 (×2).—The single specimen.

Fig. 32 (×35).—The external aspect of the specimen, viewed ventrally.



Woodland, del.

to belong to the genus Caryophyllaeus, the differences from 'C. mutabilis' (=C. laticeps) only being specific and not generic, and in view of the indefiniteness of such terms as 'subcylindrical' when applied to a pleomorphic structure like the head, of 'depressum' when applied to the body which may be flat anteriorly and oval in section posteriorly even in the same animal, and of 'single' and 'separate' when these refer to genital apertures which show all degrees of opening into a common atrium in different species which undoubtedly are to be included in one genus, it is impossible not to agree with Wagener and Monticelli. Further, as is demonstrated in the present communication, the muscular cylindrical type of head, which was the most characteristic feature of Diesing's genus Monobothrium, is to be found associated, indiscriminately, with two very different types of body, whence it is evident that the character of the head cannot serve in this group as a feature of generic value. The name Monobothrium thus also lapses.

Having thus concluded that eight of the ten Caryophyllaeid species described previously to this communication belong to the genus Caryophyllaeus, and provisionally allowing Archigetes to stand as a separate genus on the sole ground that it possesses a 'caudal' appendage in the sexual condition, it is necessary to redefine the genus Caryophyllaeus and to show reason for creating a new third genus, Wenyonia.

The genus Caryophyllaeus was apparently founded by O. F. Müller in 1787 (**18**), but since I have not been able to consult this work I will give the definition of this genus supplied by Gmelin (**7**) in 1790, which is probably fully as comprehensive as that of Müller, both being based on specimens of C. laticeps. Gmelin's definition of the genus is: 'Corpus teres: ore fimbriato. . . . Habitat in piscium, aquas dulces inhabitantium, potissimum cyprinorum, carpionis, tincae, jesis, bramae intestinis, rarior, margaritaceus, ad pollicem longus, reliquis intestinalibus vitae tenacior, fine posteriori rotundato, anteriori latiori.' This definition clearly applies to C. laticeps, but it is too special to include other species subsequently

described, and Diesing (**6**) therefore, as aforesaid, created another genus—Monobothrium—to include the 'Ligula tuba' of Wagener (**25**) and the 'Caryophyllaeus punctulatus' of Molin. Diesing's definitions of the two genera Caryophyllaeus and Monobothrium are respectively as follows: 'I. Caryophyllaeus, Gmelin. Corpus continuum elongatum depressum, vesicula pulsatoria postica cum poro excretorio. Caput dilatatum fimbriatum, bothrio terminali transverso bilabiato. Os . . . Collum nullum. Penis lateralis conicus retractilis retro medium corporis; apertura genitalis feminea pene postposita contigua. In Piscium fluviatilium praeprimis Cyprinorum intestinis. Evolutio directa.' [One species C. mutabilis, Rud.], and 'II. Monobothrium, Diesing. Corpus continuum elongatum, vesicula pulsatoria postica, caput sybcylindricum, bothrio terminali subcirculari. Os . . . Collum nullum. Apertura genitalis unica, organo musculo et femineo communis, lateralis ventralis in postico corporis triente. In Cyprinorum intestinis. Evolutio ignota. (Animalcula bothrii ope parieti intestini firmiter adhaerent. Systema vasorum e truncis longitudinalibus quatuor et vasculorum rete compositum).' [Two species: M. tuba, Wagener, and M. punctulatum, Molin.] Other and more recent authors, e.g. Lühe (**13**) and Cholodkowsky (**3**), group the species of the Caryophyllaeidae into two genera—Caryophyllaeus and Archigetes—and distinguish the former from the latter chiefly by the absence of the 'caudal' appendage in the sexual stage of the former.

Assuming the definition of the Caryophyllaeidae to be that which I shall propose in the next section, I now propose to divide these forms into three genera—Caryophyllaeus, Archigetes, and Wenyonia—with the following definitions:

Caryophyllaeus, O. F. Müller, 1787 emend.

The sexual apertures are situated within the last quarter of the body-length, and the ovary is near the posterior extremity. The longitudinal extent of the uterus is at

most one-third of that of the testes and usually much less.[1] Parasitic in the intestines of Malacopterygii and Ostariophysi (Cyprinidae, Mormyridae, and Siluridae[2]).

Archigetes, Leuckart, 1878.

The sexual apertures are situated within the last quarter of the body-length; and the ovary is near the posterior extremity. The longitudinal extent of the uterus is at most one-third of that of the testes and usually much less. The body of the mature parasite possesses a 'caudal' appendage posteriorly. Parasitic in the body-cavity of aquatic Oligochaetes.

Wenyonia, Woodland, 1923.

The sexual apertures are situated in the anterior half of the body. The longitudinal extent of the uterus is at least equal to that of the testes. Parasitic in the intestine of Siluridae.

The species known up to the present contained in these three genera are as follows :

C. laticeps, Pallas, 1781.

Length of body 11–30 mm. ; breadth of body 0·5–2 mm. Body posterior to the head oval in transverse section. Hind end bluntly pointed or rounded. Head region

[1] With the single exception of Linton's 'Monobothrium hexacotyle' (C. hexacotyle), which has eggs measuring only 38–40 microns in length, all species of Caryophyllaeus, as enumerated further in the text, possess eggs about 60 microns in length, or, as in the huge C. armeniacus, 80 microns in length. On the other hand, in all three species of Wenyonia the eggs average from 32 to 40 microns in length. These differences are remarkable (especially in view of the body-sizes of the parasites) and may possess a classificatory value. Another difference which may distinguish these two genera is that in Wenyonia the longitudinal muscles are solely contained in a peripheral subcuticular zone, whereas in Caryophyllaeus the longitudinal muscles are disposed in two zones—a subcuticular and an epi-medullary.

[2] I include this family because of the immature species of Caryophyllaeus found by me in Auchenoglanis occidentalis and described briefly at the end of this paper.

flattened, usually broader than the body and with fimbriated edges, but very contractile and variable in form; longitudinal grooves absent on head. Genital apertures in last fifth of body-length.[1] Post-ovarian vitellaria are present. Eggs measure about 66 microns in length. Parasitic in intestine of Cyprinidae, Europe.

C. tuba, Wagener, 1854 ('Monobothrium tuba').
Length of body 10–30 mm.; breadth of body 0·9–1 mm. Body and head oval in transverse section. Hind end slightly tapering and pointed. Head 'stumpy', i.e. bluntly rounded in outline and not broader than body; longitudinal grooves absent on head. Genital apertures situated anterior to the last fifth of the body.[2] Post-ovarian vitellaria are present. Eggs ? Parasitic in intestine of Tinca chrysitis, Italy.

C. fennicus,[3] Schneider, 1902.
Length of body 5–9·5 mm.; breadth of body 0·4–0·5 mm. Body and head somewhat flat in transverse section. Hind end bluntly pointed. Head rounded in contour and not broader than body; longitudinal grooves absent on head. Genital apertures as in C. laticeps. Post-ovarian vitellaria present. Eggs measure about 60 microns in length. Parasitic in intestine of Leuciscus erythrophthalmus, Finland.

C. syrdarjensis, Skrjabin, 1913.
Length of body 6·3–16 mm.; breadth of body 1–1·5 mm. Body and head somewhat flat in transverse section. Hind end bluntly rounded. Head slightly flattened and slightly broader than body, with distinct 'faserzellenstränge'; longitudinal grooves absent on head. Genital apertures apparently as in C. laticeps. Post-ovarian vitellaria are present. Eggs measure about 63 microns in length and 48 microns in breadth. Parasitic in intestine of Schizothorax intermedius, Russo-Turkestan.

[1] Statement based on measurements of four individuals in my possession.

[2] Statement based on the figures supplied by Monticelli (**15**) and Wagener (**25**).

[3] Skrjabin (**23**) is mistaken in supposing that in C. fennicus alone do the coils of the uterus ever extend anterior to the vagino-uterine aperture: I have seen this anterior extension in at least two specimens of C. laticeps (Pl. 24, fig. 10).

C. armeniacus, Cholodkowsky, 1915.

Length of body reaches 55 mm., with breadth of 5 mm. Body and head oval in transverse section. Hind end slightly tapering and bluntly pointed. Head not broader than body, truncated, with wedge-shaped anterior extension; longitudinal grooves absent on head. Genital apertures in last fifth of body-length. Post-ovarian vitellaria are probably present. Eggs about 80 microns long and 45 microns broad. Parasitic in intestine of Capoeta sp., Armenia.

C. terebrans, Linton, 1893 ('Monobothrium terebrans').

Length of body reaches 28 mm., with breadth about 2·5 mm. Body oval in transverse section. Hind end dilated at level of sexual apertures and pointed posteriorly. Head broader than body and subsagittate, wedge-shaped or bluntly rounded; longitudinal grooves absent. Genital apertures at about the anterior limit of the last fifth of the body. Post-ovarian vitellaria are present. Eggs measure 60–65 microns in length and 30–35 microns in breadth. Parasitic in intestine of Catostomus ardens, Wyoming, U.S.A.

C. hexacotyle, Linton, 1898 ('Monobothrium hexacotyle').

Length of body reaches 14·5 mm., with breadth of 1 mm. Body oval in transverse section. Hind end tapering and pointed. Head not broader than body, broad at base, pointed anteriorly and wedge-shaped, each of the dorsal and ventral surfaces of the wedge bearing three broad elongated loculi or triangular grooves which point to the anterior extremity; shape variable. Genital apertures in last fifth of body-length. Post-ovarian vitellaria are present. Eggs measure 38–40 microns in length and 20 microns in breadth. Parasitic in the intestine of Catostomus sp., Arizona, U.S.A.

C. catostomi, Cooper, 1920 ('Glaridacris catostomi').

Length of body measures 5–25 mm., with maximum breadth 0·4–1 mm. Body oval in transverse section. Hind end tapering and pointed. Head region broader than body, and similar in shape to that of Archigetes brachyurus, i.e. wedge-shaped, the dorsal and ventral surfaces of the wedge each bearing three broad loculi, the end of the wedge being truncated when viewed dorsally

or ventrally but pointed in lateral view; shape variable. Genital organs 'like those of Caryophyllaeus'. Post-ovarian vitellaria present. Eggs measure 54–66 microns in length and 38–48 microns in breadth. Parasitic in the intestine of Catostomus commersonii, Michigan, U.S.A.

C. filiformis, Woodland, 1923.

Length of body measures 7·5–24 mm., with breadth (maximum) 1–2 mm. The body is broadest in its posterior half, the anterior half (or third) being attenuated and usually filiform or ribbon-shaped; the body posterior to the head region is oval in transverse section. The hind end of the body is bluntly rounded. The head region is highly contractile, is more or less pointed in front, is narrow, always more flattened than the body posteriorly, and varies in form from a thin pointed filament or ribbon to a tapering wrinkled digitiform structure; longitudinal grooves are absent. Genital apertures in last seventh of the body-length; the cirrus aperture is distinctly separated from the vagino-uterine aperture, and there is no atrium. Post-ovarian vitellaria are entirely absent. Eggs (in balsam) measure 62·2–69·5 microns in length and 29·2–32·9 microns in breadth. Parasitic in the intestine of Mormyrus caschive, L., Anglo-Egyptian Sudan.

Archigetes appendiculatus, Ratzel, 1868, and A. brachyurus, Mrázek, 1908.

Both of these species possess 'caudal' appendages, the former an appendage about equal in length to the rest of the body, the latter an appendage only about one-sixth the length of the rest of the body. A. appendiculatus varies from 2·5 to 3 mm. in length, and is parasitic in the body-cavity of Limnodrilus hoffmeisteri and Tubifex rivulorum, Europe; A. brachyurus is 5–6 mm. long and is also parasitic in the body-cavity of Limnodrilus hoffmeisteri. The genital organs of these two species apparently resemble those of Caryophyllaeus.

Wenyonia virilis, Woodland, 1923.

Length of body varies from 11 to 52·5 mm. and maximum breadth from 1·5 to 3 mm. The body is externally divisible into four distinct regions—a broad-pointed head region, a narrow elongated testicular region, a dilated uterine region, and a normally long narrow tapering post-ovarian

region ; the body and head are oval in transverse section. The testicular and uterine regions, which are roughly equal in length, together occupy about one-third of the total body-length. The post-ovarian region is at least equal in length to the combined testicular and uterine regions. The hind end of the body gradually tapers to a fine point. The head region is normally sagittate in form, its base only being slightly broader than the testicular region, is oval in transverse section, highly muscular, and bears thirteen to twenty-six deep longitudinal grooves which are not suctorial or fixative in function ; highly contractile. Post-ovarian vitellaria about equal, as regards area covered, to the anterior vitellaria. Eggs (in balsam) measure 37·6–41·3 microns in length and 20·1–25·6 microns in breadth, thus being very small compared with eggs of Caryophyllaeus. Parasitic in intestine of the Siluroid Synodontis schall, Anglo-Egyptian Sudan.

Wenyonia acuminata, Woodland, 1923.

Length of body varies from 17·5 to 34·5 mm. and maximum breadth (in testicular region) 1·2 to 1·5 mm. The body is not externally divisible into regions but is nematodiform and oval in transverse section. The testicular and uterine regions, which are roughly equal in length, together occupy about two-thirds of the total body-length. The post-ovarian region is never more than one-third the length of the combined testicular and uterine regions, and is usually less. The hind part of the body only slightly tapers and ends in a blunt point. The head region is tapering and finely pointed and narrower than the testicular region ; it is not highly muscular, not specially contractile, and bears no longitudinal grooves. The post-ovarian vitellaria are not so numerous as the anterior vitellaria. Eggs (in balsam) measure 34·7–36·6 microns in length and 21·9–25·6 microns in breadth, and are distinguished from the eggs of all other (at present) known Caryophyllaeidae by the egg-shell being covered with minute spinelets. Parasitic in the intestine of Synodontis membranaceus, Anglo-Egyptian Sudan.

Wenyonia minuta, Woodland, 1923.

Length of body about 3·5 mm. and breadth 0·8 mm. The body is fluke-like in form, being flat, oval in outline, and with bluntly pointed extremities. The uterine region

is at least twice the length of the testicular region and the two regions together occupy about two-thirds of the entire body-length. The post-ovarian region is inconspicuous. The head region is, like the posterior regions, flattened, and much narrower than the body, is bluntly pointed, with irregular edges—in fact Caryophyllaeiform; it bears no longitudinal grooves and is very contractile. The post-ovarian vitellaria form a small group continuous with the anterior broad marginal strands. Eggs (in balsam) measure 32·9–40·2 microns in length and 20·1–25·6 microns in breadth. Parasitic in the intestine of the Siluroid Chrysichthys auratus, Anglo-Egyptian Sudan.

On the Caryophyllaeidae, Gyrocotylidae and Amphilinidae, and Cestodaria in general.

Having re-defined the genera of the Caryophyllaeidae, it is necessary to re-define the family, since previous definitions not only alone refer to Caryophyllaeus and Archigetes forms, but appear to me to be defective in emphasizing some of the more important features displayed by these genera.

The family was apparently first defined, with some degree of precision, by Claus in 1885 (**4**), stress being laid upon the elongated, unsegmented body, wrinkled anterior border, absence of hooks, eight or more main excretory canals, the general features of the genital apparatus and simple development, and subsequent authors have not improved upon this definition to any extent. In re-defining the Caryophyllaeidae we have to distinguish them from the two other families of the Cestodaria, viz. the Gyrocotylidae and Amphilinidae, and therefore the features to be emphasized are that the body is usually not flattened to the extent that the Cestode strobila is, that calcareous corpuscles are entirely absent, that they never possess true circular suckers, that sucking grooves or bothria may be present (always different in number, form, and arrangement to those found in merozoan Cestoda) in some forms (C. catostomi, C. hexacotyle, and Archigetes brachyurus), that the vagina and uterus form a complete circuit with a single common opening

to the exterior, that the arrangement of the genitalia is very distinctive, and that the larva of some species is hexacanth. Taking these features into account, the Caryophyllaeidae may be defined as

> Cestodaria, usually with a slightly flattened cylindrical elongated body but sometimes fluke-shaped, devoid of calcareous corpuscles and cuticular spinelets or hooks, with an anterior end extremely variable in form and size, both in the individual and in different species, which never carries circular suckers but may bear shallow elongated grooves (different in number, form, and arrangement to those found on the scolex of other Cestoda), with the cirrus and vagino-uterine apertures contiguous or nearly contiguous on the ventral surface of the body in the median line and occasionally opening into a common shallow atrium, with the testes situated anteriorly and entirely in front of the uterus, with the vagina and uterus forming a complete circuit with a common vagino-uterine opening to the exterior, with a network of excretory channels, the larger ones of which form irregular longitudinal canals about eight or ten in number and all of which open externally by a median posterior excretory bladder, and with a larval form known in some cases to be hexacanth. Parasitic in the intestine of Teleostome fishes (Malacopterygii and Ostariophysi) and in the body-cavity of aquatic Oligochaeta (Tubificidae).

Re-definition of the Caryophyllaeidae necessitates re-definitions of the other two families of the Cestodaria. The Gyrocotylidae, comprising four species of the well-known genus Gyrocotyle (**24, 8, 26**), may be defined as

> Cestodaria with a flattened elongated body, devoid of calcareous corpuscles but possessing cuticular spinelets, with an anterior ovoid sucker and a posterior 'rosette' in all known forms, with the cirrus and vaginal apertures adjacent but not contiguous, and situated anteriorly on the left margin of the body, with the testes situated anteriorly and to the outer sides of the median uterus, with the uterine aperture situated anteriorly on the ventral surface in or near the median line, a short distance but quite separate from the vaginal aperture, with a close network of fine excretory vessels devoid of main longitudinal

channels and not opening by a posterior vesicle, and with typical hexacanth larvae in some species but in others a ten-hooked larva similar to that of Amphilinidae. Parasitic in the intestine of Holocephali.

The Amphilinidae[1] may be re-defined as

Cestodaria with a flattened more or less elongated body, possessing calcareous corpuscles but devoid of cuticular spines and hooks, with a large anterior boring apparatus (proboscis, boring muscle and anchor cells) but devoid of suckers and bothria, with the cirrus and vaginal apertures either in close apposition or only separated by a short distance, both situated posteriorly on or near the edge of the hind end of the body, with the testes extending over, with the uterus, the greater length of the body, usually as two marginal rows lying external to the coils of the uterus but sometimes more scattered, with the vagina lying posterior to the ovary and the uterus anterior, with the uterine opening at the extreme anterior end, with a very long uterus, consisting of three limbs disposed like the letter N when viewed ventrally, with two main lateral longitudinal excretory channels opening medianly at the posterior extremity, and with a larval form possessing ten hooklets. Parasitic in the body-cavity of Acipenseridae, Osteoglossidae (Arapaima), Haemulidae (Diagramma), and Siluridae (Macrones).

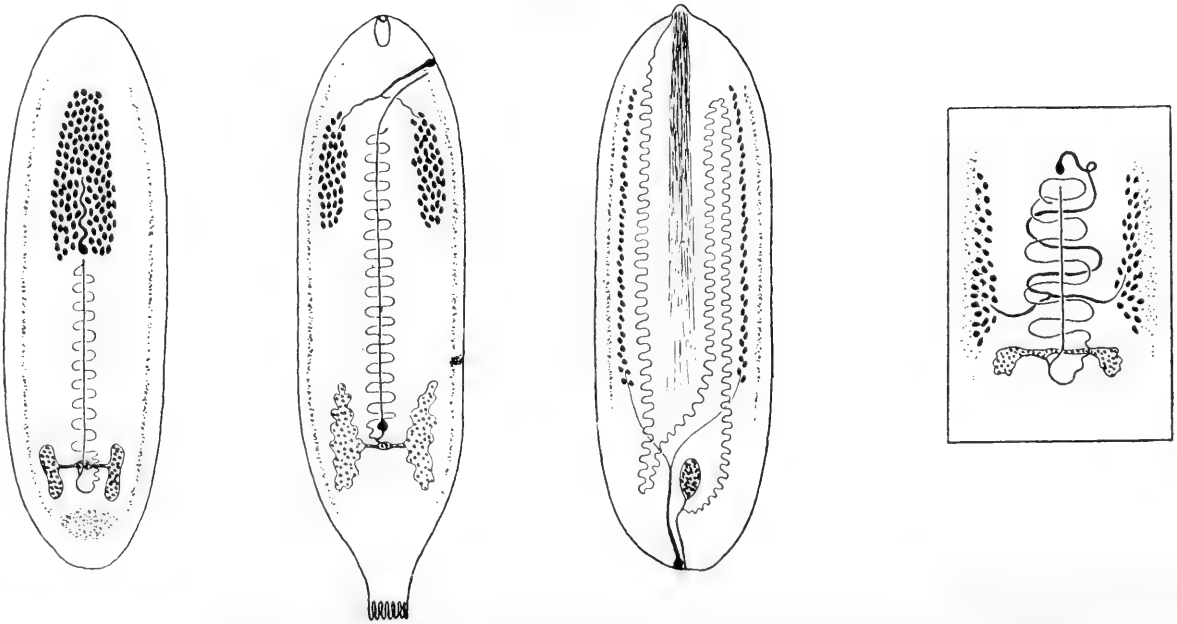
Comparing these three families, it appears to me self-evident that the Caryophyllaeidae and Gyrocotylidae are much more closely allied to each other than is either of these families to the Amphilinidae. In both of the former families (*a*) the three sexual apertures are all grouped together, and at least two of them (the uterine and vaginal) are, in both families, situated on the (ventral ?) surface in or near the median

[1] See the writer's paper on Amphilina paragonopora recently published in this journal (**28**). I may remark in this place that Poche's paper "Zur Kenntnis der Amphilinidea" ('Zoologischer Anzeiger', Bd. liv, 1922, p. 276) appeared too late for me to refer to it in my own paper, and that I am wholly unable to concur with the author's grotesque proposal to found separate families and sub-families for known species of Amphilina. The best policy to adopt with suggestions of this kind is to ignore them.

line and not on the edge of the body ; (*b*) in both families the uterus and vagina run parallel to and in the same dorso-ventral plane with each other along the longitudinal axis of the body, and in both the testes occupy only the anterior end of the body ; (*c*) in both families the ovary is bipartite, and (*d*) in both calcareous corpuscles are absent ; (*e*) in both families hexacanth embryos have been found ;[1] and (*f*) a reticulate type of excretory system is common to both. On the other hand, in the Amphilinidae, (*a*) the uterine and vaginal apertures are situated at opposite ends of the body and are in all cases situated on or near the body edge ; (*b*) the uterus and vagina run in opposite directions from the ovary, the former anteriorly and the latter posteriorly ; (*c*) the ovary is unipartite ; (*d*) calcareous corpuscles are present ; (*e*) hexacanth larvae have not been found, the larva being of the ten-hooked kind ; and (*f*) the excretory system consists of two main longitudinal canals only and minor regularly arranged looped canals. I am thus unable to agree either with Monticelli (**15**) in believing that Amphilina and 'Amphiptyches' (Gyrocotyle) are closely related forms, or with Lönnberg (**12**), who is of opinion that the Caryophyllaeidae differ essentially from the Gyrocotylidae in being secondarily monozoic forms, the Gyrocotylidae being, in his opinion, primarily monozoic. I cannot find that either of these authors have advanced any valid reasons for these opinions. It is true that Gyrocotyle differs from all Caryophyllaeidae in possessing an anterior sucker and a posterior 'funnel' (with muscular and nervous modifications to match), but the extremities of these animals can develop almost any kind of process, as we see in the Caryophyllaeidae, and this difference, as also the minor differences in the conformation of the genitalia and in

[1] Both Spencer (**24**) and Hungerbühler (**9**) found in certain species of Gyrocotyle ten-hooked larvae similar to those of Amphilina, while in G. urna Lönnberg ('Biol. Fören. Förhandl., Verhandl. d. biol. Ver. in Stockholm', vol. ii, 2, p. 55, 1890) found larvae apparently altogether devoid of hooks. The significance of these facts is as yet obscure.

TEXT-FIG.



To illustrate the orientation and disposition of the genitalia in the three families of the Cestodaria and in the Bothriocephalidae.

the excretory system, cannot be considered of much weight when the fundamental resemblances I have indicated are taken into consideration. I therefore propose, in order to emphasize these fundamental resemblances, provisionally to unite the Caryophyllaeidae with the Gyrocotylidae into one Order, the Paralinidea, in contradistinction to the Amphilinidea, which contains only one known family,[1] the Amphilinidae.

The Paralinidea may be provisionally defined as

> Cestodaria (*a*) with the three sexual apertures situated close together; (*b*) with the uterus and vagina running parallel to each other in the same dorso-ventral plane along the median longitudinal axis of the body; (*c*) with the testes restricted to the anterior end of the body; (*d*) with a bilobed ovary; (*e*) without calcareous bodies; (*f*) with a reticulate excretory system; (*g*) with a hexacanth larva (?).

The Amphilinidea possess the opposite characters, viz.

> (*a*) the uterine and vaginal apertures are situated at opposite ends of the body, the former being at the anterior and the latter posterior; (*b*) the uterus and vagina run in opposite directions from the ovary, the former anteriorly and the latter posteriorly; (*c*) the testes extend, with the uterus, over the greater length of the body; (*d*) ovary one-lobed; (*e*) calcareous corpuscles present; (*f*) excretory system consisting of two main lateral longitudinal channels with minor regularly arranged looped vessels; (*g*) with a ten-hooked larva.

Finally, it is evident that of the two Cestodarian Orders—Amphilinidea and Paralinidea—the latter is much more closely related to the Cestoda merozoa than is the former (Text-fig.). In both the Paralinidea and the Bothriocephalidae the uterus and the vagina run medianly in the same dorso-ventral plane, the vas deferens opens from the anterior side of the proglottis (though the testes

[1] Benham (1) would place Wageneria in this Order, but this organism is not yet known with sufficient accuracy to justify its inclusion in any family.

are necessarily displaced from their primitive anterior position), the three sexual apertures are all close together, the ovary is bipartite, and a hexacanth larva is present.

I would not, however, go so far at present as to follow Lühe (**13**) in actually grouping the Caryophyllaeidae with the Diphyllobothriidae in his Order Pseudophyllidea, because for me the monozoan character of the Paralinidea is a fundamental one, and to me it is inconceivable that the Paralinidea have become secondarily monozoan. It is also well to remember that the Amphilinidea may not be so far removed from the Paralinidea as their existing structure would seem to indicate, for the simple reason that this structure is, in part at least, an obvious adaptation to the mode of life of Amphilina. Amphilina is parasitic in the body-cavity and not the gut of the fish, and it can only liberate its larvae to the external world by boring through the body-wall of its host (hence the huge boring apparatus I have described (**28**) and the extreme anterior position of the uterine pore) and has to retain its larvae until it has penetrated to the outside of the fish (hence its enormously elongated uterus—a duct which would not need to be so elongated if, as in the Paralinidea, the parasite could continually shed its eggs into the intestine). These bionomic necessities must have caused a great transformation in the entire anterior end of the animal and especially in the arrangement of all the contained ducts, and it is therefore possible that even the posterior position of the vas deferens and the vagina (the openings of which must remain adjacent) is to be regarded as but another indirect concomitant of the general rearrangement. In other words, the original plan of the genitalia in Amphilina is possibly largely masked by specialization and, in the light of present knowledge, it may be incorrect to conclude that Amphilina is more separate genetically[1] from the Para-

[1] The fact that in both the Paralinidea and Amphilinidea ten-hooked larvae have been found may be called to mind in this connexion. I may here remark that Sanguinicola (still included in 1916 by Cholodkowsky (**3**) in the Cestodaria) is now known not to be a Cestode:

linidea than these are from the merozoan Cestodes. However, I concede that it is possible that this conclusion may prove to be the correct one and that, as several authors have supposed, the ' Cestodaria ' may be polyphyletic in origin.

On an Immature Caryophyllaeus sp. from the Intestine of Auchenoglanis occidentalis, Cuv. and Val., 1840.

I possess a single specimen of a species of Caryophyllaeus (Pl. 25, fig. 31) which is immature and which was found in the intestine of the Siluroid Auchenoglanis occidentalis at Khartoum, Anglo-Egyptian Sudan. The specimen measures 2 mm. in length and about 0·5 mm. in maximum breadth (posteriorly). The body is relatively thick and has the wrinkled appearance shown in Pl. 25, fig. 32. I cut this specimen into horizontal longitudinal sections and discovered that its internal structure was on the same general plan as that of C. filiformis. The ovary and vitellaria were still rudimentary, though the uterus, vas deferens, and their openings were well defined. The testes were not fully developed. Apparently the vagina was convoluted in form and not straight as is usual, and it was not easily distinguishable in sections from the uterus.

In conclusion, I wish to express my thanks to Dr. Andrew Balfour, Director of the Wellcome Bureau, for the opportunity of examining the interesting new forms of Caryophyllaeidae above described, to Professor J. P. Hill for the loan of five specimens of Caryophyllaeus laticeps, and to Mr. C. A. Hoare for his kind aid in translating certain passages in Russian.

Summary.

1. Four remarkable new species of Caryophyllaeidae from the Anglo-Egyptian Sudan are described, three of which (from Siluroid fishes) are referred to a new genus, Wenyonia —W. virilis, W. acuminata, and W. minuta—and the fourth (from Mormyrus caschive) to the original genus

vide my paper on this form in the present volume of the ' Quart. Journ. Micr. Sci.'

Caryophyllaeus—C. filiformis. The chief characteristics of the new genus are the situation of the sexual apertures in the anterior half of the body, and the elongated uterus. The family Caryophyllaeidae, after deletion of the genera Diporus, Monobothrium, and Glaridacris, thus contains three genera—Caryophyllaeus, Archigetes, and Wenyonia—all of which are re-defined, with their known species.

2. Very young immature forms of Wenyonia occur in the same (Siluroid) host as the adult and are devoid of a 'caudal' appendage, whence it would appear that the life-history of these new forms is different from that of C. laticeps.

3. The three families of the Cestodaria—Caryophyllaeidae, Gyrocotylidae, and Amphilinidae—are re-defined.

4. The Cestodaria, after eliminating Sanguinicola (a Trematode ?), are provisionally grouped into two Orders: the Amphilinidea (with one family, the Amphilinidae) and the Paralinidea (with two families, the Caryophyllaeidae and the Gyrocotylidae), the latter being closely allied to the Bothriocephalidae. These two Orders are defined.

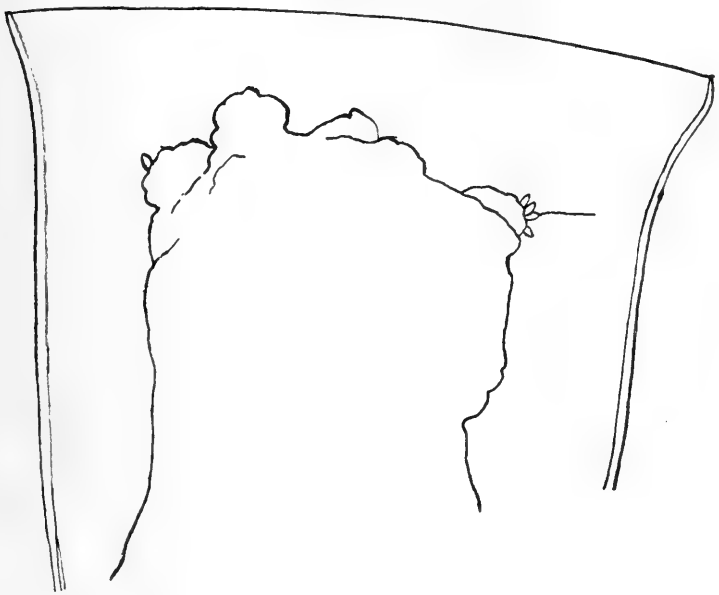
Literature References.

1. Benham, W. B., in 'A Treatise on Zoology', ed. by E. Ray Lankester, "Part IV. The Platyhelmia, Mesozoa, and Nemertini", London, 1901, p. 97.
2. Cholodkowsky, N. A.—"Notes helminthologiques", 'Petrograd Ann. Mus. Zool. Ac. Sci.', vol. xx, 1915, p. 164.
3. —— 'Explanatory Catalogue of the Collection of Parasitic Worms in the Zoological Cabinet of the Imperial Military Academy of Medicine, Petrograd', fascicle ii, 1916.
4. Claus, C.—'Lehrbuch der Zoologie', 1885.
5. Cooper, A. R.—"Glaridacris catostomi, gen. nov., sp. nov.: a Cestodarian Parasite", 'Trans. Amer. Micr. Soc.', vol. xxxix, no. 1, 1920, p. 5.
6. Diesing, K. M.—"Revision der Cephalocotyleen. Abtheilung: Paramecotyleen", 'Sitzungsb. der Math.-Naturw. Cl. kais. Acad. Wiss. Wien', Bd. xlviii, I. Abth., 1863, p. 200.

3, and 4). The stream of cells and débris can also be observed directly under the microscope while resorption is going on in the living organism.

The questions remain, how and under what conditions do the resorbed elements start the migration, and where do they eventually get to?

TEXT-FIG. 2.



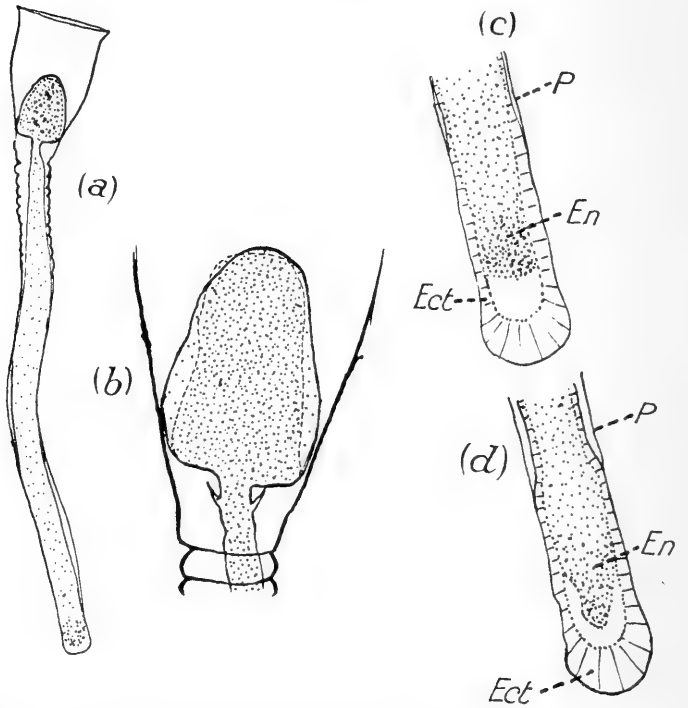
Campanularia. End of stage 2. The hypostome has disappeared, the tentacles are represented only by minute knobs, some almost resorbed. Nematocysts here and there project from the surface of the tentacles, one discharged. (Camera lucida.)

We will first study a tentacle. In the normal zooid the tentacle is almost twice as long as the hypostome, and its axis of endoderm is composed of large cells with very definite walls and flattened like a pile of discs. The endodermal axis occupies more than three-quarters of the diameter of the tentacle (Pl. 26, fig. 6).

In the first stage of resorption the tentacle has shrunk considerably both in length and diameter (Pl. 26, fig. 7). The endo-

derm cells are smaller, and the most distal ones have lost the typical disc-like structure. They are rounded and have lost their serial arrangement in a single row: at the tip it is difficult to dis-

TEXT-FIG. 3.

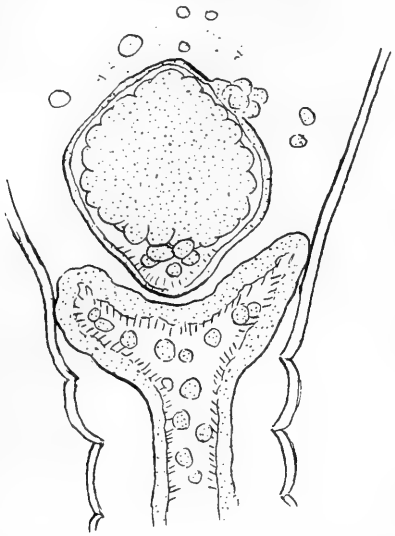


Campanularia. *a*, Stage 3. No trace of tentacles or hypostome. Considerable new growth from the stem. *b*, The same zooid magnified to show its change of shape. The dotted outline was drawn one minute later than the firm outline. *c*, Tip of the new growth from the same specimen in expanded condition. *d*, The same specimen contracted two minutes later. The ectoderm near the tip is attached to the perisarc; proximal to this the contraction is clearly visible. *ect.*, ectoderm; *en.*, endoderm; *p.*, perisarc. (Camera lucida.)

tinguish them from the ectoderm cells, which have come to present the same spheroidal appearance. Pl. 26, fig. 7, is a photograph of a single section showing progressive loss of differentiation

in the endoderm axis of a tentacle as one approaches the distal extremity. This loss of differentiation gradually extends proximally, and the whole tentacle passes into the gastrovascular cavity after rupture of the mesoglaea (confirming Thacher, 1903). In Pl. 26, fig. 4, the cavity is seen to be filled with cells and débris, the result of resorption of the tentacles.

Text-fig. 4.



Campanularia. The distal part of the zooid has separated from the proximal part. Active cilia occur in both parts, also immigrated cells, but those in the proximal part are sparser as they are migrating down the stem. Some cells of the distal part are migrating outwards. (Camera lucida.)

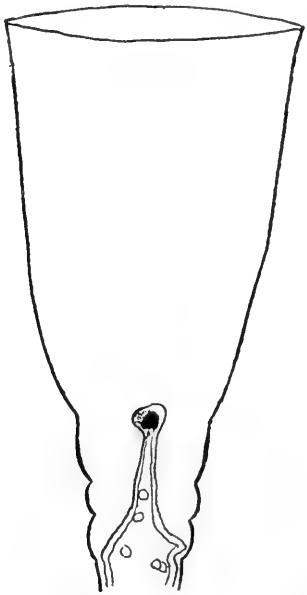
Resorbed elements from the hypostome are no doubt there also, although indistinguishable.

Examined under high powers, it appears that the boundaries of the loose cells in the gastrovascular cavity lose more of their distinctness the farther the elements are from the periphery. The spherical or ovoid bodies to be seen in the centre of the zooids containing refractive matter (but not staining very definitely) are nematocysts. Nearer the periphery

the cells appear as in Pl. 26, fig. 8, still with a definite cell boundary. Nematocysts only appear in the gastrovascular cavity in late stages of resorption, after rupture of the mesoglaea at the base of the tentacles. The bulging in of the basal mesoglaea before breaking is well shown in Pl. 26, fig. 3, left-hand tentacle.

Appearances sometimes occur in Obelia which seem to show

Text-fig. 5.



Campanularia. End of stage 4. The zooid is represented only by a small stalked knob containing a mass of pigment, mostly brown with some orange granules. (Camera lucida.)

that phagocytosis is taking place. This was corroborated on a specimen of Campanularia (the one shown in Text-fig. 6) which was accidentally ruptured: numerous cnidoblasts with contained nematocysts were then seen; but in addition, nematocysts were found inside cells much larger than cnidoblasts; frequently two would be seen within a single large cell (see Text-fig. 7).

Studies in Dedifferentiation. IV. Resorption and Differential Inhibition in Obelia and Campanularia.

By

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and

G. R. de Beer, B.A., B.Sc.

With Plate 26 and 7 Text-figures.

Introduction.

It is well known that among the processes whereby an organism is developed all are not constructive, but some may involve the demolition of certain structures which may either have ceased to subserve a useful function, or may actually hinder further development. A familiar example is the gill or tail of Amphibian larvae at metamorphosis.

In normal circumstances this retrogression only affects certain organs, but recently it has been found possible to produce this effect experimentally in whole organisms (Driesch, 1906, &c.).

Protozoa, Planarians, Sponges, Ascidians, and Coelenterates will under certain conditions give the retrogressive effect, as evidenced by the work of Lund (1917), Child (1904), Maas (1910), Huxley (1921 *b*), Loeb (1900), and others.

Following some observational work by one of us (J.S.H.), it was thought that quantitative experiments involving the subjection of organisms to different concentrations of poisons might give interesting results. One of the authors (G.R. de B.) accordingly performed some experiments on Obelia geniculata which will be described below.

The Hydrozoa are not virgin soil to the experimentalist in this connexion. Loeb (1900) observed that under certain conditions the zooids of a hydroid colony would lose all their shape and structure and retreat into the hydrocaulus. He attributed the cause of this to contact with solid objects, viz. the watch-glass in which the organisms were kept ; but this explanation is probably not correct.

Thacher (1903) investigated the process of retrogression in hydroids and called it ' absorption '. Cerfontaine (1902) says of it : ' les individus . . . dégénèrent et disparaissent.' Gast and Godlewski (1903) merely called it ' degeneration ' (' Rückbildungsprozess '). These terms are inadequate to designate a process as specific as that which Huxley (1921 *b*) has described in the case of Perophora. Strictly speaking, there are two processes at work, viz. dedifferentiation and resorption. In the following description of the experiments performed Resorption will be used to mean the process whereby the material composing the zooid is transported, and Dedifferentiation to mean other processes undergone involving a return of cells or tissues to a simpler, less differentiated condition.

In the organisms chosen for experiment there is a coëxistence of two sets of systems, the ' zooid systems ' and the ' stolon systems '. It is obvious that normally physiological equilibrium must exist between them. But if circumstances can be found whereby one system is adversely affected more than the other there will occur differential inhibition associated with resorption or dedifferentiation or both.

The experiments were performed at the Biological Laboratory, Woods' Hole, in 1916 (Campanularia, J.S.H.), and at the Marine Biological Laboratory, Plymouth, in 1920 (Obelia, G.R. de B.).

Experimental.

Given the fact that mere subjection to unfavourable conditions, viz. being kept in glass vessels in the laboratory, brings about resorption of zooids in hydroids, it was to be expected that if the toxicity of the water were increased, the process of

resorption would be accelerated and the differential inhibition made more specific. Apart from their plentifulness, Obelia and Campanularia are suitable material because :

(i) The zooids are conveniently far apart and attached to the hydrocaulus by a fairly long stem ;

(ii) The zooids in their natural condition are highly differentiated structures compared with the rest of the colony ;

(iii) The stages of resorption can be conveniently determined by reference to the hydrotheca.

On the other hand there is the disadvantage that it has poor viability, which means that resorption takes place even in the controls in clean sea-water. This, however, occurs long after it has done so in the toxic solutions.

Care was taken to ensure that the colonies were clean and healthy and free from Diatoms and Protozoa, and that all the polyps were normal and fully extended.

The experimental solutions are referred to in terms of concentrations of KCN ; but since the solvent was sea and not pure water such an expression as $\frac{N}{10}$ does not give us the actual ionic concentration.

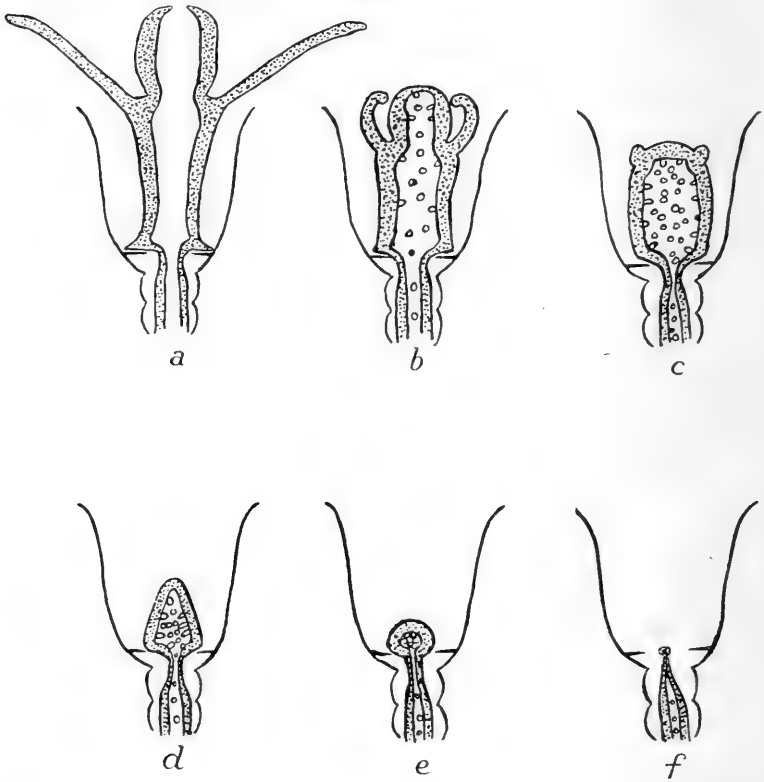
The solutions were made up in shallow glass dishes, in each of which a small number of stems of Obelia each bearing eight zooids (Table I) or one zooid (Tables II to IV) were placed. In every case the material used was fresh from the sea. KCN solutions were kept covered owing to the volatile nature of KCN, and changed every day.

Resorption can be divided into five stages (Text-fig. 1). Text-fig. 1, *a*, represents a normal zooid (also Pl. 26, fig. 1).

First Stage. The tentacles are first affected. They may become apposed to the hypostome, and may shrink so as to become shorter than the hypostome. Adjacent tentacles may fuse (Pl. 26, fig. 9), indicating an interesting change in consistency. The mouth closes, but the hypostome is still prominent. Ciliary action continues in the enteron as in the normal zooid (Text-fig. 1, *b*, and Pl. 26, figs. 2 and 3).

Second Stage. The hypostome is completely resorbed and the tentacles are represented only by a ring of tiny prominences. The ' waist ' in the body of the zooid has disappeared. The whole is well within the margin of the hydrotheca. The stalk

TEXT-FIG. 1.



Diagrams representing: *a*, normal zooid; *b*, first, *c*, second, *d*, third, *e*, fourth, *f*, fifth stages of resorption remaining in the hydrotheca.

attaching the zooid to the hydrocaulus is very thin and within it is a mass of cells and débris flowing slowly away from the zooid (Text-figs. 1, *c*, and 2).

Third Stage. The zooid has shrunk towards the bottom

of the hydrotheca ; its shape is roughly ovoid. No sign of tentacles whatsoever (Text-figs. 1, *d*, and 3, *a*, and Pl. 26, fig. 4).

Fourth Stage. Sometimes the distal portion of the zooid may become separated from the rest (Text-fig. 4). The form-determining properties of the zooid have become less powerful than the surface tension acting upon it, and it has accordingly become spherical, nowhere touching the hydrotheca and connected by a thin stalk to the hydrocaulus. At this stage and later the flow in the tube is irregular. It appears to be maintained by pulsations of the stolon (see p. 479) (Text-fig. 1, *e*, and Pl. 26, fig. 5).

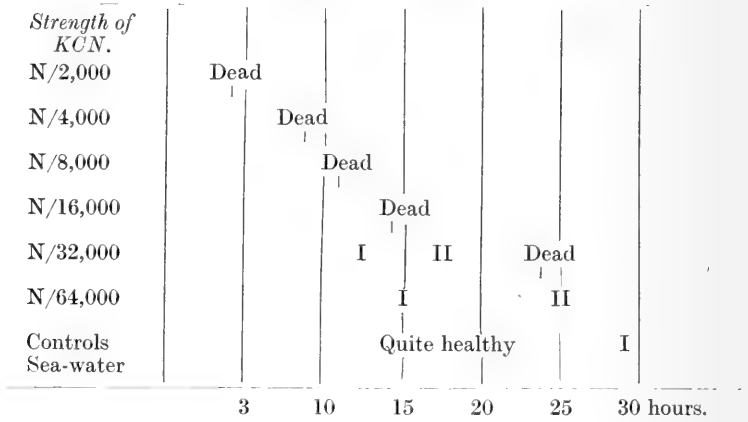
Fifth Stage. The process has been continued until the zooid is represented only by a tiny knob (often containing pigment) smaller in diameter than the hydrocaulus (Text-figs. 1, *f*, and 5). Occasionally the process is carried further and the hydrotheca becomes empty. This only occurs a considerable time after stage 5, and is mainly a mere degeneration effect.

It should be noted that in those cases where the colonies contained gonothecae, medusae were not liberated if resorption had started. During resorption the zooids are perfectly healthy and transparent. Dead tissues can always be distinguished (opacity, &c.). Small masses of dense pigment are often found in the partly resorbed zooid, representing products of degeneration.

As resorption goes on, the material derived from the zooid passes into the hydrocaulus, and from the proximal (cut) end of the latter a stolon begins to grow (Text-fig. 3, *a* and *c*). It is very transparent and clear, and may grow to the length of 10 mm. or more, affixing itself to the substratum. It sometimes happens that a small portion of what is left of the zooid in stages 3–5 is completely nipped off (by surface tension) from the hydrocaulus. It remains in the hydrotheca and dies (Text-fig. 4).

In the earlier experiments the solutions used were too strong, but even so a differential effect was obtained (Table I).

TABLE I (Obelia).



The Roman figures denote the stage of resorption at a particular time in a solution of given strength. All solutions up to N/32,000 are too strong and kill the organisms before any resorption occurs: in the N/32,000 solution resorption proceeded a little way but the organisms were then overcome by the poison.

It soon became apparent that the preparations used were not wholly satisfactory, for of the eight zooids borne by the colonies all had not been resorbed to the same extent at the same time. To meet this difficulty portions of the hydrocaulus were used bearing only one well-expanded zooid.

TABLE II (Obelia).

After: *In*	6 *hours.*	10 *hours.*	24 *hours.*	36 *hours.*
N/8,000	All dead			
N/16,000	All dead			
N/32,000	Stage I	All dead		
N/64,000	Trace of resorption	Stages I and II	All dead	
Control	Expanded and motile	Expanded and motile	Trace of resorption	Stages I and II.

The results of this experiment were much more definite, but the solutions used were still too strong.

TABLE III (Obelia).

After : In	16 *hours.*	24 *hours.*	40 *hours.*	50 *hours.*
N/64,000	Stage II. No medusae liberated	Dead		
N/128,000	Stage I. No medusae liberated	Stages II and III	Dead	
N/256,000	Trace of resorption. A few medusae liberated	Stages I and II	Stages III and IV	Stage V and Dead
Controls	Fully expanded. Several medusae liberated	Traces of resorption	Stages I and II	Stage III.

Experiments in the N/256,000 solution were repeated several times with the same results. These results indicate that a N/256,000 solution of KCN inhibits the zooids without affecting the hydrocaulus to any appreciable extent (at least for a considerable time—fifty hours). The healthy nature of the hydrocaulus is evidenced by movements of contraction and pulsation, and by growth at the proximal extremity.

The contraction of the stem is of interest, since precisely similar contraction occurs in Perophora and other Ascidians (Huxley, 1921 *b*). In addition, the partly dedifferentiated zooid also appears to contract at intervals (Text-fig. 3), although it is possible that the contraction is a mere surface-tension effect, exerted passively on relaxation of the walls of the stem. It appears that the contraction of the cells of the stem occurs when considerable internal tension has been produced through the flow of liquid and cells from the zooid. In higher forms embryonic cells which are destined to give rise to muscle appear to start contracting before differentiation, also as a result of tension (e.g. Carey, 1921 *a* and 1921 *b*, &c.).

It is possible that contraction produced by tension has no normal function in hydroid stems. It is all the more interesting to find that it occurs, being thus probably a general property of all not too highly-differentiated cells. Possibly tension acts also as a stimulus to outgrowth from the stem. (Cf. the well-known fact of better growth in regenerating Tubularia in hypotonic sea-water (Loeb, 1892).)

In order to see whether any of the results obtained were due to the specific effects of KCN, experiments were also made with $HgCl_2$. When solutions of N/1,000,000 and N/2,000,000 were used, the resorption effects were identical with those in KCN.

Table IV (Obelia).

	After 24 *hours.*
KCN $\frac{N}{128,000}$ . .	Stages II and III.
$\frac{N}{256,000}$ . .	Stage I.
$HgCl_2$ $\frac{N}{10^6}$ . .	Half dead, remainder Stage III.
$\frac{N}{2\times 10^6}$ . .	Stage I.
Control . .	Fully expanded.

After varying periods in solutions of all the strengths, some preparations were removed and placed in clean fresh sea-water with a view to inducing them to cease resorption and reform zooids. In no case was this successful. The preparations ceased resorption for a short time, but then continued. This was to be expected from the behaviour of zooids in normal sea-water.

When the zooid is severed from the stem at the base of the hydrotheca, the preliminary dedifferentiation occurs as usual; but after a certain number of cells have migrated into the interior there is no room for more. The result is an ovoid dedifferentiated mass, tightly packed with cells (Text-fig. 6). Similar phenomena were seen in Perophora. Thus the degree of resorption depends on the amount of space available. Resorption will only proceed to a limit when the migrating cells are removed. A parallel is here provided with those chemical reactions which will only proceed to a limit if the products of the reaction are removed.

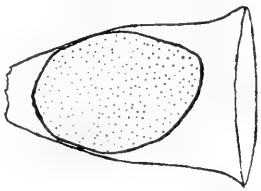
Histology.

In the previous section the various stages of resorption were briefly described. The actual route of migration of the cells is, of course, through the gastro-vascular cavity (Pl. 26, figs. 2,

Presumably the large cells are endoderm cells which still retain the phagocytic properties normally associated with intracellular digestion.

Occasionally we have noticed in sections of normal specimens appearances indicating the phagocytosis of small (presumably

TEXT-FIG. 6.



Campanularia. Zooid cut off at base of hydrotheca. Dedifferentiation to an ellipsoid mass. Many nematocysts were to be seen in the interior. (Camera lucida.)

TEXT-FIG. 7.

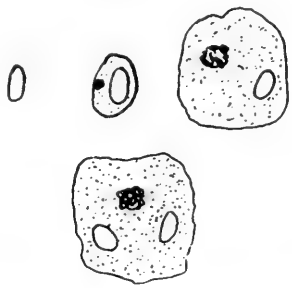


Diagram showing the manner of occurrence of nematocysts in the interior of the zooid shown in Text-fig. 6. (Observations in vivo.) The nematocysts were loose, or still in their cnidoblast, or ingested in a large endoderm cell, or two inside one endoderm cell.

interstitial) cells by normal endoderm cells. Professor G. C. Bourne, F.R.S., has kindly informed us that he has seen similar appearances in Hydra, which he also interprets as cases of phagocytosis of one type of cell by another. This process, however, is clearly much commoner in the specimens undergoing resorption than in normal zooids.

It is in any case difficult to see on what occasions phago-

cytosis of the organism's own nematocysts would occur in its normal life-history. It would thus appear that in certain circumstances the power of ingesting food-particles, possessed by certain types of cells, results in typical phagocytosis of other cells of the organism—a process unusual or abnormal for these forms, but usual and normal in higher animals.

Loeb (1900) states that the tentacles fuse in some cases. Thacher (1903) considers this to be only the appearance caused by their being crowded together and by the ectoderm being thrown into folds by excessive contraction. After careful study of serial sections of a zooid in this condition we can state that this explanation will not suffice. The tentacles are, it is true, contracted and crowded, but there is actual fusion in several places (Pl. 26, fig. 9).

The ectoderm cells of the tentacles and hypostome become more cubical during resorption.

Of the endoderm cells, the large mass of glandular cells in the hypostome very soon disappears, the cells passing into the cavity.

In the last stage of resorption before the hydrotheca is evacuated altogether (stage 5), what is left of the zooid is still bounded by a definite epithelium (Pl. 26, fig. 8) of flattened cells, beneath which are others losing their differentiation. The behaviour of the ectoderm cells may be compared with that of a rear-guard, continually retreating yet always maintaining an unbroken front. But as the zooid is resorbed, its volume and surface decrease, and so the front diminishes.

At the start the cavity is not too congested and the cells and débris pass down; but in later stages the cavity is almost blocked up (Pl. 26, fig. 8), and it is then that pulsation can be observed. This obviously facilitates the evacuation. It is presumably due to tension on the walls of the stolon.

In Obelia it is possible to observe the cells actually leaving the tissues, a process which has often been taken for granted in other forms (see Pl. 26, fig. 2, left side).

Where do the resorbed elements go when they pass down into the hydrocaulus out of the zooid? It appears that they

are ingested by the endoderm cells of the hydrocaulus, or break down and in that condition are absorbed by those cells.

The walls of the hydrocaulus even in the last stages of resorption appear normal when seen alive under the microscope and in sections, though its cavity may be filled up with cells and débris.

But is it possible that the new growth which takes place at the proximal cut end of the hydrocaulus consists of the very elements derived from the resorbed zooids? Loeb makes the observation that this growth is like the motion of a protoplasmic mass, and such it certainly appears to be in our experiments. But this would be the appearance presented by normal growth proceeding at the rate at which this stolon was produced—10 mm. in forty-eight hours or less.

In structure the new growth is similar to an ordinary piece of hydrocaulus (Text-fig. 3).

This growth starts only after a certain stage of resorption has been reached.

The new growth adheres to the substratum, thus resembling the normal creeping stolon. We were unable to observe whether it could give rise to buds in Obelia, as the preparations died. In Campanularia, pieces with several zooids might give rise to one or several buds during or after resorption of the original zooid.

Discussion.

Loeb (1900) attributes resorption to contact with solid objects. According to him the transformation must be due to liquefaction of the more solid constituents of the zooid. Contact with the fluid, sea-water, makes for the production of the more solid portion of the colony, the zooid; whereas conversely contact with a hard surface makes for the more fluid stem. Accordingly if a zooid be subjected to the stimulus to which the production of the stem-system is the reaction, the result will be the conversion of the zooid into something resembling the stem.

But resorption of zooids takes place even when the colony is maintained in an erect position and no portion of the zooid

is allowed to touch any hard object (Thacher, 1903). Clearly then, contact cannot be the only cause of resorption. It is rather to be interpreted in terms of equilibrium between two systems with different physiological reactions: if one wishes to use Child's phraseology one may say that they possess different metabolic rates, and the one with the higher rate is, normally, physiologically dominant over the other.

In the case of these hydroids there are two systems, the zooid and the stem (hydrocaulus).

Normally, the more highly differentiated zooid is able to maintain itself, but being more specialized and less plastic than the stem the zooid will not be able to maintain itself in the face of conditions which, though adverse for the zooid, do not appreciably affect the stem. Such are, e.g., a N/256,000 solution of KCN, or laboratory conditions after fifty hours. The result of the adverse conditions is inhibition of normal function; and within limits it is differential, affecting the zooid before and more than the stem.

By interfering with general metabolism, as is done by exposure to toxic agencies, the output of energy is reduced. Energy is needed to maintain differentiated form against surface-tension. Thus one of the first results of non-lethal interference will be the loss of typical form by cells and their reversion to a spheroidal or cuboidal shape. This is found in all cases of dedifferentiation known, and often leads to the assumption of spheroidal form by the whole organism (see Huxley, 1922).

The fact that exposure to laboratory conditions, to KCN and to $HgCl_2$, all bring about identical reactions indicates that the effects of the poisons, &c., are not specific, but that all act in a general way, by affecting the energy-production of the tissues.

It may be asked how the process we have called dedifferentiation in Obelia differs from simple degeneration. The answer is to be found in observation of the process. At no time can it be said that the zooid is dead; during the whole process of resorption what is left of it is just as alive as the normal zooid or stem. If the zooid dies, as it does if the poisons are too strong, the cells acquire a characteristic semi-opaque appearance

which cannot be mistaken. There is then no more resorption and the cells macerate, and later disintegrate, without dedifferentiation.

Resorption is a result of the process of migration and it could not take place were the elements to be resorbed to remain in their differentiated condition. Resorption then is consequent on dedifferentiation. It occurs in many forms when a cavity is present into which the migration may occur (Child, 1904; Huxley, 1921 *b*).

In Obelia, as in Perophora and probably in many other cases, if the cavity into which migration can occur be by some method or other limited, dedifferentiation with no or slight resorption may take place (Text-fig. 6, p. 485).

It is then dedifferentiation plus resorption that Loeb means by 'liquefaction'. But this is not a specific result of contact with hard surfaces. Whether such contact by itself can produce the effect we do not know, but as an unfavourable condition it can and does accelerate it. Contact stimulates tentacles to contract, and constant stimulation must be unfavourable; oxidation must also be reduced in proximity to the substratum. Loeb's analogy between the liquefaction of the zooid and the clotting of blood, both due to contact with solid objects, thus cannot stand.

It may be said that dedifferentiation implies potential subsequent redifferentiation. There is, however, no reason why dedifferentiation should be reversible any more than differentiation. If we lay down that dedifferentiation is reversion to a morphologically simpler state with lower energy-requirements, the simpler condition being preserved for a considerable time and not merely a stage in the process of dying, we have a good working definition.

Dedifferentiation is the accepted term to denote the simplificatory processes undergone by differentiated cells in tissue culture; and in this case there is usually no redifferentiation, the tissues merely remaining alive for a longer or shorter time in their simplified condition. Smooth muscle grown in culture solutions dedifferentiates to a condition in which the cells divide

actively (no division normally occurs in adult smooth muscle). If such a preparation be grafted back into its former position it is just possible to arrest the mitoses, but redifferentiation proceeds no further (Champy, 1913). (Strangeways informs us verbally that he has been more successful.) On the other hand, redifferentiation of dedifferentiated tissue has been obtained by Drew (1923) in vitro.

Redifferentiation of the zooid was not obtained in Obelia as it was in Clavellina (Driesch, 1906; Huxley, unpub.), Pennaria (Cerfontaine, 1902), or Sycon (Huxley, 1911). But in those cases where redifferentiation does occur, we must ask whether the new adult structure is formed from the redifferentiation of the dedifferentiated cells, or from indifferent cells which have all along retained the full hereditary potentialities. The study of budding and asexual reproduction, especially Hadzi's work (1910) on Hydra, suggests that the latter is usually the case. If this is so, then the failure to redifferentiate is in no way due to the inactivity of the dedifferentiated elements, but of the indifferent cells. Vandel (1921), however, shows that in regenerating Planarians (Polycelis), the new pharynx is produced from cells of other organs which dedifferentiate and then redifferentiate along new lines, scarcely any mitoses being observed. This is a good example of pluripotent dedifferentiation (Adami and Macrae, 1914; Huxley, 1921 *a*). The usual process, however, in colonial forms, is for the dedifferentiated tissues to provide material for new outgrowths of the nature of stolons, from which later new zooids may arise (cf. Ascidians, Huxley, 1921 *b*; Hydroids, Müller, 1913).

When the metabolic requirements of the zooid have decreased, the equilibrium between it and the stem is upset and the balance is now in favour of the latter (Huxley, 1921 *b*). In the higher animals complete resorption of systems does not usually occur. For one thing the cells are too solidly packed in tissues, and they are usually attacked by phagocytes before they have even had time to be resorbed. But a concurrence of both processes is seen in the absorption of the tail in Ascidian tadpoles. Here, according to Delage and Hérouard (1898), ' ses

éléments se désagrègent,' after which process the phagocytic action commences. Most authors are also agreed that a process of dedifferentiation initiates the resorption of the tail in Anuran tadpoles, phagocytosis being secondary (cf. Naville, 1922).

Phagocytosis here only occurs after resorption, i.e. after the tissue elements have migrated from the tissues. It would appear not to be a normal process in Hydroids, but to be a result of (*a*) the power of the endoderm cells to ingest solid particles, (*b*) the presence of abnormally situated cells which have migrated out of the tissues in the neighbourhood of the endoderm cells. Phagocytosis of this nature appears to occur both in the case of emigrated endoderm cells of the zooid, and of normal endoderm cells in the walls of the stem.

Resorption (as a result of emigration of dedifferentiated cells) may be regarded as the most primitive method of eliminating tissues in Metazoa. Even at the outset it may be secondarily accompanied by a low form of phagocytosis. Later the function of phagocytosis is assigned to special cells, and the dedifferentiating tissues are attacked by these at a much earlier stage in the process. The limited extent of phagocytosis in low forms is also to be seen in Planarians (Vandel, 1921).

Resorption is in the first instance a direct result of exposure to unfavourable agencies, but may be utilized later as a method of accomplishing normal processes of the life-history. This appears to be the case in Echinoderm metamorphosis (Huxley, 1922).

The stimulus in the case of Obelia is a certain concentration of toxic products in the water. It is the same stimulus which causes dedifferentiation in Clavellina and Perophora, and also in Echinoderm larvae, Planarians, Sponges, and Protozoa (Lund, 1917).

Hunger is another stimulus which may cause dedifferentiation ; and of course may act differentially. Dedifferentiation caused by hunger has been found in Hydra (Schultz, 1906), Echinoderm larvae (Runnström, 1917), &c. Starvation will again act by interfering with general metabolic processes. As an example of the differential action of hunger, it may be mentioned that in starved tadpoles, localized dedifferentiation

often takes place in one or more places on the tail (unpublished observations, J.S.H.). Dedifferentiation is not, however, followed by resorption in this case, at least before death.

Müller (1913, 1914) has conducted an elaborate series of experiments with various species of Hydroids. He finds that dedifferentiation and resorption may occur not only in hydranths but also in gonophores and in portions of hydrocaulus. There is a delicately balanced equilibrium between various parts of a system; in a compound system, whether gonophore, hydranth, or stem shall be resorbed depends (*a*) on the relative sizes and (*b*) on the ages of the sub-systems (cf. Perophora, Huxley, 1921 *b*). Wounds will induce gonophores to dedifferentiate and be resorbed.

The quantitative action of poisons in accelerating dedifferentiation and resorption, and the fact that in severed zooids dedifferentiation may proceed independently of resorption, are points on which we would like to lay stress.

Summary.

1. Confirmation is given of the results of Loeb, Thacher, Godlewski and Gast, and others, in showing that the hydranths of hydroids (in this case Obelia and Campanularia) when exposed to unfavourable conditions proceed to dedifferentiate and to be resorbed, wholly or mainly, into the stem.

2. Exposure to toxic agencies accelerates the process. Too great concentration of poison kills the zooids before dedifferentiation starts. Below the death-point, the acceleration is proportional to the concentration.

3. The effect is non-specific, both KCN and $HgCl_2$ producing the same result as prolonged exposure to laboratory conditions.

4. When zooids are separated from the stem, resorption is impossible. Dedifferentiation, however, proceeds until an ovoid undifferentiated body packed with cells is produced.

5. The tentacles are first affected, then the hypostome. In early stages, separate tentacles may fuse locally. Stumps of tentacles are, however, still present after the hypostome has quite disappeared. The body becomes ovoid, then spherical, and is finally reduced to a minute pigmented dot.

6. The surface tension of the dedifferentiated zooid causes the emigrated zooid cells to flow into the stem. In later stages spontaneous pulsations of the stem and of the zooid (these possibly not spontaneous) occur.

7. Dedifferentiation of the tissues of the tentacles starts at the tip. Progressive histological dedifferentiation of the endoderm cells can thus be clearly followed in a single section.

8. Only after the mesoglaea at the base of the tentacle has ruptured can the contents be resorbed (confirming Thacher).

9. Cnidoblasts with nematocysts can be distinguished within the gastrovascular cavity as resorption proceeds. They may also be seen phagocytosed within large cells, presumably immigrated endoderm cells.

10. The dedifferentiation is regarded as due to interference with general metabolic processes, and especially with the production of the energy needed to maintain form against surface-tension.

11. Resorption is regarded as the natural result of dedifferentiation when there are adjacent cavities into which the cells can migrate. In higher forms it has been largely replaced by phagocytosis.

EXPLANATION OF PLATE 26.

Acknowledgements are due to Mr. Chesterman, of the Anatomy Department, Oxford, for assistance in the preparation of the microphotographs.

Obelia geniculata.

Fig. 1.—Longitudinal section through a normal zooid. ×100.

Fig. 2.—First stage of resorption. ×115.

Fig. 3.—First stage of resorption (slightly later than Fig. 2). ×130.

Fig. 4.—Third stage of resorption. ×146.

Fig. 5.—Fourth stage of resorption. ×240.

Fig. 6.—A tentacle of a normal zooid showing the differentiation of the endoderm. ×300.

Fig. 7.—A tentacle of a zooid in the first stage of resorption showing beginning of dedifferentiation in the distal endoderm cells. Also note especially the reduction in width of the endoderm cells. ×420.

Fig. 8.—Cells and nematocysts in a zooid in the fourth stage of resorption. ×420.

Fig. 9.—Fusion of tentacles. First stage. ×270.

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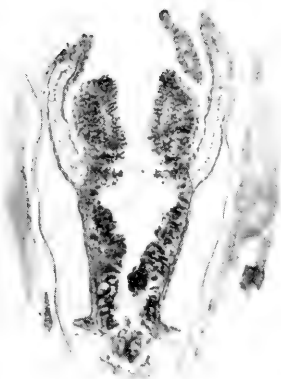

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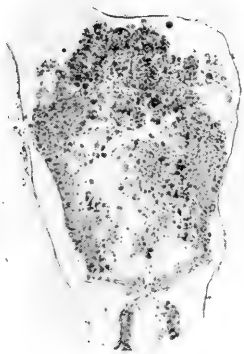

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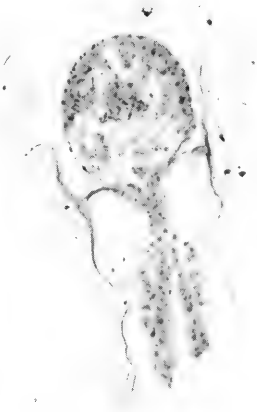

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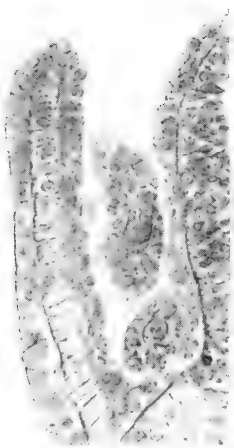

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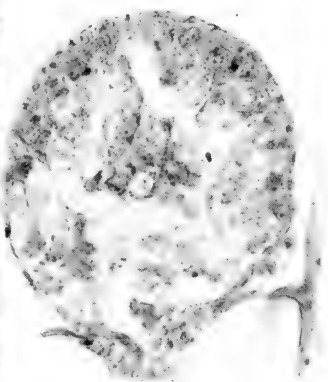

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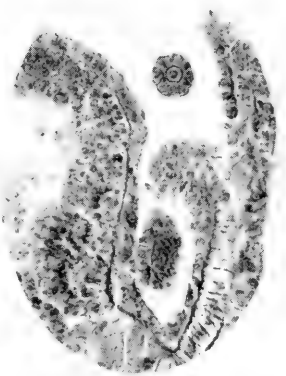

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The Cleavage of the Egg of Lepidosiren paradoxa.

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With 12 Text-figures.

The process of cleavage in Lepidosiren was first described by Professor Graham Kerr in his paper on the development of the external features of this animal in the 'Philosophical Transactions of the Royal Society', B, vol. excii, 1900. Since the date of Professor Kerr's expedition additional material has become available from which it has been possible to make up a more completely graduated series of segmentation stages. The following paper deals with this material and may be regarded as supplementing Professor Kerr's paper. In referring to the various stages I use the same numbers as Professor Kerr used in the paper already mentioned and also in his 'Normentafel'.[1]

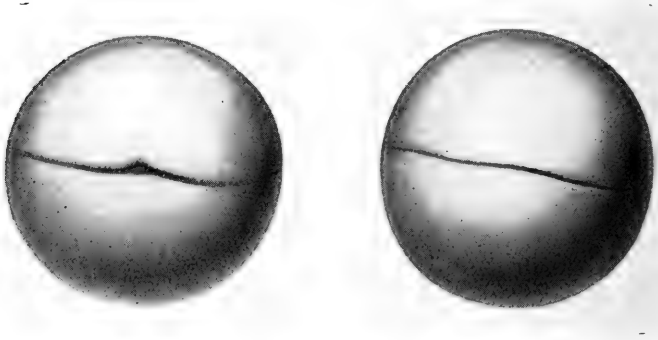
Stages 2–3.—Professor Kerr's earliest cleavage stage (2) showed the first meridional furrow bisecting the finer-grained apical cap but not extending beyond its margin; while in the second stage figured by him (3) the first meridional furrow had spread down to the equator of the egg, while the second meridional furrow at right angles to the first extended to just beyond the margin of the apical cap.

Amongst the new material is an egg (Text-fig. 1) in which the first meridional furrow has extended right to the abapical

[1] Keibel's 'Normentafeln zur Entwicklungsgeschichte der Wirbeltiere', Zehntes Heft, 1909.

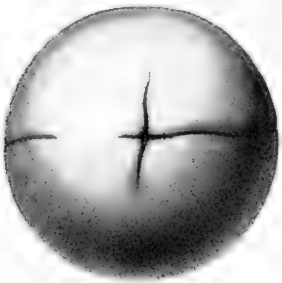
pole while the second furrow has not yet made its appearance. Another egg shows the first meridional furrow extending nearly to the abapical pole and a very faint second furrow

TEXT-FIG. 1.



a. View of apical hemisphere. *b*. View of the abapical hemisphere.

TEXT-FIG. 2.



Apical hemisphere.

intersecting it and extending as far as the margin of the apical cap. A third egg (Text-fig. 2) is somewhat peculiar, inasmuch as the first meridional furrow shows a break in continuity in the region of the apical pole.

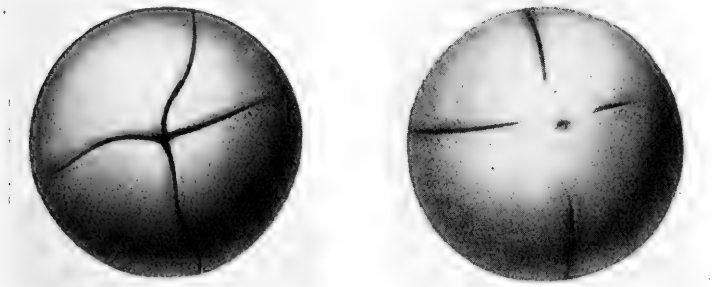
Three other eggs of the same stage of development agree in the main with those already described, except that the

first furrow is not strictly meridional but is displaced outwards some little distance from the abapical pole.

On the whole the evidence of the seven eggs obtained of this stage goes to indicate that the normal procedure of the egg of Lepidosiren is that the first meridional furrow is completed before the second furrow at right angles to it begins to make its appearance.

Stages 3–4.—Five eggs belong to the commencement of this period during which the egg is normally sub-divided by the completion of the first and second meridional furrows into four

TEXT-FIG. 3.



a. Apical view. *b*. Abapical view.

approximately equal parts, and the third set of furrows—vertical—make their appearance. In two eggs out of the five the first and second meridional furrows are alone present and perfectly normal. Two other eggs exhibited a feature mentioned in an earlier stage, the continuity of the furrows being very faintly marked and in some places quite obliterated. In one of the two eggs one of the furrows in the region of the apical pole shows quite faintly marked; in the other egg (Text-fig. 3) in which neither the first nor the second furrows had quite reached the abapical pole, the continuity of the first meridional furrow is broken at one side and again at the abapical pole.

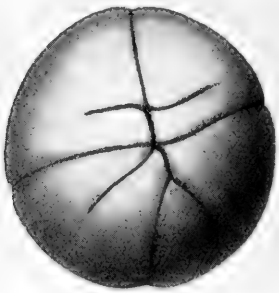
The last specimen of this particular stage shows the tendency

before mentioned of displacement outwards of the meridional furrow from the abapical pole.

Towards the end of this period the greater rapidity of the segmentation at the apical pole becomes more marked, and the four vertical furrows make their appearance.

Four eggs at about stage 4 were available and two showed similar development, two of the new furrows showing the normal ' vertical ' position and maintaining a vertical course, while the other two furrows, instead of being vertical, have their point of origin displaced towards the apical pole so that

Text-fig. 4.



Apical view.

they have become meridional, bisecting two of the quadrants. The study of sections show that all the eight nuclei are in the metaphase stage of mitosis ; therefore, up at least to this stage, mitosis is synchronous throughout the egg. This paper is not intended to deal with nuclear phenomena, but it will be of interest to show an accurate drawing (Text-fig. 5) of a nucleus from the segmenting egg of Lepidosiren as illustrating the extraordinarily favourable nature of these nuclei for cytological investigation.

In the egg shown in Text-fig. 6 the two meridional furrows are complete. On one side of the egg (that shown in the upper half of Text-fig. 6) two vertical furrows are present, exactly in line with one another so that they have the appearance of

one continuous furrow cutting across the meridional furrow; while on the other half of the egg (Text-fig. 6, lower half) two small furrows, likewise vertical, are just beginning to develop,

TEXT-FIG. 5.

Nucleus of segmenting egg of Lepidosiren in early stage of mitosis. The nucleus was traced in outline under a Zeiss $\frac{1}{12}$″ homogeneous immersion objective with ocular 18, and then worked up in detail under 3 mm. apochromatic homogeneous immersion objective with Bitumi binocular eye-piece. An attempt has been made to bring out the stereoscopic relief of the original by shading the nucleus as if it were isolated.

The divisions of the scale represent hundredths of a millimetre.

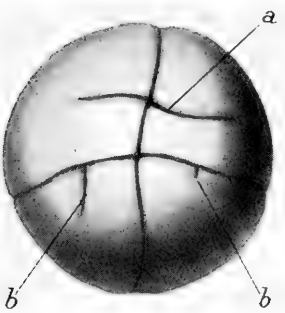
taking their origin however from the other meridional furrow than that from which the first two started.

In the fourth egg at this stage the segmentation furrows at

the apical pole are for the most part obliterated, although the fact that down at the abapical pole the two meridional furrows quite visibly intersect indicates that the egg belongs to this stage.

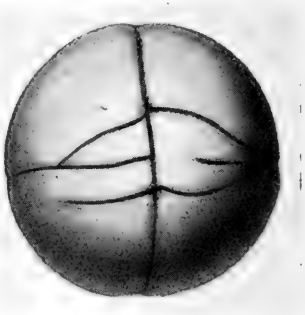
Stages 4–5.—Altogether six eggs were found to be approximately at this stage, and five gave evidence of further irregularities in development. One which belongs to the beginning of this period (Text-fig. 7) showed, again, lack of continuity of one of the primary meridional furrows in the region of the apical pole. Two secondary furrows arising

TEXT-FIG. 6.



Apical view.

TEXT-FIG. 7.



Apical view.

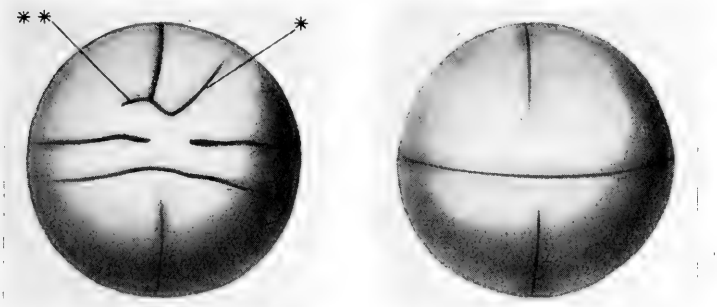
from the same meridional furrow (see upper half of Text-fig. 7) proceed in a latitudinal direction and reach the other meridional furrow, while of the corresponding furrows of the opposite side one is actually vertical in direction (see left-hand furrow in lower half of Text-fig. 7), and the other, assuming a rather latitudinal direction, runs into the meridional furrow of that side.

A second egg similar to the one already described showed again the discontinuity of the meridional furrows; neither at the apical nor the abapical poles is there any sign of intersection. Vertical furrows appear in one-half of the egg (lower half of Text-fig. 8, *a*), and in the other (upper half in Text-fig. 8, *a*) a short latitudinal groove grows out from the meri-

dional furrow as seen on the right side of the figure. Before this furrow has progressed very far another furrow * (Text-fig. 8, *a*) [1] grows down from it towards the abapical pole bisecting the quadrant; on the other side of that same meridional furrow there is an indication of a similar latitudinal furrow (* *).

The third specimen showed practically the same arrangement and development of furrows as the egg shown in fig. 5 of Professor Kerr's 'Philosophical Transactions' paper. One

TEXT-FIG. 8.



a. Apical view. *b*. Abapical view.

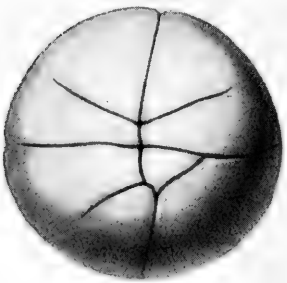
meridional furrow is rather displaced, and one of the furrows of the third set growing from it is definitely latitudinal (Text-fig. 9, right lower quadrant).

To quote one more example illustrative of irregularity of segmentation: in the egg represented in Text-fig. 10 one of the two primary meridional furrows (the one which is horizontal in the figure) is perfectly normal but the other shows a distinct break at the apical pole, the portion next the apical pole in one hemisphere (that which is above in Text-fig. 10) having undergone a distinct displacement. As seen in the figure the amount of this displacement increases as the apical pole is approached.

[1] Which is however in this case uncomplicated by any branching or distortion.

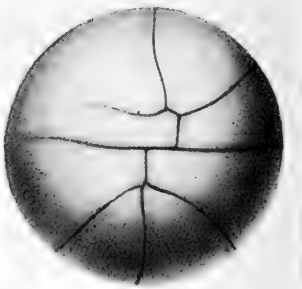
The fifth specimen, illustrated by Text-fig. 11, is interesting mainly from the fact, not shown in the drawing, that one of the meridional furrows is markedly displaced outwards from the region of the abapical pole.

Text-fig. 9.



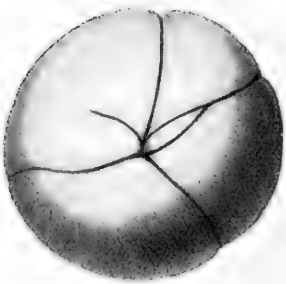
Apical view.

Text-fig. 10.



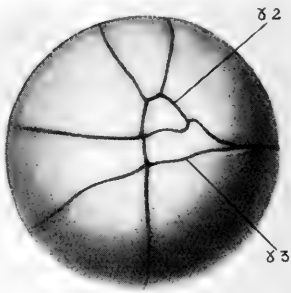
Apical view.

Text-fig. 11.



Apical view.

Text-fig. 12.



Apical view.

The last egg belonging to this period of development (Text-fig. 12) illustrates very clearly the kind of irregularities in the position of the furrows which do so much to obscure the process of segmentation from now on. In the right half of the figure the two furrows which would normally be vertical

(ɣ 2 and ɣ 3) are seen to be displaced in each case so that their outer ends reach one of the meridional furrows, and in the case of one of them the furrow has become practically latitudinal. Owing to the increasing frequency of such displacements combined with increasing ' loss of step ' of the various blastomeres in their successive fissions, the regularity of the segmenting process becomes from now onwards completely obscured.

In conclusion I desire to thank Professor Graham Kerr both for providing me with the material on which these notes are based, and also for his kind supervision during the course of the work. I should like further to thank the Carnegie Trust for providing the illustrations to this paper, and Mr. A. Kirkpatrick Maxwell for the care and skill with which he has carried them out.

The Morphology of the Nudibranchiate Mollusc Melibe (syn. Chioraera) leonina (Gould)

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With Plates 27 to 37.

Contents.

I. Introduction.

A considerable part of this work was done under the direction of Professor Trevor Kincaid, at the University of Washington (Seattle), 1914–15. It was continued at Columbia University, 1918–20, and at Woods Hole, 1919 (summer). Dr. T. C. Frye, the Director of the Puget Sound Biological Station at Friday Harbour, Washington, had some new material collected during the summer of 1919, and sent to me at Woods Hole. Upon this material, and the previous data together with new material collected at Friday Harbour, during the summer of 1921, this paper is concluded.

In three previous communications (1919, 1921) I described the method of feeding, the kind of food, method of swimming (1922), and the colour, of Melibe leonina (Gould) as well as the distribution of the family Tethymelibidae[1] Bergh (1890, 1892: 1039–43). I also made an extensive review of the literature on the nudibranchiate molluscs, particularly the Cladohepatica. Several misprints, relative to year, volume, and page, occurred in the literature. These have been corrected. I hope that the references as printed in my papers may be of help to other workers in this field on the molluscs.

[1] Attention should be called to the fact that the name Tethys as applied to a Nudibranch is incorrect. This was established in 1895. (Vide H. A. Pilsbry, "Classification and Phylogeny of Tectibranchiata", 'Manual of Conchology', 16: i–vii, 1–262, 74 plates.) The name Tethys was first given to a Tectibranch by Linnaeus. (Vide 'Systema Natura', 10th Edition, 1758, p. 653.) Consequently the family name Tethymelibidae must be rejected from the nomenclature of Nudibranchiata. The Nudibranch 'Tethys' has apparently no name of its own.

II. Acknowledgements.

The writer wishes here to express sincere thanks to Professor Trevor Kincaid for helpful suggestions during the first period of this work; to Dr. H. L. Osterud for collecting and fixing new material in 1916; to the Curator of Books and Literature, Dr. R. W. Tower, of the American Museum of Natural History, for unfailing kindness during the review of the literature; and to the Director of the Puget Sound Biological Station, Dr. T. C. Frye, for every co-operation and assistance while at the station, in the summer of 1921.

III. On the Status of Chioraera Gould.

Bergh's description of various species of Melibe (1875*b*, Melibe capucina, M. rangii; 1879*a*, M. vexillifera; 1884, M. papillosa; 1888, 1890*b*, M. ocellata; 1902, M. bucephala; and 1908, M. rosea Rang), emphasizes the following as Melibean characteristics: 'Bulbus pharyngeus cum mandibulis ut in Phylliroidis; margo masticatorius mandibulae fortiter dentatus' (1875*b*: 362). Perhaps the only exception to this may be found in the species collected at the mouth of the Columbia River, in the State of Washington (1904), in which case the author is not sure of the mandibles. He says: 'Bulbus pharyngeus lingua destitutus. . . . Die Mundröhre und der Schlundkopf schienen sich wie sonst bei den Meliben zu verhalten. . . .' I have previously called attention (1919, 1921) to the possibility that this species may be the same as the one described by Gould (1852) from the Puget Sound region. Not all Melibes have the same characteristics, as indicated by Bergh; this is also shown by Alder and Hancock (1864), and substantiated by Eliot (1902). The generic characteristics as enunciated by Bergh (1875*b*) do not necessarily hold, even though this author thinks that Hancock's (Alder and Hancock, 1864) description is incorrect. Bergh (1875*b*: 363, 364) says: 'Es kann kaum bezweifelt werden, dass die von Hancock untersuchte Form mit der von mir besprochenen congenerischist. Es werden sich daher die bei

dem englischen Verf. vorkommenden, von den untenstehenden abweichenden anatomischen Angaben wahrscheinlich als unrichtig erweisen . . . Besonders wird solches wohl der Fall sein, wo Hancock den Anfang des Verdauungskanals bespricht: "The buccal organ is provided with neither tongue, jaws nor collar; it is not by any means very distinctly marked, formed as it were by a mere enlargement of the oesophagus, and having little or no increase of muscular power." '

But Eliot (1902) verifies Hancock's claim when he writes: 'I also found Alder and Hancock's description of the internal anatomy correct, particularly as regards the absence of jaws. . . . Mr. Crossland and I have . . . dissected several specimens of Melibe fimbriata, and in all failed to detect any trace of jaws.'

Gould's Chioraera leonina (1852) corresponds very closely in the general anatomy to that of Melibe fimbriata Alder and Hancock, (1864); this is also true in regard to the species discovered by Rang (1829) and subsequently described by Bergh (1863, 1871, 1875*b*, 1879*a* 1884, 1888, 1890*b*, 1902, 1904, and 1908). The only difference is on the point in regard to mandibles. Some authors, Rang, Gould, Pease, Cooper, and Fewkes, do not touch on this point, and for that reason one cannot tell whether the particular specimens with which they dealt actually had such organs. With the exception of the mandibles, all the generic characteristics as set forth by the earliest writers on this type of the molluscs agree (Rang, 1829; Gould, 1852; Pease, 1860; Cooper, 1863; Alder and Hancock, 1864; de Filippi, 1867; Tapparone-Canefri, 1876; and Fewkes, 1889; as well as the numerous descriptions of Bergh, 1863–1908). The discovery of the genus Melibe by Rang (1829) seems to have been unknown to Gould (1852), who created a new genus (Chioraera) for this type. Cooper (1863) and Fewkes (1889) employed the nomenclature of Gould. The generic characteristics as enunciated by the original author for Melibe, Rang (1829), are practically identical with those set forth by Gould twenty-three years later for Chioraera. Tryon, Jr. (1883: 328), without

stating a reason, classifies Chioraera as a synonym of Melibe. Owing to the fact that Gould and also Cooper were ignorant of the actual discovery of the genus Melibe, the name Chioraera was invented by Gould and subsequently used by Cooper. The name is, in fact, a mythical term that is related in meaning to the former. Bergh (1904), describing a species from the territory of Gould, Cooper, and Fewkes, does not hesitate to employ the nomenclature of Rang (1829), so similar is this form to the Melibes from other parts of the world. No other author, except Bergh, gives mandibles as a generic characteristic. That is, this feature is not observed by Rang (1829), Gould (1852), Pease (1860), Cooper (1863), de Filippi (1867), Tapparone-Canefri (1876), or Fewkes (1889). Although Melibe Rang (1829), and Chioraera Gould (1852), differ somewhat in shape, they are very similar in most other respects. Both have a series of papillae on each side dorsolaterally; a large hood, cowl, or veil; a pair of tentacles (the so-called rhinophoria) on the veil; the veil fringed with at least two rows of cirrhi; and a narrow grooved foot which is blunt in front and pointed behind; the head distinctly separated from the body, and in each case it is very large; the gizzard is lined with a 'keratinized' secretion which protects the delicate epithelium, the so-called stomach-plates of Alder and Hancock, or 'Magenzähne' of Bergh; these two types are carnivorous; both are pelagic; and both are distinctly cladohepatic. Therefore the species of the American west coast which falls within this description must be the same genus, i.e. Melibe. The effort, therefore, to build further on the nomenclature of Gould, as was done by Cooper (1863), Fewkes (1889), and more recently by Heath (1917), seems to me to be indefensible. And, owing to the fact that the genus Melibe may either possess mandibles (Bergh, 1875*b*) or not, (Alder and Hancock, 1864; de Filippi, 1867; Tapparone-Canefri, 1876; Eliot, 1902), the generic description may be modified to read, in part, Bulbus pharyngeus aut cum mandibulis aut sine mandibulis; radula et lingua destitutus. None of the authors (Gould, Cooper, Fewkes, Heath) who has not

employed the nomenclature of Rang for this type, has described mandibles, and O'Donoghue (1921) states: 'The radula and jaws or any representatives of such structures are entirely absent.' Although O'Donoghue (1921) also employed the nomenclature of Gould for the genus Melibe, in a subsequent letter to me he states: 'I have quite given up Chioraera as a name.' In recent publications by this author (1922: 125; 1922*a*: 165) and by O'Donoghue (1922: 134) it is suggested in a foot-note that Melibe leonina would be a 'better' name than Chioraera leonina. Neither Cooper, Fewkes, nor Heath made an intensive study of the type; this is evident from their descriptions. A careful study of Gould's Chioraera has brought out sufficient reason to merge it with Melibe as indicated by Tryon, Jr. (1883), Bergh (1908), and Agersborg (1921 *a*). The structures and the general characteristics of Chioraera leonina Gould, correspond in many details with those of the Melibes of Rang, Bergh, et al. For this reason I have adopted the name as indicated by Tryon, Jr., and also suggested by my friend, Professor Trevor Kincaid, viz. Melibe leonina (Gould) as indicated in the title of this work. (Vide Agersborg, 1921 *a*, 1922 *a*, 1923.)

IV. Melibe leonina (Syn. Chioraera leonina Gould).

The type of the genus Melibe was discovered at the Cape of Good Hope and was described by Rang in 1829. Since that time a number of species (vide supra) have been added by various authors.[1] In 1852 Gould described Melibe (s. Chioraera) leonina from Puget Sound, founding for it the genus Chioraera, now merged with Melibe. In 1914 I observed this animal at the Puget Sound Biological Station (vide Agersborg, 1916, 1919, 1921, 1921 *a*, 1922, 1922 *a*, 1923).

[1] My designation of Melibaea australis Angas (1864), as Melibe australis (Agersborg, 1919, 1921) is not justified, as indicated by the description of Angas. His description seems to fit the genus Doto (vide Kjerschow Agersborg, 1921).

Since the descriptions of Gould and also of Cooper and Fewkes, each of whom described a species from the American west coast, are rather incomplete, and since the anatomy had not been worked out, I felt that there was sufficient reason to engage in such an investigation upon this very interesting animal. As a result of this work I have succeeded in bringing to light some points of considerable zoological interest.

The body-substance of Melibe leonina appears as a mass of brown jelly, when the animal is alive or freshly caught: in the aquarium it turns practically transparent; when it has been preserved in alcohol or formaldehyde it gradually loses its brown colour, and becomes almost white. O'Donoghue (1922: 125) says: 'Hundreds of individuals of this species have been seen, but there is practically no variation in colour. In some forms the yellowish or whitish-grey jelly-like body may be tinged with pale brown but hardly sufficient to notice.' However, while this is really the general colour of Melibe leonina, I have also seen it deep green in colour (vide Agersborg, 1922*a*: 439–42). Gould (1852), in his original description of this species, says: 'Body limaciform, smooth and of a pearly and whitish colour, finely reticulate with orange.' The colour of the animal is caused by an extensive ramification of the brownish-coloured liver in the body-wall (Pls. 1 and 30, figs. 1, 2, 4, 7, 18–23, 25); a ramification which extends to the hood, the tentacles, the papillae, and to the ectoderm of the rest of the body. The colour of nudibranchs has sometimes been attributed to their food, Hecht (1895), Eliot (1910); but Alder and Hancock (1845) ascribe the colour to the liver and the gonads. Bergh (1879*a*: 163, 165), describing Melibe vexillifera, says in fact: 'Durch die dünnen Körperwände schimmerten, besonders an den Seiten, die denselben angehörenden, dicht an einander liegenden, schmalen, weisslichen, parallellaufenden Längsfasern hindurch; ferner undeutlich die Eingeweide, besonders Theile der Leber und die vordere Genitalmasse; . . . Die Leber wie bei anderen Meliben eine lose, gelblichweisse Masse, welche vorne an den Magen reicht, hinten sich bis an das Ende der Eingeweidehöhle

erstreckt.' From this one might infer that the author was describing the species from preserved material; for the liver is brown in colour as a rule, and from it Melibe obtains most of its colour. The internal organs, however, may be partly seen through the body-wall in living specimens of M. leonina (Pl. 27, fig. 2). Gould describes the brownish ramifications of the papillae as vascular; these, however, are branches of the liver. He also says that the tentacles are 'destitute of venation', referring no doubt to the hepatic branches which are very abundant everywhere. The hepatic branches are less abundant in the tentacles but they are not wholly absent.

1. The Head or Veil.

The head of M. leonina is very prominent because of its exceedingly large veil or hood. This is modified by two rows of cirrhi (Pls. 27, 28, figs. 1, 3, 10, 17) which fringe its edge, and by a pair of ear-like tentacles (Pl. 27, figs. 1, 2, 3). The cirrhi of the outer row are much larger and far less in number than those of the inner row. The average, taken from a number of specimens, was 48 in the outer row, and 123 in the inner row, or 2·56 small cirrhi to 1 large. The outer row does not extend entirely around the rim of the hood, but, in an animal about 6 centimetres long, terminates about 1 centimetre from the mid-ventral line. The inner row extends all around the periphery of the hood, although the last three cirrhi, on each side of the mid-ventral, are rudimentary (Pl. 28, fig. 17, *Icr*, *Icr*[1]). A large veil or hood, except in M. ocellata Bergh (1890*b*), is a common thing for Melibe Gould (1852), Alder and Hancock (1865), Bergh (1875*b*, 1902, 1904), Eliot (1902). In M. bucephala, Bergh (1902), 'The edge of the head is rather thin above and almost smooth; its outer parts, however, are thick, inwardly somewhat refolded or convoluted, and provided with several, mostly perhaps about five, close-set series of cirrhi, which are displaced among each other; these cirrhi are conical, somewhat constricted at the base, the innermost ones are the larger, toward the outside they decrease

regularly in length.' Gould (1852), describing M. leonina, says: 'The mouth is inferior and surrounded by a series of long cirrhi, each of which has an independent motion.'

(1) The Cirrhi.

The cirrhi (Pl. 27, figs. 1, *C*, 10, *Ic*, *Oc*, Pl. 28, 17, *Irc*, *Orc*) have an inner median axis (Pl. 28, figs. 11, 12, *Cg*, 13, *Cga*) from which radiating fibres pass to the periphery (*Rf*). The inner axis seems to consist of a series of nerve ganglia from which fibres radiate to the periphery of the cone-like cirrhus, ending in the basal layer of the epidermis (Pl. 28, fig. 11, *Oe*, *Ie*). In other words, a cirrhus is a conic structure having an epithelial wall resting upon a delicate connective-tissue framework, with an inner axis which passes from the apex to the base of the cirrhus, and from this axis fibres radiate to the epithelial walls of the cone. These fibres have the resemblance of nerve-fibres, particularly in their relation to the cells in the ganglionic axis and to the epithelium of the epidermis. In one of the angles, formed by the radiating fibres at the axis, is a large bladder-like structure around which the fibres pass, and at the periphery are placed large and small cells (Pl. 28, fig. 13, *Pc*) the inner part being reticular in structure (*Ir*) and containing apparently no cells, or a few only (Pl. 28, figs. 11, 12, 13, *Ir*). The cirrhi are probably tactile or gustatory in function. Something to that effect was demonstrated experimentally (Agersborg, 1922*a*: p. 441). Sedgwick (1898: p. 366) writing on the gasteropods in general and opisthobranchs in particular says: 'Tactile organs are represented by the tentacles, the edges of the lips, which are often folded (labial palps), the tentacular and lobe-like prolongations which are found here and there on the head, mouth, and foot.' It should be remembered that there are sometimes two pairs of tentacles in nudibranchs; the anterior pair being the one referred to here; the posterior is the so-called rhinophoria of many authors, which, however, do not seem to be olfactory in function (Agersborg, 1922*a*: 423–44).

(2) The Dorsal Tentacles or 'Rhinophores'.

On the outside of the veil, in M. leonina, is a pair of tentacles, supposed to be equal to the posterior pair in, e.g., Aeolidia Cuvier (1798). According to the original description of the genus Melibe by Rang (1829) the tentacles are 'au nombre de deux, situés à la base du voile, très allongés, coniques, terminés par une petite capsule, de laquelle sort un organe conique et rétractile'; Gould (1852), in his description of Chioraera leonina (s. Melibe leonina), points out: '. . . tentaculae cephalicae foliatae, retractiles;' and Pease (1860) for M. pilosa says: 'Tentacles on the posterior portion of the veil rather remote, small, ovate, closely and transversely lamellated and retractile into long trumpet-shaped sheaths, which are furnished with laciniated appendages.' Again, Tapparone-Canefri (1876) for Jacunia (s. Melibe) papillosa de Filippi, states: 'Tentacula (Rhynophoria) laminata, tenuia, apice obtusiuscula, retractilia, e vagina caliciformi angusta vix proeminentia.' Cooper (1863) and Fewkes (1889) are content with the description of Gould; neither of them mentions tentacles. Alder and Hancock (1864) refer to these organs as dorsal tentacles. The largest part of the tentacles in Melibe is the tentacular stalks; they are wedge-shaped bodies (Pl. 27, figs. 3, 8) somewhat rounded at their base. The wedge is like a broad axe, the edge of which is a little curved. They are arranged at right angles to each other, and this angle would intersect posterior to their base in the mid-dorsal line of the hood. Along the edge of the curved wedges are slits, in one of which, on the inner part of the curve, i.e. not on the apical part of the tentacle—except when it is expanded—is a small organ (Pl. 27, fig. 8, *Rh*), the real tentacle, that may be retracted below the surface of the wedge-shaped tentacular stalks (vide Agersborg, 1923, figs. 4, 5, *pa*). Gould, in describing these, says: 'On the top of the head are two foliate expansions destitute of venations, which answer to the true tentacles; on their anterior edge is an opaque whitish papilla, presenting something of a spiral or lamellar

structure; they are sometimes wholly retracted within a permanent sheath.' At the base of these tentacular organs, when they are retracted (Pl. 27, fig. 8 and Pl. 29, fig. 15, *K*), is a small knob. In sections (Pl. 29, fig. 15) the lamellar structure is indicated by certain lobations along the outer part (*S*). The knob (*K*) seems to be made up of a mass of large and small nerve-cells (Pl. 29, fig. 16), which fibres connect with similar cells distal in position to the former (*Nc, Nes, Nfb, Nfi*), and finally by innervation in the epithelium of the lamellar external parts of the organ (*Nfp*). From the distal border of the knob-like ganglion, fibres communicate with the base of the tentacle; these fibres are not made up of nerve-fibres only but also of muscle-fibres (*Nmf*). This is known from their staining reaction and also from the fact that some of these fibres communicate with fibres within the organ which are decidedly nervous, while the other fibres terminate on the organ. The muscle-fibres help to retract the organ below the surface of the tentacle, that is, to withdraw the tentacle within its stalk, which in that case serves as a sheath; the nerve-fibres to convey stimuli. There is no permanent sheath, as indicated by Gould, save that part of the tentacular stalk which surrounds the organ, and acts as a sheath when the tentacle is retracted.

The function of these tentacular organs in nudibranchs according to various authors is olfactory. Thus, Alder and Hancock (1845: 19) say: 'The dorsal tentacles are the organs of smell, and, judging from their development, this sense must be more acute in most of the nudibranchs than it is in many other molluscs, with the exception, perhaps, of Nautilus.' Hancock and Embleton (1852: 242), discussing a Doris, say: 'The dorsal tentacles, which have never been observed to be used as tactile organs, we believe to be the seat of the sense of smell; and this belief is strengthened when we reflect that these sense organs are most highly developed and minutely laminated; that they are plentifully supplied with nerves from the ganglia placed in front of all the rest of the cerebral masses; that they are externally covered with

vibratile cilia, and so placed on the head as easily to receive impression from any odorous particles that may be mingled with the circumambient water.' Likewise, Jeffreys (1869) claims olfaction for the dorsal tentacles, but he says : '. . . olfaction in these animals probably is not so much to assist in the discovery of alimentary matters, as to give warning of the unhealthy state of the surrounding medium, arising from putrescence or other causes. . . . and its outer surface, in all the nudibranchs, is provided with vibratile cilia.' The tentacles in M. leonina, however, are not ciliated. Bergh adopts the term 'rhinophoria' for the dorsal tentacles, not only indicating in that way the function, but he claims, in fact olfaction to be their function. Later writers, Lang (1896 : 48, 103), Sedgwick (1898 : 366), seem to agree on this point. Copeland (1918 : 177–227) demonstrated experimentally that the monotocardiate prosobranchs Alectrion obsoleta and Busycon canaliculatum respond to stimulations by dilute food extracts and materials emanating from distant food ; he thinks that the snails do not find food by coming upon it accidentally, but are directed to it by movements brought about through stimulations of the olfactory organs with odorous substances conducted to the receptor in varying concentrations by the moving siphon. By means of an olfactory apparatus consisting of a single organ of smell associated with a siphon terminating in a shifting 'nostril', for sampling the surrounding water and its contents, the snail is as successfully directed toward distant food as an animal which, like the dogfish, possesses paired olfactory organs and fixed nostrils. After the osphradium in Busycon was destroyed the snail failed to respond to dilute food materials, but a year later, when the lamellae of the organ were partly regenerated, the scenting responses returned. The osphradium, therefore, is an olfactory organ. This author claims further that taste in the snail (Busycon) is a diffused sense as compared with olfaction, and that a large portion of the surface of the snail possesses this sense. But Arey (1918 : 531) distrusts the capability of snails to analyse chemical stimuli as discrete sensations, and thinks that it would be safer to avoid referring in their case

to a sense of taste and smell at all, or even to a common chemical sense, but rather to designate the particular senses in question a general chemical sense. Moreover, Arey working experimentally on several nudibranchs, Chromodoris zebra, Facelina goslingi, Elysia crispa, and Fiona marina, found that there was nothing in the tests which he applied to these animals that connected the rhinophores with olfaction, with the exception perhaps of Facelina whose non-retractile rhinophores react by a lashing withdrawal and more vigorously than the oral tentacles when stimulated by oil of pennyroyal, carbon bisulphide, and anilin oil. All these forms, however, responded to tactile stimuli. Like Copeland, he found that the general body-surface also responded to chemical stimuli. Crozier and Arey (1919 : 301) elaborated on this by stating that in Chromodoris the rhinophores and the oral tentacles are in a general way the parts most sensitive to chemical stimuli. Again, as regards the rhinophores, these authors (1919 : 278–81) found that while Chromodoris zebra may creep in an entirely normal fashion after the rhinophores have been removed, it loses its power of orientation to the water current. In other words, these authors claim that to currents of adequate velocity the nudibranchs are negatively rheotropic and that the rhinophores are the prime receptive organs for this kind of reaction. However, as I have pointed out elsewhere (Agersborg, 1922*a* : 432, 439), the dorsal tentacles (rhinophores) of Hermissenda opalescens (Cooper),[1] do not seem to have a rheotropic function, because specimens with one or both of the dorsal tentacles removed oriented as easily and moved against the current as did the normal individuals. It did not seem to make any difference whether the dorsal tentacles were present or not. At any rate, the rhinophores or dorsal tentacles do not seem to be ' rheotropic ' in Hermissenda. Copeland, Arey, and Crozier found that the types with which they worked were more sensitive around the anterior than elsewhere on the body. Such a specialization of the integuments is also the case

[1] The correct name is Hermissenda crassicornis Eschscholtz. Vide C. H. O'Donoghue (1922), ' Nautilus ', 35 : 74–7.

in Hermissenda, Dendronotus, and Melibe (Agersborg, 1922*a*: pp. 423–44). Indeed, as recently brought to light by Gross (1921), on Nereis virens Sars, the general integuments of this organism are sensitive to chemical stimulation with a localization or concentration of the chemical sense in the palps and tentacles, a circumstance correlated with the rich innervation of these appendages and the relation of their nerves to the brain. However, I have not yet found any specialized receptors either in the dorsal tentacles or in the cirrhi of nudibranchs. Their function, therefore, as far as the nudibranchs are concerned, may not be so definite as previously indicated. For this reason, and because of the facts brought to light by experimental evidence (Copeland, 1918; Arey, 1918; Arey and Crozier, 1919; Agersborg, 1922*a*), I have used the original name tentacles, as employed by Alder and Hancock (1845, 1864), Hancock and Embleton (1848), and Gould (1852), rather than the suggestive 'rhinophore' as adopted by Bergh and freely used by subsequent writers. For, although the tentacles of the hood are highly specialized as indicated by Alder and Hancock, Hancock and Embleton, and by my drawings (Pl. 27, fig. 8, Pl. 29, figs. 15 and 16), it is now very doubtful whether their function is olfactory per se, or even slightly so.

The remainder of the hood is apparently smooth, but upon close examination it is found to be covered with tubercles, a feature so common to the ectoderm all over the body of Melibe leonina; these tubercles are macroscopic in M. fimbriata Alder and Hancock (1864), Eliot (1902). The ventral side of the cowl (Pl. 27, fig. 1, Pl. 29, fig. 17) is concave in M. leonina; muscle-fibres, radiating from the muscles of the neck, support the veil. In the middle of the concave area between the bases of the tentacles (Pl. 29, fig. 17, *R*) is a marked depression (*Mdp*). The ventral side of the cowl (Pl. 27, fig. 6, *En*) is tuberculate like the external side, but it has no odoriferous glands (Pl. 27, fig. 4, *Oo*, Pl. 30, fig. 25, *Og*): to be discussed below. The head is set off distinctly from the body by a neck (Pl. 27, figs. 2, 3).

2. The Papillae or Epinotidia.

Among the Cladohepatica, where the papillae are mostly foliaceous or lobate structures, the denomination of papillae is preferable to the term cerata, branchiae, or gills. Many English authors have adopted this term: Hancock and Embleton (1848), Jeffrey (1869), Gamble (1892), et al. Others use a different nomenclature: Alder and Hancock (1845), branchial papillae; Parona (1891), Viguier (1898), dorsal appendages; Parker and Haswell (1910), secondary branchiae; Lang (1898), dorsal respiratory appendages (cerata); and still others, Herman and Clubb (1892), Sedgwick (1898), Hertwig (1912), Arnold (1916), Pratt (1916), use the term cerata for the cladohepatic nudibranchs, and branchiae for the Holohepatica. The following authors, dealing with Melibe in each case, designate the papillae as follows: Rang (the founder for the genus) (1829), branchiae; Gould (1852) (Chioraera s. Melibe), foliaceous branchial expansions; Pease (1860), tuberculated lobes; Cooper (1863), branchiae; Tapparone-Canefri (1876), branchial lobes; Fewkes (1889) (Chioraea leontina s. Chioraera leonina, Melibe), branchial appendages; Bergh (1908), epinotidia; Heath (1917) (Chioraera dalli s. Melibe leonina), lappets; and O'Donoghue (1921) (Chioraera s. Melibe), branchial cerata. The last-named author employs the term cerata for the following cladohepatic genera: Dendronotus, Aeolidia, Coryphella, Hermissenda, and Doto; but he applies the word branchiae to the Holohepatica. This is in keeping with the usage of many authors (vide supra). Boas (1916, 1920) employs the term gills for both the Aeolidiidae and the Doridiidae, while Bergh (1879*c*: 73) says that he uses the term papillae for the Aeolidiidae partly because it is a Linnean term, partly because the organs do not exclusively serve for respiration, which is partaken of by the whole surface of the skin, that over the papillae as well as elsewhere, among all the Nudibranchiata. This fact was pointed out earlier by

Hancock and Embleton (1848: 103), who wrote: 'The function of respiration we believe to be performed by the whole surface of the skin, including the papillae, the skin of the back and of the sides between the papillae, and the entire surface of the latter organs. . . .' It may, therefore, be quite incorrect to designate these 'branchial lobes' cerata; moreover, this term stands nearer ctenidium or true gill in meaning, and on that account, and for the reason stated by Bergh, and the facts recorded by Hancock and Embleton, papillae may be the most appropriate term. The Tethymelibidae being cladohepatic nudibranchs, of course, come under this terminology. Bergh also uses the term papillae for the Tethymelibidae, and is the most consistent writer in this as well as in other respects relative to the nomenclature he employs.

Melibe leonina has six pairs of papillae, alternating in position (Pl. 27, figs. 1, 3). They appear smooth to the naked eye, but fundamentally they are tufted or fimbriated as in M. fimbriata Alder and Hancock, M. bucephala Bergh, and Tethys leporina Linnaeus, although in M. leonina (Gould) the fimbriated condition of the papillae is hardly distinguishable. The first pair is located dorsad and a little posterior to the genital pores, and approximately in line with the hepatic junction to the stomach. The arrangement, the size, shape, and structure of the papillae may be seen in Pl. 27, figs. 1, 3, and Pl. 30, figs. 18–25 respectively. Microscopically, the papillae show two principal morphological constituents, viz. (1) terminating branches of the liver (the brownish vascular ramifications of Gould), and (2) smooth muscle-fibres; but also vascular spaces (Pl. 30, fig. 25, *Osp*), odoriferous glands (*Og*), and a tubercular surface (*Tbr*). But the papillae are, however, subject to variation in their structure, depending on the age and the position of the papilla. The two anterior pairs (Pl. 30, figs. 18–21) are far more profusely supplied with hepatic diverticula and muscle-fibres than are the remaining pairs (Pl. 30, figs. 22–4). The last pair does not seem to have any appreciable amount of muscle-fibres or liver-branches.

The muscles ramifying in the papillae form a sort of supporting wall; underlying this are the hepatic branches (Pl. , fig. 25, *Cshb*), with many transparent spaces between (*Osp*). On the outside of the muscle-wall, just beneath the ectoderm, are odoriferous glands (*Og*). The size of the papillae decreases gradually from the anterior to the posterior pair, the last pair being very small, and its hepatic as well as muscular contents are reduced accordingly. Regenerating pairs of the anterior papillae show a great number of hepatic branches and muscle-fibres; this is a striking contrast to old posterior papillae which apparently have no muscle-fibres and no hepatic diverticula. It has been observed quite frequently, by different authors, that the papillae of Aeolidia when cast off swim through the water like worms, propelled by the vibratile cilia, and occasionally by the spasmodic action of the muscles (Jeffrey, 1869). I have myself kept papillae of Aeolidia, at Woods Hole, and of Hermissenda, at Friday Harbour, alive in glass dishes for weeks at a time, the papillae being in constant motion, swimming in a circle owing to ciliary action on their curved surface. This phenomenon is not so extraordinary as it seems. It is found in other invertebrates (Planaria, Echinoderms, &c.). Gamble (1892) reports that the papillae of Lomanotus show remarkable co-ordinative movements when they are touched gently. Autotomy and regeneration of the dorsal appendices, according to Parona (1891), is a common occurrence among Tethys and Aeolidia. Pease (1860), referring to M. pilosa, says: 'When slightly disturbed they would cast off one or all of their lobes . . . : they may be consequently reproduced, after being cast off.' This is also true for M. leonina, for it frequently throws off some or all of its papillae, and yet I have kept specimens of this species for a number of days without autotomy taking place; I have also kept preserved specimens for years without the papillae having dropped off, even though they were subjected to considerable handling (see Pl. 27, figs. 1, 2, 3). Lang (1896) also records the fact that if the papillae fall off they are regenerated. It is believed that the papillae serve as organs

of respiration, that is, they are at least partly respiratory in function; for this purpose large intercellular sinuses are present which communicate with the heart through the efferent branchial veins (Pl. 30, figs. 25, *Osp*, 54, *Aur*). Such sinuses, however, are not present in the papillae only.

3. The Foot.

The foot (Pl. 27, fig. 1, Pl. 28, fig. 9) extends a little beyond the trunk both anteriorly and posteriorly. Its general form is like an inverted flat-bottom dory, with the anterior end wider than the posterior, curved, and extended forward about 1 cm. from its base, the so-called keel. The posterior end, also curved but narrower than the front, projects about 1·5 cm. from the base in an adult. The edges bend considerably outward, making the width from rim to rim about twice as wide as the base. Between the edges, on the ventral side, is a depression so deep that the comparison with a dory is quite fitting. This groove is highly tuberculate and ciliated (Pl. 30, figs. 26, 27, *Cil*, *Tbr*). The internal structure of the foot varies. The anterior end has a fine network of nerve-cells spread throughout its length, and at the posterior end is an aggregation of nerve-cells into a ganglionic centre (Pl. 30, figs. 26, 27, 28, 29). The anterior end of the foot has also a great number of small glands which open to the outside all along the foot by fine crypts through the ciliated ectoderm (Pl. 30, figs. 28, 29, *Mug*) and decrease in number and size toward the posterior end. The secretion of these glands is perhaps of use to the animal in helping it to move over fronds of marine vegetation and other solid objects. Very fine neural fibres extend from the pedal ganglion (Pl. 30, fig. 29, *Nt*) to the base of the ciliated columnar epithelial cells and to the glands. This suggests that the cilia of the ectoderm of the foot and the glands in the foot are under nerve control. And, as I have previously recorded (1919, 1921), M. leonina may move without any visible bodily contortions, the foot being then either in touch with the surface tension of the water, or with the fronds of marine vegetation or some other solid. In the

laboratory it moves along the side of the glass aquarium as other nudibranchs do, e.g. Aeolidia. Such movements seem to be caused by the ciliary action of the foot. In this respect my observations differ decidedly from those of Pease (1860) on M. pilosa, who writes: 'Their foot cannot be used for creeping on a flat surface, but it is well adapted for clasping sea-weed;' and O'Donoghue (1921: 194), who says in part: 'It does not creep about on the eel grass but only seems to adhere for the purpose of laying its eggs. In the laboratory, too, it does not creep on the sides of the aquaria and only partly clings to them. It has not been observed creeping on anything after the manner of other nudibranchs, and if not entirely a pelagic form like Phyllirhoe it is beyond doubt very nearly so and is a most interesting form.' Although O'Donoghue thinks Melibe is mainly pelagic, it is quite evident, judging from its habitat, that the pelagic habit is periodic at the most, i.e. its recurrence is spasmodic (Agersborg, 1916, 1919, 1921, 1921*a*, 1922, 1922*a*, 1923). M. leonina occurs not only as a pelagic form, but may be found at a considerable depth, which is perhaps its habitat the greater part of the year. Gould's specimen, 133 mm. long, 17 mm. high, and 32 mm. wide, was dredged at about $5\frac{1}{2}$ metres depth; Cooper's, 70 mm. long and 17 mm. high, at a depth of 38 metres. Presumably, when it is at the bottom, it crawls on the bottom, for it has a well-developed foot not only for clinging to sea-weeds but also for actual creeping and, indeed, for 'galloping', to use a term employed by previous writers for other forms (Agersborg, 1923).

On one occasion, as I was trying to feed M. leonina, it dropped to the bottom of the aquarium and commenced gliding along the bottom. I continued the feeding experiment, when to my astonishment this nudibranch, Melibe leonina, suddenly elongated to nearly twice its normal length, showing a method of creeping similar to that described by Parker (1917) for the sea-hare, Aplysia californica Cooper (Pl. iv, fig. 9). When elongating the body, the anterior one-third of the foot was lifted above the substratum and

then let down; the posterior third then passed toward the middle of the body which became much wider at the base and along the sides, and then the forward stretching was repeated. This sort of creeping was accomplished by a large muscular wave which passed from the anterior to the posterior, i.e. by direct monotaxic waves. In ordinary locomotion (creeping) the cilia of the foot may play an important part because the locomotor waves are almost indiscernible (Agersborg, 1923: 93–6). M. leonina is, indeed, pelagic, but it is a poor swimmer as compared with Dendronotus giganteus O'Donoghue (Agersborg, 1922: 264); it is less pelagic than Phyllirhoe, which has lost its foot, and it is perfectly able to use its foot both for clinging to sea-weed and other solids and for creeping.

A ciliated foot is a common thing among the gasteropods and other molluscs. This was recorded by Flemming as early as 1869 for Helic hortensis; List (1887) for Tethys fimbriata; later by Stempell (1899) for the lamellibranch Solemya tagota Poli; and recently by Copeland (1918) for Alectrion obsoleta, et al. I have myself examined the foot of various cladohepatic nudibranchs (Aeolidia olivacea, Ae. coronata, Ae. concinna, Ae. diversa, Doto coronata, et al.) at Woods Hole, Massachusetts, and found a uniformly ciliated foot in each case. Pedal glands are recorded by various authors: Leydig (1876), List (1887), Lang (1896), Sedgwick (1898), Stempell (1899), Lankester (1906), Parker and Haswell (1910), Hertwig (1912), but no one has described the pedal gland in Melibe. Lankester (1906) comes the nearest to describing the condition as it exists in this species. That is, the pedal gland is not an aggregation of glands or a simple branched invagination of the integuments opening in the mid-ventral line of the foot as in Triton nodiferus in particular and other gasteropods in general according to the records of Parker and Haswell, and Hertwig, but consists of a number of unicellular glands, apparently equally distributed all over the foot, that open by small crypts through the ciliated columnar epithelial surface

of the ventral side. List finds three kinds of glands in the foot of Tethys fimbriata Linnaeus; two of these are unicellular and the third is multinuclear. The unicellular glands with one nucleus are located on the dorsal side of the foot; the multinuclear glands are found on the ventral side of the foot. Some of the mononucleate unicellular glands contain a fatty substance. The foot is covered by a layer of ciliated columnar epithelium with basally placed nuclei. Between these epithelial cells unicellular and multinucleated glands open to the outside through an individual pore or crypt. The structure of the foot of M. leonina conforms very nearly to that recorded by List for Tethys. It is not, however, my present intention to make a critical physiological or morphological comparison of the foot in these two types. The 'mehrkernige Drüsen' of List I am unable to recognize in this species. Pl. 30, figs. 26, 27, 28, and 29, show the relation of the unicellular mucous gland to the ectoderm. These glands are highly granular in structure with a central nucleus (Pl. 30, fig. 28, *Gmug, Nu*). They are basophil in their staining and there is a marked contrast between them and the nerve-cells which are scattered all through the foot as a net. The latter, however, is firmly aggregated into a pedal ganglion in the posterior end of the foot (Pl. 30, figs. 27, *Gl*, 29, *Pdgn*).

The function of the pedal glands seems to be that of secreting a mucus for the purpose of aiding the animal in its progressive movements when creeping on the surface of any object. This, in fact, is also practised among terrestrial gasteropods, some of which may use the pedal secretion to spin themselves from the limb of a tree or some other plant to the ground. In the Aeolidia the activity of the pedal glands is so great that a few specimens (circ. $1\frac{1}{4}$ cm. long) confined in a finger-bowl for a few hours may produce a complete film of slime on the surface of the water, to which the organisms adhere.

4. The Body-wall.

(1) The Odoriferous Glands.

The entire surface of the body of M. leonina is fimbriated or tufted, although it is not recognizable to the naked eye, but it is easily detectable with the aid of a lens (figs. 6, *Ex*, 25, *Tbr*, 26, 27, *Tbr*, 31, *Pec*). In this respect it resembles M. rosea Rang, M. pilosa Pease, M. papillosa de Filippi, M. fimbriata Alder and Hancock, M. bucephala Bergh, and also Tethys fimbriata Linnaeus (s. fimbria, Bohascht, Delle Chiais). Closely associated with the fimbriated ectoderm are several kinds of glands (Pl. 31, fig. 30, *Glo*, *Sm*, *Um*). There are at least three kinds of these glands : (1) odoriferous (*Glo*), (2) saccular mucous (*Sm*), and (3) unicellular mucous (*Um*). The former is the largest of the three and most numerous (Pl. 27, figs. 4, *Go*, 7). In structure, the odoriferous and saccular mucous glands are similar, but the latter react to Delafield's haematoxylin very much like that of the mucous glands of the foot, while the former seem to be like serous glands. The odoriferous glands, in addition to being larger than the mucous glands, are also a little more complex, i.e. compound saccular (Pl. 31, figs. 30, 31, *Glo*). One reason for assigning the odoriferous function to the largest and most numerous of the cutaneous glands is the fact that this species exudes a rather strong odour, which in a previous paper (1921) I have designated as a means of defence, when it is touched, and at the same time mucus secretion is not noticeable. The unicellular mucous glands are typical glands of its kind (Pl. 31, fig. 30, *Um*). The skin of M. vexillifera Bergh (1879*a*) is also noted for its numerous ' ähnliche Drüsenzellen '. And according to Hancock and Embleton (1848 : 103), referring to Aeolidia, ' The outer or dermal layer of the skin appears to secrete the abundant tenacious matter that exudes from the animal, and to be the seat of an exquisite sensibility. This layer is thin but continuous with the next or muscular layer, which might be called the cellular for its structure.' But Flemming (1870) thinks the subepithelial connective tissue in Helix pomatia secretes slime.

(2) The Muscular System.

The muscular system of M. leonina is one of the most striking features of the animal. When the skin and the caecal endings of the liver (Pl. 27, fig. 3) are removed, the main arrangement of the muscles may be seen to be like that of the interwoven fibres of a basket, the sides of the animal being supported by a network of muscular fibres. One set runs parallel to the median axis from the anterior to the posterior ends, terminating anteriorly in the periphery of the hood. Dorsally these fibres end by branching in the ridge of the back and in the papillae ; posteriorly they end in the ridge and in the base of the papillae of that part of the body ; ventrally they run parallel to the foot, ending anteriorly and posteriorly in the base of the foot ; the last parallel fibres end in the groove of the foot. Another set runs diagonally, also parallel to each other, and ends in fine fibres anteriorly, posteriorly, dorsally, and ventrally. Hartmann (1880: 11) describes the muscles for Tethys fimbriata as follows : 'Die Oberseite des Kopfsegels und des Rückens zeigt auch öfters gedüpfelte, manchmal wieder weiss gesäumte Schräg- und Querbänder. Dies hat bereits G. Cuvier recht gut abgebildet (Mollusques, Tab. VII, Fig. 1).' It is then seen that Tethys and Melibe resemble each other in the arrangement of the muscles of the body. In Melibe the muscle-fibres are located midway between the ectoderm and the boundary of the visceral cavity (Pl. 31, fig. 30, *Mb*). Between the ectoderm and the muscles are a great many connective-tissue cells (*Ct*) and fibres (Pl. 31, fig. 34, *Pmc*), ends of the branching hepatic system (Pl. 27, figs. 4, 7, *Hep*), and plasma (Pl. 31, fig. 31, *Sp*). The finer structure of the muscles is of the type common to molluscs : a circular arrangement of finer fibrils in each individual muscle-cell (Pl. 31, fig. 33, *My*). The beautiful picture, however, presented by a transverse or longitudinal section through a muscle-bundle needs to be commented on a little further.

Schneider (1908), describing the muscle structure of Chiton siculus, says : 'Ueber die feine Structur der Muskelzellen ist nicht viel auszusagen. Die langen Fasern

sind Bündel von Myofibrillen ohne innere Sarcaxe. Der längliche Kern liegt der Faser dicht angepresst.' Continuing on the same subject, but dealing with a different type, i.e. Helix pomatia, he says: ' Die Fasern sind von rundlichem Querschnitt, langgestreckt, glattfibrillär und zeigen den Kern seitlich in einem geringen Sarcrest (Zellkörper) anliegen.' His comment on the muscles of Anodonta mutabilis is much the same as for those already cited, i.e.: ' Die glatten Muskelfasern zeigen gewöhnlich nichts Auffallendes. Jede Faser besteht aus parallel verlaufenden Fibrillen. Der längliche Kern liegt der Faser einseitig an, innerhalb einer geringen Sarcmenge die als Zellkörper zu bezeichnen ist.'

Schneider's description of molluscan muscle is rather generalized and, in fact, fails to bring out the facts concerning the finer structure. The muscle-bundles of M. leonina are surrounded by a thin membranous sheath, the perimysium (Pl. 31, fig. 33, *Ms*), and each individual muscle-cell by an exceedingly thin membrane, the endomysium (*Mt*). Between the muscle-fibres, or muscle-cells, are primitive connective-tissue cells (*Inct*). The muscle-cell or -fibre (Pl. 31, fig. 33, Pl. 37, fig. 82,) is differentiated into two sarcoplasmic regions : an outer finely granular and an inner coarsely granular region with a transparent ground substance. At the periphery of each region, very coarse granules are arranged in such a way so as to give the appearance of a granular network enclosing each (figs. 33, *Sar*, 82, *Myf*). The outer of these may be in close relation with the sarcolemma if such a one is present ; the inner granular network holds a similar relation to the finely granular region as the outer granular network holds to the periphery of the cell, each enclosing, as it were, two different cytoplasmic regions of the cell. There are thus four kinds of granules in the muscle-cell relative to their location and size. These granules may be designated as myofibrillae because of their linear arrangement. The nucleus is placed centrally within the ground substance of the inner region surrounded by the coarser myofibrillae, immediately, and ultimately by the outer and more finely granular substance. In staining capacity,

the ground substance (hyaloplasm or sarcoplasm) of the inner region shows less affinity for cytoplasmic stain than that of the outer region. The granules (macromeres) of the inner region are farther apart than those (micromeres) of the outer region of the muscle-fibre. This is perhaps also the reason why the muscle-cell of molluscs appears as being, according to Schneider, ' innerhalb einer geringen Sarcmenge '. Chromatin bodies are distributed throughout the nucleus, either in the meshes of the linin and around the nuclear periphery, or around the peripheral part only (figs. 33, *K*, 82, *Kar*). The peripheral granular net (sarcolemma ?) may be seen, in the whole mounts of muscle-fibres stained by Congo red, as a fine granular structure around the periphery of the fibre (Pl. 31 fig. 32).

The structure of the inner body-wall as found beneath the basket formation (Pl. 27, fig. 3) of the muscle arrangement in the body-wall, shows apparently no regular arrangement of fibres as in the case of the muscles ; the fibres here, which are of connective tissue, seem to extend in every conceivable direction (Pl. 27, fig. 5), and this layer is continuous until some visceral organ or the pericardial chamber is reached. In this plexus of irregularly arranged connective-tissue fibres and cells are the visceral bodies : the brain, the heart, the stomach, the intestine, the organs of reproduction with their adjuncts, and the renal organs. The amount of connective tissue in the body-wall does not render it opaque. This is partly due to the loose arrangement of the various kinds of tissues and to the presence of numerous sinuses (Pl. 31, fig. 31, *Sp*) containing a transparent fluid. This fluid contains characteristic, primitive connective tissue cells and strands (Pl. 31, fig. 34, *Pnc*).

5. The Visceral Cavity.

According to Lang (1896: 211) the Mollusca are said to have primary and secondary body-cavities. The former is the system of lacunae and sinuses, into which the arteries open, and out of which the veins, where these are present, draw their blood. It has no epithelial walls of its own. Its

boundaries are formed by connective, nerve, or muscle tissues, or by epithelia, which, however, belong to other organs, such as the intestine, the kidneys, or the body-wall. The latter, the so-called secondary body-cavity or coelom, is in most Mollusca very much reduced, usually consisting of only two small cavities, the pericardium and the cavity of the gonads. The coelom is always lined by an epithelium of its own, the coelomic epithelium, and corresponds with the true coelom of the Annelida, which also possesses such an epithelium.

The primary body-cavity of Lang corresponds with the perivisceral cavity of Sedgwick (1898: 375), who says: 'In Gastropoda there is usually a well-developed perivisceral cavity in relation with the alimentary canal or with the anterior part of it.' The secondary body-cavity of Lang corresponds to the pericardial cavity of Sedgwick. 'There is also another cavity, which has no connexion with the perivisceral, and is called the pericardial because it is related to the heart. By most anatomists the perivisceral is regarded as haemocoelic in nature. It is part of the vascular system, and therefore haemocoelic.'

In regard to the Tethymelibidae Bergh (1908: 97), writing on M. rosea Rang, says: 'The cavity of the body reaches to the region of the last of the papillae.' In M. leonina Gould, I find that the cavity extends anteriorly to the oesophagus, dorsally to the back, ventrally beyond the genital ducts, posteriorly as far as the anus, back of which the branching of the liver and the kidneys is so profuse, together with the crossing of connective-tissue fibres, as to render it very difficult to tell whether the perivisceral cavity extends beyond the anus. This cavity is not a true coelom. It corresponds with the primary body-cavity of Lang or the perivisceral cavity of Sedgwick. There is no definite termination of an inner body-wall, although the muscle-wall seems to represent one, but that is really superficial. Beyond the muscle-wall the connective-tissue fibres run in all directions, all through the cavity. It is, therefore, not a well-defined cavity. It is in this so-called cavity that all the visceral

bodies are located, hence the visceral, or perivisceral, cavity. The pericardial chamber, discussed below, is a true coelom.

6. The Alimentary Canal.

(1) The Buccal Cavity.

As in other organisms the buccal cavity of M. leonina is the beginning of the alimentary canal. It corresponds very closely with Alder and Hancock's (1864) and Eliot's (1902) descriptions of M. fimbriata. The mouth is bounded by the two lateral, slightly furrowed lips (Pl. 27, fig. 1, *L*, Pl. 29, fig. 17, *M*, Pl. 32, fig. 35); the furrows are not seen in fig. 1 as they are smoothed out by the swelling of the lips, but they may be seen in fig. 35 which is a photograph of a transverse section through the buccal cavity. Within the mouth there is a uniform invagination of the ectoderm (fig. 35), and this invagination produces a number of folds or corrugations which increase in depth and finally merge with those of the oesophagus. The food, as it is engulfed, passes directly through the oesophagus into the proventriculus. No masticatory process is carried on in the mouth for the simple reason that this species is absolutely void of tongue, radula, or mandibles. The food is swallowed whole, as evidenced by the contents of the alimentary canal including the intestine, and is disintegrated by the digestive processes only. The jawless condition of this species is a character common with that of its relative, Tethys Linnaeus. Jeffreys says: 'Tethys has neither jaw nor tongue.' And Vayssière (1901) finds for Tethys fimbriata (S. leporina): 'Buccal bulb absent. Large anterior chamber; having exterior circular muscle-fibres probably used in mastication. (Cette région offre à sa surface extérieure un anneau musculaire, auquel correspond intérieurement un anneau de plis longitudinaux presque tendineux, que l'on peut considérer comme un organe masticateur.)' However, this jawless condition does not prevent Tethys or Melibe from being carnivorous, as is shown by the contents of their stomach (von Jhering, 1876: 37; Berg, 1882; Vayssière, 1901: 84–5; Eliot, 1902: 69; Agersborg, 1919: 272; 1921: 228, 232).

a. Mandibles and Radula.

Bergh (1902: 207) reports the presence of mandibles for the species M. bucephala, saying: 'The mandibles joining above are of a form like that of other Melibes. . . . The masticatory edge is finely dentate in the upper part, in the lower part provided with coarser, rounded teeth.' For the species M. pellucida (1904: 13) he reports: '. . . die gelblichgrauen Mandibel ganz zerbröckelt.' And for M. rosea (1908: 94–9) he writes: '. . . through the walls the outlines of the mandibles were very distinctly visible (fig. 3*b*). The clear yellow mandibles (fig. 5) resembling those of *f. ex.*, the Tritoniadae or Pleurophyllidiae; . . . very plump denticulated masticatory edge, the denticles reaching a height (fig. 5) up to 0·08 mm. (The bulbus pharyngeus with its mandibles agree very likely in other species of Melibes; in general with that of the typical species.)' It is thus seen that Bergh finds mandibles in many of his Melibes, in fact, in all species of Melibe which he described, or nearly so. He even took issue with Hancock's description. It really seems strange that Bergh should be so insistent on this point. I have my doubt as to the correctness of his description of one of the species (M. pellucida), collected from the mouth of Columbia River in the State of Washington, as no other authors (Gould, 1852; Cooper, 1863; Fewkes, 1889; Heath, 1917; Agersborg, 1916, 1919, 1921, 1921*a*, 1922, 1922*a*, 1923; and O'Donoghue, 1921, 1922, 1922*a*) who have collected the species from the same coast, i.e. off the coast of Santa Barbara, at Monterey, South-eastern Alaska, Puget Sound, and the Vancouver Island region, have recorded mandibles for the types with which they dealt. A number of other authors who have described several species from different parts of the world also, do not record mandibles for this genus: Rang (1829), M. rosea; Pease (1860), M. pilosa, 'mouth proboscidiform, and the orifice vertical'; de Filippi (1867), Jacunia papillosa (s. Melibe papillosa de Filippi); Tapparone-Canefri (1876), M. papillosa '. . . nel suo interno ha nè lingua, nè radula, nè mascelle.' On the

findings of Bergh, however, Lankester (1906: 175) characterizes Melibe as having mandibles; but Lang (1896: 180) says: 'Jaws are wanting or rudimentary in . . . many Nudibranchia (Tethys, Melibe, Doridopsis, Phyllidia).' According to this, it would be best to state, at least in part, as a generic characteristic for Melibe: the pharyngeal bulb is either with or without mandibles; radula and tongue always absent.

The alimentary canal is remarkably straight (Pl. 28, fig. 9), in fact there is no coiling or looping whatever; only the intestine curves a little from the median position and to the anus, which opens on the right side, a little out of the median line. In this way, the alimentary tract extends somewhat diagonally through the body-cavity, and only the intestine curves. This corresponds with Lang's (1896: 33) statement for the Nudibranchia: 'The anus lies either dorsally in the median line, or laterally to the right.' In M. leonina, the anus is on the right side of the body (Pl. 27, fig. 2), a little ventral to the base of the second papilla of that side. This is also its position in M. bucephala Bergh (1902), though in this case it is midway between the first two anterior pairs of the papillae. In M. leonina the ureteric pore is laterodorsal to the anus.

b. The Buccal and Salivary Glands.

The buccal cavity is highly corrugated (Pl. 32, figs. 35, 36, *Oe*), but it is without jaws or radula. Specialized organs for chewing are substituted by the folding and invagination of the ectoderm. The folds are non-glandular, but just beneath this ectodermal layer are numerous glands, even in the external parts of the mouth. These glands correspond to the buccal glands of various authors. Lang (1896: 185) distinguished clearly between these glands and the salivary glands and says, referring to the Opisthobranchiata: 'The salivary glands, of which only one pair is almost always found, here vary in size and shape still more than in the Pulmonata. Those glands which enter the pharynx must not be confounded with

other glands which in many Opisthobranchiata enter the buccal cavity, and are sometimes more strongly developed than the salivary glands.' Sedgwick (1898: 371), referring to the same organs, says: 'In addition to buccal glands, sometimes found round the buccal opening, there is always a pair of salivary glands opening into the buccal cavity. The buccal cavity leads into the oesophagus which is followed by a dilated stomach, and is usually provided with a caecal appendage.' In M. leonina the glands of the oesophagus consist of a series of small, simple, saccular glands (Pl. 32, fig. 41, *Sg*) arranged in rows along each side of the swallowing tube. They open directly into the oesophagus by small crypts (*V*). Heath (1917: 147) reports that the salivary glands are absent in Chioraera dalli (s. Melibe leonina), but, of course, he is mistaken about this. The activity of the salivary glands in gasteropods was beautifully demonstrated by Lange (1902: 85–153), who showed there is a close relation between the structure of the nucleus and cytoplasm and the physical condition of the organism relative to starving and feeding of the animals.

(2) The Oesophagus.

The length of the oesophagus in an animal circ. 10 cm. long is 3 mm. (Pl. 32, fig. 36, *Oe*). The oesophagus itself is simply a narrow part of the alimentary canal between the mouth and the proventriculus. Corrugations, which begin at the lips and increase progressively in the mouth, deepen still more in the oesophagus. The lining is still non-glandular, but the glands in the underlying tissue increase until the anterior part of the proventriculus is reached, when they end quite abruptly. The corrugations of the oesophagus are largely longitudinal, which suggests that the oesophagus is capable of expansion in case the animal swallows some large food particle. In some nudibranchs, e.g. the Tritoniadae, according to Vayssière (1877), the oesophagus is very long.

(3) The Stomach.

a. Proventriculus.

Following the oesophagus, the alimentary canal enlarges into a chamber 3 mm. long, 1·51 mm. in diameter, and is constricted posteriorly by extensive evaginations of its epithelial lining. This chamber, the glandular stomach or proventriculus, constitutes the preliminary digestive cavity of the alimentary tract. Its epithelial lining is distinctly different from that of the oesophagus, by being highly glandular (Pl. 32, fig. 38, *Gl*). The food is probably kept here until acted on by secretions of the unicellular glands of its columnar epithelial lining.

b. Gizzard.

The remaining part of the stomach is the gizzard. Its length in an animal 12 cm. long was 10 mm., with a diameter of 4 mm. at its widest part. The structure of the gizzard is variable. Its walls consist of two coats (Pl. 32, figs. 37, 42, 43) or layers, each of which may be divided into two parts. The outer layer consists of a thin outer cover of connective tissue and occasional muscle-fibres, which run longitudinally with the organ (*Cc*), and of a thick median circular layer (*Mus*). The inner layer consists of a single layer of tall columnar epithelial cells of glandular nature (*Ept*) and a false epithelial cell border (*Stpl*), formed from the secretion of the underlying glandular epithelium. This secretion fuses into a homogeneous mass giving the appearance of a transitional epithelial layer of cornified type (Pl. 32, fig. 42, *Trs*, 43, *Trp.pl*). This cornified border is about as thick (Pl. 32, fig. 37) on the dorsal side of the middle part of the gizzard as the other two layers together, and gradually decreases until, on the ventral side, . . . it becomes very thin (*V*). It is far more marked in the anterior part of the gizzard than in the posterior. The glandular nature of the epithelial border is well marked in the anterior part of the gizzard but decreases in the ventral region of this part of the stomach, where the cornified border also entirely disappears.

In the extreme posterior part of the stomach the epithelial lining becomes ciliated, a feature which is continuous throughout the remainder of the alimentary canal. In this respect the alimentary canal of M. leonina differs greatly from that of Neritina fluviatilis, a prosobranch, which according to Lensen (1899) is ciliated from the oesophagus to the anus. The stratum corneum of the epithelial border of the gizzard represents in M. leonina the so-called stomach-plates of various authors, which are supposed to be a common characteristic of Melibe and related forms. Thus, Alder and Hancock (1864), in their description of M. fimbriata, say: 'The stomach is a rather large pyriform pouch, with its small extremity placed backwards. It lies diagonally across the anterior portion of the visceral cavity and is divided into an anterior and posterior chamber by a slight constriction near the centre. The anterior of the lower portion of the chamber is encircled transversely by an almost complete belt of horny, compressed, lancet-shaped process, similar to those in the gizzard of Scyllaea.' Bergh (1875*b*), referring to Tethys and Melibe, says: '. . . und das in der Tiefe des Kopftrichters hervorstehende Mundrohr leitet unmittelbar in die Speiseröhre und in den ersten Magen hinein, der, wie bei den Meliben, mit starken (Cuticular-) Falten bewaffnet ist,' p. 346. And again on p. 356: 'Die Innenseite der mittleren kleineren Abtheilung des Magens zeigt . . . eine Masse von sehr kleinen und niedrigen Längsfalten (fig. 1 *c*) oder eine geringere Anzahl (15–20) von stärkeren; die Falten sind mit einer horngelblichen Cuticula überzogen, die im ersten Falle nur von geringer Dicke, im anderen viel stärker, mit Leisten sich zu einer Höhe von etwa 0·5–0·75 mm. erhebt; an den dickeren Cuticula-Falten tritt eine (Taf. XLV, fig. 22) deutliche Querstreifung oder mehr unregelmässige Theilung hervor. Die Unterseite des Cuticula-Ueberzuges zeigt ein fein körniges Aussehen, das auch an der Oberfläche der unterliegenden Schleimhaut hervortritt und von den zahlreichen, dicht gedrängten Papillen derselben hervorgebracht wird.' Again, on p. 366, referring to M. capucina, he says: 'Magenzähne . . . 10 starken Kielen

gebildet.' Finally, referring to M. rangii, he finds: '... die Magenzähne viel zahlreicher als bei der vorigen Art, schmäler und im ganzen von einem Individuum 26, bei dem anderen 31.' Also von Jhering (1876), writing on Tethys, says: 'Der Magen trägt nach innen von der Faser- und Muskelschicht ein einfaches Epithel 0·02 bis 0·03 mm. grosser Zellen, und darauf folgt nach innen eine oft mehr als 0·3 mm. dicke Schicht, welche aus einfachen, schlauchförmigen 0·014 mm. dicken Drüsenschläuchen besteht, deren histologischer Bau ... nicht sagen lässt ob es gestattet ist, sie mit der bekannten Cuticularschicht im Muskelmagen der Vögel zu vergleichen. Diese Schicht erinnert sowohl in ihrem Aussehen als in ihrer Consistenz an Knorpel, indem sie zwar weich und sehr elastisch ist, aber doch durch diese an Gummi erinnernde Elasticität dem Magen denselben Schutz gewährt, wie eine harte Kalk- oder Chitinauskleidung.' And Vayssière (1877: 300), in his description of a new genus of the family Tritoniadae, writes: 'L'œsophage, qui est très long, aboutit à une première dilatation qui est le gésier: c'est dans l'intérieur de cette cavité que se trouvent près de quarante dents cultriformes, placées côte à côte et formant un anneau complet. Ce caractère ne se montre parmi les Nudibranches que dans le genre Scyllaea.' And again (1911: 102), working on the species Bornella digitata, he records the following: 'Cette poche dans son tiers antérieur possède des parois musculaires assez épaisses, muscles transverses et longitudinaux; cette forte musculature est destinée à soutenir à l'intérieur une quinzaine de rangées longitudinales de longues épines un peu recourbées, de nature chitineuse. Cette partie de la poche constitue un véritable gésier dont l'armature sert à broyer les aliments qui y arrivent.' This is further considered by Bergh (1879*a*: 165) in his description of M. vexillifera: 'Der hintere Teil des Magens ist kürzer als der vorige und hat festere Wände; an seiner Innenseite (fig. 9 *c*) 14 starke Magenplatten und zwischen denselben meistens eine (seltener) kleinere und meistens kürzere (fig. 9 *c*); die Platten, die Falten darstellen, an denen die Cuticula stärker entwickelt ist, ...

schwach harngelber Farbe eine Länge bis beiläufig 0·8 bei einer Höhe bis etwa 0·20, und einer Breite bis 0·25 mm. erreichend. Das hintere Ende des Magens (fig. 2) mit von etwa der Mitte eradiirenden Falten, neben dem Pylorus hier eine taschenförmige Erweiterung (fig. 2*fg*) (etwa wie in Tethys) mit starken Falten der Innenseite.' In 1888: 691, the same author, describing M. ocellata, writes: '. . . die Magenplatten schimmerten undeutlich durch; diese letzteren fast wie in der M. papillosa.' And for the last-named species (1884*a*): '. . . hinter der Mitte (des Magens) der Länge nach schimmert der Zahngürtel undeutlich hindurch.' And in 1902: 107, for M. bucephala: 'The belt of the stomach-plates shines through in about the first half of it, and immediately before the belt the foremost liver-branch is attached on either side somewhat upwardly. . . . The belt of plates consists of twenty-eight faint lemon-coloured firm plates partly alternating in height.' Eliot (1906), describing Tritoniopsis, says in regard to the stomach: '. . . into a rather small membranous and fragile stomach, almost entirely covered by the liver, and no trace of plates.' And in 1910: p. 40 he writes: 'The liver secretions harden in the stomach and form a protecting membrane which is found to cover the stomach.' On the previous page he stated: 'Into the posterior part open four or five liver-ducts and also a pear-shaped gastric pouch, whose orifice in the stomach-wall is closed with a more or less distinctly developed flap. This pouch is often called the gall-bladder, but nothing indicates that its functions correspond to this name. Its walls are glandular, and appear to secrete globules of a glistening material which is also found in the intestine. It is possible that this secretion subsequently dissolves and forms a membrane which is found to cover the walls of the stomach and intestine, and probably serves to protect these delicate surfaces against the spicules abounding in the sponges on which most Dorids feed.' Stomach-plates constitute a common feature among species of Melibe and other related forms; something similar to stomach-plates is present in other species (Eliot,

1910). The origin of the stomach-plates in M. leonina, which no one seems to have described, is not the same as indicated by Eliot (1910: 39–40) for the Dorids. This may be plainly seen in sections which are represented in my drawings (Pl. 32, figs. 42, *Trs*, 43, *Trp.pl*). In the case of Melibe the plates originate as a secretion-product of the epithelial lining of the gizzard. This secretion accumulates within the cell and is voided, little by little, by the cell into the cavity of the stomach. As these secreted droplets pass into the stomach they become attached to their predecessors, harden, and form into a continuous layer resembling the stratum corneum of the human epidermis but, of course, arises differently. The secretion droplets seem to originate around the nucleus of the epithelial cells; they then coalesce into larger droplets and finally break up into smaller ones that pass out of the cell and into the stomach and then form by a keratinization (?) process into the hard or protective lining of the stomach. This protective lining, the stomach-plates of Melibe, no doubt serves a double function: to protect the living cells against the spines of the crustaceous food; to help in masticating the food before it passes into the intestine. This latter use seems to be necessary, particularly in those cases in which the organism is void of mandibles or radula of any kind.

c. The Pyloric Diverticulum.

The pyloric diverticulum (Pl. 27, fig. 9, *Pd*,) is described by Alder and Hancock (1845: p. 14) as pancreatic in function. Whatever function it may have, it seems logical to think that it plays a specific part in the process of digestion because of its internal structure. This part of the alimentary canal is situated at the constricted posterior part of the stomach. Externally it consists of an elaborate evagination into a number of folds, beginning laterally and continuing ventrally, until meeting on the opposite side. Internally (Pls. 32 & 33, figs. 39, 44) the pyloric diverticulum is considerably corrugated, being thrown into much larger folds than the remainder of the tract. Ciliation of the mucous layer begins here. The corrugations are formed,

as in other animals, by the connective tissue which intervenes between an outer muscular layer (although in this case the muscular layer is very thin) and an inner mucous layer. The latter consists of ciliated columnar epithelium, and, as in the stomach, is glandular in nature (Pl. 33, fig. 44), but the secretion of the intestine does not harden after it reaches the lumen of the tract. The epithelium contains large and small vacuoles which are formed in the neighbourhood of the nucleus but in the distal region of the cell. As in the stomach, these vacuoles or secretion droplets coalesce into larger ones which either break up into smaller droplets before leaving the cell, or pass directly into the lumen. But in no case does the epithelium form into regular goblet-cells, nor does the secretion form into protection-plates of the lining as in the stomach.

(4) The Intestine.

That the intestine is the principal digestive region of the alimentary canal is well shown by the different conditions of the food in the stomach and in the intestine. In the former, the food seems to have undergone little or no disintegration, while in the latter, only the skeletal parts of the food remain. As the food passes through the pyloric diverticulum, it is perhaps acted upon in such a way that the intestinal juices more easily complete the digestion that takes place in the posterior part of the alimentary canal. The slightly enlarged part of the anterior portion of the intestine has parallel corrugations externally, which may go to show that it is capable of considerable enlargement. In specimens 7 cm. long the corrugated enlargement was circ. 7mm. in diameter.

The absorptive surface of the intestine is increased very greatly by the presence of a typhlosole which extends from the pyloric diverticulum to the anus. The typhlosole is very large in the anterior portion of the intestine, where it protrudes into the intestinal cavity from the ventral side until there is little free space left between it and the rest of the intestinal walls. Posteriorly, the typhlosole gradually decreases in size, and just before reaching the anus it is obliterated in the ventral part of

the tract. Only the ordinary corrugated part of the intestinal lining remains. The extent of the typhlosole, however, varies, because one specimen, whose intestine was sectioned, had no typhlosole in the smaller part of the canal.

The structure of the intestine is similar throughout. There is an outer fibrous layer and an inner glandular one. Between these are fine connective-tissue fibres and small cells and colourless lymph. The glandular layer consists of tall ciliated columnar epithelium (Pl. 33, figs. 45–7). There is no difference in the glandular layer of the typhlosole and of the remainder of the intestine, or in these parts of the pyloric diverticulum. A close study of the internal layer of the intestine reveals some interesting morphological facts, viz. this layer consists of very tall columnar cells with the nucleus located, in most cases, in the middle (Pl. 33, figs. 45, 47) with vacuoles either around the nucleus, on either side, or on the distal side only. The vacuoles arise as a confluence of smaller vacuoles which arise in the neighbourhood of the nucleus, and again break up into smaller ones and then pass into the lumen of the intestine. The epithelial secretion does not keratinize here as it does in the gizzard. Eliot (1910) suggests that the hepatic secretion hardens in the intestine and the stomach of Dorids. The hard substance, however, which covers the endoderm of the alimentary tracts of Eliot's Dorids and of M. leonina, is at least in the case of the latter of an entirely different origin. Sometimes more than one nucleus may be present in the same cell (Pl. 33, fig. 46). The most striking feature of this epithelium is the regular fibrillar structure, and the linear arrangement of the cytoplasmic granules from border to base of the cell running parallel with the cilia ; these do not converge on the nucleus but pass to the base of the cell. The cell rests on a distinctly granular basement membrane (Pl. 33, fig. 45, *Bm*). There are two distinct rows of basal granules, or terminal bars (desmochondria), the proximal being the larger of the two (*Bg*). The cilia (*Cil*) may be seen readily between the two terminal bars. Beneath the basement membrane (*Bm*) is a loose connective-tissue layer (*Lcc*) which is covered by a denser

fibrous coat with a few occasional muscle-fibres (*Cc*). This same cover, in the pyloric diverticulum, is very loose (Pl. 33, fig. 44, *Vas*), and suggests a possibility of interchange of body-intestinal fluids in this part of the alimentary canal. The outer coat of the remainder of the intestine, though much thicker, is, however, so loose that it may allow ready interchange of intra- and extra-intestinal fluids.

There are then, five distinct regions of the alimentary canal each differing from the other in structure and function. These are (1) the oesophagus with the non-glandular lining below which are the oesophageal or salivary glands (Pl. 33, figs. 36, *Oe*, 41, *Sg*) ; (2) the proventriculus with distinct glandular lining (Pl. 33, figs. 36, *G*, 38, *Gl*) ; (3) the gizzard with its stomach-plates (Pl. 33, figs. 37, 42–3, *Stpl*) ; (4) the pyloric diverticulum with its glandular and ciliated internal surface, which secretions, as in the glandular stomach, do not keratinize (Pl. 33, figs. 38, 39, 44) ; (5) the intestine with its large typhlosole and glandular ciliated epithelial surface (Pl. 33, figs. 40, 45, Pl. 34 figs. 56, 57).

(5) The Liver.

The gastro-hepatic apparatus, or hepatic caeca of M. leonina, does not arise as in M. fimbriata, '. . . a little in advance of the belt of horny processes ' (Alder and Hancock, 1864), but it arises from the anterior portion of the gizzard and consists of a very extensive arborization which passes to all parts of the body (figs. 1, 2, *Hp*, 7, 9, *Hc*, 40, *Hep*). As in other members of this genus, the liver consists of three principal tubular trunks which start at the anterior end of the gizzard and pass to the various parts of the body. Two of these trunks, situated opposite each other, send out branches as follows : the one on the left side runs in the main to the gonads, branching very profusely in that region ; it also sends out other but minor branches, some to the papillae, some to the body-wall, and one to the mucous gland. (The mucous gland = (1) albuminous gland, and (2) nidamental gland.) The right trunk sends out several major and minor branches. Of the

former, one goes to the mucous gland and the other to the gonads and prostata ; of the latter, one goes to the veil and others to the papillae and the body-wall. Both of these trunks branch profusely in the posterior part of the body-cavity. A third trunk situated in front of the other two on the left anterior side of the stomach sends out two main branches, one to the hood and the other to the left papilla of the first pair. Besides these there are many minor branches which go to the body-wall. The extreme branches, and particularly those lying within the body proper and surrounding the gonads, are highly glandular. The contents of the gizzard are affected by the secretion of the liver, which is shown by the fact that particles in the stomach near the hepatic openings stain very similarly to the hepatic tracts through the stomach-plates. The arrangement of the hepatic system in the different species of this genus is considerably variable, and still in some of them it is very similar to that of M. leonina. Thus Bergh (1875*b*: 366) finds for M. capucina: 'Die Leber scheint eine lose, aus mehreren grossen, unregelmässigen, lose mit einander verbundenen Lappen gebildete, etwas gelbliche Masse zu sein, die sich durch die grösste Strecke der Eingeweidehöhle hinzieht, fast überall an den Lappen der Zwitterdrüse angeheftet ist und, wie es scheint, auswärts gegen die Körperwand (gegen die Papillae ?) kurze dicke Aeste ausschickt. Aus dem vordersten Theile der Leber entspringt der ziemlich weite Gallengang (fig. 19*e*), der in die Rückenseite des (Cardeatheils des) Magens einmündet.' In 1879*a*: 165, for M. vexillifera: '... die Leber wie bei anderen Meliben eine lose, gelblichweisse Masse, welche vorne an den Magen reicht, hinten sich bis an das Ende der Eingeweidehöhle erstreckt ; sie ist eine sehr stark verästelte, mit gerundeten, dünnwandigen Endkolben und Ausbuchtungen versehene Drüse (fig. 10), von welcher sich aber zwei Lappen ganz abgelöst hatten, die sich im ersten Papillenpaare verbreiteten und in die vordere Abtheilung des Magens einmündeten. Diese Lebermasse ging vorn in einen ziemlich weiten, kurzen, dünnwandigen, gemeinschaftlichen Gallengang über.' The hepatic system in M. papillosa Bergh (1884)

is not so completely broken up as in M. leonina. Again (1890*b*: 283) he calls attention to the fact that the liver of M. ocellata opens into the stomach behind the stomach-plates, and in that way it is seen to differ from M. leonina. But the distribution of the hepatic branches is similar: 'Dicht hinter dem Gürtel der Magenplatten münden die dicken Leberstämme ein, rechts der besonders dicke aus der ersten rechten Papille, links der aus der entsprechenden linken, und dicht neben demselben der grosse Hauptleberstamm, längs der Rückenseite der Zwitterdrüse und über dieselbe hinaus verlaufend.' Finally, describing the species M. rosea Rang, he shows (1908: 98) that it is quite similar to Chioraera Gould, when he writes: 'The three principal liver-branches with their ramified hepatic ducts and the principal branchlets to the dorsal epinotidia as usual. . . . Network of liver-branches is interwoven with the much branched renal tubes (figs. 9, 10) the branches reaching the root of the epinotidia, but did not seem to ascend into them.'

Pease (1860: 34) refers to the liver in M. pilosa, only by stating: '. . . body punctured with brown, which are most conspicuous along the flank.' And Alder and Hancock (1845: 13), writing on this subject, say in part: 'In the greater number of Eolididae (Aeolidiidae), however, the liver has entirely disappeared from the abdomen and is broken up into numerous minute portions or glands which are thrust into the branchial papillae. The delicate ducts from these glands pass onward and unite to form great hepatic ducts or trunk channels, which open into the stomach.' Hertwig (1912: 335) concurs in this by saying: 'In Aeolidae (Aeolidiidae) branches of the digestive tract enter the cerata, expand distally to small sacs filled with nettle-cells used for defence; they are derived from hydroids on which these animals feed.' So also Lang (1900: 300) writes: 'Bei zahlreichen Nudibranchiern löst sich die Verdauungsdrüse in sich verästelnde Darmdivertikel auf, die sich fast nach Art der Gastrokanäle oder Darmäste der Tubellarien in Körper ausbreiten und bis in die Rückenanhänge des Körpers emporsteigen (cladohepatische Nudibranchier), wo sie mit den

Nesselkapselsäcken communiciren können Diese Form der "Leber" macht wahrscheinlich, dass sie nicht etwa bloss verdauende Secrete absondert, sondern sich auch selbst bei der Verdauung und bei der Resorption der Producte der Verdauung betheiligen wird. In der That weiss man schon lange, dass bei den Nudibranchiern Speisebrei in diese Verästelung des Darmes hineingelangt; aber auch für eine Form mit ganz compacter Leber, nämlich für Helix pomatia, wurde kürzlich der Beweis erbracht, dass in der That in der "Leber" Aufsaugung oder Resorption der verdauten Nahrung stattfindet.'

Quatrefages (1844, 1844*a*, 1848) maintained that the liver in Nudibranchs is of a threefold function; hence his term 'Plebenterism' to designate that species of gradation which consists in the union of different functions in one system of vessels. That is, he maintained the absence of anal opening, heart, and blood-vessels, adopting the term gastro-vascular system introduced by Milne H. Edward (1842, 1845) for the digestive organs in the family Aeolidiidae, the true significance of which has since been the subject of much controversy. It is now, however, a well-established fact that the group of molluscs with which de Quatrefages dealt (Aeolidiidae) has a well-established circulatory system, i. e. heart and blood-vessels, and alimentary tract with anal opening. The liver branching off from the digestive tract forms into many parts and ramifies to various parts of the body. One unquestionable function of the liver, as far as Aeolidia is concerned, is an exit for harmful and indigestible parts taken in with food (Alder and Hancock, 1845; Glaser, 1903; Hertwig, 1912). Glaser describes the hepatic caeca as secondary exits in nudibranchs which feed on hydroids whose nematocysts produce indigestible formic acid; the mollusc rids itself of its useless stomach contents through these secondary openings of the liver-branches which end in the dorsal papillae. M. leonina does not feed on hydroids, but on crustaceans (Agersborg, 1916, 1919, 1921, 1921*a*, 1922*a*, 1923); and, although the hepatic system is tubular (Pl. 30, figs. 25, *Cshb*, 31, *Ch*; Pl. 33, figs. 51, 53), it does not end with openings

through the ectoderm (Pl. 27, figs. 1, 2, 4, 7 ; Pl. 31, fig. 30), but caecally between it and the muscle-wall (Pl. 27, figs. 2, 3).

Frenzel (1886 : 273) believed with other authors that the liver of molluscs performs a double function : (1) as in Crustacea it is a digestive gland, ' d. h., dass sie ein Secret bildet und ausscheidet, welches zur Verdauung der in den Darmkanal aufgenommen Speisen verwendet wird.' (2) In addition, this gland is according to Max Weber (1880) for the Crustacea, and according to Barfurth (1883) for the Gasteropoda, of ' excretorische Function '. They think that the liver of these forms is analogous to that of vertebrates. They describe cells that have special functions, such as secretory and excretory. Frenzel points out three kinds of epithelial cells of the liver of Tethys: (1) ' Kornzellen ', (2) ' Keulenzellen ', (3) ' Kalkzellen '. To these different cells he ascribes the various functions of the organ. These cells are further described by Hecht (1895 : 675), as follows : ' La présence de trois types bien définis de cellules : (1) Cellules vacuolaires excrétrices caractérisées par leurs grandes dimensions et leurs grandes vacuoles (Frenzel : Fermentzellen, Keulenzellen) contenant chacune une granulation. . . . ; (2) Cellules excrétrices à grosses sphères brunes (Leberzellen, Kornzellen) ; (3) Cellules à ferments. Leur coloration en gris par les réactifs osmiques ; on y joindra ; (4) Cellules indifférentes qui, je le suppose, peuvent évoluer dans un sens ou dans l'autre.' The structure and function of the liver in a Doridiform cladohepatic nudibranch, is still further commented on by Eliot and Evans (1908) as follows : ' The cells which line the hepatic lobules are columnar or cuboidal and highly granular. Some are in a distended condition, others are attached to the wall of the lobule only by a strand or are free in its cavity. It would seem, therefore, that some of the liver cells are excretory in function, and are dropped into the follicle as they become extended with excreted material.' Eliot (1910 : 39) attributes to the liver the function which, in the case of M. leonina, I have shown to be the function of the epithelium of the posterior chamber of the stomach, i.e. the gizzard, viz.:

'Into the posterior part open four or five liver-ducts and also a pear-shaped gastric pouch, whose orifice in the stomach-wall is closed with a more or less distinctly developed flap. This pouch is often called the gall-bladder, but nothing indicates that its functions correspond to this name. Its walls are glandular, and appear to secrete globules of a glistening material which is also found in the intestine. It is possible that this secretion subsequently dissolves and forms a membrane which is found to cover the walls of the stomach and intestine, and probably serves to protect these delicate surfaces against the spicules abounding in the sponges on which most Dorids feed.' Finally, Arnold (1916: 353–4), referring to the Cladohepatica, thinks that the liver, which in most nudibranchs is extremely large and completely surrounds the stomach, in Dendronotus also extends into the dorsal cerata (papillae), so that they may have some digestive function.

The hepatic diverticula of M. leonina consist structurally of two main layers: an outer fibrous coat and an inner columnar epithelial membrane. The fibrous layer, consisting of connective tissue, is thrown into corrugations—inwardly—so that the surface of the lumen of the hepatic appendages is greatly increased (Pl. 33, figs. 51, 53). The cytoplasm of the epithelium is highly glandular and one may notice considerable variation in the contents of the cells. Some of the cells show a similarity to the 'Keulenzellen' of Frenzel, or 'Cellules vacuolaires excrétrices' of Hecht. In fact, as is shown in Pl. 33, figs. 48, 49, 50, some of the cells have large vacuoles containing granules of different sizes (Pl. 33, figs. 48–9), and some are vacuolated and contain no granules, while others have no vacuoles but their cytoplasm is highly granular. Others, again, show a remarkable linear arrangement of the granules, basal to the nucleus (Pl. 33, fig. 49). The greatest activity of the cell seems to take place around or near the nucleus with a progressive differentiation toward the border, where the cell in many cases is more homogeneous than in the remaining part. Sometimes a large secretion vacuole may contain threads of darkly stained substances with a darkly

staining cap toward the periphery. Such threads may be present in the cell without the cell being vacuolated. The secretion product, at other times, aggregates from a number of small vacuoles at the border of the cell and passes out en masse, when it seems to be basic in staining reaction. That is, the basophil reaction is shown in material which has been fixed in an osmic acid mixture and stained with Heidenhain's haematoxylin. The nucleus, as a rule, is basal in position and contains one or two nucleoli (Pl. 33, fig. 48). The morphological aspect of an actively functioning liver of Physa gyrina Say, and Planorbis trivolvis Say, does not differ much from that of Melibe leonina (Gould) (vide Agersborg, 1923*a*, figs. 35, 36, 38, 41, 42). It is not my purpose at this time to discuss the function of the hepatic system of M. leonina; but it is evident from the facts observed, and as pointed out above, that at least some of its products passes into the stomach, and for this reason it is secretory in function. Perhaps, owing to the peculiar granular nature of the cytoplasm of the epithelial layer, it may be absorptive in function also. In that connexion it is interesting to note the extensive distribution of the liver to the various organs of the body, the significance of which at the present time may only be conjectured. The peculiar granular nature of the cytoplasm of the epithelium shows that its function is different from that of the epithelium of the alimentary tube proper.

7. The Circulatory System.

The work of Milne Edwards (1842) on Aeolidiidae; Alder and Hancock (1845) on the Aeolidiidae and Tritoniidae; Hancock and Embleton (1848) on Aeolidia, (1881) and (1882) on Doris; Hancock (1865) on Doris tuberculata, D. reponda, D. bilamellata, Tritonia hombergii, Bornella, and Scyllaea; Bergh (1875) on Melibe and Tethys, (1884*a*) on M. papillosa; Lansberg (1882) on Neritina; Boas (1886) on Pteropodes (Limacina, and Cleodora acicula); Sedgwick (1888) on Peripatus, (1898) on gasteropods; Bouvier (1891) on

Opisthobranchiata; Lankester (1893) on gasteropods and other molluscs; Goodrich (1895) on nematodes, chitons, Peripatus, &c.; Hecht (1895) on nudibranchs; Lang (1896) on the Mollusca, (1900) nudibranchs; Shipley and Macbride (1915) on the molluscs; and others, have thrown a great deal of light on the nature of the organs of circulation in Invertebrata, particularly the works of Sedgwick (1888), Lankester (1893), Goodrich (1895), and Lang (1900).

(1) The Pericardium.

According to Hancock (1864 : 513) the so-called pericardium in Dorids lies immediately above the renal chamber and directly below the dorsal skin in front of the branchial circle. It is, with the exception of the opening leading into the pyriform vesicle, a closed membranous sac, formed apparently by what has been designated the peritoneum, and is just sufficiently large for the accommodation of the dilated auricle and ventricle. It is lined with its own proper membrane which is closely adherent to and intimately confounded with the peritoneal membrane, but can be observed reflected upon the heart at the root of the aorta. It has just been stated that this cavity is closed—previous communications to the contrary were erroneous owing to defective material used.

Goodrich (1895 : 484–6) maintains that the vascular system or blood system is simply a liquefaction, as it were, of the mesoblast (Lankester's view). This corresponds with facts as found in Diploblastica, e.g. (adult) Coelenterata, where blood-spaces are entirely absent, '. . . while as to the nematodes, . . . it seems probable the body-cavity is a blood-space, corresponding in relation to the parenchyma of the planarians.' He shows, following Erlanger, how the pericardium in Paludina arises as two coelomic sacs on either side—a hollowing out of the mesoblast. These coelomic cavities then fuse, and later by processes of special growth form a peritoneal funnel that opens to the outside on either side. 'The gonad develops from the wall of the coelom; then together with the rudimentary left peritoneal funnel, it

becomes constricted off from the main division of the coelom (the pericardium), forming a small genital sac. From the wall of this sac the genital duct grows out, and joins an epidermal invagination like the peritoneal funnel of the right side.' In Melibe the gonads are situated ventrally with openings on the right side; the single kidney (nephrocoel) is situated dorsally and communicates internally with the pericardium through the renal syrinx and externally through the ureter on the left side of the anal pore. The perigonadial coelom (perigonadium) and pericardium are completely separated by the visceral cavity or vascular (haemocoels) cavities. These cavities are blood-spaces into which the blood percolates from the atrial vessels bathing the visceral organs. According to Goodrich (1895), in Chitons, a separation has taken place in the genital region of the coelom from the renal; the gonads then require special ducts which may not be homologous with the peritoneal funnels. In Peripatus, soon after the metameric somites have been hollowed out from the coelomic follicles, the upper half of each coelomic cavity becomes nipped off from the lower half. From the wall of each of these lower coelomic sacs a peritoneal funnel is formed as an outgrowth which fuses with the epidermis. While these organs have developed in this way, the dorsal or genital halves of the somites in the posterior segments have become fused, forming two genital tubes communicating posteriorly with the undivided coelomic follicles of the last segment. The peritoneal funnels of this segment retain their primitive function and develop into the genital duct; Sedgwick (1898: 375) finds that the coelom of the Gasteropoda is in three sections. (1) The pericardium; (2) the nephridia; (3) the gonads. The pericardium is in relation with the heart; it normally communicates with the nephridial system, and part of its lining is generally glandular and forms the pericardial gland. It has no connexion with the blood system. Finally, Lankester (1893: 428) points out the following: the perigonadic spaces and the pericardial space are, then, the coelom of the Mollusca. It is quite distinct from the haemocoel. In cephalopods, and

in the archaic gasteropod Neomenia, the pericardial and perigonadial coelomic remnants are continuous, and form one cavity. There is strong reason to believe that in ancestral molluscs the haemocoel was more completely tubular and truly vasiform than it is in living molluscs. In the later molluscs the walls of the vessels have swollen out in many regions (especially in the veins) and have obliterated the coelom, which have shrunk to the small dimensions of the pericardium and perigonadium. There are, however, many molluscs with complete capillaries, arteries, and veins, in certain regions of the body.

(2) The Heart and Arteries.

While the intestine in M. leonina, by its diagonal course through the visceral cavity, disturbs the apparent bilateral symmetry, the heart is situated in the median line, just anterior to the anus (Pl. 31, fig. 33), and to the left of the intestine. The heart consists of two chambers, a dorsal and a ventral (Pl. 34, fig. 55, *Au*, *Vent*). The dorsal chamber is the smaller of the two; it is partitioned off into small spaces through which the blood is returned by the efferent branchial veins (*Au*). These chambers may be called auricular chambers; they are perforated (*Av*), and the partitions (*Ar*) which may serve as valves may also close the perforations. The partitions or valves with their apertures are so arranged that the openings do not coincide with each other, and are therefore easily closed. The ventricle or the larger of the two cardiac chambers has a regular valve at its lower and constricted portion (Pl. 34, fig. 59, *Valve*). The heart, therefore, may be completely closed upon the contraction by the valves of the two chambers. The heart is enclosed within the pericardium which also encloses the efferent branchial veins (Pl. 27, figs. 9, *Au*, *V*; Pl. 34, fig. 54, *Au*, *Per*, *Vent*). The aorta passes from the floor of the ventricle (figs. 9, 54, *Ao*) to the ventral region of the visceral cavity where it divides into two anterior and posterior trunks (fig. 54, *Aa*). Just ventral to the ventricular valve is an enlargement of the wall, the structure of which

is like that of a lymph-node (Pl. 34, fig. 59, *Bgl*). Within this gland (node) are a number of free cells (Pl. 34, fig. 56), which vary considerably in size and structure (Pl. 34, fig. 58, *a*, *b*, *c*). Some of the cells of the inside of the gland are pseudopodic (*a*), which is also perhaps the condition of the cells free in the lumen (Pl. 34, figs. 56, *L*, 58, *c*). The structure of these cells may also be the same, but the cells within the gland remain in a more stable environment during the time of death, and on that account may be less subjected to physical shock than the cells within the lumen of the blood-vessels at the time of the killing. In fact, the cells from the lumen (Pl. 34, fig. 58, *c*) show a considerable morphological difference in that they are highly vacuolated. It is known from the study of invertebrate blood that the cells contained in the blood-fluid are exceedingly unstable (Tait and Gunn, 1918). In fact, these men were able to destroy the blood-cells of the circulation of the fresh-water crayfish, Astacus fluviatilis, by injecting india ink into the circulation of the living animal; the cells explode very easily upon contact with foreign solids. The cells of the circulation of Melibe may not be as easily destroyed or as sensitive as the blood-cells of Astacus. Whether the difference in environment relative to that of the blood-plasma and the node is sufficient to produce this difference in post-mortem structure by the same killing method cannot be determined here.

Boas (1886) finds for Cleodora acicula that the ventricle is constructed of a few, large, short, flattened, perhaps muscle-cells, which touch each other by their edges. Each cell has a nucleus which lies on the outside of the contractile substance, surrounded by a small protoplasmic mass. The contractile substance consists of fine fibrillae, which are visibly transversely striped. In M. leonina the cardiac wall does not consist of muscle-cells exclusively, but of large nucleated fibrillated epithelioid cells (Pl. 34, fig. 57, *Cfm*), but I cannot at this time tell definitely whether the fibrillae are striped transversely; it is quite evident, however, that these cells are different from the muscle-cells of other organs and of the body-wall of this animal.

(3) The Venous System.

The venous system seems to consist of a number of very thin-walled sinuses so that the blood easily exudes through them, bathing the surrounding organs. The efferent branchial veins collect the blood from the sinuses of the papillae, and perhaps also from the larger sinuses of the body-cavity. The so-called pericardium lies closely below the mid-dorsum, and in front of the intestine and the ureter, and above the anterior branches of the kidney. I have not at this time determined the exact nature of the pericardium and its relation to the blood, whether it is a completely closed chamber or not; whether it is invested with its own peritoneal membrane, or whether it is fenestrated, allowing blood to enter it from the surrounding sinuses. It is, in fact, usually thought that the pericardial space in the molluscs contains blood, and is in free communication with veins; but Lankester (1893) has succeeded in showing by observations on the red-blooded Solen legumen, and by more recent careful investigation on Anodonta cygnea, Patella vulgata, and Helix aspersa, that the pericardium has no communication with the vascular system and does not contain blood.

8. The Organs of Excretion.

(1) The Kidney.

According to Pelseneer (Lankester, 1896: 111) the kidney is a compact mass, as a rule, without external projections, but it is divided into lobes in Stenoglossa in general, and in some Taemoglossa, viz. Paludina and Cypraea. In a fairly large number of nudibranchs (Doridomorpha, Janus, &c.) the kidney is divided into ramifications which extend between the visceral organs of the greater part of the body. Shipley and MacBride (1915) say the kidney is a vesicle, into the cavity of which numerous folds project covered by the peculiar cells which have the power of extracting waste product from the blood, which flows in spaces in the kidney wall. The kidney in Mollusca varies a good deal in structure, but

is always built on the same fundamental plan as that of the snail. The excretory system of M. leonina consists of a bilateral structure with two main renal trunks, ureter, and renal syrinx. The trunks extend anteriorly and posteriorly, dividing into two sub-trunks, each of which pass into primary and secondary branches. The anterior trunk divides much earlier than the posterior, and the spread of the anterior bifurcation is much larger than that of the posterior (Pl. 35, fig. 60, *Ab*, *Pb*).

(2) The Ureter.

The ureter (Pl. 35, fig. 60, *U*) follows the intestine very closely and empties a little to the anterior and left side of the anal opening. This corresponds to Pelseneer's description (vide Lankester, 1906), where it is recorded that the external opening of the kidney is situated near the anus and sometimes the two open together into a sort of common cloaca, as may be seen in Gymnosomata and in certain Pulmonata, such as Limax. In rare cases, he says, such as in the nudibranch Janus, the excretory aperture is distant from the anus. The anterior bifurcations of the renal organ (*Ab*) extend just beneath the pericardium with one sub-trunk on each side of the aorta. The posterior renal trunk sends its branches among the hepatic arborizations and connective tissues in the posterior region of the animal, caudal to the ureter and the intestine.

(3) The Renal Syrinx.

On the side of the ureter, midway between the junction of the ureter to the renal trunks and the ureteric pore, is a bilobed and somewhat convoluted whitish body which empties into the ureter (Pl. 35, fig. 60, *Rs*). This body is described by Hancock (1865) as the pyriform vesicle; von Jhering (1876) as the 'Pericardialtrichter'; Bergh (1884*a*) as renal syrinx that drains the pericardial chamber. The renal syrinx is quite peculiar in structure (Pl. 35, figs. 61, 64, 67). In sections, it is shown to be extensively plicated, but its walls are not muscular as observed by Hancock (1865) on Tritonia hombergii. The plicae are strongly ciliated. The cilia of the individual cells are kept

together in such a way that under a low magnification they appear to form tufts which give to the lining the appearance of flask-shaped cells (Pl. 35, figs. 61, 64). Higher magnification brings out their true nature, that they are moderately columnar cells with large cilia nearly four times longer than the cell (Pl. 36, figs. 67, 68, 69). The renal syrinx communicates with the pericardium by a cyncitial plate with the nuclei scattered, but as a rule nearer the base, i.e. toward the syringeal side (figs. 66, 67, *Sypl*). It communicates with the ureter, however, by a rather wide opening (fig. 61). There is then no reno-pericardial pore between the kidney and pericardium, through the renal syrinx. This I have determined by the study of serial sections of the organ. Elysia, according to Lankester (1906 : 110), is exceptional in that the kidney is placed below and partly surrounds the pericardium, and the reno-pericardial orifices are multiple, some ten being present. And, according to Shipley and MacBride (1915) there is a reno-pericardial canal, a narrow ciliated passage, between the kidney and pericardium in the molluscs. In Melibe leonina on the pericardial side, the renal syrinx narrows into a neck (Pl. 35, fig. 60, *P*), which internally is formed into two channels by a plica or villus which extends from the cyncitial plate and into the organ (Pl. 35, figs. 64, 65, Pl. 36. fig, 67, *Pl*). Only about one-third of this villus is ciliated, that is, its tip, or the part farthest away from the cyncitial plate. The sides of the syrinx opposite the non-ciliated portion of the villus are also non-ciliated. The non-ciliated part of the walls has a large number of nuclei situated near the surface. The structure of the ciliated columnar cells of the renal syrinx, or pyriform organ, in M. leonina, shows the same remarkable feature as in the intestine, viz. the individuality of the cilia as they pass into the cell. In the renal syrinx there is first the plainly visible terminal bars, but unlike the condition found in the intestine, the terminal bars (basal granules) are shown only as one row, or one for each cilium. From the terminal bar the cilium continues to the base of the cell as a distinctly granular fibrillar structure. As in the intestine (Pl. 33, fig. 45, *Bm*), the basement

membrane is prominent, but unlike the condition here, where it seems to be granular, the appearance of the basement membrane in the ciliated cells of the renal syrinx is a continuous, non-granular line, or the granules if present are fused. However, this may also be the condition in the intestinal cells (Pl. 33, figs. 46, 47), reflecting, perhaps, the fact that the bringing out of certain cytological features depends, at least, on two things: (1) the physical condition of the organism at the time of killing, (2) the method and kind of chemicals employed in the killing. Another differential feature of the ciliated cells of this organ, is, as pointed out above, the independent arrangement of the cilia. That is, the cilia are not mingled with the cilia of neighbouring cells, as in the case of the intestine. Still another feature is the size of the cilia both in length and diameter. This specialization of the cilia may point toward a special function of the organ. For example, it may be that of creating a suction within the organ in order to draw the pericardial fluid toward and through the cyncitial plate. The cyncitial plate, then, with the action of the specialized cilia of the plicae may function as an extracting organ, and one may expect this process to be that of ridding the pericardial fluid of waste. This is also the opinion of Hancock (1864: 520) for Dorids.

Von Jhering (1876: p. 49), applies the name 'Pericardialtrichter' to the renal syrinx. By this name its function is indicated also. The case in question is that of Tethys, for which the author finds that the syrinx communicates with the lumen of the ureter: '. . . in weiter Communication steht, andererseits durch eine kleinere runde Oeffnung mit der Pericardialhöhle zusammenhängt. Die letztgenannte liegt in einer Membran, welche quer zur Axe des Pericardialtrichters steht und sein Lumen von dem des Pericardium trennt. In dieser Membran liegen um die Oeffnung herum zahlreiche ringförmig angeordnete Muskelfasern, die also einen Sphincter bilden durch welchen die Communication zwischen Niere und Pericardium nach Belieben aufgehoben werden kann.'

Bergh (1884*a*: 76) does not describe the exact relationship

of the renal syrinx to the ureter and pericardium. Dealing with a number of types: Phylliroe, Acura, Rizzolia australis, Bornella, Tritonia challengeriana, and Marionia (pp. 3, 8, 30, 41, 47, and 51, respectively), he only describes the shape and size and a few of its finer structures, and also that it opens into the pericardium on the one hand and the ureter on the other. For the holohepatic form, Chromodoris striastella, he says: the renal syrinx is bulb-shaped of 0·75 mm. greatest diameter; the folds of the interior can easily be seen from the outside; the ciliated cells are as usual. The duct of the renal syrinx is about 1·5 mm. long, opening into the chamber; in the anterior are the usual villi and papillary outgrowths.

Pelseneer (1893: 458): 'Je crois que c'est le plus antérieur ou ventral qu'on doit considérer comme tel: les Nudibranches les plus voisins de Elysia (Hermaea, Cyerce) m'ont en effet montré l'orifice réno-péricardique à la même place, ventralement et à gauche. Les autres conduits seraient secondaires ou cénogénétiques et résulteraient vraisemblablement de la multiplicité des points de contact entre le péricarde et le rein, celui-ci entourant plus ou moins le premier.'

Stempell (1899: 142) finds for Solemya togata, Poli: 'Von histologischem Interesse ist zunächst die Beschaffenheit der Nierenspitzen. Dieselben besitzen nämlich nicht wie diejenigen der Nuculiden ein flaches, mit langen Geisseln besetztes Epithel, sondern ein gewöhnliches mittelhohes Cylinderepithel, welches nur mässig lange Cilien trägt.'

And MacFarland (1912: 527), for Dirona picta, writes: 'The reno-pericardial opening is found in the renal syrinx, a conspicuous pyriform body situated midway of the animal's length, upon the right dorsal surface of the visceral complex. It communicates below with the pericardial cavity, opening through the floor of the right side. Its lumen is divided by numerous folds of the wall, many of which in turn bear secondary folds. The complicated opening thus formed is lined in its upper portion by high columnar cells, bearing very long cilia, which are directed downward.'

Thus it is seen that, while the communication between the kidney and the pericardium differ, the internal structure of the renal syrinx seems to be similar as far as being lined with ciliated columnar epithelium.

The cilia of the renal syrinx of M. leonina discontinue in the region of junction with the ureter (Pl. 35, figs. 61, *A*, 62, *A*). The cells are indistinct in this region (Pl. 35, figs. 61, *B*, 63). The structure of the ureter is unique in itself. The epithelium shows a cyncitium (Pl. 36, figs. 71, 72), some parts being conspicuous by the presence of large vacuoles which seem to have formed by the confluence of smaller ones that arise around the nucleus. These larger ones, then, as in the case of the intestine, pass into the lumen of the organ. The ureter is covered by an exceedingly fine fibrous cover (Pl. 36, figs. 71, 72, *Ex*). This cover as well as the epithelium vary according to their position. That is, nearer the renal syrinx it is more glandular in its feature (Pl. 36, fig. 72) than near the end of the ureter (Pl. 36, fig. 71). The renal chamber proper consists of a corrugated glandular lining with a small amount of fibrous tissue covering it (Pl. 36, fig. 70, *Pr. nph, Ct*). The structure of the epithelium suggests that the organ is one of periodic function. According to Stempell (1899 : 142), ' Das Epithel der Nierenschläuche selbst hat eine ziemlich typische Form. In den distalen Abschnitten der vergleichsweise hohen Zellen finden sich helle Vacuolen, welche . . . regelmässig kleine Concrement - Klumpen enthalten. Nach Conservirung mit Flemming'scher Flüssigkeit gelang es mir auch, deutliche Cilien auf den Zellen nachzuweisen.' The kidney of M. leonina, as far as I have seen up to the present time, is not ciliated. But the epithelium is highly glandular in structure, which also is the nature of the lining of the ureter, but there is considerable difference in structure, nevertheless.

The function of the kidney is supposed to be that of extracting waste from the blood (Shipley and MacBride, 1915). Ward (1900 : 152) finds that among the variable types of excretory cells two appear to be constant : the first absorbs indigo-carmine and refuses ammonium-carminate, while the second

precisely reverses this action. Rarely excretory cells do both, but even then an excretory cell absorbs the one substance more freely than the other, or vice versa. These two types are associated with voluminous organs. The indigo kidneys produce urea, uric acid, and urates, while in carminate kidneys, thus far known, none of these substances are formed, though some non-indigo excretory cells contain urates. Referring to special cases he states: 'In two groups of molluscs the nephridia instead of being lined throughout their entire extent by a single type of excretory cells present noteworthy differences: in Amphineura the reno-pericardial ducts of acid reaction eliminate actively carminate and litmus; while the rest of the nephridium, formed of different cells, and with alkaline reaction, eliminate indigo. Both nephridia of Patella eliminate equally indigo. The most numerous non-ciliate eliminate indigo; the others, ciliated, eliminate only carminate—the single nephridium being thus a physiological equivalent of two nephridia in the Diotocardia (Trochus, &c.).'

9. The Organs of Reproduction.

It was pointed out by Lankester (1881, 1893), Sedgwick (1888, 1898), Goodrich (1895), and Lang (1896), that the true coelom in molluscs is much reduced, being divided into three parts: (1) the pericardium, (2) the perigonadium, and (3) the nephrocoel; the remaining body-cavities being haemocoels which are derived in part from a system of spaces which arise between the ectoderm and the entoderm (Sedgwick, 1888: 383). It is not my purpose to discuss here the homology of these cavities in M. leonina, but only to call attention to their relation to one another and their relative duct systems. As stated above the pericardium is a closed cavity which communicates with the nephrocoel by the renal syrinx which seems to be closed at its point of communication by what I have called a cyncitial plate. The perigonadial coelom lies directly below the nephrocoel. It was shown by Erlanger, 1891–2, for Paludina that the pericardium arises as two coelomic sacs on either side by a hollowing out of the mesoblast.

These coelomic cavities then fuse and later by a process of special growth form a peritoneal funnel that opens to the outside on either side. The gonad develops from the wall of the coelom ; then, together with the rudimentary left peritoneal funnel, it becomes constricted off from the main division of the coelom (the pericardium), forming a small genital sac. From the wall of this sac, the genital duct grows out, and joins an epidermal invagination like the peritoneal funnel of the right side.

In M. leonina the perigonadium is situated caudoventrally in the perivisceral cavity. It has a complex duct-system which opens on the right side near the anterior end of the trunk of the body (Pl. 27, figs. 2, 3, *P* ; Pl. 37, fig. 81). The single kidney (nephrocoel) is situated dorsally and communicates internally with the pericardium through the renal syrinx and externally through the ureter which opens on the left side of, and close to, the anal pore. The perigonadial coelom (perigonadium) and pericardium are completely separated by the visceral cavity or vascular (haemocoels) cavities. These cavities are blood-spaces into which the blood percolates from the arterial vessels, bathing the visceral organs. In Chitons, 'A separation has taken place in the genital region of the coelom from the renal ; the gonad then acquires special ducts which may not be homologous with the peritoneal funnels' (Goodrich, 1895 : p. 486).

(1) The Hermaphrodite Gland.

It is a well-known fact that the gonads among nudibranchs and many other molluscs are hermaphroditic, but the duct system of the two different functional regions varies relative to their complete development. Some authors who have worked on the Elysiadiidae (Allmann, Hancock, Souleyet, Gegenbaur, &c.) maintained that the male and female parts of the hermaphrodite gland are separate as are the ducts (vide Pelseneer, 1891). It has been shown by Pelseneer (1891), who worked on several genera of the group in question, that the same part of the gland is both male and female ; some of the follicles of the hermaphrodite gland among certain

Doridiidae and Aeolidiidae, he found, were distinctly male or female; among the Elysiidae all the follicles, in fact, contained two genital products. Mazzarelli (1891 *a*) found that, while the hermaprodite gland is divided into lobes with subdivisions somewhat deeply placed, each lobe presents a great number of acini contrary to that which up to that time had been observed in tectibranchs—referring in particular to the works of Lucaze-Duthier on Pleurobranchus, of Moquin-Tandon on Umbrella, of Vayssière on Aphalaspidea, and by himself on the Aplysiidae—each acinus produces at the same time and contains ova and spermatozoa. In Pleurobranchiaea, however, there are both male acini and female acini.

Lang (1896) characterizes three types of genital ducts in the molluscs as follows:

Type I.—'The hermophrodite gland has a single undivided efferent duct opening through a single aperture—Gastropteron, Pteropoda, Cephalospedae (Bulla, Dorium).'

Type II.—'The hermaphrodite gland gives rise to a hermaphrodite duct which soon divides into two parts, the vas deferens or seminal duct, and the oviduct. The former runs to the male copulatory apparatus, the latter to the female genital aperture. The male aperture and the penis lie in front of the female . . . both lie on the right. This second type may be deduced from the first, if we assume that the common duct of the hermaphrodite gland divided into a male and female duct, but also that the seminal furrow closed to form a canal in continuation of the male duct. To this type belong:

1. A few species of Dendebardia
2. Basommatophora,
3. Oncida, and
4. Vaginulidae

} of the Pulmonata.'

Type III.—'In all Nudibranchia and a few Tectibranchia (e.g. Pleurobranchiaea), the hermaphrodite gland gives rise to a hermaphrodite duct, which, as in the

second type, sooner or later divides into a male and female duct. These, however, do not open through distinct apertures, but again unite to form a common atrium genitale or genital cloaca.'

The first type is also demonstrated by Bonnevie (1916) for the pteropod Cuvierina columnella Rang, where the hermaphrodite duct arises first as a groove (Rinne) and later forms into a thin duct (Rohr) which makes its exit from the left dorsal edge of the hermaphrodite gland.

Type IV.—This is a new type. It is represented by the reproductive system of Melibe leonina (Gould) and is equivalent to types ii and iii plus a more complete duct system. The organs of reproduction in this species (Pls. 28 and 37, figs. 9, 81) consist of a well-defined pair of gonads, each consisting of many lobes or acini; an oviduct, a 'prostate gland' (convoluted portion of the female duct), a uterus (enlarged distal portion of the oviduct), a spermatotheca, and a vagina; a vas deferens with its ampulla, a penis, and a mucous gland. The male duct is further modified into vasa efferentia. Each of these parts is peculiarly modified, and together furnish a unique system of reproduction. The relative position of these organs in the body-cavity is shown in Pl. 28, fig. 9.

The male and female ducts in M. leonina do not unite to form a common genital atrium as set forth by Lang for 'all Nudibranchia and a few Tectibranchia', but open close together through separate apertures (Pl. 37, fig. 77, *Mgp*); that is, the penis lies in front of the vagina (Pl. 28, fig. 9, *P*); in that way, it resembles the second type of Lang. Both branch, i.e. a vas efferens and an oviduct pass to the same acinus, which is to say: the hermaphrodite gland gives rise to a double genital duct system which passes from the respective male and female germ-cell area of the various acini. In this respect the reproductive system of M. leonina differs from all the three types of Lang, and for this reason I have designated the genital duct system of this mollusc as constituting a fourth type. However, since the oviduct is still connected to the ampulla of the vas deferens by a duct,

it is evident that this type is derived from the third type of Lang.

The hermaphrodite gland lies in the caudo-ventral region of the perivisceral cavity and consists of a great number of bilobed acini (Pl. 37, fig. 81, *Ot*). The eggs and the spermatozoa are situated in different regions of the same acinus, which is easily demonstrable in the neutral phase, that is when the male or female germ-cells are in a regressive or progressive stage of growth ; as soon as the one has gained the ascendency either a male or a female phase appears which seems to take over the entire acinus. At such a time the small duct system which leads from the indifferent or resting region of the acinus is nearly crowded out by the actively employed ducts, and then the acinus may give the appearance as having only a single duct arising from it. That is, during the ripe male phase, the female germ-area with the ducts of any acinus may be crowded to the periphery or to one side in such a way so as to give the appearance of only one duct system leading out from that acinus. However, in any stage of either male or female phases, the two duct systems may be discerned in some of the acini. I cannot determine with any certainty whether this species is protandrous or not, as I have not studied sufficiently young individuals on this point. All the individuals, whose glands I have sectioned, have shown ripe spermatozoa in the acini and also ova.

Pelseneer (1895 : 31) states that protandry ought to be regarded as a general phenomenon in Euthyneurous gasteropods. That this is notoriously the case in pulmonates, and that it has been recognized in various opisthobranchs which have been studied from this point of view, viz. Lohiga, the Thecosomatous pteropods, e.g. Cliostriola, &c. ; nudibranchs, among which he observed it in Aeolidia and Elysia ; and lastly Clione limacina (Gymnosomata), in which he noticed that individuals of a length of 15 mm. (or less) do not as yet show any ova in their genital glands, but stages in the development of spermatozoa only. He also found that the ovogenous and spermatogenous

regions of the acini in Onchidiopsis are not demarcated with any regularity, that in the middle portion male and female acini can be seen in sections lying side by side. Also, that the products of the two sexes either do not arise in the same caecum or they do not arise in the same region of a caecum.

Bonnevie (1916) finds that Cuvierina columnella is protandric; the spermatozoa pass into the 'Zwittergang' and then the eggs develop; there being only one ripening of the germ-cells in the life of the individual. If this be also the case among individuals of Aeolidia (Coryphella) landsburgii, as reported by Pelseneer (1895: 23), that the acini of the hermaphrodite gland produce ova in their distal portion and spermatozoa in their proximal portion, it is not so difficult to understand how the product of the peripheral region of the acinus with only one duct system may gain access to the duct, the ripe spermatozoa simply passing out of the way, giving its former position to the oncoming mass of the ripening ova. Pelseneer reports that the same condition as noticed in Aeolidia landsburgii has been recognized as general in all the Elysioidea (Cyerce, Hermea, Elysia, and Limapontia). Mazzarelli (1891*a*) found male and female germ-products present at the same time in the acini of Aplysiidae, and in Pleurobranchaea separate male and female acini. 'In the former there are only spermatozoa and spermatids in varying stages of development, and spermatozoa with sheaths (fascetti) seem joined together by cytophors. In the latter (female acini) there occur only ova. The tiny ova in diverse grades of development lie distributed all around the internal aspect of the wall of the acinus as an epithelium. Of the original germinative epithelium there remains slight traces. The ova which are larger and nearly being expelled are found more or less in the centre of the lumen. This fact, that of the formation of spermatozoa and of ova in separate acini in the male-female gland which is abnormal in the Tectibranchs, is seen to be true or ordinary in effect in many Nudibranchs.'

(2) The Hermaphrodite Duct.

Mazzarelli (1891 *a*) found that the hermaphrodite duct which leaves the male-female gland is very minute in diameter at its beginning, but later, after a certain descent, dilates abruptly to a greater lumen. From this point it gradually narrows again only to bifurcate, giving origin to two minute ductuli. From this point one passes straight to the penis (oedagus), in which it constitutes the deferent channel. The other ductule which is the oviduct enlarges rapidly after its origin and here presents a tiny caecum, then the oviduct contracting gradually, only again abruptly to dilate, develops the first ampulla. In M. leonina, however, I find that the hermaphrodite duct has separated into two distinct male-female ducts, i.e. from the time an exit is formed in the acinus it is double. It should be pointed out, however, that the male duct caudad of the ampulla is larger than the corresponding female duct.

(3) The Oviduct.

The oviduct, after it leaves the last acinus (Pl. 37, fig. 81, *Od*), passes into the prostata (*Pr*), which, in fact, is a part of the female duct. It stands in relation with the ampulla of the vas deferens by a biluminate duct (*Bil.dpr*) (Pl. 37, figs. 73, 79, *Pro*) ; the so called prostate gland is a much-coiled portion of the oviduct, consisting of two kinds of coils, a large and a small coil system coiled upon itself. The ampulla-prostate duct shows that the female-duct system formerly was in functional relation with the male-duct system at the ampulla ; the genital-duct system from the ampulla and to the gonads constituted the hermaphrodite duct. At the point of exit from the prostata the oviduct dilates into a much saccular portion (Pl. 37, figs. 79, *Ut*, 80, *F*, 81, *Ut*), which after some distance passes into a narrow portion. From the distal part of this (Pl. 37, fig. 81, *Osp*) there is a large sac, the spermatotheca, and from this point to the orifice the duct dilates a little (*Va*) forming into what I have called the vagina. The most distal portion of the duct lies in communication with the mucous gland (*Mgl*).

The prostate portion of the oviduct is not very glandular. The wall of the uterus consists mainly of fibrous and of some muscular tissue. Its lining consists of glandular epithelium (Pl. 37, fig. 80, *Ms*, *Gl*).

(4) Ovispermatotheca.

The ovispermatotheca has a most unique internal structure (Pl. 37, fig. 82). Its outer part consists of a loose vascular connective tissue (*L*) and a muscular layer of circa two cells in depth. Its middle part is a connective-tissue layer (*Sm*) upon which rests a papillated epithelial layer. The muscle-cells of the muscle-layer show the interesting structure already described. The epithelium retains Delafield's haematoxylin stain very well. The papillated epithelium consists of cells that are free (*Ept*) at their two-thirds distal portion, abutting (Pl. 37, fig. 74, *Spt*) into the cavity as finger-like processes (Pl. 37, fig. 82, *Spt*). The larger of these seem to be supported by the underlying basement layer (*Sm*, *Bm*). In the cytoplasm there is a distinct micromeric network (*Mic*). The nucleus is situated at the base in the smaller cells, and midway between the base and the free end in the larger cells. The cells of the larger papillae are wider at their free or distal portion, so that they actually approach each other. The spermatotheca contains both semen and ova, and, since in some cases this organ is filled with eggs, I have called it ovispermatotheca.

Mazzarelli (1891 *a*) found that the structure of the spermatotheca was plical. He says: 'Indeed the entire aspect of the wall of this presents a great number of longitudinal folds (or plicae) highly developed and disposed in such a manner as to constitute a series of correlated passages or channels (rooms) ('concameragioni'), on the periphery of the lumen of the ampulla which are commonly engorged with the sperm.'

Eliot and Evans (1908: 287) write: 'The walls of the spermatotheca in Doridoides gardineri are thick and produce a secretion. In some specimens small clumps of spermatozoa are imbedded in this secretion. In others all the spermatozoa form a central mass in the main cavity of the

spermatotheca. It is possible that the secretion serves to form small pockets of spermatozoa or spermatophores.'

MacFarland (1912) found, in the Dironidae, that the spermatotheca was almost rudimentary, and that the dilated oviduct seemed to have assumed in part the function of a spermatotheca, 'for it is frequently crowded with spermatozoa, while the spermatotheca itself contains relatively few.' On the contrary, 'in Diron albobineata the oviduct is short and slender: spermatotheca very large, reaching a diameter of 1·3 mm., total length being 4 mm. in a large specimen'. In this connexion it is well to note that, in M. leonina, which has a large spermatotheca, the male germinal product also frequently passes into the distal regions of the female genital duct system even as far as and including the prostata.

(5) The Male Genital Duct.

The male genital duct starts in the hermaphrodite gland as small tubules (Pl. 37, fig. 81, *Ve*), which join into a common median duct that enlarges into a round bulb-like part (*Amp*) at the anterior region of the ovitestes (gonads). This enlargement I have called ampulla. From the ampulla the male duct passes anteriorly as a large organ of fibrous tissue (Pl. 28, fig. 9, *Vd*). It is surrounded by a sheath of its own (Pl. 37, figs. 78, *Iglp*, 74, 76, 77, 79, 83, *Oc*). Intervening between the penal sheath and the penis itself, is lymph or mucus, a colourless, structureless substance (*M*). The penis is covered with cells of epithelial structure, perhaps both of these, and the cells of the lining of the penal chamber just described, secrete the mucous substance also mentioned. The organ itself is made up mainly of fibrous connective tissue (Pl. 37, figs. 75, 78, 83, *Int*, *Ml*). In fig. 75 the biluminate effect as shown here is due to the coiled condition of the organ at the point of section. The main bulk of the organ consists of this fibrous tissue (Pl. 37, fig. 76, *P*). The lumen of the organ is lined with ciliated cuboidal epithelium (Pl. 37, fig. 83, *Iepl*, *cil*). The ampulla of the penis seems to be quadroluminate; anteriorly these lumina converge into three, then two, and finally into one, which becomes the seminal tube

of the penis. The structure of the seminal vesicle and of the ampulla is alike ; it consists of a heterogeneously arranged cell-mass, so well welded together that the whole structure is quite compact. The penis is, indeed, so large that when it is withdrawn it fills a large part of the body-cavity. The penis is simply an extension of the seminal vesicle. The sheath of its anterior portion is firmly lodged on the mucous gland (Pl. 37, fig. 81, *Cl.p*) with the penis in its pore. The penal pore merges with the body-wall, adjacent to the vaginal orifice (Pl. 37, fig. 77, *Mga*, *Fv*). The penis is sometimes extended to the outside, and is then curved like a screw (Pl. 27, fig. 3, *P*). In copulation the penis, which is long, twisted like a screw and of tough musculature, is inserted into the posterior (female) genital pore of the mate, and so firm is the union that separation may not occur even though the couple be dipped from their natural abode and placed in a vessel (Agersborg, 1921 : 238). I have not found mutual coitus effected at the same time in this species, although it is supposed to be a common practice among nudibranchs, according to different authors : Alder and Hancock (1845 : 25), Mazzarelli (1891 *a* : 237), Crozier (1919) et al.

(6) The Mucous Gland.

The mucous gland constitutes the albuminous and nidamental glands (Pl. 37, figs. 74, 76, 79, *Mg*, 81, *Mgl*, and 84, *A*, *B*, *C*, *D*). It consists of laminations so arranged that sections through the side of it have a six-layered aspect ; continuous sections soon bring out the true conditions. The gland is made up of simple, tall, ciliated columnar epithelium, highly glandular in nature, and which rests on thin connective-tissue fibres with cells which connect the gland to the body-wall. The gland extends almost to the outside of the vaginal orifice (Pl. 37, figs. 74, 76, 77). It functions when the animal spawns ; the mucus and the capsulated eggs pass out together.

Mazzarelli (1891), in Pleurobranchaea, finds that the vagina at the back of the oviduct's terminal point prolongs itself remarkably dorsad becoming sacculated, its walls being

formed of robust folds and studded with glands which are the glands of the nidamento; near the opening of the oviduct into the vagina but still more dorsad opens the gland of the albume. This is contrary to that noted in other tectobranchs in which the albume gland terminates in a vast number of minute ductuli with blind origins arranged in such a way as to constitute un fitto gomitolo. The albume gland of Pleurobranchaea resembles much in structure the albume gland of some nudibranchs, e. g. Ercolania (? Hercolania) as described by Trinchese.

The albuminous gland of M. leonina seems to be more uniform in its physiological condition relative to that of the mucous gland. Unfortunately, I have not at the present time worked out its exact relation to the oviduct, but in general it is somewhat like that described by Mazzarelli for Oscanius and Acera, i.e. the nidamental gland is nearer the orifice of the vagina than is the albuminous gland. The epithelium of the nidamental gland which secretes a great deal of mucus at the time of oviposition shows some very interesting things relative to its activity during such a time (Pl. 37, fig. 84, *A–C*):

1. The nucleus (*B*) presents no visible membrane, the nucleoplasm being filled with almost uniformly sized granules which seem to be formed by the nucleolus and then pass as a liquid into the cytoplasm of the cell where from small, minute micromeres the cell becomes entirely filled with darkly staining macromeres which seem to have grown from these smaller ones so that the ordinary and less stainable cytoplasm is practically obliterated, i.e. obscured by these granules. These granules are very strongly basophil in their staining reaction. The macromeres then liquefy and pass out of the cell and into the lumen of the gland.

2. This leaves the cell in a condition strongly contrasting to the one before the liquefying of the mucus. The cell is now very vacuolated, containing a non-stainable, or rather oxyphil, substance (*A*, *Rs*) with relatively few granules. These granules are micromeric, and are aggregated in the meshes of the reticular net-work of the cytoplasm.

3. In the proximal region of the cell may be seen the nucleus, small and shrunken, containing a small nucleolus, and lodged at the base of the cell.

4. A state of refilling now ensues (*C*). The nucleus begins to enlarge and then a homogeneous cytoplasmic substance is accumulated around it, which then spreads throughout the cell. At the same time the micromeres of the vacuolated cytoplasm of the cell increase in size.

That the nucleus takes a very active part in the formation of the mucinous substance is clearly evident, but the micromeres of the cytoplasm seem also to take an active part in the refilling of the cell as indicated by the growth of the micromeres formed in the meshes of the reticular network of the cytoplasm before the nucleus has assumed its normal condition. In semi-vacuolated cells, i.e. cells which are in the state of refilling, the nucleus is intermediate in size and granulation to those described under (*B*). It is of interest to note some of the findings of Lange (1902) from his studies of the structure and function of the ' Speicheldrüsen ' of gasteropods. This author found that the cells of the glands showed great differences during feeding as compared with periods of starvation. Some of these phases of activity of the cell are quite similar to those recorded above for the mucous gland of M. leonina. Lange says :

In allen Stadien der Fütterung und auch des Hungerzustandes finden sich nie sämtliche Sekretionszellen auf derselben Sekretionsstufe. Es kommen in jedem Stadium der Fütterung und des Hungers alle Stadien vor, doch in verschiedener Häufigkeit. Der Kern nimmt innigen Anteil an der sekretorischen Thätigkeit, indem im Anfang seine Membran sich auflöst und sein Inhalt sich mit dem Protoplasma vermischt, sodass der erste sekretorische Vorgang sich am Kern bemerkbar macht. Die von Barfurth als ' Speichelkugeln ' bezeichneten Gebilde sind Sekretvakuolen, welche angefüllt sind mit muciginer Substanz. Man kann deutlich verfolgen, wie sich das Mucigen in diesen Sekretvakuolen zu Mucin umwandelt. Ist das Mucin gebildet, so verliert der Kern seinen Turgor wohl durch Austritt von Kernflüssigkeit. Dabei steht das Kerninnere stets im offenen Zusammenhang mit dem

Protoplasma. Es lassen sich zwei Teile an dem Zellich unterscheiden : der den Kern umgebende protoplastische Teil und der periphere parablastische mit den Sekretvakuolen ; dieser letztere wird mitsamt dem in ihnen gereiften Mucin bei der Sekretion ausgetossen. Es bleibt in der Bindegewebskapsel allein der protoplastische kernhaltige Teil übrig, von dem aus die Neuproduction des Zelleibis vor sich geht, sodass die Sekretion zunächst in der Bildung von Sekretionsvakuolen, sodann aber in der Ausstossung des ganzen peripheren Teils der Zelle mit dem gebildeten Sekret aus der bindegewebigen Kapsel besteht.

In the epithelium of the mucous gland of M. leonina, as in the epithelium of the intestine and of the renal syrinx, basal granules or desmochondria are demonstrable ; but in the mucous gland the linear fibrillar arrangement of the micromacromeres of the cytoplasm is absent. The reason for this is obvious ; the cilia also seem to end on the distal basal granular border (Pl. 37, fig. 84, *Bg*, *Fcb*).

V. Summary.

1. It is evidenced by the work of various authors that Gould's Chioraera (1852) is identical with Rang's Melibe (1829) ; Chioraera, therefore, is a synonym of Melibe ; Gould, at the time of his description, did not know of the genus discovered by Rang. For these reasons I have consistently named it throughout all my works on this species Melibe leonina (Gould) in spite of the attempt of certain authors to build on the nomenclature of Gould.

2. Melibe leonina is absolutely void of masticatory organs ; the generic description of Gould may be augmented, therefore, to read in part, Bulbus pharyngeus aut cum mandibulis aut sine mandibulis ; radula et lingua destitutus.

3. The anterior end of M. leonina is formed into a large cowl ; this has a pair of stalked foliaceous tentacles which may be retracted below the edge of the stalk which then acts as a sheath. The tentacles are very complex in structure, being innervated with nervous tissue. The tentacles are not ciliated, as claimed by Jeffreys (1869) for all nudibranchs. The cowl

is fringed with two rows of cirrhi which also are highly complex in structure. From an inner ganglionic axis, nerve-fibres radiate to the peripheral ectoderm of the cirrhus. The exact function of the tentacles, as well as of the cirrhi, is not known. The tentacles are commonly called rhinophoria but, since the exact function is not known, I have employed the original term tentacles (dorsal tentacles) instead of the commonly used term 'rhinophoria'. The cirrhi are more sensitive to tactile stimulus than are the dorsal tentacles (Agersborg, 1922*a*: 441–3).

4. The body-surface of M. leonina appears smooth, but upon close examination it is found to be everywhere tuberculate, including the sole of the foot and the ventral side of the hood; in that way this species corresponds to other members of this genus.

5. The dorsal appendages, which in M. leonina consist of six pairs of foliaceous lobate structures, I have called by the Linnean term papillae, instead of cerata, or branchial papillae for the reason as stated by Bergh (1879*c*): 'Respiration takes place all over the surface in Nudibranchs,' &c. The papillae alternate in position; they are subject to variation in structure relative to position and age (Pl. 27, figs. 1, 2, 3, and Pl. 30, figs. 18–25).

6. The foot projects in front of, and behind, the main body. It is highly tuberculate and ciliated. Internally, a fine nerve network is seen spread throughout its length and breadth, and at the posterior end it aggregates into a ganglionic centre. Fine nerve fibrils are seen to pass to the ciliated ectoderm. A great many mucous glands are present all through the foot, which open independently through small crypts between the ectoderm cells. These glands are the pedal glands which are scattered all through the foot (figs. 1, 2, 3, 9, 26, 27, 28, 29).

7. There are three kinds of glands in the body-wall: (1) the largest and most numerous are the odoriferous glands; (2) the next in size and number are the saccular mucous glands; and (3) the unicellular mucous glands (Pl. 31, figs. 30, 31).

8. The muscle system lies below the glandular fimbriated

ectoderm ; the muscle-fibres are arranged in a fashion like the fibres in a basket (Pl. 27, fig. 3).

9. The muscle-cell consists of two sarcoplasmic regions each containing an abundance of micromeres : those in the inner region are larger (Pl. 31, fig. 33, *Ca*) than those in the outer region. The larger are called in the text macromeres. Each of these micro-macromeric sarcoplasmic regions is invested by a coarsely granular net-work (*My*, *Sar*). The nucleus is placed centrally within the cell. Its chromomeres (*K*) are scattered differently, i. e. sometimes around the periphery of the nucleus (Pl. 31, figs. 32, 33, Pl. 37, fig. 82) and sometimes less so.

10. There is no definite body-cavity. The body-cavity as it exists corresponds to the primary body-cavity of Lang (1896) or the perivisceral cavity of Sedgwick (1898).

11. The alimentary canal consists of five regions :

(1) The oesophagus with the non-glandular lining, and back of it the oesophageal glands or salivary glands (Pl. 32, figs. 36, *Oe*, 41, *Sg*).

(2) The proventriculus with distinct glandular lining (Pl. 32, figs. 36, *G*, 38, *Gl*).

(3) The gizzard with its stomach-plates which are formed by the secretion of the epithelial lining (vide supra), (Pl. 32, figs. 37, 42, 43, *Stpl*).

(4) The pyloric diverticulum with its glandular and ciliated and much corrugated surface, which secretion does not keratinize as that of the gizzard (Pl. 32, figs. 35, 39, 44).

(5) The intestine with its large typhlosole protruding into the cavity from the ventral side, and glandular ciliated surface (Pls. 32 and 33, figs. 40, 45, 46, 47). The structure of the intestinal lining is unique as concerns the fibrillar nature of the cytoplasm, the clearly visible terminal bars, and the basement membrane ; also, the non-convergence of the cytoplasmic portion of the cilia on the nucleus.

12. The liver ramifies all the parts of the body. Its secretion

into the gizzard does not harden in the alimentary canal. The glandular structure of the epithelium of the liver exhibits that it has an active function in vivo, owing to the presence of a variable series of granules and vacuoles in the adjacent cells fixed at the same time in the same way (Pl. 27, figs. 1, 2, 4, 7, Pl. 33, 48–53).

13. The heart consists of two chambers enclosed within the pericardium. These chambers are separated by valves from the efferent branchial veins and the afferent aortic trunk-vessel. In the aorta, just below the valve of the ventricle, is a blood-gland or node which contains pseudopodic cells. The cells found on the outside of this node, i.e. within the lumen of the aorta, are different in structure from those found within the node (Pl. 34, figs. 54–9).

14. The wall of the heart consists of epithelioid and some semi-musculofibrilloid cells (Pl. 34, fig. 59).

15. The kidney is much branched, and is situated between the pericardium and the gonadium. It communicates with the pericardium through the renal syrinx which is closed at the point of junction with the pericardium by what I have called a cyncitial plate. The lining of the kidney is glandular; so is also that of the ureter, but neither is ciliated. The renal syrinx, however, is ciliated. The cells of the renal syrinx are peculiar in that the cilia are very large and independent in position, i.e. they do not mingle with those of adjacent cells. The renal syrinx is plicated, and from the cyncitial plate a ciliated villus protrudes into the organ (Pls. 35 and 36, figs. 64, 65, 67, *Pl*).

16. The organs of reproduction represent an additional type to the three types enumerated and described by Lang (1896). The male and female ducts in M. leonina do not unite to form a common atrium genitale as set forth by Lang for all nudibranchs and a few tectibranchs, but open close together through separate apertures (Pl. 37, fig. 77, *Mgp*); the penis lies in front of the vagina (Pl. 28, fig. 9, *P*); in that way it resembles the second type of Lang. Both genital ducts pass independently to the same acinus; in this respect it differs from all three types of Lang, and, for this reason, I have

designated the genital duct system in this mollusc as constituting a Fourth Type. During any ripe phase of the gonads the duct leading from the inactive area of an acinus may be quite obscured by the ripening mass of germ-cells. The organs of reproduction are represented in Pl. 37, fig. 81.

17. The spermatotheca I have called ovispermatotheca because it is frequently filled with eggs from the oviduct. The structure of the ovispermatotheca is quite peculiar owing to the plicated nature of its lining. The cells lining this organ are flask-shaped, the neck being longer than the body and abutting into the cavity (Pl. 37, fig. 82).

18. The mucous gland is relatively rather large. A great deal of mucus is formed by this gland at the time of oviposition (Agersborg, 1919, 1921, 1923*a*). Sections of the gland which I have studied show that during the act of mucus-formation the nucleus takes an active part. The nuclear membrane is then very obscure or apparently absent, the nucleus goes through fragmentation, the smallest cytoplasmic granules of basophil nature are the nearest to the nucleus; after the mucous granules have liquefied and passed into the lumen or cavity of the gland, the nucleus of the gland-cells is small, shrunken, and non-granular, with a small nucleolus, and basal in position within the cell. The cell then passes through a period of refilling during which time the nucleus first grows in size, and at the same time the micromeres of the cytoplasm, which are lodged in the meshes of the reticular structure of the cell, also grow.

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VII. Note to Explanation of Figures.

Figs. 11, 12, 13, 14, 15, 16, 46, 47, 48, 49, 50, 52, 53, 56, 57, 59, 61, 62, 63, 64, 65, 66, 67, 69, 72, and 74 were drawn by the aid of Spencer's compound microscope, tube length 16 mm., and camera lucida, angle of mirror 35. The others are either free-hand drawings, microphotographs, or photographs from preparations.

n.p.r. = low power with front lens removed. l.p. = low power. h.p. = high power. o.i. = oil immersion. c.l. = camera lucida.

VIII. EXPLANATION OF PLATES 27–37.

Fig. 1.—Photograph of Melibe leonina (Gould) from the ventral side. Specimen preserved in 70 per cent. alcohol. *C*, cirrhi; *F*, foot; *Hp*, hepatic diverticula; *L*, lips; *M*, mouth; *Ml*, marginal edge of hood. × 1.

Fig. 2.—Photograph of a preserved specimen from the right side showing

the profuse arborescence of the liver. *Ao*, anal pore; *Ap*, anterior right papilla; *F*, foot; *P*, penis; *R*, tentacle. ×1.

Fig. 3.—Photograph of a preserved specimen from which the ectoderm and hepatic arborizations of the body-wall have been removed to show the arrangement of the muscles of the body-wall. *P*, penis. ×1.

Fig. 4.—Microphotograph of the skin to show the odoriferous glands (*Go*); *Hep*, hepatic branches; *M*, muscle-fibres.

Fig. 5.—Photograph of a whole-mount of the skin with the underlying fibrous tissue to show the heterogeneous arrangement of the connective tissues.

Fig. 6.—Photograph of a trans-section of the hood to show the tufted or tuberculate surface of both the external side (*Ex*) and ventral side (*En*) of the hood. In the ectoderm of the external side of the hood are also shown the odoriferous (...) glands, which are absent from the ventral side. ×24.

Fig. 7.—Microphotograph of the body-wall seen from the inside. It shows the caecal endings of the hepatic diverticula. Contrast with fig. 4, which is of the skin, photograph taken from without; note the relative position of the odoriferous glands in the body-wall.

Fig. 8.—Photograph of dorsal tentacle with the sense organ (papilla of Gould) (*Rh*) retracted. *C*, lamellated part; *K*, neural knob. See also *K* in fig. 15. (Vide Agersborg, 1923, figs. 4, 5, *pa*.)

Fig. 9.—Schematic drawing of a dissected adult to show the general arrangement of the visceral organs. *A*, anus; *Au*, efferent branchial veins; *Br*, brain; *hc*, hepatic trunks; *Ft*, foot; *G*, proventriculus; *Li*, larger part of the intestine; *M*, mouth; *Mg*, mucous gland; *Oe*, oesophagus; *Od*, oviduct; *Ospt*, ovispermatotheca; *Ot*, ovitestes; *P*, penis; *Pc*, pericardium; *Prg*, prostate gland; *Si*, smaller part of the intestine; *Vd*, vas deferens; *V*, ventricle; *Vg*, vagina; *Sto*, gizzard; *Pd*, pyloric diverticulum.

Fig. 10.—Part of the rim of the hood to show the arrangement of the cirrhi. *Ic*, inner row; *Oc*, outer row; *M*, muscle-fibres.

Fig. 11.—Cross-section of a large cirrhus, showing the axis at *Lcm* from which fibres radiate (*Rf*) to the sub-epithelial (*Ie*) layer of the periphery. *Oe*, outer epidermal layer. (l.p., c.l.)

Fig. 12.—The axis of the cirrhus shown in fig. 11. *Ccm*, smaller central cell-masses with a reticular structure; *Cg*, central ganglion; *Lcm*, large central cell-mass with a few cells scattered; *Rf*, radiating fibres. (h.p. c.l.)

Fig. 13.—Same as fig. 12. *Ir*, inner reticular network of the large central cell-mass (*Lcm*); *Pc*, peripheral cells; *Cga*, central ganglion. ×1,013·4; (o.i., c.l.).

Fig. 14.—Part of the periphery of cirrhus as shown in fig. 13. *Ie*, sub-epithelial layer with which the radiating nerve-fibres (*Rf*) communicate; *Oe*, super-epithelial layer. ×1,013·4; (o.i., c.l.)

Fig. 15.—Longitudinal section through the sense organ of the dorsal

tentacle (lamellar papilla of Gould). *Nfb*, nerve-fibres of a highly ganglionic knob; *Nfl*, the same fibres in the lamellated part; *Nfp*, nerve-fibres which communicate with the peripheral ganglion below (*S*); *Nc*, small nerve-cells surrounding central fibres; *Ncs*, ganglionic knob, consisting of nerve-cells only; *Nmf*, neuro-muscular fibres which intercommunicate between the sense organ and the base of the tentacle; *Owr*, the outer wall of the tentacle which serves as a sheath to the organ; *S*, lamellae. ×75.

Fig. 16.—Nerve-cells from '*K*' in figs. 8 and 15. *Nc*, larger nerve-cells; *Ncs*, smaller nerve-cells. Notice the large granular nucleus. ×1,013·4.

Fig. 17.—Front view of Melibe leonina. *Fh*, rim of hood; *Fr*, front; *Ft*, foot; *Irc*, inner row of cirrhi; *Irc*^{1}, mid-ventral rudimentary cirrhi; *L*, lip; *M*, mouth; *Mf*, muscle-fibres; *Mdp*, mid-dorsal depression; *Orc*, outer row of cirrhi; *R*, base of tentacle. ×1½.

Figs. 18–24.—Papillae. Figs. 18, 19, first pair; 20, one of the second pair; 21, one of the third pair; 22, one of the fourth pair; 23, one of the fifth pair; 24, the sixth pair. *Hep*, caecal terminal branches of the liver. The parallel lines represent muscle-fibres. ×1.

Fig. 25.—Longitudinal section of an anterior papilla. *Cshb*, cross-section of a hepatic branch; *Mf*, muscle-fibres; *Og*, odoriferous gland; *Osp*, vascular space or sinus; *Tbr*, tubercles. ×2.

Fig. 26.—Cross-section through the anterior region of the foot. *Cil*, cilia; *Lv*, liver; *Mc*, muscle-bundle; *Og*, odoriferous gland; *Tbr*, tubercles. ×8.

Fig. 27.—Cross-section through the posterior region of the foot. *Bc*, connective tissue; *Gl*, pedal ganglion. The other labelling as the preceding. ×8.

Fig. 28. Section of a single tubercle from the anterior end of the foot. *Cec*, ciliated columnar ectoderm; *Cil*, cilia; *Gmug*, granules of mucous glands; *Grc*, granular border; *Mf*, muscle-fibres; *Nf*, nerve-fibres; *Ng*, nerve-cells; *Nu*, nucleus.

Fig. 29.—Section through the pedal ganglion of the foot (vide fig. 27). *Cec*, ciliated columnar ectoderm; *Cil*, cilia; *Gl*, ganglionic cells; *Mf*, muscle-fibres; *Mug*, mucous glands with crypts passing through ectoderm; *Nf*, nerve-fibres; *Ng*, nerve-cells; *Nt*, neural fibrillae ending on to ciliated ectoderm; *Pdng*, pedal ganglion.

Fig. 30.—Section through the body-wall. *Bv*, body-wall; *Cr*, crypt; *Ct*, connective tissue; *Ec*, ectoderm; *Sm*, saccular mucous gland; *Mb*, muscle-bundle; *Mf*, muscle-fibres; *Glo*, odoriferous glands; *Um*, unicellular gland.

Fig. 31.—Section from part of the wall of a large papilla (see fig. 25). *Cc*, connective-tissue cells; *Ch*, cross-section of hepatic branches; *Ct*, connective-tissue fibres; *Glo*, odoriferous gland; *Pec*, ectoderm of fimbriated surface.

Fig. 32.—From a whole mount of muscle-cells taken from the body-wall. The fine granular appearance along the periphery of the cells is represented in the next figure by '*Sar*'; *Nu*, nucleus.

Fig. 33.—Cross-section of muscle-bundle showing section of a few fibres only. *Ca*, axial sarcoplasm; *Intc*, inter-fibre connective-tissue cell; *K*, chromatin granules; *Lin*, linin; *Mc*, muscle-cell cut through the centre; *Mcp*, peripheral micromeric region of cell; *Mfe*, muscle-cell cut near its tapering end; *Ms*, perimysium; *Mt*, endomysium; *My*, myofibrillae; *Nuco*, nucleolus; *Nu*, nucleus; *Nus*, nuclear sap; *Sar*, sarcolemma.

Fig. 34.—A few connective-tissue cells and muscle-fibres from the body-wall. *Mf*, muscle-fibres; *Pnc*, granular connective-tissue cells.

Fig. 35.—Photograph of a cross-section of the pharynx near the mouth to show the corrugations (*Cor*) of the lining.

Fig. 36.—Microphotograph of a median sagittal section of the oesophagus. *Oe*, oesophagus; *G*, proventriculus; *S*, gizzard; *Br*, brain. ×8.

Fig. 37.—Microphotograph of a cross-section through the gizzard. *Hep*, hepatic canals into the gizzard; *V*, ventral side. ×14.

Fig. 38.—Microphotograph of a cross-section of the posterior part of the proventriculus showing the end of the glandular lining. *F*, remnants of food; *Cm*, circular muscle-layer; *Gl*, glandular mucous lining; *M*, stomach-contents (mucus ?). ×22.

Fig. 39. Microphotograph of a cross-section through the anterior part of the pyloric diverticulum. *Mu*, mucous coat; *Mus*, muscle-layer; *Ty*, typhlosole. ×18.

Fig. 40. Microphotograph of a cross-section of the larger portion of the intestine. *Ex*, mucosa; *Ty*, typhlosole; *Veb*, ventral blood-vessel. ×37.

Fig. 41. Drawing of a longitudinal section of the oesophagus. *Bc*, connective-tissue cells; *Ept*, epithelial lining; *Mst*, muscle-fibres; *Nu*, nucleus; *Sg*, salivary glands; *V*, crypts of salivary glands.

Fig. 42.—Drawing of a cross-section of the gizzard. *Bm*, basement membrane of the endoderm lining of the gizzard; *Cc*, external cover; *Ept*, epithelium of the gizzard; *Mus*, circular muscle-layer; *Stpl*, stomach-plates; *Trs*, transitional parts of the stomach-plates, a product of the epithelial lining.

Fig. 43.—Longitudinal section of the gizzard. *Ct*, connective tissue, and a few muscle-fibres; *Cs*, circular muscle-layer cut transversely; *Ept*, epithelial lining showing several secretion vacuoles in the cytoplasm and nuclei in various conditions of the so-called resting stage; *Kar*, chromatin granules; *Lin*, linin; *Nuco*, nucleolus; *Nu*, nucleus; *Ol*, external cover; *St.pl*, stomach-plates; *Sv*, secretion vacuoles; *Trp.pl*, transitional part of stomach-plates.

Fig. 44.—Drawing of one of the corrugations from the pyloric diverticulum (vide fig. 39). *Bg*, basal granules (terminal bars); *Bm*, basal

membrane; *Cil*, cilia; *Sb*, sub mucosa; *Vas*, highly vascular cover of the organ; that is, the tissue is very loose and seems to contain many spaces or sinuses; *Vac*, mucus vacuoles.

Fig. 45.—Cross-section of the wall of the smaller intestine. *Bg*, terminal bars; *Bm*, basement membrane; *Cc*, connective-tissue cover; *Cil*, cilia; *Kar*, chromatin granules; *Lcc*, loose connective-tissue layer or sub mucosa with many vascular sinuses; *Nuco*, nucleolus; *Nu*, nucleus; *Nus*, nuclear sap; *Sv*, secretion vacuoles.

Figs. 46, 47.—The same as fig. 45. The secretion vacuoles are not so large. *Bg*, terminal bars; *Cil*, cilia; *Sv*, secretion vacuoles. ×1,013·4; (o.i., c.l.)

Fig. 48.—Longitudinal section of one of the main branches of the hepatic caeca. *Nu*, nucleus; *Sc*, secretion cap (product). ×1,013·4; (o.i., c.l.)

Fig. 49.—Longitudinal section of hepatic branch, showing the very variable condition in adjacent cells. (h.p., c.l.)

Fig. 50.—Cross-section of hepatic branch in the body-wall of the hood. *Sg*, secretion product. ×1,013·4; (o.i., c.l.)

Fig. 51.—Cross-section of hepatic branch from the body-wall. *Dsc*, darkly staining cells; *Gc*, vacuoles; *L*, lumen; *Nu*, nucleus.

Fig. 52. From 'x' in fig. 53. (h.p., c.l.)

Fig. 53.—Cross-section of a branch of the liver in the tentacle. ×107. (l.p., c.l.)

Fig. 54.—Schematic drawing of the heart and part of the pericardium. *Aa*, anterior arteries; *Ao*, descending aorta; *Aosh*, very fine transparent sheath of the aorta; *Au*, efferent branchial veins; *Per*, pericardium; *Vent*, ventricle.

Fig. 55.—Longitudinal section through the auricular part of the heart. *Au*, efferent branchial veins; *Av*, auricular apertures in the auriculo-ventricular valves (*Ar*); *Bc*, blood-cells; *End*, epicardium; *Vent*, cardiac wall of the ventricle.

Fig. 56.—Blood-gland from the wall of the aorta just below the ventricular valve (vide fig. 59). *Aosh*, sheath of aorta; *L*, lumen. ×107. (l.p., c.l.)

Fig. 57.—Longitudinal section of the aortic wall opposite the blood-gland seen in figs. 56 and 59. *Cmf*, cardiac muscle-fibres. Section 15 micra thick. ×1,013·4; (o.i., c.l.)

Fig. 58.—Blood-cells from the lumen of the aorta. *a*, *b*, from the inside of the blood-gland (node), fig. 56; *c*, from the lumen close to the gland. ×1,013·4; (o.i., c.l.)

Fig. 59.—Longitudinal section of the lower part of the ventricle of the heart. *Bgl*, blood-gland (node); *Pc*, pericardium; *Valve*, valves between the aorta and the ventricle; *Vw*, ventricular wall; *Wda*, wall of the aorta. (h.p., c.l.)

Fig. 60.—Schematic drawing of the kidney. *Ab*, anterior branches; *Pb*, posterior branches; *P*, point of communication with the pericardium; *Rs*, renal syrinx; *U*, ureter; *Up*, uretero-connexion with the renal syrinx.

Fig. 61.—Section through the renal syrinx showing its connexion with the ureter (*Ur*) and pericardium (*Rs*). Note the arrangement of the cilia of the renal syrinx; *Pw*, pericardium. (n.p.r., c.l.)

Fig. 62.—From *A*, in fig. 61, showing the transition of the epithelium from ciliated to non-ciliated. (h.p., c.l.)

Fig. 63.—From *B*, in fig. 61, showing the cyncitial relation of the cells. (h.p., c.l.)

Fig. 64.—Section through the renal syrinx showing complete connexion with the pericardium, *A*–*A*. *Crs*, cavity of renal syrinx; *Pl*, plica or villus of the cyncitial plate; *Ur*, ureter. (n.p.r., c.l.)

Fig. 65.—*A*–*A* in fig. 64. *Pl*, villus of the cyncitial plate. ×107. (l.p., c.l.)

Fig. 66.—From *B* in fig. 64. *Pl*, plica or villus. (h.p., c.l.)

Fig. 67.—The cyncitial plate with villus (*Pl*). (h.p., c.l.)

Fig. 68.—From the wall of the renal syrinx. *Cil*, ciliated columnar cells lining the organ; *Sw*, syringeal wall covering the epithelium. (h.p., c.l.)

Fig. 69.—A single cell from the wall of the renal syrinx showing its remarkable structure. *Bg*, basal granules; *Bm*, basement membrane. ×1,013·4; (o.i., c.l.)

Fig. 70.—Cross-section of a renal branch. *Ct*, connective-tissue capsule; *Pr.neph*, periodically functioning cells. ×267.

Fig. 71.—Section from the wall of the ureter. *Ent*, internal border; *Ex*, external cover; *Nu*, nucleus; *Nuco*, nucleolus.

Fig. 72.—Longitudinal section of the ureter nearer to the kidney than that part shown in fig. 71. Note the glandular condition. ×1,013·4; (o.i., c.l.)

Fig. 73.—Microphotograph of a cross-section of the body in the region of the prostate coils of the female genital tube. *Hep*, hepatic branch; *Int*, intestine; *Pro*, large coils of the prostata; *St*, stomach; *Vd*, vas deferens. ×14.

Fig. 74.—Microphotograph of a cross-section through the region of the mucous gland. *Fd*, food in the stomach; *Hep*, section of the liver; *Mg*, mucous gland; *P*, penis; *Sept*, ovispermatotheca; *Vg*, uterus. ×14.

Fig. 75.—Microphotograph of a cross-section of the posterior part of the penis. *Lm*, lumen. Two lumina are shown owing to the coiling of the organ in this region. ×15.

Fig. 76.—Microphotograph of a trans-section of the body in the region of the brain. *Cg*, cerebral ganglia; *P*, penis; *Mg*, mucous gland. ×14.

Fig. 77.—Microphotograph of a trans-section of the body in the region

of the genital pores. *Fv*, female genital vestibule; *Mga*, male genital aperture; *St*, stomach. This section is of particular interest since it shows the two separate openings. ×15.

Fig. 78.—Microphotograph of a cross-section of the penis. *Ig.lp*, glandular lining of the penal cavity; *Lm*, lumen; *Og.lp*, glandular cover of the penis; *Ms*, musculo-fibrous part of the penis.

Fig. 79.—Microphotograph of a cross-section of the body in the region of the female genital duct, showing the many corrugations of the uterus (*Ut*). *Mg*, mucous gland; *Pro*, prostata; *St*, stomach. ×14.

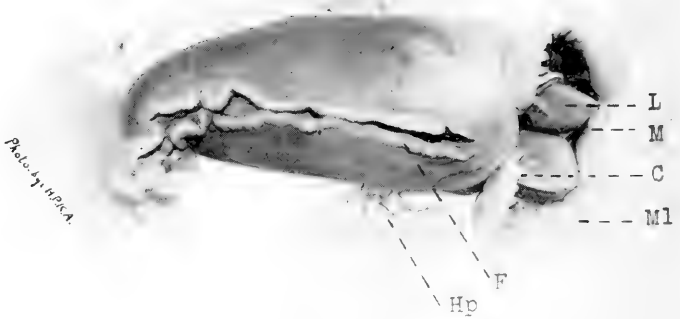
Fig. 80.—Microphotograph of a cross-section of the uterus, showing semen in its lumen (*Sem*). *F*, uterine pocket; *Gl*, glandular lining; *Ms*, muscular wall. ×42.

Fig. 81.—The organs of reproduction in Melibe leonina. *Bil.dpr*, biluminate ampullo-prostate duct; *Amp*, ampulla; *Cl.p*, penal cleft in the mucous gland; *Mgl*, mucous gland; *Od*, oviduct; *O.sp*, ovispermatotheca; *Ot*, ovitestis; *P*, penis; *Pr*, prostata; *Sv*, seminal vesicle of the penis; *Ut*, uterus; *Va*, vagina; *Ve*, vasa efferentia. ×2.

Fig. 82.—Drawing of portion of a cross-section of the ovispermatotheca. *Bm*, basement membrane; *Ept*, tall columnar epithelium lining the organ; *Mf*, muscle-cell cut longitudinally; *Nu*, nucleus; *Nuco*, nucleolus; *Kar*, chromatin granules; *Lin*, linin; *Mic*, micromeres; *Myf*, myofibrillae; *Ret*, reticular structure or axial myofibrillae; *L*, loose connective-tissue cover.

Fig. 83.—Drawing of part of a cross-section of the penis. *Cil*, cilia; *Cc*, connective-tissue cells; *Excpl*, external epithelial cover of the penis; *Iepl*, internal epithelial lining of the organ; *Int.ml*, internal fibrous layer; *M*, mucous substance; *Oc*, lining of the penal cavity. ×36.

Fig. 84.—Drawing of a few cells from the mucous gland, showing the gland to be composed of tall columnar epithelium with a short ciliated border. *Bg*, basal granules; *Bm*, basement membrane; *Ctc*, connective-tissue capsule; *Fcb*, free ciliated border of the internal surface of the gland; *Mic*, micromeres; *Nu*, nucleus, small and shrunken; *Pcnu*, nucleus of actively filling cells; *Rs*, reticular structure of cells in the state of refilling. The cells in *A* are in a state of exhaustion. They stained poorly with Delafield's haematoxylin. *B* represents a condition prior to liquefaction of the granula. Note the nuclear membrane seems to be wanting and there are a number of small chromatic granules near the nucleus. The nucleus (*Pcnu*) is filled with uniformly sized chromatic bodies. The nucleolus being in some cases irregular in shape. *C* represents cells like *A* and *B*, but in *C* the micromeres (*mic*) are larger than in *A*, and the nucleus not quite so shrunken. That is, these cells are in a state of refilling. *D*, cuboidal epithelium from a loop of a non-glandular part of the organ.



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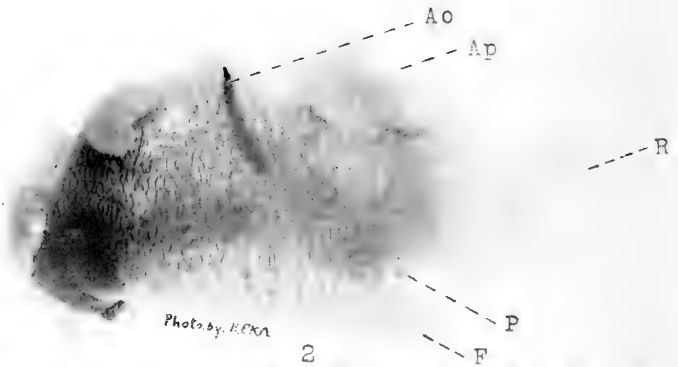

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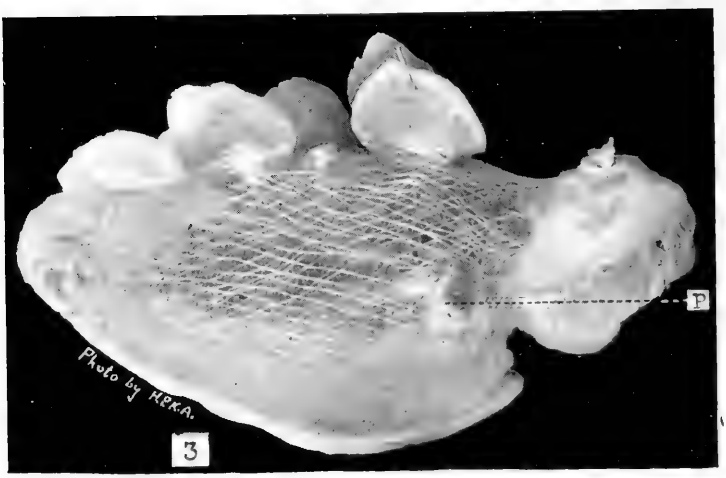

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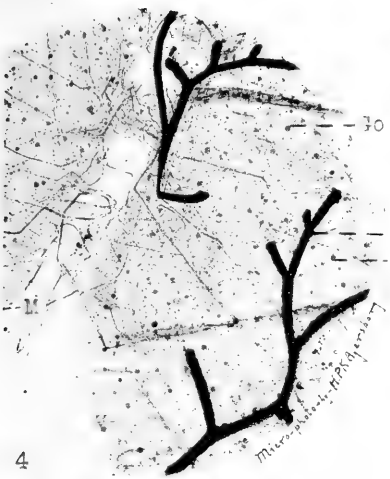
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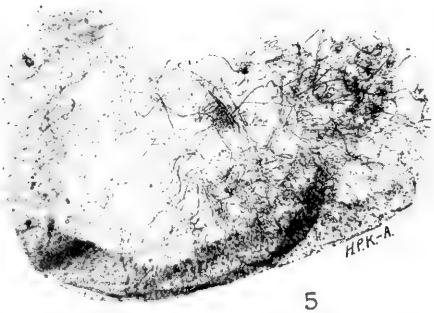
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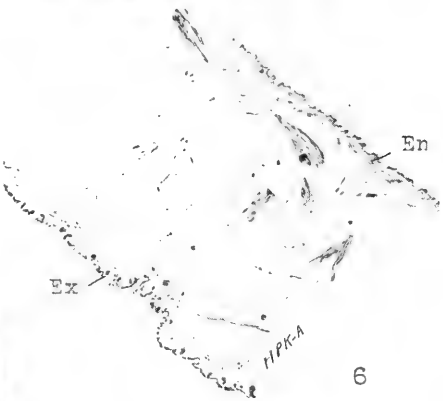
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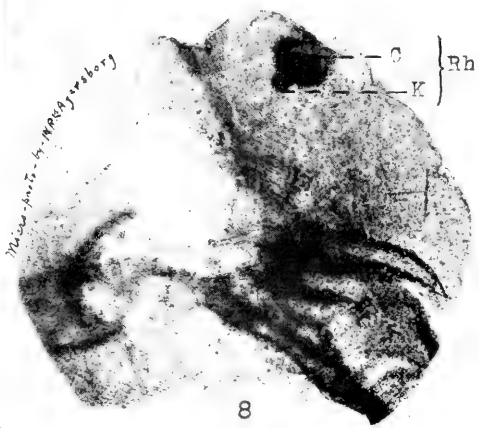
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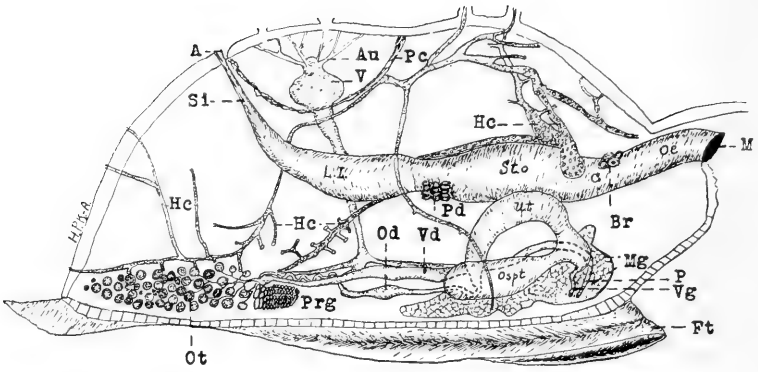
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Ot

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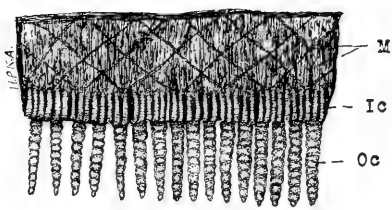
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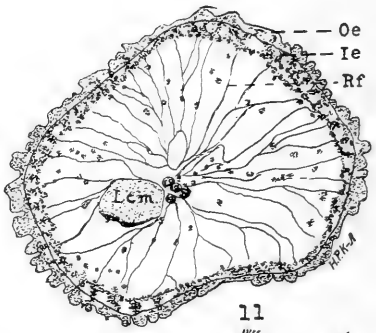
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Ie
Rf
Lcm

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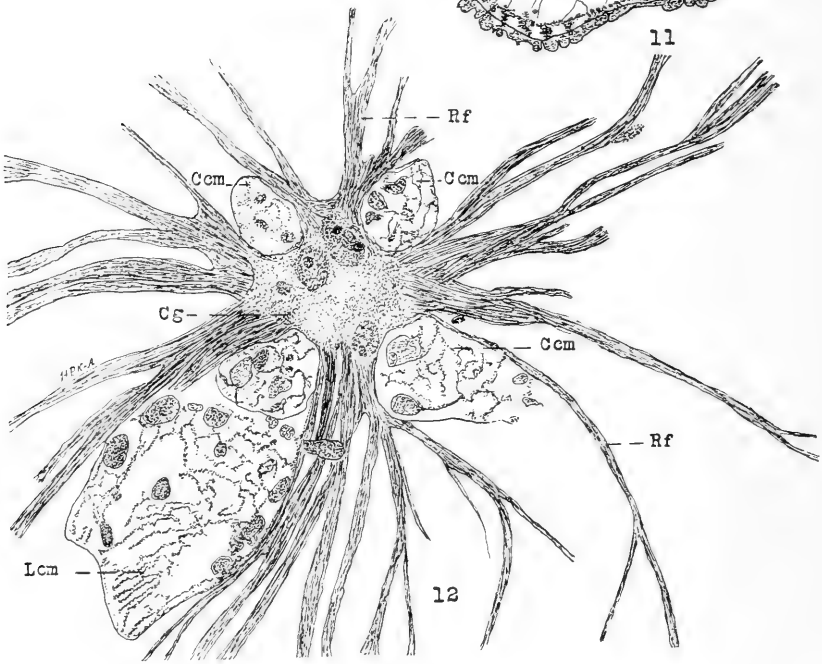
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Ccm
Ccm
Cs
Ccm
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Lcm

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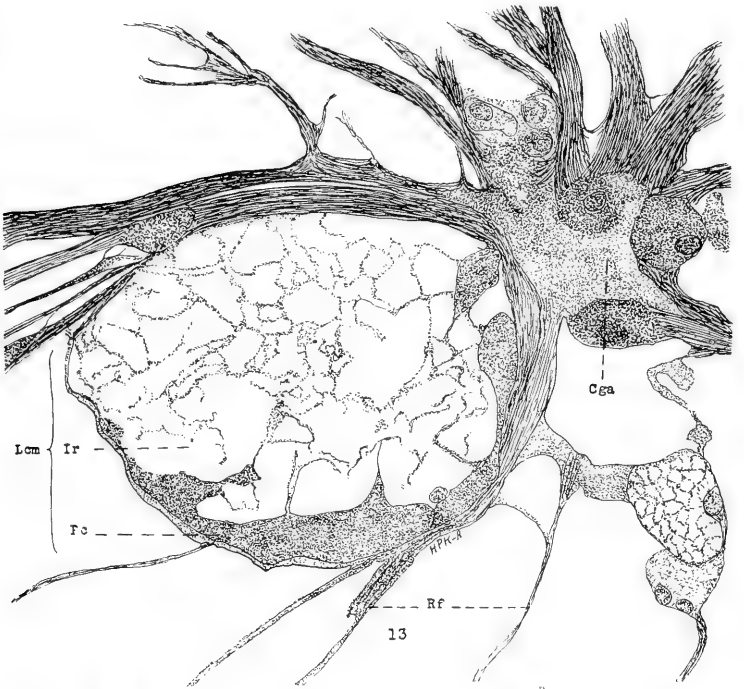
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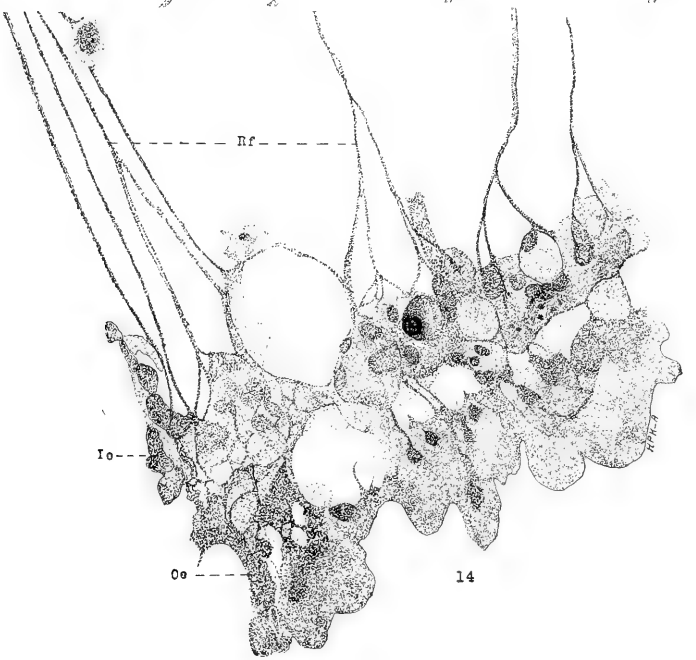
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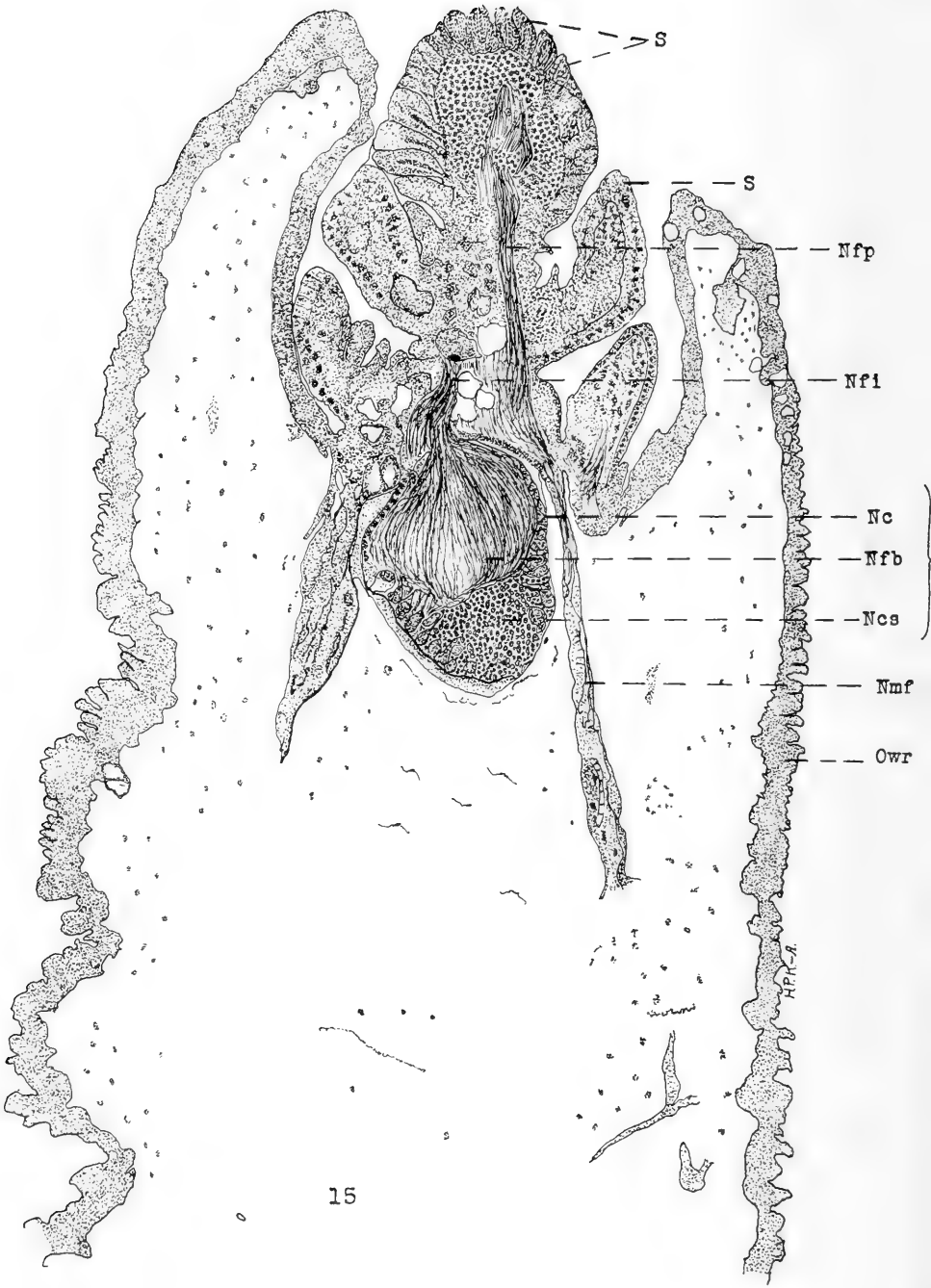
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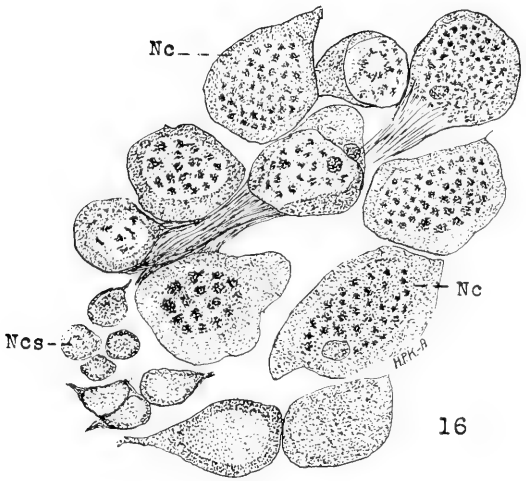
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Nc
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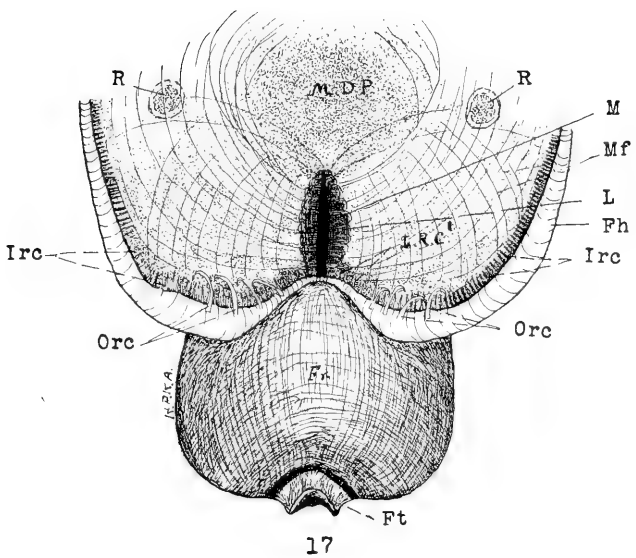
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Irc
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Irc
Orc
Orc
Ft
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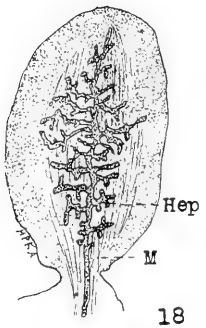

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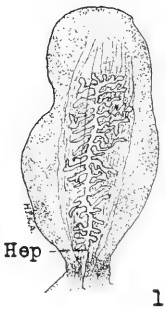

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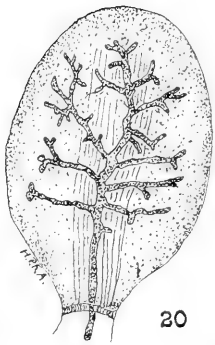

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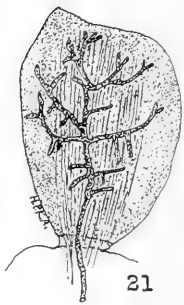

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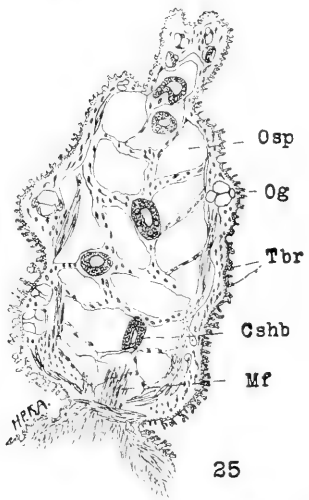

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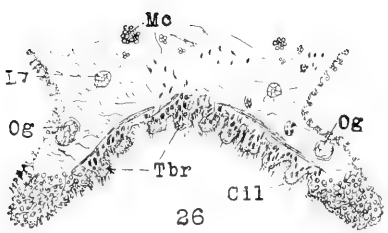

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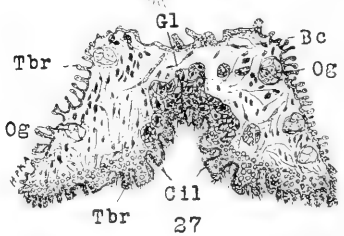

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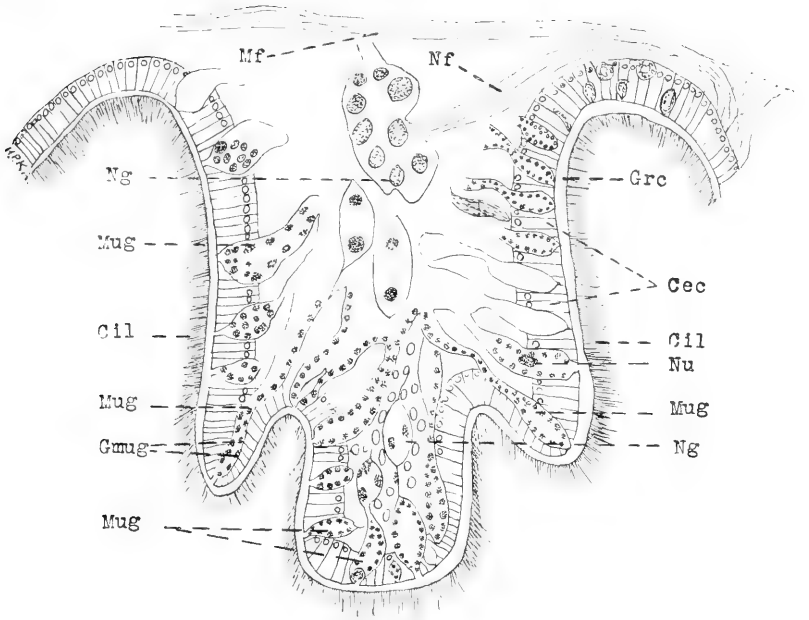
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Gmug
Ng
Mug

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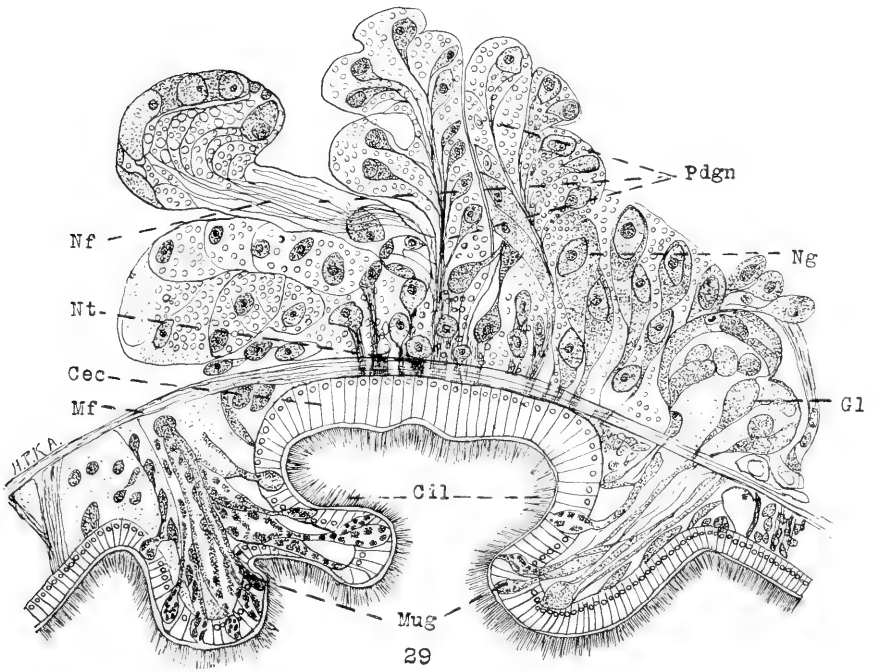
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Ng
Nt
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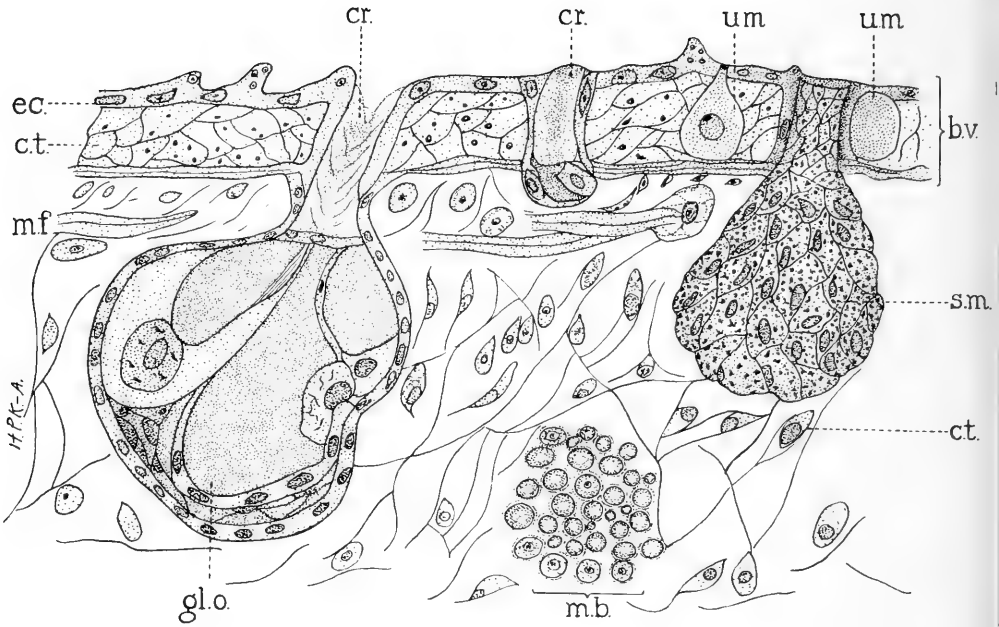
Gl
Cil
Mug

29



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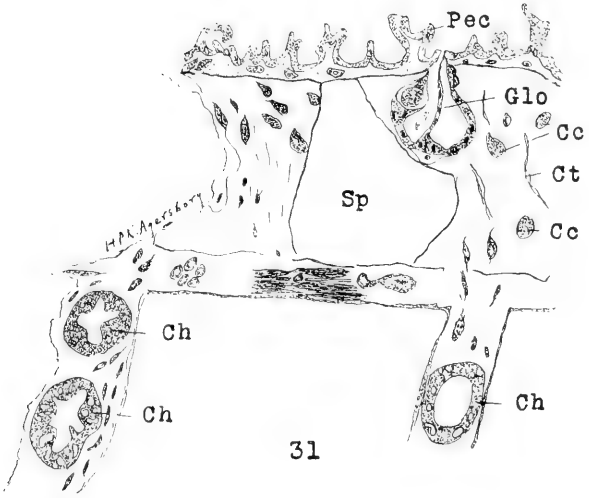

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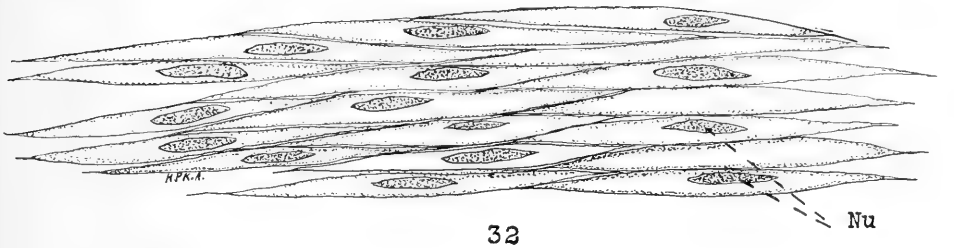
H.P.K.A.
Nu

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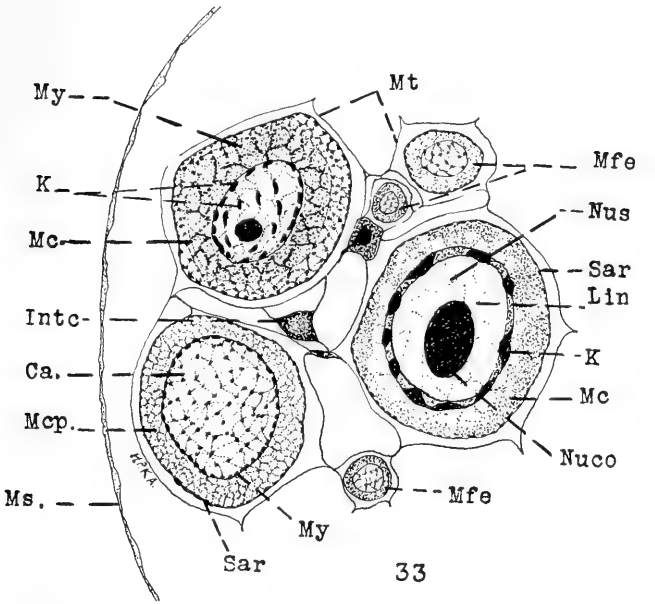
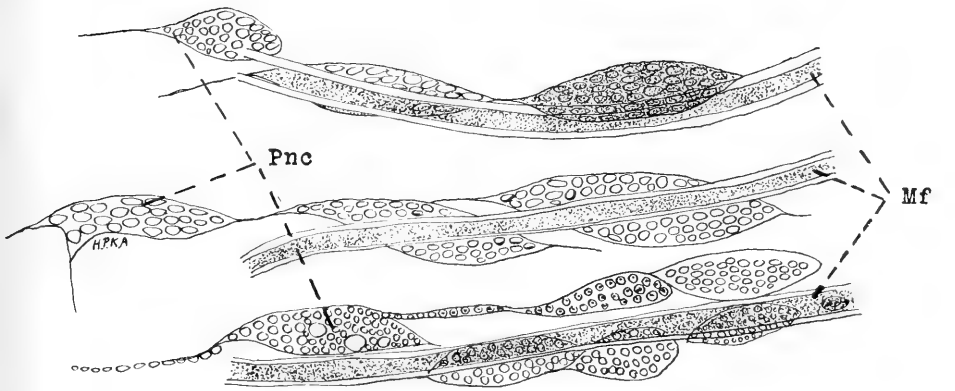

My
Mt
Mfe
K
Nus
Mc
Sar
Lin
Intc
K
Ca.
Mc
Mcp.
Nuco
H.P.K.A.
Ms.
Mfe
My
Sar

33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Mf
H.P.K.A.

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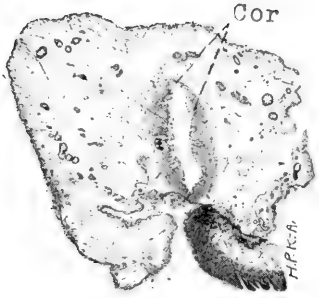

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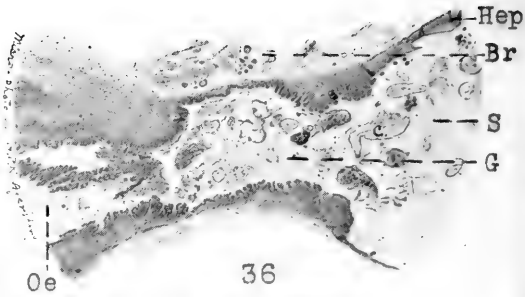

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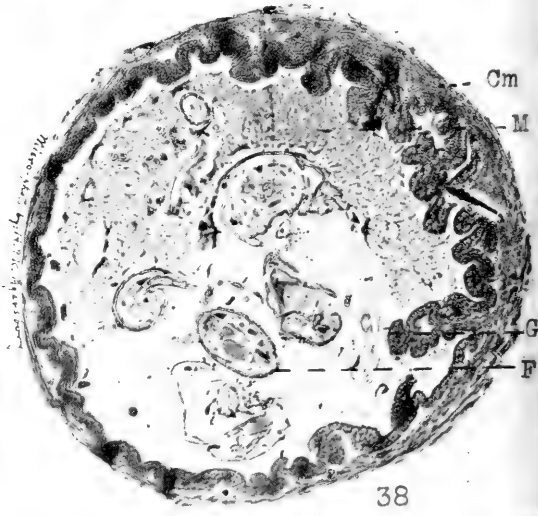

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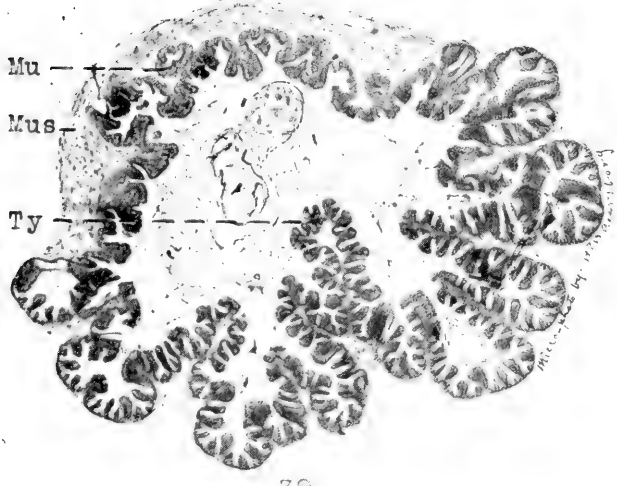

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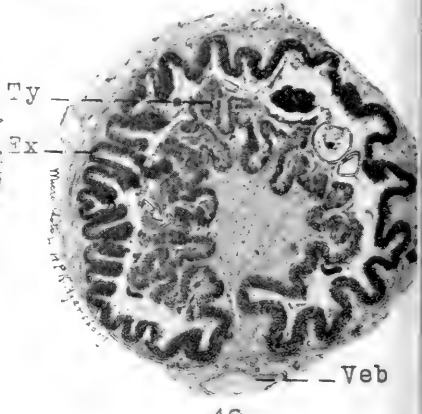

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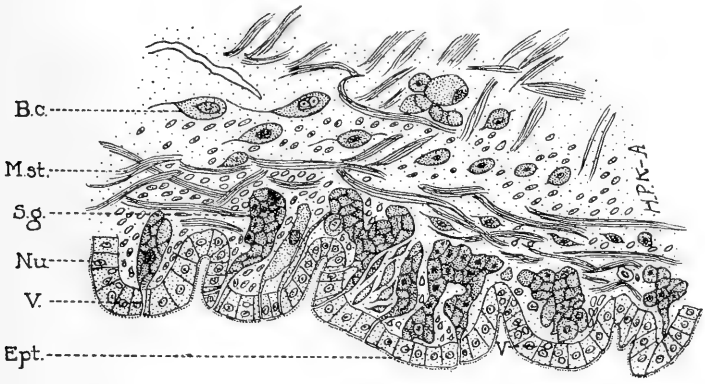
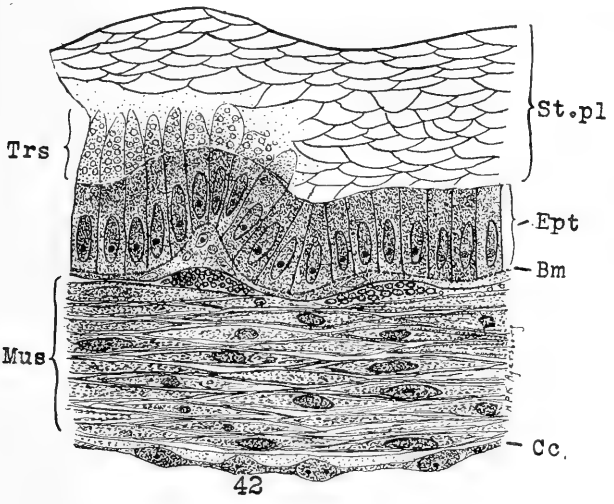

B.c.
M.st.
S.g.
Nu
V.
Ept.
H.P.K-A

41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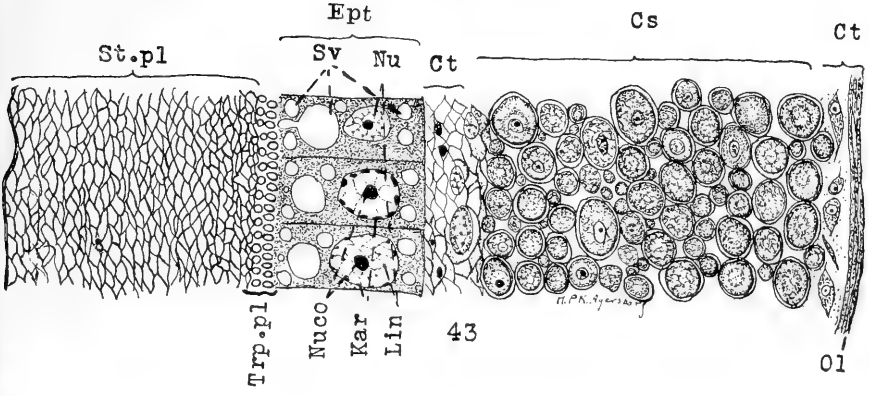
Ept
Bm
Mus
Cc.

42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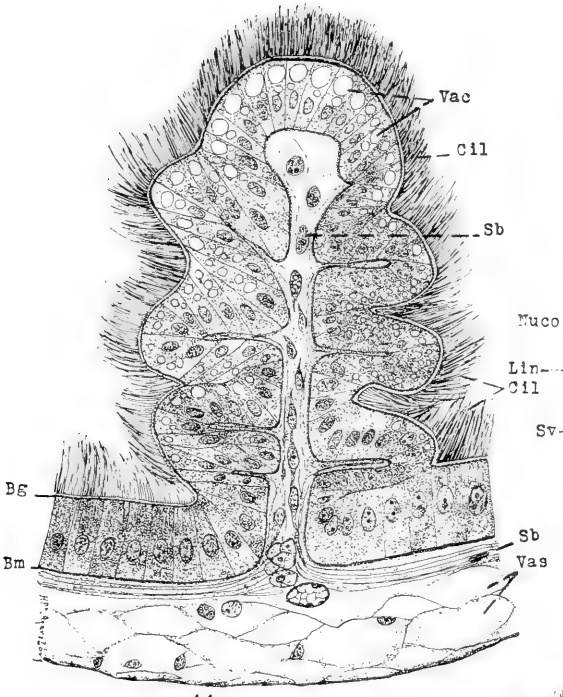
St.pl
Sv
Nu
Ct
Cs
Ct
Trp.pl
Nuco
Kar
Lin
Ol

43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Cil
Sb
Bg
Bm
Sb
Vas

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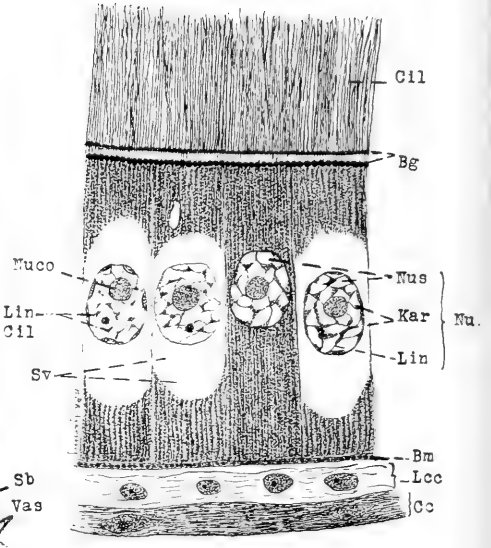
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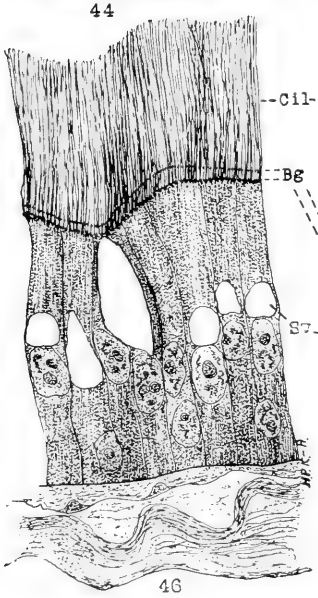
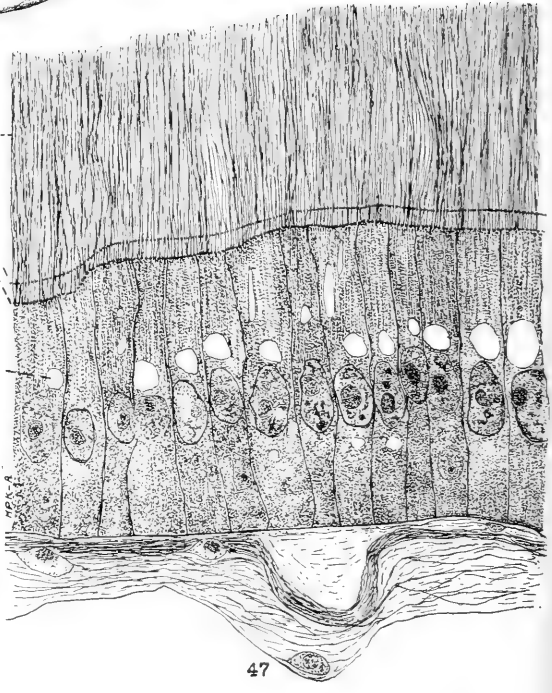
Bg
Muco
Lin
Cil
Sv
Nus
Kar
Lin
Nu.
Bm
Lcc
Cc

45


Cil
Bg
S7

46



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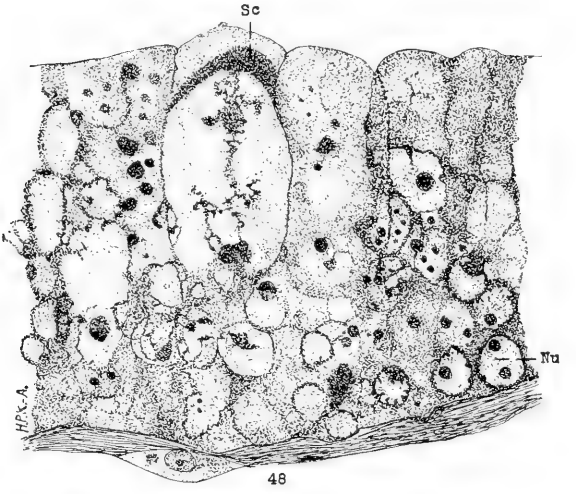

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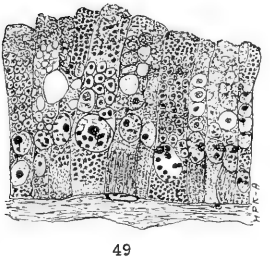

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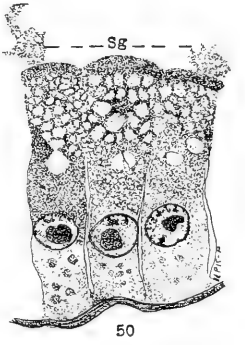

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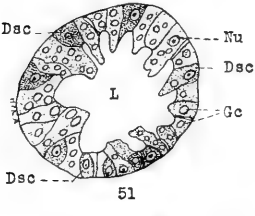

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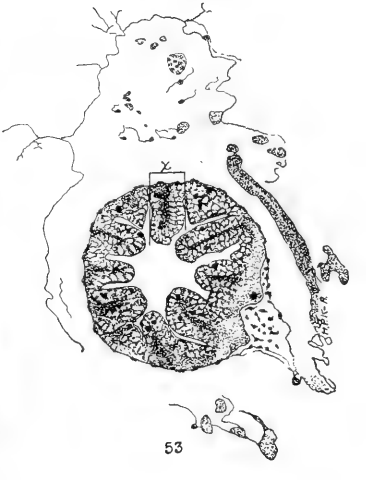

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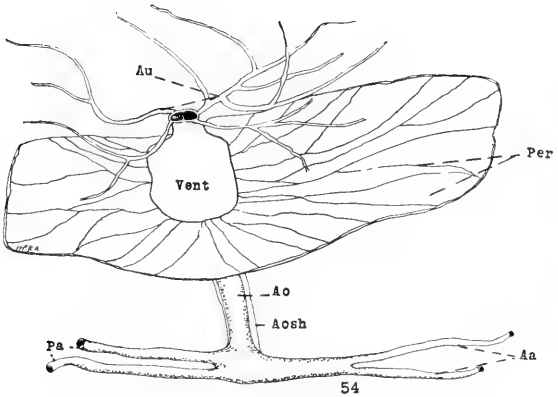
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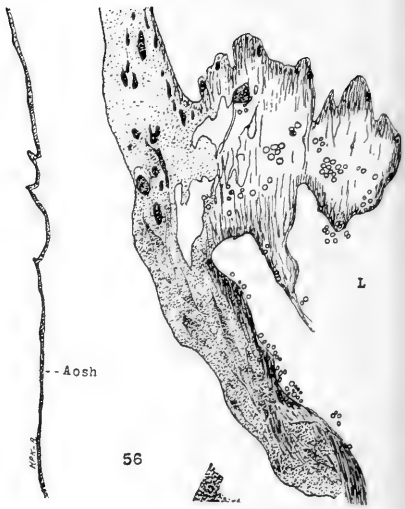
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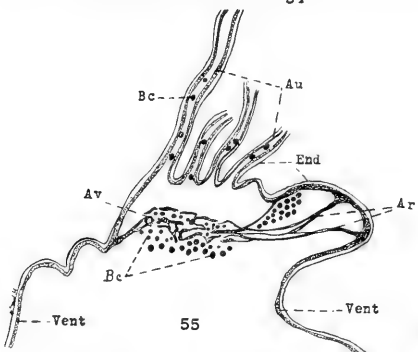
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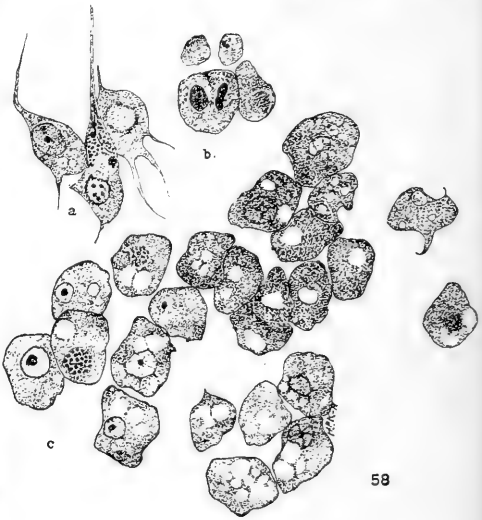
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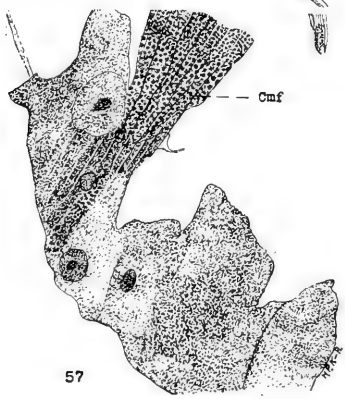
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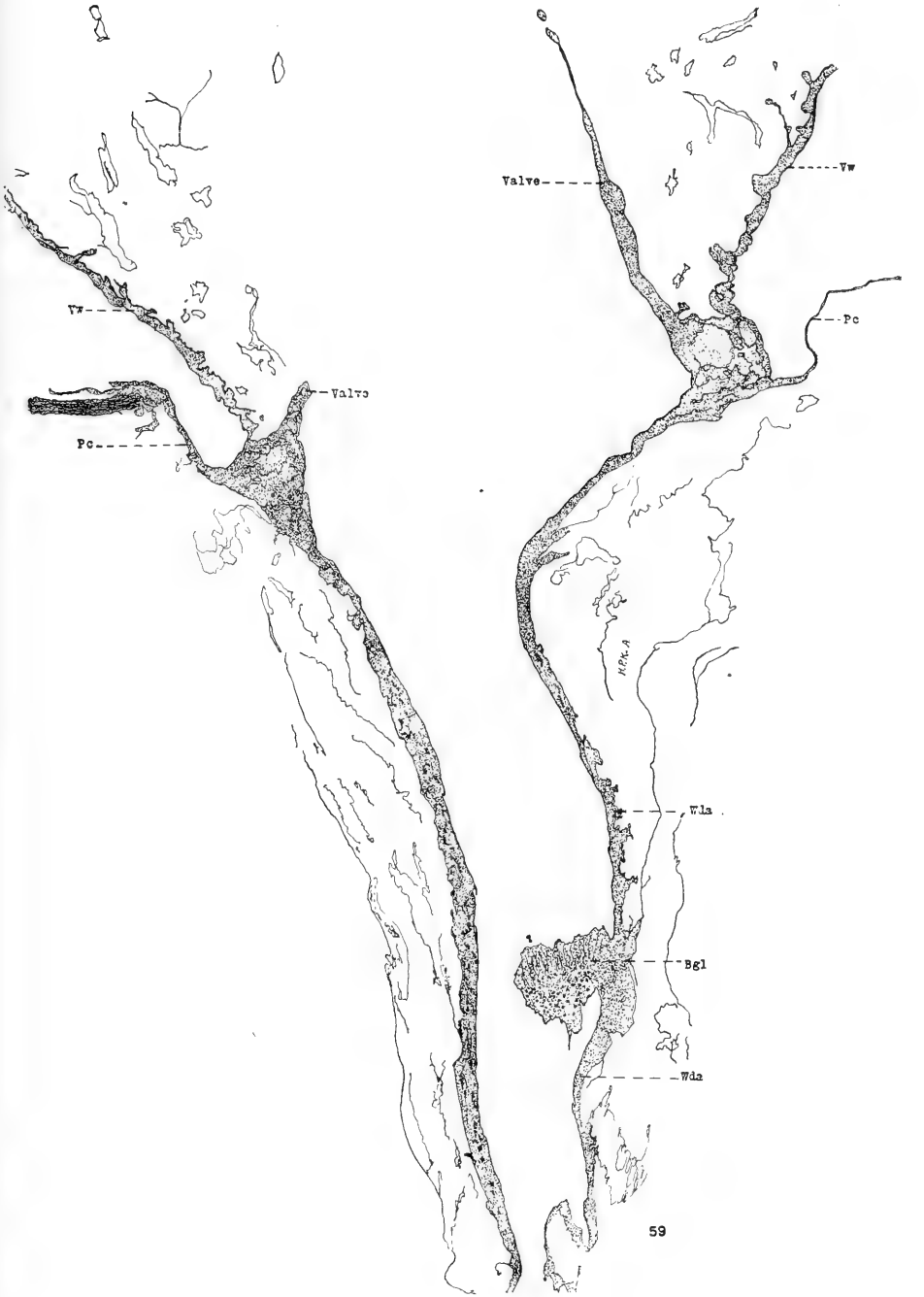
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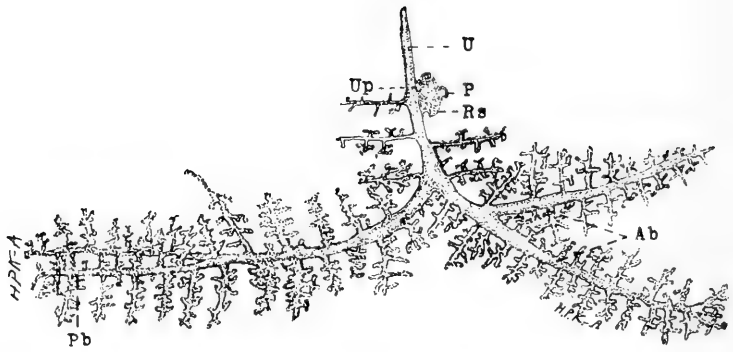

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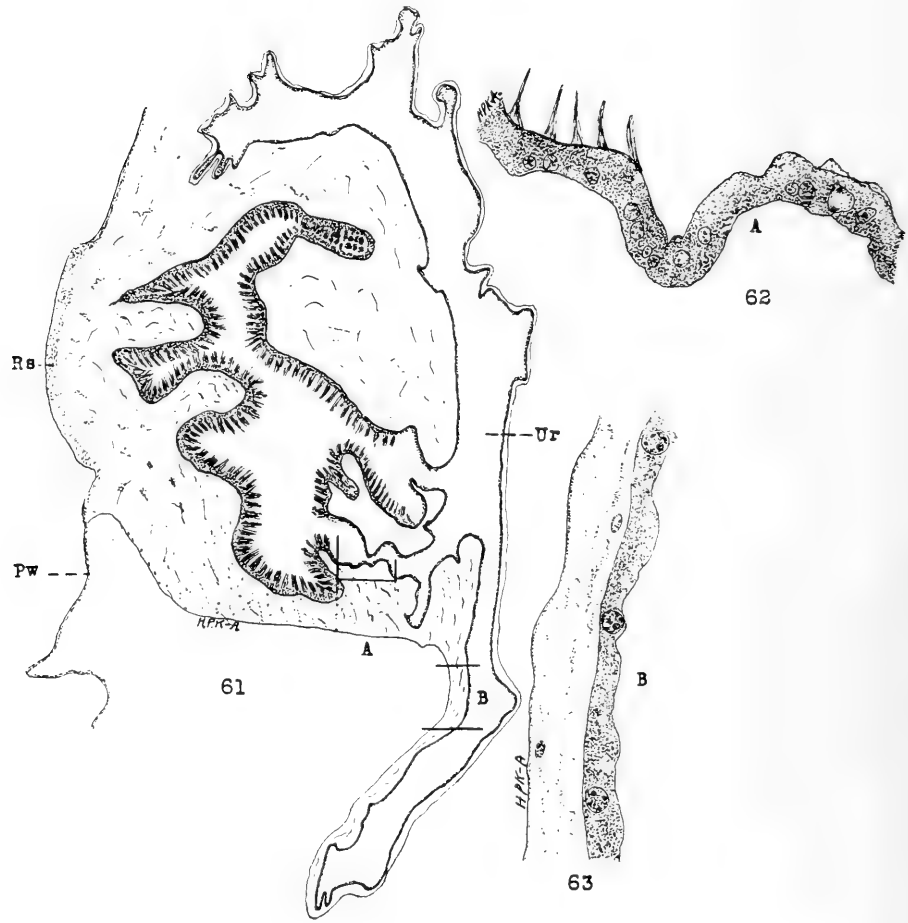

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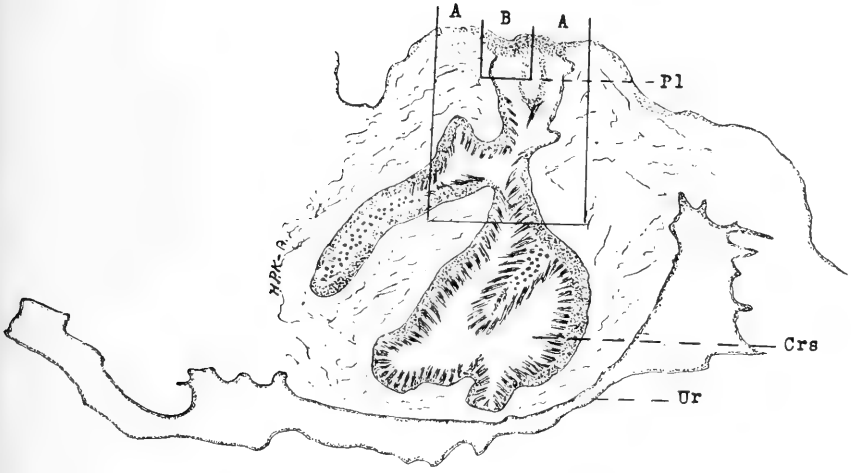

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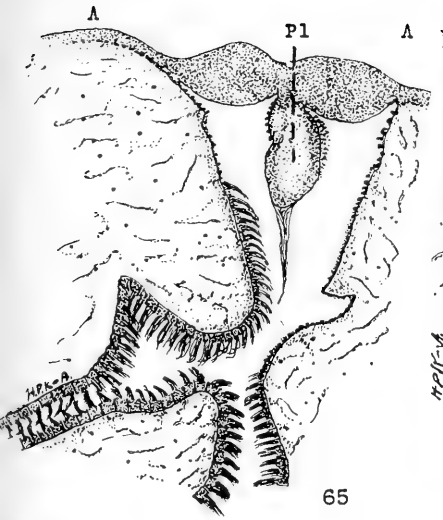

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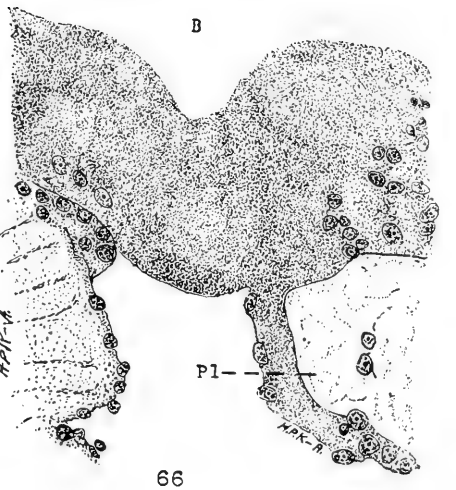

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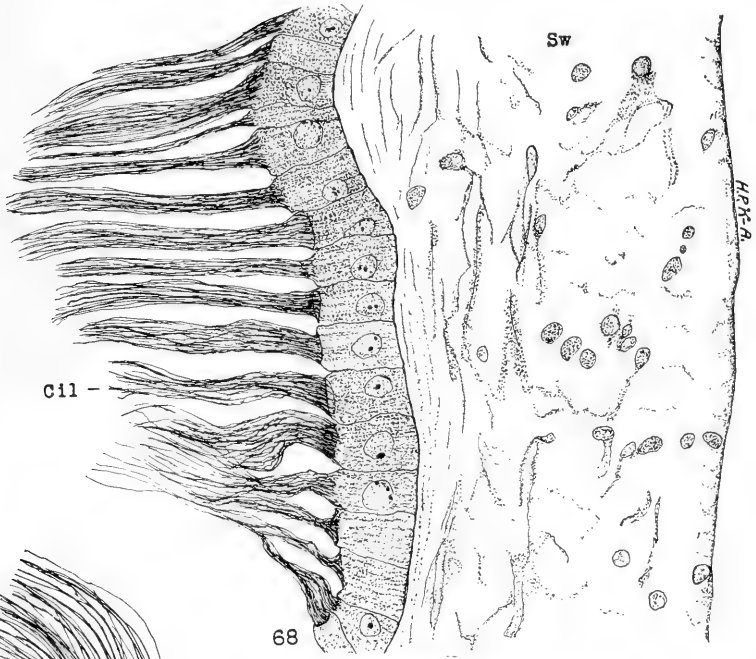
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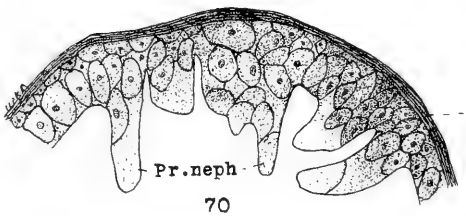
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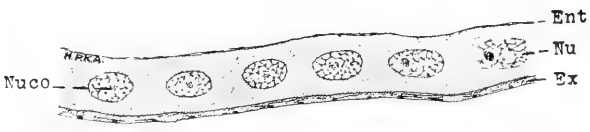
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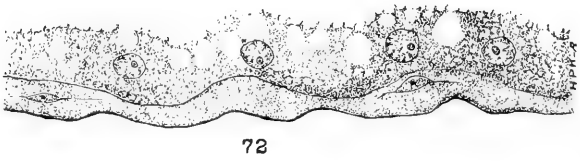
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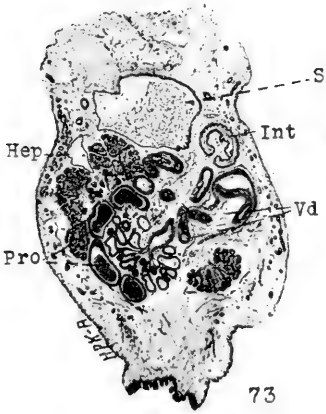
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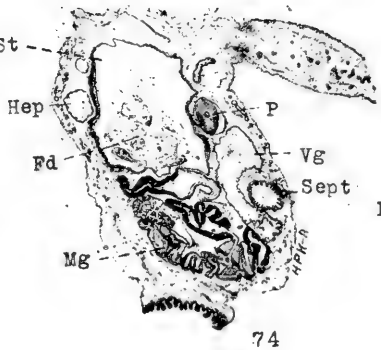
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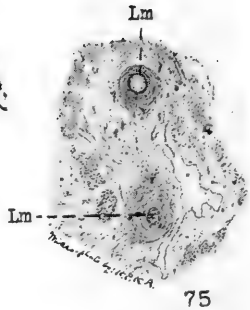
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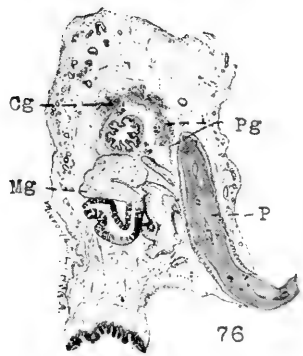
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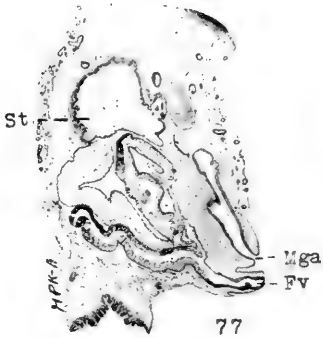
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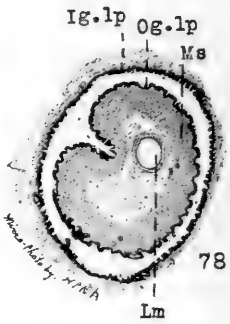
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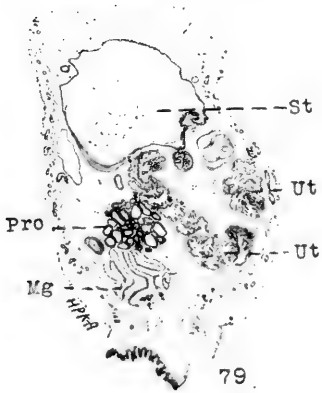
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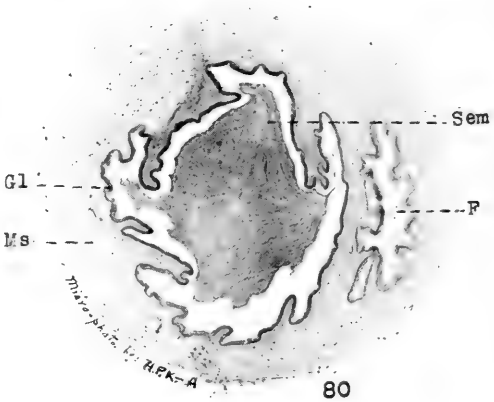
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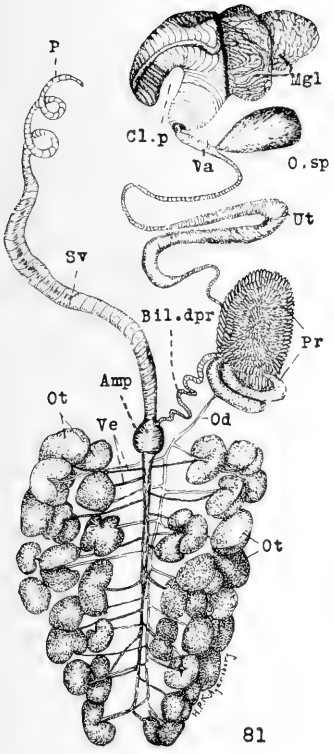
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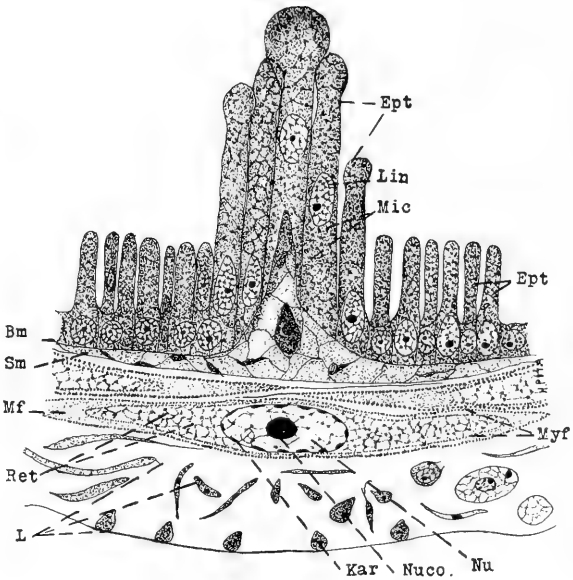
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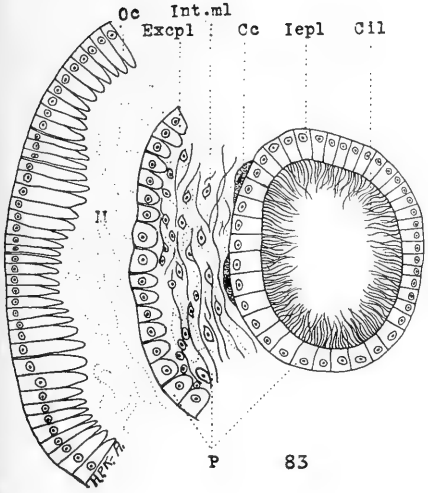
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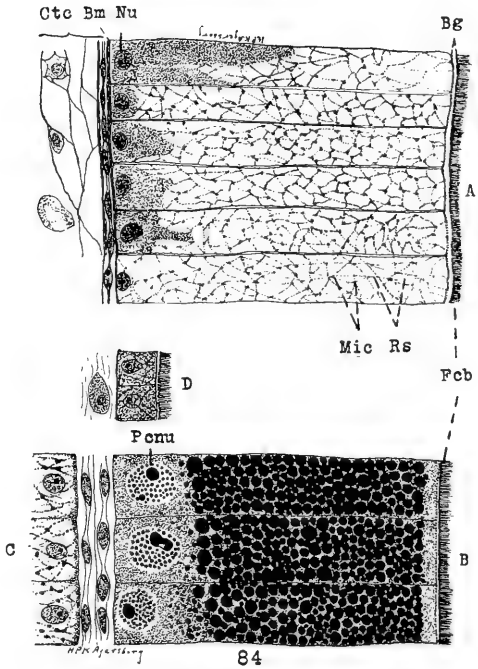
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Observations upon the Behaviour and Structure of Hydra.

By

Sheina Marshall, B.Sc.,

Assistant Naturalist, Scottish Marine Biological Station, Millport.

With 4 Text-figures.

Contents.

Introduction.

The Hydras upon which the following observations were made were obtained from various sources, chiefly through the kindness of Dr. Monica Taylor, from the Convent of Notre-Dame. They were kept in covered, half-pint glass tumblers, in water from a large tank in which there was a fair quantity of weed and a variety of animal life (Isopods, Cladocerans, Planarians, &c.). This was used because the Hydra would not live for more than one or two days in tap-water. The water was changed and the tumblers cleaned when necessary. This was about once a week in summer, as food-remains became foul very quickly then, but less frequently in winter. The water was never aerated artificially. The Hydras were fed on a culture of Daphnia twice a week, and the remains and excreta removed, as far as possible, the following day. Under

these conditions the Hydras lived and remained healthy for months. Occasionally one or two, for no apparent reason, would decrease in size and finally degenerate and die, but in only one case was a whole tumbler attacked by a ' depression period '. Even here a fair proportion remained healthy throughout. When well fed the Hydras budded actively. None of my specimens carried more than four or five buds at a time and the number was usually less.

Sexual reproduction took place in autumn and in early summer. The animals were hermaphrodite. With the exception of four specimens, not differing outwardly from the rest, all those which produced eggs produced testes at the same time. Testis formation usually began before egg formation in any individual. Many specimens showed testes without eggs, but as egg production entails a considerably larger expenditure of energy and food material than testis production, this is not surprising. The four exceptional, apparently female, specimens were kept under observation for about eight weeks, but died before undergoing another sexual period. Three or four eggs were sometimes formed at one time, although they might not all attain full size and break through the ectoderm.

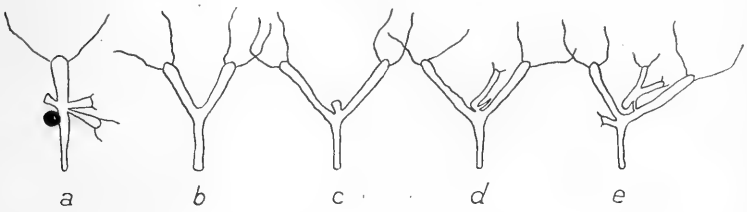
One large and healthy specimen showed four eggs developing and a number of testes. Three of the latter had developed in the ectoderm of three tentacles. One testis was just at the root of a tentacle, another a little further out, and the third at a distance from its base of about quarter the length of the tentacle. The testes were ripe and spermatozoa were swarming in them. In this animal interstitial cells must have been present in the tentacles, which is not usually the case.

The period from the freeing of the egg to the hatching of the young Hydra varies with the season. A batch of eggs set free in November hatched in January, while some produced in summer took only about three weeks to develop. In many cases the eggs failed to hatch owing to the attacks of bacteria or fungi. The young Hydra emerges by a crack in the shell, usually equatorial. It is oval and almost colourless, but in

a few minutes it stretches out and extends little projections which develop into tentacles during the next twenty-four hours. It is then able to feed. The rupture of the shell suggests the formation of a hatching ferment, such as has been described in Lepidosiren and Teleostean fishes (**15**).

Abnormalities are not uncommon among newly hatched Hydras, possibly due to injuries during hatching. Several two-headed specimens appeared, the double part varying in length. Such specimens are not infrequent among adult Hydras. In one or two cases the division appears to be growing gradually deeper, so that eventually the compound would split

Text-fig. 1.



into two separate Hydras, but in most cases the specimens remained without change for weeks and died without further division. In no case was there any suggestion that a process of fusion was going on. In two specimens the hypostome only was double and several of the tentacles belonged to both rings.

The origin of a double Hydra can sometimes be observed (Text-fig. 1). One normal specimen produced two buds close together (Text-fig. 1, *a*), and in the course of development these grew out on a common stalk. Although one of the buds was a day or two younger than the other, they soon grew to equal size. This double individual then separated (Text-fig. 1, *b*) from the normal parent, and a week later both of its limbs produced normal buds which separated off. Two days later a third bud was formed near the junction of the two limbs (Text-fig. 1, *c*). The next day a curious pointed projection grew up between the bud and one limb (Text-fig. 1, *d*). Eventually this grew into a second bud joined to the first (Text-

fig. 1, *e*), and the whole was separated as a double Hydra. Unfortunately this specimen died before reproducing itself further. It should be noted that the mode of origin of the second half of the compound is distinctly abnormal.

Leiber (**8**) reports a case in which one component of a double Hydra, just before the division had reached the foot and the two were about to separate, itself divided again at the hypostome. It died before further observations could be made. These cases indicate that a tendency towards doubleness may be inherent in some Hydra stocks. From the infrequency and origin of these abnormalities and the frequent death of the compounds, it does not seem probable that longitudinal fission is a normal method of reproduction.

I have seen transverse fission take place only in obviously unhealthy specimens.

Hydras are sometimes found in which two or more tentacles are in process of fusion from the base upwards. This seems to be a method of regulating the number of tentacles to the size of the Hydra, for it is found chiefly in animals which are decreasing in size, or in those which have an exceptionally large number of tentacles for their size. The commonest numbers of tentacles were five, six, or seven, but some specimens showed as many as ten.

The appearance of an animal undergoing ' depression ' has been so often described that it is unnecessary to do so here. In the early stages the Hydra may take on an appearance very different from its usual, the differences being sometimes those described as characteristic for another species.

For several months the Hydras I had were overrun with Kerona and Trichodina, but they seemed none the worse for it. This is contrary to the observations of P. Schulze (**9**), who states that Kerona caused depression in his animals.

Feeding.

The Hydras were usually fed on a culture of Daphnia, but were occasionally given Cyclops, Cypris, or small

insect larvae, all of which they ate readily. Simocephalus was also tried as a food, but the Hydra seemed unable to kill it. The Simocephalus were frequently caught and held struggling for an hour or more, but in the end they freed themselves and escaped uninjured. If killed and presented to the Hydra, they were eaten as readily as Daphnia. On three occasions a Simocephalus was captured and eaten by a Hydra. In the first case the animal remained alive inside the Hydra for a considerable time and could be seen moving its antennae. A second was caught and digested immediately after ecdysis. I have, however, seen a Hydra catch a Simocephalus which then underwent ecdysis and was immediately recaptured, yet eventually freed itself. When caught, the contrast between the behaviour of Daphnia and of Simocephalus is remarkable. The Daphnia struggles violently for a few minutes, then the heart stops beating and the animal soon succumbs although the antennae may keep up a quivering movement for some time longer. If freed from the Hydra, it does not recover. Simocephalus, on the other hand, continues to live and to struggle at intervals until it frees itself. The heart continues to beat the whole time. The cuticle of Simocephalus is not appreciably thicker than that of Daphnia. It may be more resistant to the entry of the nematocysts, or the tissues of the animal to the action of their poison. When both Daphnia and Simocephalus are immersed in dilute solutions of poisons (such as formic acid, or chloroform) Simocephalus succumbs first.

Schulze (**9**) mentions that in a culture of H. circumcincta a Daphnia sometimes stuck on to the tentacles and was dropped again uninjured. It is perhaps possible that these were really Simocephalus also, for the two genera are closely similar and may be mistaken for one another unless carefully examined.

The capture of the food generally seems to be a more or less passive action, any small object presented to the Hydra being seized and carried to the mouth. Indifferent substances

are usually dropped after a few seconds, although a few Hydras were induced to swallow pieces of white of egg (which were returned undigested in the course of twelve hours) and pieces of blotting-paper when soaked in blood.

If Daphnia is left in a weak solution of litmus for eighteen to twenty-four hours, the alimentary canal in the head-region usually takes on a pink tinge. If these stained animals are fed to Hydra, a colour-change from pink to blue takes place in those animals which show nematocysts sticking into the head-region. This may indicate an alkaline reaction for the nematocyst poison. The change takes place slowly, often after the death of the Daphnia, but does not occur when the animal is killed with a needle. Simocephalus does not take up the colour well, and often dies if left in the litmus solution for twenty-four hours.

The digestive juices of Hydra are alkaline, but have no effect on Hydra itself. I have seen one Hydra completely ingested by another, in whose cavity it remained for more than twelve hours. It was returned again none the worse. A tentacle is frequently swallowed along with the food to which it is sticking, and remains in the coelenteron for some hours, but comes out quite unaltered. One Hydra even went as far as to engulf about half its own body, beginning at the foot, where a Daphnia had stuck.

In many cases, particularly where the animal was attempting to swallow something exceptionally large, the hypostome was turned inside out over the tentacle-bases and remained so for some time. It rarely went further than this, but in one small regenerating specimen the process went on till the whole animal had turned inside out. It righted itself in the course of an hour. The converse also takes place sometimes, and the hypostome is turned inwards till it hangs down into the coelenteron. Both of these performances are of interest, inasmuch as a condition is assumed which has become permanent and normal in other types of Coelenterata, e.g. the trumpet-shaped hypostome of Obelia and the invaginated hypostome of the Actinozoa.

Reaction to Stimuli.

Hydras were tested to find out their reaction to mechanical and chemical stimuli.

Mechanical Stimulation was carried out with a glass rod drawn to a fine point.

Slight stimulation of a tentacle leads to contraction of that tentacle only. The tentacle often adheres to the rod for a few seconds.

Strong stimulation of a tentacle leads to its contraction over the mouth, when the other tentacles bend up till they meet and the head turns to one side, exactly as when catching prey (capture response). This response is sometimes obtained by rubbing on the insides of the tentacle bases, and sometimes by touching the oral cone. Strong stimulation of the oral cone leads to contraction.

Stimulation of the outsides of the tentacle bases and of the body in that region sometimes leads to contraction, but often has no result.

Gentle stimulation of the body generally has no result, but sometimes leads to contraction, as strong stimulation always does.

Stimulation of the foot always leads to contraction of the body (not of the tentacles, unless strong). This is probably an adaptive reaction to movements of the object to which the Hydra is attached. When contracted, the resistance to water will diminish, and the animal will be less liable to be torn off.

Gentle touches repeated at intervals of five seconds or two and a half seconds sometimes have no effect even when carried on for several minutes. More often they lead, first, to a swinging away of the body, and finally to contraction after a few minutes. In swinging away the body is bent just above the foot region. As the body remains quite straight the action must be confined to a small number of muscle-fibres in the region of the bend, which is usually in a different part of the body from the stimulation. This shows the existence of a conducting mechanism.

Chemical Stimulation.—When food, such as a piece

of Daphnia, is made to touch a tentacle tip, the capture response immediately takes place. The food is thus brought in to the hypostome; and the tentacles, bending over, would prevent escape were it alive.

Chemical stimulation was also carried out with weak acetic acid coloured with methylene blue. The strength used was 0·025 per cent., but this was probably much weaker by the time it actually reached the Hydra. It did not injure the tissues appreciably.

Stimulation applied to Tentacle.—In about 60 per cent. of the cases the tentacle contracted and the capture response followed in about twenty seconds. In 30 per cent. of the cases the whole Hydra swung away after forty seconds, and in the remainder the animal contracted after about thirty seconds. In one case a repetition of the stimulus immediately after the response led to a second response without any pause.

Body.—The body, when stimulated, either contracted or swung away after thirty to forty seconds. In several cases there was no result.

Oral Cone.—When the oral cone was stimulated the body contracted, sometimes immediately, sometimes after thirty to forty seconds.

Foot.—Stimulation of the foot resulted in a general contraction of the body, but it was very insensitive, and out of eighteen stimulations twelve had no effect. In other cases the body contracted after an interval varying from fifteen to thirty seconds.

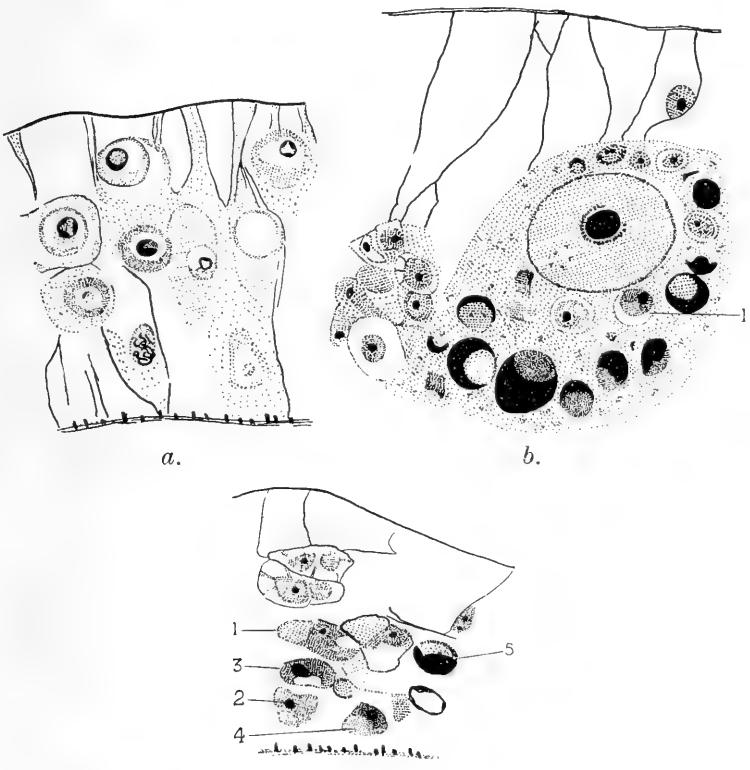
From the above it will be seen that the head and foot regions are much the most sensitive parts of the Hydra to mechanical stimuli, while the foot appears to be comparatively insensible to chemical stimuli, at least to acetic acid.

Egg Formation.

When the Hydra is about to form an egg the interstitial cells are multiplied enormously and form a mass bulging out the ectoderm. Characteristic changes take place in their

nuclei. The chromatin collects round the periphery of the nucleus, leaving the nucleolus in the centre of a clear space (Text-fig. 2, *a*). The nucleolus sometimes becomes difficult to stain, or only part of its periphery stains. Wager (**13**)

TEXT-FIG. 2.



Sections of ectoderm showing development of ovum.

believes that vacuoles form in it at this stage. Secondary nucleoli may make their appearance, often in large numbers. These may be droplets of food material as Wager suggests, for the cytoplasm is also full of darkly staining particles. At a stage just before the definite egg-cell becomes recognizable

several interstitial cells, much larger than the rest, may be seen undergoing nuclear changes. The chromatin has formed a thick spireme thread, and in some cases the nuclear membrane has disappeared. The nucleolus takes no part in this, but lies unchanged in the midst of, or to one side of, the spireme. This is in accordance with the observations of Wager, who states that 'the egg begins its growth by the coalescence of a group of the primitive ova; this process is frequently attended by a peculiar nuclear degeneration'.

Later the egg appears as a hemispherical mass of protoplasm with lobed edges, which lies with its base in contact with the mesogloea. The protoplasm is reticular, and at this stage contains none of the so-called 'pseudo-cells'. The nucleus corresponds in size with the cell and contains a large nucleolus and a number of smaller secondary nucleoli varying in size. The egg grows by the absorption of other interstitial cells, but at this stage the nuclei of the latter break down completely before absorption. The protoplasm of the egg is filled with minute deeply staining dots, probably, at least in part, the remains of the chromatin of the absorbed cells.

At a later stage (Text-fig. 2, *b*) the egg protoplasm is filled with a mass of degenerated cells ('pseudo-cells') and interstitial cell nuclei. Intermediate steps can be traced showing the process of degeneration of an interstitial cell. The process goes on both inside and outside the egg. In groups of interstitial cells, usually at some distance from the egg itself, the nucleus is seen in the centre of the cell, surrounded by a dense mass of protoplasm. The nuclear membrane is hardly visible or has disappeared entirely (Text-fig. 2, *c*, 1 and 2). This mass moves to one side and applies itself to the cell-wall as a densely staining mass (Text-fig. 2, *c*, 3 and 4). The nucleus is at first visible as a darker body, but eventually the whole mass stains so deeply that the constituent parts are indistinguishable (Text-fig. 2, *c*, 5). Groups of these degenerate cells are found in the ectoderm after the egg has separated and are possibly used up by a subsequent egg.

When the degenerative process takes place within the cyto-

plasm of the egg, it may go on much as described above (Text-fig. 2, *b*, 1), or the cytoplasm may, to all appearance, be absorbed directly into that of the egg and the nucleus alone undergo visible change. Wager (**13**) describes such degenerative products formed from whole cells, from nuclei, and from nucleoli. I have not seen any of the last named in process of formation, but from the small size of some of the masses it seems probable that this is the case in my specimens also. In several cases a nucleolus seemed to be breaking through the nuclear membrane of an interstitial cell.

These degenerative cells are looked on as stores of energy to be used up by the embryo during development. Tannreuther (**11**) states that they divide by amitosis after their absorption into the egg-cell, but I have seen no signs of this. It has been stated that they are all used up before the young Hydra hatches, but this is not the case, for large numbers are present in the tissues of newly hatched Hydras and they do not entirely vanish till some time after hatching.

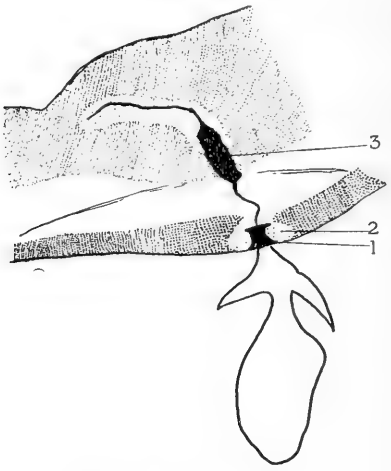
In my specimens the egg-shell is formed by a kind of vacuole formation at the surface of the egg. The edges of the vacuoles come in contact with one another, and their limiting membranes then harden to form the spicules. This is in accordance with the observations of Brauer (**2**) and Kleinenberg (**7**). The eggs then drop off and fall to the bottom.

Nematocysts.

The nematocysts have been much studied, and of late have been used largely to distinguish the different species of Hydra. There are four main types of nematocyst, large pear-shaped, small pear-shaped, and two types of cylindrical nematocysts, which differ from each other in size and in the way the thread is coiled inside. As the two last are not always strictly cylindrical, and as the names in other respects are not suitable, Schulze (**9**) has proposed to name the different types from their functions as Penetrant, Volvent, and Glutinant. Otto van Toppe (**12**) was the first to study the functions of all three types in detail.

The large pear-shaped nematocyst is, as the name penetrant implies, used for piercing the chitinous exoskeleton of the prey. When the hard shell has been pierced, the thread is everted into the soft tissues beneath. Usually only that part of the nematocyst immediately above the large barbs succeeds in penetrating, but as the thread itself has a spiral coil of small hairs or barbs upon it, it is firmly held in place. The large barbs, which are so conspicuous a feature of the exploded

Text-fig. 3.



Discharged nematocyst.

nematocyst, are never found imbedded in the tissues of the prey. Iwanzoff (**6**) says that the three, just at the moment of their eversion, form a stiletto which pierces the cuticle of the prey, making a hole by which the thread can enter. With the further eversion of the thread the barbs swing outward to their final position. Some chemical action is exerted on the chitin, as can be seen from a study of sections. Immediately around the point of entry is a deeply staining area, irregular in form (Text-fig. 3, 1). This probably corresponds to an outpouring of the poisonous fluid contained in the nematocyst, for the thread stains in the same way. Outside this dark patch is

a bowl-shaped space which stains less deeply than the normal chitin (Text-fig. 3, 2). The thread can be seen lying in the soft tissues, the first part at right angles to the external surface. The distal part usually curves to one side. It is filled with a darkly staining mass which is extruded either at the end or along its course (Text-fig. 3, 3). In preparations of unfixed, exploded nematocysts stained in methylene blue, the fluid inside the penetrants stains deeply and can be seen partly inside the capsule and partly in the form of tiny drops on the outside of the thread. The thread has, apparently, rows of minute pores or permeable areas through which the fluid can escape, as well as by the opening at the end.

The threads of the small pear-shaped nematocysts, or volvents, when exploded, wind tightly round any protruding hairs or bristles on the prey, and so hold it captive till it has been killed by the poison of the penetrants. The thread is coiled inside the capsule in two loops which lie on one another so that they appear in section like one thick ring. There is a row of small hairs on the thread arranged in a very open spiral. When exploded, the thread coils up tightly, and in optical section there is seen to be a narrow space along the axis of the coils which is closely beset with hairs. This forms an efficient mechanism for grasping the bristle, and there is some evidence that the secretion in the capsule and thread, which stains deeply with methylene blue, is sticky. Van Toppe (**12**) states that the stimulus for the explosion of this type of nematocyst is different from that exploding the penetrants, as the latter explode when their cnidocils come in contact with flat surfaces, while the former do not.

The third and fourth types of nematocyst, the glutinants, are usually cylindrical. One type is usually larger than the other. In the former the thread shows four or five turns almost at right angles to the long axis of the capsule and below this is wound irregularly. In the latter the thread is wound in an irregular figure of eight. Schulze (**9**) therefore calls them streptoline and stereoline respectively. In some Hydras (e.g. H. attenuata, van Toppe, H. circumcincta,

Schulze, H. stellata, Schulze, and Pelmatohydra braueri, Schulze) the streptoline is not cylindrical but pear-shaped. When Hydra sticks on to glass or to any other surface by means of its tentacles or hypostome, it uses these nematocysts. If one of the adherent tentacles is examined, there are seen numerous exploded glutinants whose threads are firmly attached to the glass. They are so firmly fixed that if any pull is exerted on them the cell protoplasm of the Hydra is drawn out into a thread with the nematocyst at its tip. Zygoff (**16**) was the first to notice these, and looked on them as pseudopodia by which the animal moved. Toppe discovered their true nature. These processes can withstand a considerable strain. I have seen a Hydra apparently trying to free a tentacle which was held at the tip by one of these nematocysts only. The tentacle was given several tugs, was twirled round rapidly, first in one direction and then in the other, the animal contracted tightly once or twice, and finally the tentacle was torn away. It was striking to watch an animal like the Hydra exhibiting such apparently purposeful movements. The twirling movements are much more complex than any the Hydra usually shows, and must have called into play a different mechanism. The nematocyst is always left sticking to the substratum while the protoplasmic process is gradually withdrawn into the cell. A similar process is sometimes drawn out when a tentacle is pulled away from some bristle on which a volvent has wound itself.

In unfixed, exploded glutinants stained with methylene blue, numerous droplets can be seen on the outside of the thread, as in the case of the penetrants. This secretion is probably sticky, and possibly hardens in contact with water. When used, it is extruded not only by the pore at the end of the thread but also by the side pores, for the thread can often be seen adhering at a point about half-way down its course.

It seems probable that the secretion of the other types of nematocyst has also to some degree the property of sticking firmly. I have observed tentacles adhering both by the penetrants and by the volvents, though not so firmly.

The size of the nematocysts has been cited as a characteristic difference for the various species. Most authors, however, give relative sizes only, and all measurements are given for the penetrants alone, which, as will be seen, are much the most variable in size of all the types. Steche (**10**) states that the penetrants of H. fusca are at most 8–8·5 μ, and those of H. grisea at least 10·5 μ and usually 13–13·5 μ. Toppe (**12**) says that those of H. fusca are the smallest, H. grisea next, and those of H. attenuata the largest. Schulze (**9**) gives 25 μ for his H. attenuata (which is not the same species as Toppe's) and 13 μ for Pelmatohydra oligactis.

In my Hydra the size of the penetrant varies greatly. Some forms measure 10 μ or 11 μ and others may be as large as 22 μ. Even in one individual the sizes may vary by as much as 8 μ. The small penetrants are found in the tentacles as well as in the body and are not merely incompletely developed. It is difficult to speak with certainty, but the most frequent size seems to be about 15 μ; 12–13 μ and 18–19 μ are commoner than the intermediate sizes. In small Hydras the smaller size seems to be more frequent than the large.

The glutinants vary much less, the ranges of individual variation not being more than 3 or 4 μ. The larger type (streptoline) measures about 11·5 μ (maximum 13 and minimum 10 μ), and the smaller (stereoline) about 9 μ (7–11 μ).

The volvents measure from 5–10 μ. The usual size is about 8 μ and the smaller sizes are more numerous than the larger.

In order to see whether the size of the nematocysts varied from time to time with changes in the size of the individual, I took a large well-grown Hydra, and after removing two of the tentacles in order to measure the nematocysts, starved it for seventy-three days. The measurement of the width of the foot when the animal was fully extended was, before starvation, about 0·275 mm., and, after, about 0·100 mm. It had therefore decreased to almost a third of its original width, and this gives a rough indication of the general effect. Whereas, before starvation, the nematocysts were of normal size (penetrants

19 μ), the penetrants now measured only 11–15 μ. This was due, in the first place, to the disappearance of the large type. The other nematocysts, however, also showed a similar though slighter decrease in size. The same experiment with another Hydra (starved forty-seven days) yielded similar results. If the size of the nematocysts varies with the size or condition of the individual it is obvious that this cannot be used as a trustworthy characteristic in differentiating between the various species.

In Hydra viridis the penetrants measure 8–10 μ; the streptoline glutinants are kidney-shaped and are as large as the penetrants, being 10–11 μ. The stereoline glutinants are much smaller and rarer, being only 6–7 μ, and the volvents are about 5 μ.

To obtain the nematocysts freely the Hydra was at first macerated in weak chloroform water. Latterly I used Schulze's phenol-glycerine mixture (phenol, crystallized, 1 grm., glycerine 200 c.c., distilled water 200 c.c.), as giving the same results and being more convenient to handle.

Nervous System.

The distribution of the nervous elements is such as one would expect from the reactions of the living animal. They are most numerous in the foot and in the head and tentacles, and are much scarcer in the middle part of the body.

The nervous cells are best seen in maceration preparations. I have found the most useful method to be maceration in Hertwig-Schneider's mixture (0·02 per cent. osmic acid, 1 part : 5 per cent. acetic acid, 4 parts) for fifteen to twenty minutes and subsequent staining in a strong filtered solution of methylene blue for three-quarters of an hour or longer. The methylene blue is dissolved until a deep blue solution is obtained. The specimen is then well macerated and several gentle taps on the coverslip are sufficient to separate the cells. By dividing the Hydra before maceration has begun a fair idea of the relative distribution of the nervous cells may be obtained.

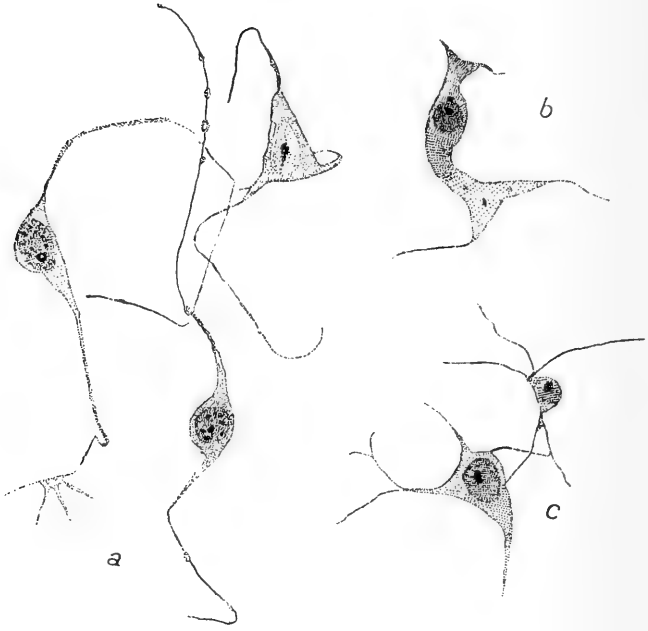
The maceration method has been largely used by Hadzi (**4**) in his work on the nervous system of Hydra. I cannot, however, confirm all his findings.

The nervous cells may be roughly divided into two types, (1) the ganglion cells and (2) cells which are more or less intermediate in form between the ganglion cell and the epithelial cell.

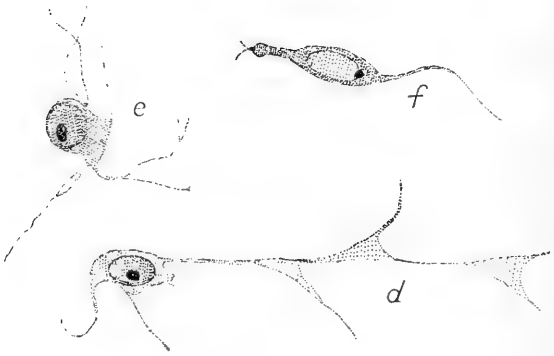
The former are of various shapes according to the number of processes they possess (Text-fig. 4, *a*, *c*, *d*, *e*). The nucleus stains much less deeply with methylene blue than that of the ordinary interstitial cell, as does also the nucleolus. There may be two of the latter. The cell-body usually consists of a thin layer of protoplasm surrounding the nucleus, but it is frequently prolonged at either end into a short thick process before giving off the long nerve-threads. These latter are long thin processes, often branching. Where they branch there is usually a slight swelling, and these swellings are seen along the course of unbranched processes as well (Text-fig. 4, *a* and *d*). The processes of one cell can often be seen to unite with those of another, so that the cell-body appears to lie in a network of interlacing threads. Many of the processes apparently end freely, sometimes in a small knob, while others are attached to the muscle-fibres of myoepithelial cells from which they cannot be detached by tapping on the cover-glass or by irrigation under it. The ganglion cells of the body are usually simpler and less branched than those of the head or foot.

The second type of nervous cell is long and narrow in shape, with the nucleus about the middle of the cell-body, and has a nervous process only at one end (Text-fig. 4, *b* and *f*). This may branch and sometimes it comes into connexion with another nervous cell. The other end is flattened (Text-fig. 4, *b*) or knob-like. These cells correspond to Hadzi's ' sensory cells ' or ' sensory nerve-cells ', but I have, as a rule, not been able to find a short projecting hair on the flattened end. In one case a knob-shaped end bore two fine hairs each ending in a little swelling, and in several other cases there were one or two short hairs (Text-fig. 4, *f*). On examining the surface

TEXT-FIG. 4.



a.



b.

Nerve cells.

of a deeply stained Hydra with an oil-immersion lens, I have not been able to find any number of projecting hairs (apart from the cnidocils) such as one would expect if sensory cells bearing hairs played a large part in the stimulation of the Hydra.

I have also tried Hadzi's vital methylene blue method (**4**) but without success. Hadzi states that his method gives good results only with H. viridis and in sunshine. In my specimens the nematocysts took up the stain strongly and the ectoderm faintly, but there was never any differential staining of the nervous elements.

It is considerably more difficult to recognize nervous cells in section, since the processes are cut short. Hadzi figures, and describes as nervous, cells which stain more deeply than the other interstitial cells and lie basi-epithelially, sending processes to the surface and in other directions. I have seen such appearances in section, but find it impossible to say whether these apparent threads are not merely strands of protoplasm belonging to the myoepithelial cells, or cut edges of cells. I have not found the sensory apparatus which he describes at the surface.

The nerve-cells probably originate from interstitial cells. Some of the latter may often be found connected to one another by short strands, and interstitial cells with short or long processes are not uncommon especially in the tissues of newly hatched Hydras. These differ little from the nervous cells except in their nuclei and in the larger amount of cell protoplasm which they possess.

The effect of various nerve poisons was tried.

Chloroform.—A weak solution of chloroform in water (which anaesthetized a Daphnia completely in a few minutes) caused a curious rhythmic contraction. The animal contracted down into a tight spiral quickly, and then slowly straightened out again. This was repeated at intervals which gradually increased from about two minutes up to fifteen or twenty minutes. Eventually it became motionless and insensitive to contact. This occurs in two hours or longer, after which

the Hydra can recover if removed to pure water. The chloroform has, however, a macerating effect, which begins to act at the tentacles.

Chloretone.—The effect of this was to make the Hydra throw out masses of endodermal cells by the mouth. It usually died.

Cholin.—Weak solutions (1 : 1,000) of this were used but were ineffective. Stronger solutions led to a half-contracted state, but the animal soon recovered in fresh water.

Curare.—A weak solution of curare was prepared by grinding up 0·1 grm. curare with 20 c.c. water and filtering. A clear yellowish solution was obtained. A Hydra was put in a small dish and this solution added till it was about half strength. For some hours the Hydra remained quite normal. After that a stimulus on any part of the body was responded to by a general contraction, and the tentacles remained half contracted. It was left in the solution over night and in the morning was found to have eaten a Daphnia which had been present; but it was very much contracted and did not respond at all to stimulation. Removed to fresh water it expanded but remained very insensitive. On examination the tentacles were found to be degenerating from their tips downwards.

The experiment was repeated with another Hydra and the same result obtained much more quickly, for in two hours the animal ceased to react to stimulation and its tentacles began to degenerate.

It is noteworthy that necrosis always begins at the tentacle tips and works down gradually, the body remaining apparently normal for some time after the tips of the tentacles have disappeared entirely.

In one case the whole head suffered necrosis and the animal remained as a closed tube for some days. It then produced two buds about the middle of the body and a third near the head region but slightly to one side. These developed and constricted off and a fourth bud appeared, again to one side of the head. As it developed it swung round so that eventually

it was in line with the main axis of the body, and acted as the true head. The curare thus appears to destroy the power of regeneration in the affected parts: possibly the interstitial cells are killed off.

Symbiotic Cells.

Since the differences between the various species of brown Hydra seem less marked than their resemblances, it is of interest to know whether, under any circumstances, a H. viridis deprived of its green cells would grow to resemble a brown Hydra.

Goetsch (**3**) obtained brown Hydras showing pathological features, which, when fed with algae, turned green. As they did so they diminished in size, budding ceased, and under natural conditions they died. Some which were fed with freshly killed Daphnia lived, and produced testes or ovaries. The symbiosis was easily lost, disappearing after four weeks in darkness. Goetsch suggests that this Hydra is a new mutant, capable of receiving the alga, which is a large form of Chlorella.

Whitney (**14**) describes a method of ridding H. viridis of its green inhabitants. He kept his specimens in a weak solution of glycerine (0·5 per cent.) for a few weeks. During this period the endodermal cells swelled up and extruded the algae, which were then thrown out by the mouth. Eventually he obtained several colourless Hydra which lived normally in the aquarium for some time without being reinfected. They retained all the features typical of H. viridis except the colour.

On January 26 I set five Hydras in a jar of 0·5 per cent. glycerine, where they were fed as usual. They budded actively. On February 13 they had increased to nine and were removed to 0·75 per cent. glycerine as the weak solution had had no effect. On March 1 there were twenty-three Hydras, still quite green, and they were removed to 1 per cent. glycerine. One was fixed and sectioned. The endoderm appeared to be quite normal in size, and the green algae were arranged as

usual all through the cell and were not collected at the distal end. On March 9 thirty-one Hydras were removed to 1·5 per cent. glycerine. In the middle of April they were still quite green.

Several Hydras were kept in the dark for the same purpose, but although the colour grew somewhat paler they all died before the green cells had been entirely lost. Hadzi (**5**) has also noted that they do not survive in darkness. Eggs, apparently colourless, which were produced in the dark, died before hatching.

Some brown Hydras were induced to swallow pieces of H. viridis by slipping the latter inside the carapace of Daphnia, but they were ejected along with the remains of the food and had no effect.

Daphnia were also fed on a pure culture of Chlorosphaera and were then given to the Hydras, but with no effect.

There have been many attempts to make a pure culture of the green organism inhabiting H. viridis. In Beyerinck's (**1**) paper on the culture of algae and lichens he states that he has been unable to obtain a pure culture of the zoochlorella from Hydra, but he adds a foot-note to the effect that he had obtained such a culture, and that the organism was indistinguishable from Chlorella vulgaris.

Later writers have made damp cell-cultures and have seen division taking place, but so far as I know there has been no large culture obtained.

I washed H. viridis in several changes of sterile water and then teased it up with needles till practically all the green cells were freed. They were then sown on Miguel solution in tubes and sporulation dishes, on Amoeba-agar, and on agar made up with Miguel solution, but in none of these was any culture obtained.

In one tube of Miguel, Chlorosphaera limicola (Beyerinck) appeared. The Daphnia on which the Hydras were fed were themselves fed on a mixed green culture which proved to contain Chlorosphaera, and the organism may

have remained alive inside the Hydra after the Daphnia had been eaten.

In damp chambers and on sterile slides the green cells remained alive for a week or more and some divided, but eventually they all died off or bacteria appeared. The cells divided either into two or three. Radais states that in a culture of Chlorella vulgaris the cells divided into four when healthy, but as the culture grew older the rate of division slowed down, and the cells divided into three or two.

When stained, the organism from H. viridis shows a large and distinct pyrenoid. The nucleus is less distinct and usually appears as an irregular ring of darkly staining material. No division stages were seen.

Taxonomy.

The number of species of Hydra has been much discussed ever since the foundation of the genus. Schulze (**9**) has lately divided it into three genera and about ten species. The Hydras on which I worked do not exactly correspond to any of Schulze's species but come nearest to his H. attenuata, from which they differ in being hermaphrodite. It seems to me improbable that the genus Hydra is justifiably divided up into so many definite species. Some of Schulze's species are founded on the examination of preserved specimens only. The general habit, colour, size, and so on, are used as differentiating characters, while the nematocysts are always treated as important diagnostically. The Hydras on which I worked varied considerably in size and habit, but all possessed the same kind of nematocysts. In some the egg was stuck on the side of the glass and in some it fell freely to the bottom. Considering the great variation in appearance which may take place within the lifetime of one individual, it seems unsafe to separate off as distinct species animals whose whole life history has not been completely followed through.

This work was done during my tenure of a Carnegie scholarship from 1920–2, in the Natural History Department of

Glasgow University. I should like to express my gratitude to Professor Graham Kerr for the help he gave, and the interest he took in my work.

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Head Length Dimorphism of Mammalian Spermatozoa.

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With 3 Text-figures.

Contents.

Introductory.

Work on mammalian spermatogenesis has in a large number of cases shown that the spermatozoa are of two types, one type possessing the accessory chromosome, whilst the other type has no sex chromosome or a mere vestigal complement. As the spermatozoon head is constituted almost entirely of nucleus it might be expected that the additional chromatin possessed by the one type would slightly increase the size of the head. In three cases this correlation has been found. Wodsedalek (**5** and **6**) has shown that in the horse and bull the spermatozoa are of two types, and that in each case a frequency polygon of the head lengths shows distinct dimorphism. In the case of the dog, Malone (**2**) found an unpaired accessory chromosome in spermatogenesis, and

Zeleny and Faust (**8**) have demonstrated dimorphism of the head lengths. The work recorded in this paper was an attempt to extend the application of this correlation to other mammalian spermatozoa for which chromosomal dimorphism has been shown.

Methods and Material.

The new work recorded here deals with man, the rat, the cat, and the mouse. I have to thank Mrs. R. Sellars of the Manchester Medical School for procuring the human material for me from the Manchester Royal Infirmary. In the other cases the material was obtained by dissection of the epididimis. In each case smear slides were made, as this method has advantages compared with using testis sections. Some difficulty was at first experienced in making satisfactory smears owing to the tendency for the spermatozoa to drop off. Increased experience in manipulation, however, was found to surmount this. By teasing out the epididimis in salt solution and fixing, it was found possible to make the spermatozoa adhere without using egg-albumen cement. For fixing Zenker's fluid was used to start with, as recommended by Zeleny and Faust (**8**), but finally the ordinary corrosive and aceti-solution (90 per cent. saturated solution corrosive sublimate and 10 per cent. glacial acetic acid) was found to be quite efficient. Various stains were tried, but Delafield's haematoxylin was eventually found to be by far the most satisfactory.

The measurement of the spermatozoa was found to present great difficulty, especially in the case of the rat and mouse where the head-piece is sickle-shaped. This fact, together with the minute size of mammalian spermatozoa, makes measurement with an ocular micrometer almost impossible. Both these difficulties were alleviated by using the Zeiss-Greil drawing apparatus possessed by the department. This consists of a lantern throwing light through a horizontal photo-microscope and projecting the image on to a screen. By placing a mirror at 45 degrees in front of the eye-piece the image can be thrown down on to a table. This apparatus can be used

with an oil immersion, and the resulting image thrown on the table, even of such a small object as a spermatozoon head, is sufficiently large to admit of measuring round a curve with a pair of compasses. The co-efficient was worked out previously by putting a stage micrometer in the microscope and finding out how many centimetres on the table corresponded to 10μ on the slide. The subsequent calculation was as easy as that necessary when using an ocular micrometer. By this means the unavoidable margin of error in the measurements was very greatly reduced. On one occasion when the drawing apparatus was out of order some measurements were made with the aid of a camera lucida. In both cases extreme dimensions were marked on paper, connected by a line, and the whole number measured afterwards. An 18 Zeiss ocular could be used with the camera lucida, but not with the drawing lantern, owing to the excessive diffusion of light, and so the nett magnification came to about the same in both cases.

Wodsedalek measured the spermatozoa in testis sections by the camera-lucida method, and Zeleny and Faust used smear slides with the ocular micrometer. It will be seen, therefore, that the method described above is a combination of the two, and it was found to be the most satisfactory. The error involved by the use of the ocular micrometer is avoided, as is the possibility of getting immature spermatozoa on the slide.

As the whole value of the work depends on the degree of accuracy which can be achieved, the following remarks may not be out of place. Three sources of error arise :

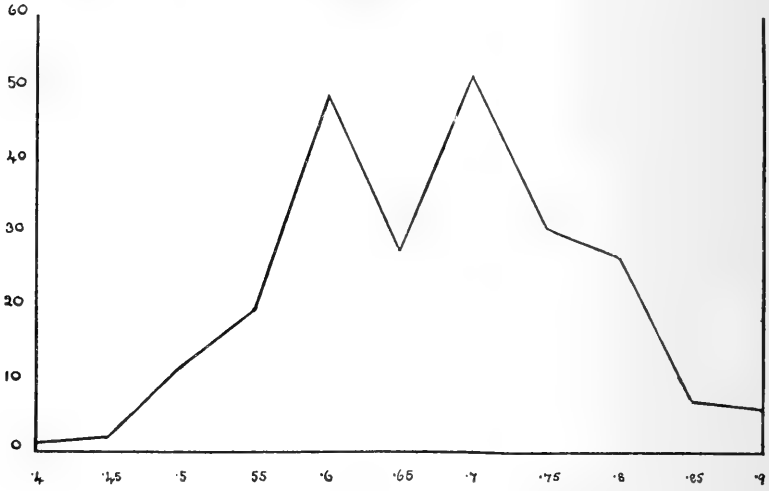
(*a*) Distortion of the spermatozoa during fixing and preparing.
(*b*) The personal factor in measuring.
(*c*) The unavoidable error in measuring.

With regard to the first point, general shrinkage of the spermatozoon head must almost inevitably occur ; but only one slide was used for each set of measurements, and no attempt has been made to mix measurements from different slides. As the spermatozoa on one slide would all be affected in the

same manner, this precaution should remove the first source of error.

Secondly, bias particularly easily arises in such work and may almost unconsciously detract from the accuracy. A conscious attempt to discount this bias may lead to the opposite extreme and cause an equal inaccuracy. Also, the work is very trying and strain seriously disturbs the measurements. In general, however, the personal factor was discounted, as far as possible, by only working for very short periods at a time.

Text-fig. 1.



Frequency polygon of head lengths of human spermatozoa.

Thirdly, the unavoidable margin of error must be considered. Fortunately, however, this is a calculable quantity, and the following tests were made. In the first case the same spermatozoon head was measured under three different magnifications, the co-efficients of which were known, and the results compared. The following table sums up the results:

Ob. and Oc.	*Size in μ.*
Bausch and Lomb $\frac{1}{6}''$ and Zeiss 18 .	4·09
Koristka $\frac{1}{12}''$ and Bausch and Lomb 10	4·10
Koristka $\frac{1}{12}''$ and Zeiss 18 . . .	4·14

It will be seen that the range of the variation in the three measurements is only $0{\cdot}05\mu$ or $\frac{1}{20}\mu$.

The second manner of testing accuracy consisting in measuring the same spermatozoon several times under the same magnification, and the results gave a range of variation in six measurements of $0{\cdot}10\mu$ or $\frac{1}{10}\mu$. Both these margins of error are very much smaller than the least fraction of μ usually dealt with.

I should like to take this opportunity of acknowledging my great obligation to Mr. J. T. Wadsworth for his invaluable assistance in the technique of this work.

Spermatozoa of Man, Rat, Cat, and Mouse.

Von Winiwarter (**4**) has described chromosome dimorphism of human spermatozoa according to the presence or absence of an unpaired accessory. In my material, measurement of the head lengths was found to give the following results :

Table I. Frequency of Head Lengths of Spermatozoa of Man.

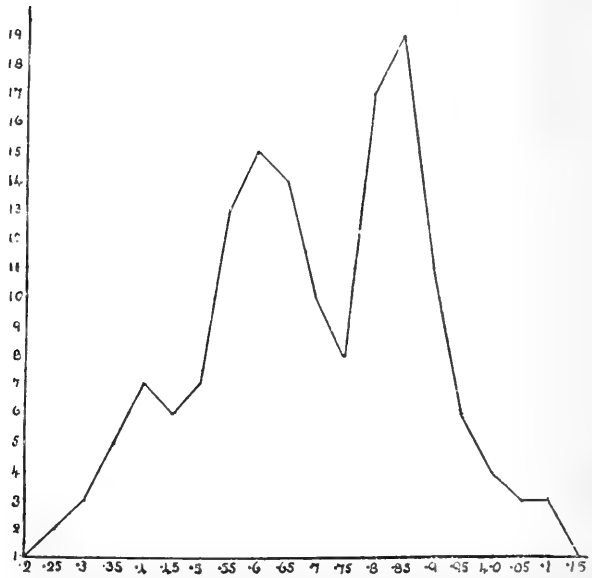
Head lengths ×1,460 (*in cms.*)	*Number found.*	*Head lengths* ×1,460 (*in cms.*)	*Number found.*
0·4	1	0·7	52
0·45	2	0·75	31
0·5	12	0·8	27
0·55	20	0·85	7
0·6	49	0·9	6
0·65	28		

In the case of the rat Allen (**1**) has demonstrated an accessory chromosome in spermatogenesis, the spermatozoa having eighteen or nineteen chromosomes. The dimorphism again appears to communicate itself to the head sizes of the spermatozoa, for dimorphism was found in the head lengths of the spermatozoa of the rat. The frequency polygon given below (Text-fig. 2) was made from measurements of the head lengths (× 4,000) of 155 spermatozoa.

Table II. Frequency of Head Lengths of Spermatozoa of Rat.

Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
3·2	1	3·7	10
3·25	2	3·75	8
3·3	3	3·8	17
3·35	5	3·85	19
3·4	7	3·9	11
3·45	6	3·95	6
3·5	7	4·0	4
3·55	13	4·05	3
3·6	15	4·1	3
3·65	14	4·15	1

Text-f g. 2.



Frequency po'ygon of head lengths of spermatozoa of rat.

Von Winiwarter (**3**) has shown that the spermatozoa of the cat are cytologically dimorphic, one type having eighteen chromosomes and the other seventeen. In measuring the head lengths, however, no clear dimorphism was found. The results were as follows :

TABLE III. FREQUENCY OF HEAD LENGTHS OF SPERMATOZOA OF CAT.

Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
1·2	2	1·7	32
1·25	2	1·75	20
1·3	4	1·8	28
1·35	2	1·85	13
1·4	7	1·9	22
1·45	9	1·95	6
1·5	17	2·0	5
1·55	20	2·05	1
1·6	31	2·1	1
1·65	26	2·15	1

Yocum (7) has shown that the spermatozoa of the mouse are cytologically dimorphic, one type having nineteen and the other twenty chromosomes. I found this reflected in the head lengths, as the following results show:

TABLE IV. FREQUENCY OF HEAD LENGTHS OF SPERMATOZOA OF MOUSE.

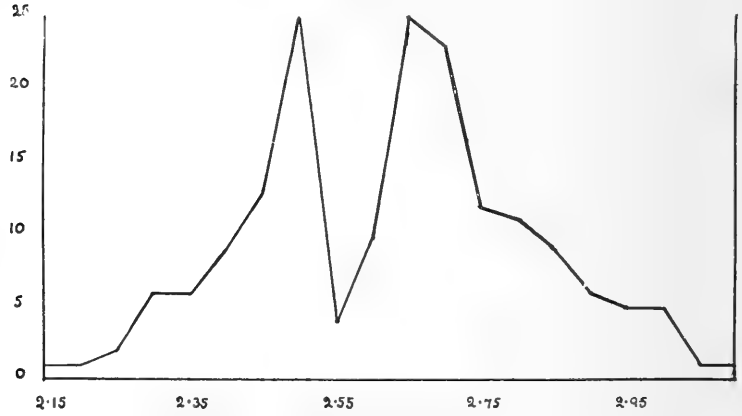
Head lengths ×4,000 (*in cms.*)	*Number found.*	*Head lengths* ×4,000 (*in cms.*)	*Number found.*
2·15	1	2·65	25
2·2	1	2·7	23
2·25	2	2·75	12
2·3	6	2·8	11
2·35	6	2·85	9
2·4	9	2·9	6
2·45	13	2·95	5
2·5	25	3·0	5
2·55	4	3·05	1
2·6	10	3·1	1

CONCLUSION.

It would thus appear that in three more species of mammals the spermatozoa show dimorphism in the head length, while in a fourth species, the cat, the evidence is uncertain. The chief interest in these conclusions lies in the bearing which the size dimorphism of the spermatozoa might have in determining the proportion of the sexes at conception. If the potentially male and the potentially female-producing spermatozoa are of different size, their activity and vigour may also be relatively

different, causing more of one type than of the other to survive the severe journey through the female organs to the ova (as T. H. Morgan has suggested, ' Physical Basis of Heredity ', 1919). It is most probable that the disproportion which exists between the sexes at conception in most mammals is connected with this point.

Text-fig. 3.



Frequency polygon of head lengths of spermatozoa of mouse.

Summary.

1. Chromosome dimorphism of the spermatozoa has been shown for a variety of mammals, and in some cases this has been shown to be correlated with dimorphism in the head lengths of the spermatozoa.

2. In the present paper this correlation has been extended to the spermatozoa of man, the mouse, and the rat, in which chromosome dimorphism of the spermatozoa had previously been shown, and in which head length dimorphism seems to exist.

3. The interest of these results lies in the probability that the histological difference in the X- and Y-spermatozoa may account for the inequality of the sexes at conception in mammals.

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On Brinkmann's System of the Nemertea Enopla and Siboganemertes Weberi, n.g.n.sp.

By

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With 26 Text-figures.

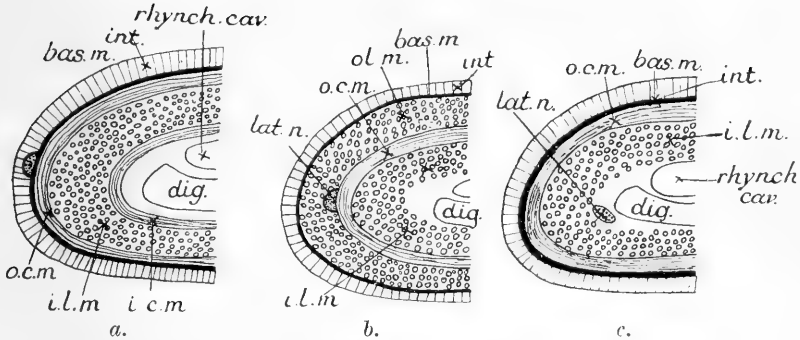
All text-books of zoology give the Nemertean system as Bürger published it several times in his monographs on this subject (**6**, **7**, **8**). The old systems of Schultze, McIntosh, Hubrecht have been forsaken, and our text-books do not divide the Nemertini any more in Anopla and Enopla, or Palaeonemertea, Hoplonemertea, and Schizonemertea. The armature of the proboscis and the arrangement of the cephalic slits are believed to be of secondary importance, and Bürger divided the Nemertini into four orders, three of which, his Protonemertini, Mesonemertini, and Metanemertini are supposed to be closely related by having a body-wall that consists of an epithelium, a basement membrane, and two muscular layers. Benham, in Ray Lankester's 'Treatise on Zoology', unites them as Dimyaria, and his Trimyaria consist of one order only, the Heteronemertini Bürger. The Protonemertini are McIntosh's family Carinellidae, Mesonemertini are his family Cephalotricidae and the genus Carinoma (Hubrecht); Metanemertini is a new name for Hoplonemertini (Hubrecht) or Enopla (Max Schultze), and Heteronemertini are Hubrecht's Schizonemertini and his families Eupoliaidae and Valenciniaidae. Everybody agrees that the last two families are more nearly related to Lineids and Cerebratulids than to the Protonemerteans, and their enclosure in the order Heteronemertini seems to be well

founded. The following schema gives the synonyms in the different systems.

Max Schultze.	McIntosh.	Hubrecht.		Bürger.	Blaxland Benham.
Enopla	fam. Amphiporidae	Hoplonemertini.		Metanemertini	Dimyaria.
Anopla	fam. Cephalotricidae	fam. Cephalotricidae	Palaeonemertini	Mesonemertini	
		gen. Carinoma		Protonemertini	
	fam. Carinellidae	fam. Carinellidae			
		fam. Eupoliaidae		Heteronemertini	Trimyaria.
		fam. Valencinidae			
	fam. Lineidae	Schizonemertini			

The combination of Bürger's three other orders in the Dimyaria was not well founded. Bürger meant them to be two stages in the development of the third, the Metanemerteans or Hoplonemertini; the Protonemertini with their epithelial nervous system (Text-fig. 1, *a*) being the most primitive ones. Carinoma and Cephalothrix, the Mesonemertini, give the development that leads to the central position of this system in the body parenchyma (Text-fig. 1, *c*). Now Bergendal, in his treatise on Carinoma armandi (**2**), made it clear that this genus is closely related to Carinella and the Heteronemerteans (Text-fig. 1, *b*), and has no affinity at all to Cephalothrix nor to the armed Nemerteans. In another Swedish article he shows (**1**) that Bürger's order Mesonemertini is quite unnatural, and that both genera Cephalothrix and Carinoma are true Protonemertini; so Hubrecht's order Palaeonemertini ought to be restored. A study on Cephalothrix taught me (**16**) that this genus is not nearer related to the armed Nemerteans than to the unarmed, and so we get instead of Proto- and Meso-nemertini the old order of Palaeonemertini, nearly related to the Heteronemertini and without any special relation to these groups the armed Nemerteans. In 1912 (**17**) I proposed to return to the old division of Max Schultze. The class Nemertini is subdivided into two sub-classes: Anopla and Enopla. Each sub-class contains two orders: the Anopla with Palaeonemertini (Hubrecht) and Heteronemertini (Bürger), of which diagnoses were given and schemes are given here in Text-fig. 1, *a* and *b*; and the Enopla

TEXT-FIG. 1.

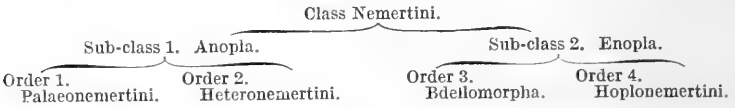


Schemata of the musculature, nervous system, and body-wall in: *a*, Palaeonemertini; *b*, Heteronemertini; *c*, Enopla. In transverse section.

LIST OF ABBREVIATIONS.

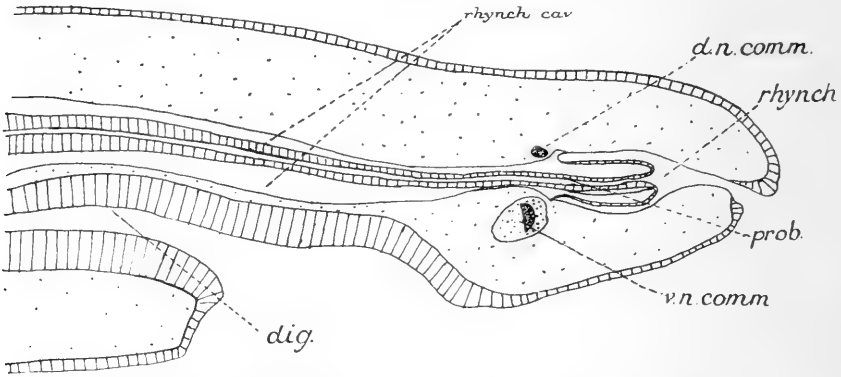
acc.st., accessory stylet; *an.bl.v.*, unpaired anal blood-vessel; *an. l.*, anal commissure of blood-vascular system; *atr.*, atrium; *bas.*, base of stylet; *bas.m.*, basement membrane; *b.g.*, entodermal blind-gut; *bl.v.*, blood-vessel; *bod.par.*, body parenchyma; *br.*, brain; *cer.bl.c.*, cerebral blood commissure; *cer.can.*, cerebral canal; *cer.sac.*, sac of cerebral organ; *circ.m.f.*, circular muscle-fibres; *c.m.*, new circular musculature of proboscis; *d.b.v.*, dorsal blood-vessel; *d.g.*, dorsal ganglion; *dig.*, digestive tract; *d.n.comm.*, dorsal nerve commissure; *ej.d.*, ejaculatory duct; *gangl.*, ganglionic part of cerebral organ; *gastr.*, gastric cavity; *gl.*, glands; *g.p.*, gonopore; *g.s.*, gonadial sac; *i.c.m.*, inner circular muscle-coat; *i.l.m.*, inner longitudinal muscle-coat; *int.*, integument; *int.p.*, intestinal pouch; *lat.n.*, lateral nerve-cord; *l.b.v.*, lateral blood-vessel; *l.m.f.*, longitudinal muscle-fibre; *m.*, mouth; *m.bl.c.*, metamerical blood-vessel commissure; *musc.sept.*, muscular septum; *nephr.*, nephridium; *o.c.m.*, outer circular musculature; *oes.*, oesophagus; *o.l.m.*, outer longitudinal musculature; *ov.*, ovary; *p.e.*, proboscidian epithelium; *p.end.*, proboscidian endothelium; *p.p.*, proboscis pore; *prob.*, proboscis; *prob.cav.*, proboscidian cavity; *prob.n.*, proboscidian nerve; *prob.w.*, proboscidian wall; *pyl.*, pylorus; *rad.m.*, dorsoventral musculature; *rh.c.m.*, normal circular coat of rhynchocoelomic wall; *rh.l.m.*, normal longitudinal coat of rhynchocoelomic wall; *rhynch.*, rhynchodaeum; *rhynch.bl.v.*, rhynchocoelomic vessel; *rhynch.cav.*, rhynchocoelomic cavity; *rhynch.div.*, rhynchocoelomic diverticula; *rhynch.end.*, rhynchocoelomic endothelium; *rhynch.m.*, rhynchocoelomic musculature; *rhynch.w.*, rhynchocoelomic wall; *sac*, sac with accessory stylets; *st.*, stylet; *t.*, testis; *v.g.*, ventral ganglion; *v.g.s.*, V-shaped gonadial sac; *v.n.comm.*, ventral nerve commissure of the brain.

with Bdellomorpha (Verrill) and Hoplonemertini (Hubrecht) (Text-fig. 1, *c*).



The sub-class Enopla (Text-fig. 1, *c*) shows a tendency to have the digestive system and the proboscidian apparatus opening to the exterior by one aperture. In the Anopla both openings

TEXT-FIG. 2.

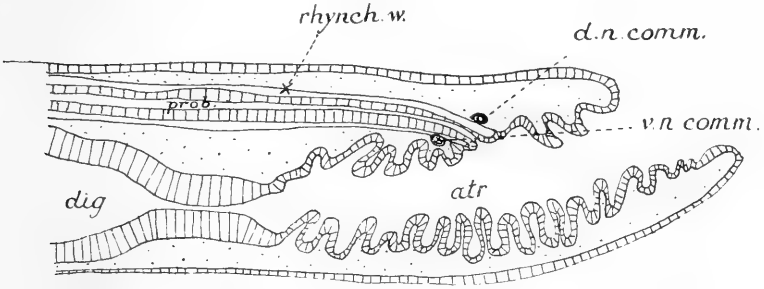


Schematic longitudinal section of an unarmed Nemertean after Bürger (**6**, Pl. xxi, fig. 1, Cerebratulus marginatus).

are always widely separated, as shown in Text-fig. 2; in the Enopla the common aperture is obtained in different ways. The Bdellomorpha, containing the parasitic genus Malacobdella, which Bürger included in his Metanemertini, though it lacks an armed proboscis and shows great differences in the structure of almost every organ, has its proboscis inserted in the wall of the stomodaeum (Text-fig. 3). In the Hoplonemertea the same result, one common mouth, is developed in two other ways, as will be shown afterwards. As Bürger's Metanemertini are only a newer name for Hoplonemertini his subdivision of this order into Pro- and Holo-rhynchocoelomia might be followed in our system as well. The main difference between these

sub-orders exists in the length of the proboscis sheath, which in the first group is developed in part of the body only, in the Holorhynchocoelomia, however, is present from the snout to the tail. That this division is unnatural Brinkmann showed in his monograph on the pelagic Nemerteans (**4**). In this most interesting paper, that contains the minute anatomical description of eighteen genera of pelagic Nemerteans with thirty-two species, the greater part of which are new, Brinkmann describes nearly related species of one genus, Balaenanemertes, that might be types of Bürger's

Text-fig. 3.

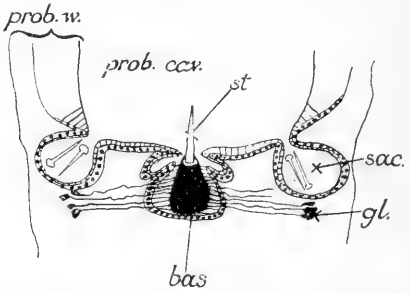


Schematic longitudinal section of Malacobdella grossa after Bürger (**6**, Pl. xviii, fig. 2).

two sub-orders. In other genera the difference is less great but still exists. This fact alone is sufficient to demonstrate the unnaturalness of the subdivision of Bürger's Metanemertini. Another fact of interest was that all pelagic forms are nearly related, and show a peculiarity in the armature of the proboscis that we knew only from the genus Drepanophorus. This genus is one of Bürger's Holorhynchocoelomia. Though at first included in the family Amphiporidae, the family Drepanophoridae was later established; and Bürger believed this genus with its paired rhynchocoelomic diverticula to be the most highly specialized one of his sub-class. In his study on Uniporus, a nearly related genus, Brinkmann (**3**) came to the conclusion that the Drepanophoridae in many respects are very primitive forms of Hoplonemerteans, which

conclusion I share. All these facts show evidently that Bürger's system of Metanemertini does not give the real relationship of the genera. Brinkmann gives another system, that seems to suit much better our present state of knowledge. The armature of the proboscis is the distinctive character. In most armed Nemerteans the proboscis has one stylet on the top of a somewhat pear-shaped handle (Text-fig. 4). The only known exception to this rule was till fifteen years ago Drepanophorus ; then the Valdivia material showed that some of the pelagic Nemerteans have a crescent-shaped handlelike Drepanophorus with many small stylets (Text-fig. 5)

Text-fig. 4.

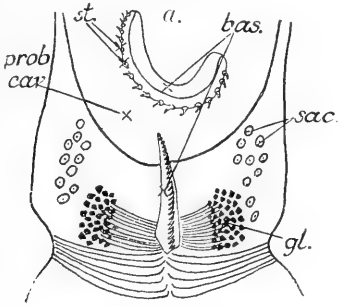


Armature of the proboscis of Stichostemma eilhardi after Montgomery, 1894 ('Zool. Anzeiger', Jahrgang 17, fig. 3) (Monostilifera).

and Brinkmann (**4**) confirmed this discovery of Bürger for all pelagic forms. He divides the Hoplonemertini into two sub-orders, Polystilifera and Monostilifera (**4**, p. 145). The Monostilifera contain all genera of Metanemertini (Bürger), with the exception of (1) Malacobdella (=Bdellomorpha, Verrill), (2) the pelagic genera, and (3) Drepanophoridae. The Polystilifera consist of all pelagic Nemerteans and the genera Drepanophorus (Hubrecht), and Uniporus (Brinkmann). There can be no doubt as to the naturalness of these sub-orders. Both contain a great number of genera and species, which are widely different in structure, but still are more closely related to each other than to any other form. This is shown by the position of the mouth, which in the Anopla lies behind

the brain. The proboscis pore is found in front of it, as a rule at the tip of the snout (Text-fig. 2). I remarked already that in Enopla both structures stand in connexion with each other ; in the Bdellomorpha the rhynchodaeum is absent and the proboscis cavity opens into the digestive tract (Text-fig. 3). In the Hoplonemertini, it is said, the digestive tract opens into the rhynchodaeum (Text-fig. 6). This last fact is only true as far as concerns the Monostilifera. That this connexion of the two systems is not primitive is shown by the embryology.

Text-fig. 5.

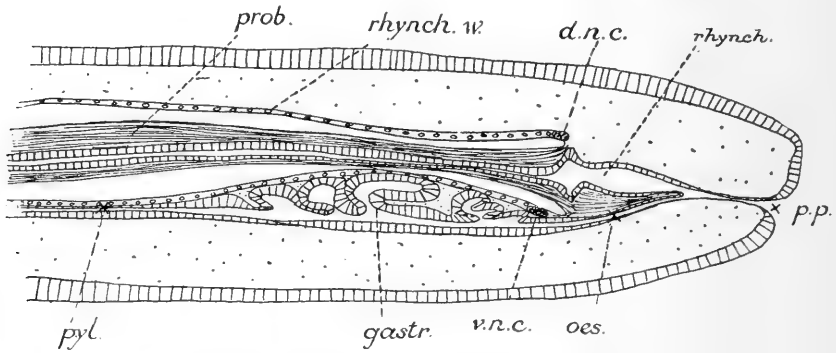


Armature of the proboscis of Drepanophorus spectabilis (Polystilifera) after Bürger (**6**, Pl. viii, fig. 2). *a*. Base and stylets of D. crassus.

In my article on the proboscidian system in Nemertines (**18**) I put the facts together in the following way (p. 304) : ' Drepanophorus, the genus in which oesophagus and rhynchodaeum open separately, shows no connexion at all between the two systems, not even in embryology ; for here the blastopore is closed, the narrow endoderm part giving rise to the blind gut by being removed forward. The primary ectodermic oesophagus invaginates near the proboscidian system, but perfectly separately. . . . In all other Hoplonemertea the primary oesophagus originates in exactly the same way ; the mouth closes afterwards and the primary oesophagus gets a new opening to the exterior through the rhynchodaeum ' (Text-fig. 7).

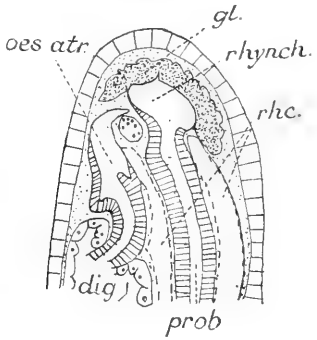
What has been said of Drepanophorus is not true for all Polystilifera. We know species in which mouth and proboscis pore are widely separated and the mouth even lies under the

TEXT-FIG. 6.



Schematic longitudinal section of a Monostilifer after Bürger (6, Pl. xv, fig. 1, Nemertopsis peronea).

TEXT-FIG. 7.

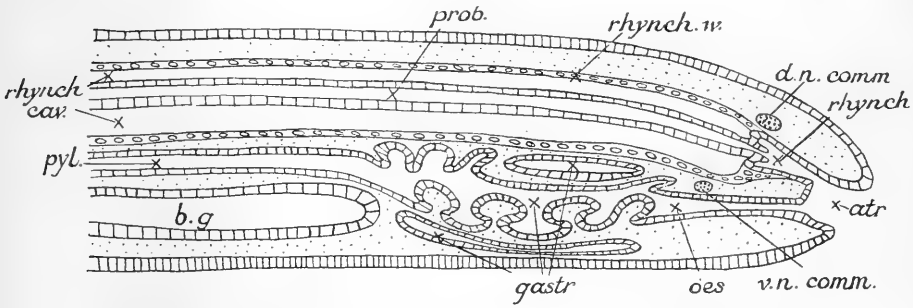


Longitudinal section through Prosorochmus after Salensky ("Über die embryonale Entwicklung des P. viviparus," 'Bull. Acad. Imp. Sciences', Petersb., 1909, fig. 8).

brain. But we also know species in which they communicate by one pore; we even know all the stages between these extremes in the Polystilifera. Brinkmann (**4**) showed the development of an ectodermal atrium, in which rhynchodaeum and mouth open separately in the Pelagica. He also describes

(3) a large ectodermal atrium in Uniporus, one of his Reptantia ; Drepanophorus lankesteri (Text-fig. 8) exhibits the same feature, and so do some genera of the East Indian archipelago. In the Drepanophorus species of the Channel, known as D. spectabilis, these openings lie quite near to each other—I might say, they touch each other ; the species known under the same name from Naples has a large space between the two, but never does the mouth open into the rhynchodaeum, nor vice versa. So there is another distinctive character between Poly- and Mono-stilifera. A difference in habits and

Text-fig. 8.



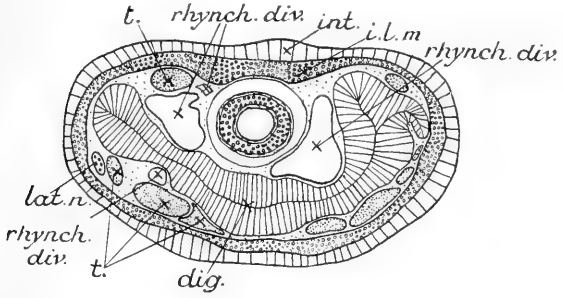
Schematic longitudinal section of Drepanophorus lankesteri from sections.

manner of life accompanies the great differences of structure in Brinkmann's divisions of the Polystilifera. The Pelagica are free-swimming or hovering pelagic Nemerteans that live at a great depth, without eyes, without the, for Nemerteans, so characteristic cerebral organs, without a nephridial system, without rhynchocoelomic diverticula, without metamerical vascular communications. They might be considered related to the Monostilifera as well as to the Polystilifera with all these negative characters if we had not known the structure of the proboscis armature and of its sheath. Another positive character is the place of the male gonads. Though the ova develop in metamerically placed sacs between each pair of intestinal diverticula, the sperm-cells

develop only in front of this region, at the side of or directly behind the brain, a unique fact in Nemerteans. The Reptantia, containing the genera Drepanophorus and Uniporus, and, as the Siboga material shows, quite a number of other genera, that crawl about at the bottom of the sea and its coasts, have as a rule eyes and metamerical blood-vessels, but always they possess cerebral organs, nephridia, rhynchocoelomic diverticula, and metamerically placed ♂ gonads. Especially the cerebral organs are different from those of the Monostilifera and the development of a sac in this organ as well as the presence of diverticula of the proboscis sheath show that the Reptantia are widely different from the Monostilifera. Almost all Polystilifera that the Siboga expedition brought home belong to the Reptantia. About one form only there can be any doubt, as it is collected by the deep-sea trawl to the south of Timor at a depth of 883 metres. This is the depth in which most pelagic forms occur and, as occasionally pelagic Nemerteans can and have been caught by the trawl, we might be in doubt as to the manner of life of this Nemertean. Moreover the inner structure of Siboganemertes weberi reveals such peculiarities that we cannot with certainty decide anything. It has no eyes, but Uniporus, a genus of Reptantia of the Norwegian sea, living in the dis- and aphotic regions, lacks them as well. It possesses cerebral organs, but they are quite minute and of a much more primitive structure than anything known. Nephridia are present, but metamerical blood-vessels fail as in Polystilifera and Uniporus. Rhynchocoelomic diverticula are present, but instead of lying peripherically at the outside of all organs as in all Reptantia (Text-fig. 10) they lie inside between the proboscis sheath and the digestive tract (Text-fig. 9). The testes are placed metamerically, but they display features that we do not know in other Polystilifera. The mouth lies under the brain, which in its structure shows a great resemblance to the Pelagica and differs greatly from the Reptantia. The digestive tract, which lacks an oesophagus in the pelagic forms and has a well-developed one in the Reptantia, has quite a short balloon-like

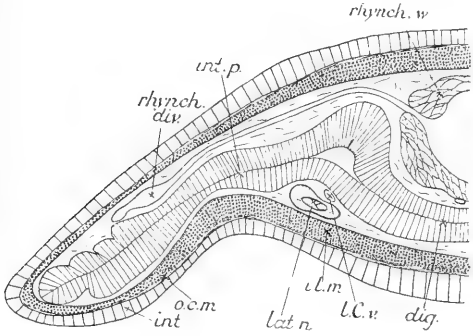
oesophagus that does not reach the brain ; on the other hand it differs greatly from that in both groups, as the different parts of the stomodaeum, that as a rule gradually pass into each other (Text-fig. 6), are sharply separated. The narrow

TEXT-FIG. 9.



Section through Siboganemertes weberi, n. gen. n. sp.

TEXT-FIG. 10.



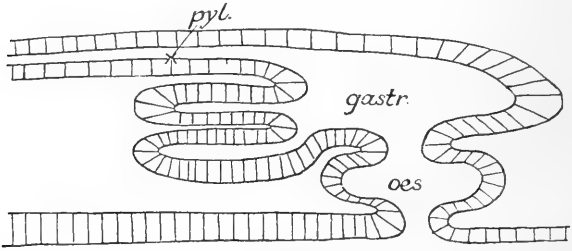
Section through Drepanophorus albolineatus after Bürger (**6,** Pl. xvii, fig. 10).

pylorus opens by a hole in the wall of the gastric cavity ; oesophagus and gastric cavity communicate by a narrow opening (Text-fig. 11). It seems evident that, though the presence of rhynchocoelomic diverticula, cerebral organs, and metamerically placed genital organs might suggest the enclosure of Siboganemertes weberi in the Reptantia, the

structure of the digestive system and the arrangement of the diverticula of the proboscis sheath separate them.

The most interesting feature seems to be the structure of the cerebral organ that is so highly developed in the Reptantia. This sense organ consists in the Monostilifera (Text-fig. 12, *a*) of three different parts: a channel, a ganglion, and glands. These are joined together to a more or less rounded organ with its own neurilemma. In the Reptantia the same constituents are present, but as a rule the different parts are more free from each other and partly lie outside the rounded circumference of the organ, as in Drepanophorus cerinus and wil-

Text-fig. 11.



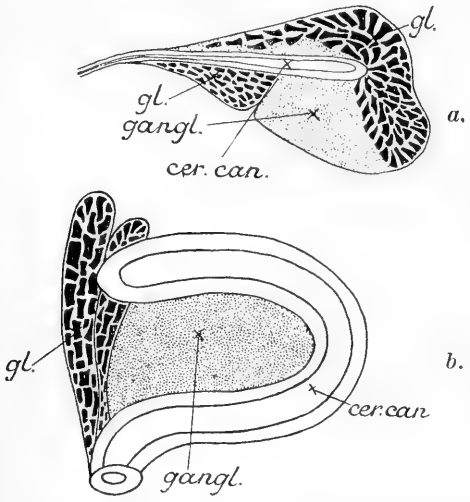
Schematic longitudinal section of the digestive tract of Siboganemertes weberi after transverse sections.

leyanus (**14**, **15**) and in Uniporus (**3**). Moreover, the duct that leads from the cephalic furrows into the cerebral organ bifurcates in the organ; one part gives rise to a more or less spacious sac, characteristic of Reptantia, and the other part ends as a narrow duct in the glandular portion of the organ (Text-fig 13). Both sac and glandular tube can lie embedded in the body parenchyma. In Siboganemertes there is no bifurcation of the cerebral canal (Text-fig. 12, *b*). When the channel gets to the ganglion two small bunches of glands open into it which lie quite free. The epithelium is sensory and never gets glandular. The channel is as primitive as possible; it turns backward at the contact with the ganglion and at its end bends upward and forward on its first part, where it ends blindly. This is the most primitive cerebral

organ we know in Enopla, and makes it very probable that we stand near the origin of this organ.

As to the brain it displays very primitive features too. Brinkmann writes in his monograph (**4**, p. 165): 'Die den meisten Drepanophoriden so characteristische starke Vergrösserung der dorsalen Gehirnganglien, die dazu führt, dass sie

Text-fig. 12.

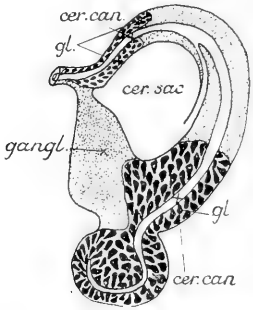


Cerebral organs of *a*, Prostoma cephalophorum after Bürger (**6**, Pl. viii, fig. 28); *b*, Siboganemertes weberi (Schema).

wie grosse, kuglige Gebilde den kleinen, ventralen, birnförmigen Ganglien aufsitzen und bei weitem die grösste Masse des Gehirns bilden, kommt bei den pelagischen Nemertinen nicht vor. Die hier stattgefundene Reduktion, die dazu führt, dass die dorsalen Ganglien höchstens nur wenig grösser sind als die ventralen, ja gar nicht selten kleiner als diese werden können, ist zweifelsohne durch das Verschwinden der Cerebralorgane verursacht, denn es sind ja diese Organe, die vor allem von den dorsalen Ganglien aus innerviert werden.' Siboganemertes weberi exhibits the same structure of brain as certain Pelagica, though a small cerebral organ is present.

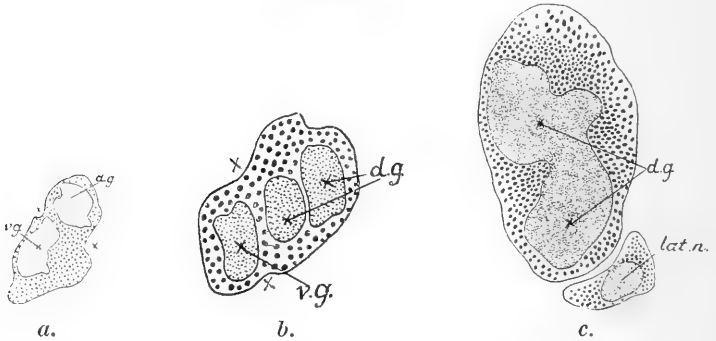
It is comparable with those in which the dorsal ganglia lie quite outside the ventral. Both have the same length, but the position of the dorsal lobe is somewhat more forward than the

TEXT-FIG. 13.



Cerebral organ of Drepanophorus spectabilis after Bürger (6, Pl. viii, fig. 23), Schema.

TEXT-FIG. 14.



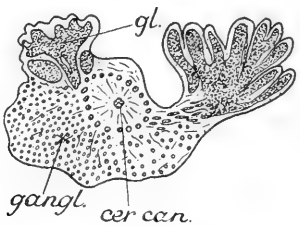
Transverse sections through the brain in the hinder region of the ventral ganglion: *a*, of Siboganemertes weberi; *b*. of Drepanophorus albolineatus of Bürger (6, Pl. xvii, fig. 3), Schema. The two crosses give the boundary of the ganglia and the corresponding places in the two genera; *c*, Drepanophorus latus, just after the origin of the lateral nerve-cord (6, Pl. xxiv, fig. 43).

ventral. In Text-fig. 14, *a*, the ventral lobe obtained its greatest diameter, as the nerve-cells of the nerve-cord are partly included. The two crosses give the limit of the lobes. In

Reptantia the dorsal lobe attains its greatest development in its hinder part, when the ventral lobe has disappeared. A section of Drepanophorus albolineatus (Text-fig. 14, *b*) to be compared with Text-fig. 14, *a*, reveals the difference between the two. The proportions have changed ; instead of the ventral the dorsal ganglion exceeds in Drepanophorus, and if we compare a section of another form, in which the nerve-cord is seen instead of the ventral lobe, the contrast is still more obvious (Text-fig. 14, *c*). I cannot agree with Brinkmann that the disappearance of the cerebral organs caused a reduction of the dorsal brain-lobe in Pelagica. Brinkmann considers these to be descendants of true Drepanophoridae that have lost eyes, cerebral organs, the great development of the brain, the nephridia, the rhynchocoelomic diverticula, the anastomosing blood-vessels. As to the eyes I might agree with him ; it seems quite plausible that species or even genera that live in the aphotic regions of the sea lose their eyes, as most Pelagica, Siboganemertes, and Uniporus hyalinus, though for Uniporus acutocaudatus and U. borealis this reason cannot exist as they live in the dysphotic zone just as well. The presence of atrophied eyes in some pelagic genera, however, makes it probable that they got lost in the other. But certainly the Pelagica never possessed cerebral organs. These have developed in different ways in armed and unarmed Nemerteans. In both sub-classes we know genera without them, and these in both are primitive forms. Callinera, Carinesta, Cephalothrix belong to the most primitive Palaeonemerteans and they have no cerebral organs. In the Enopla this sense organ is absent in the Pelagica and in Malacobdella. The parasitic genus Gononemertes has them and in Carcinonemertes they seem to fail. Why must it have got lost in Malacobdella and Carcinonemertes, when it is present in the third parasitic genus ? As to Carcinonemertes, that belongs to a non-parasitic family of Monostilifera with well-developed cerebral organs, it seems natural to consider the parasitic habits of the genus as the cause of their absence, though nothing is less certain. In Bdellonemertea this

reason is quite absent, and so it is in pelagic forms. When we can, moreover, follow the development, as is the case (1) in the Anopla, from stages like Tubulanus pellucidus, Procarinina, and other Tubulanus species through Hubrechtia to the Heteronemerteans, and on the other hand in Enopla from Siboganemertes (Text-fig. 12, *b*) to several Monostilifera (Text-fig. 12, *a*) and to Drepanophorus (Text-fig. 13), from (2) the irregular organ with partly free and simple constituents through the composite and irregular organ of several Reptantia as Uniporus (**3**, Pl. i, figs. 6 and 7) and Drepanophorus cerinus, willeyanus, indicus **15**, 14), to the well-

Text-fig. 15.



Transverse section through the cerebral organ of Emplectonema gracile after Bürger (**6**, Pl. xxvi, fig. 41).

defined organ of D. spectabilis (Text-fig. 13), or from (3) Siboganemertes (Text-fig. 12, *b*) through stages as Emplectonema (Text-fig. 15) and Prostoma cruciatum to Prostoma cephalophorum (Text-fig. 12, *a*), it seems to be rather probable that this organ has developed in Nemerteans and has not been inherited from now extinct ancestors. The great development of the dorsal brain-lobe is a characteristic feature of the Reptantia, but need not have been a possession of all Polystilifera. In other forms with a well-developed cerebral organ, as in Amphiporus, the difference of the proportions of the brain-lobes is not great, and I am rather inclined to think that the development of the sac caused the different structure of the dorsal brain-lobe of the Reptantia. Paired rhynchocoelomic diverticula are absent in all Nemer-

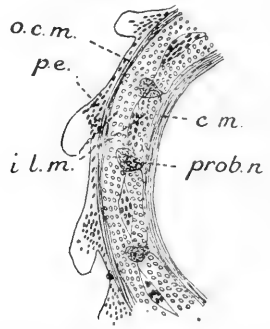
teans with the exception of Reptantia and Siboganemertes and in these they developed in different ways.[1] As in the

[1] Here seems to be the right place to mention the fact that in one species of Monostilifera the presence of paired rhynchocoelomic diverticula is noted by Bürger, i.e. Amphiporus stannii. However, these diverticula are quite other structures, or at least much more primitive; they are present in the nephridial region alone and are very small though wide. The musculature of the rhynchocoelomic cavity widens out at certain places. These are the sacs that have a wide lumen and open with a wide mouth into the rhynchocoelomic cavity. The muscular walls of these sacs are the regular continuations of, and just as thick as, the rhynchocoelomic wall, and a difference of structure seems not to exist. The wall of these diverticula in Polystilifera is as a rule much thinner, even when contracted, and we know that the mouth is provided with a sphincter, that is absent in A. stannii. In some unarmed genera other rhynchocoelomic diverticula, paired and unpaired, exist, but they never are comparable with those of the Polystilifera. Amphiporus stannii is a Monostilifer, as its stylet is well known. In Drepanophorus valdiviae (Bürger), which species has exactly the same rhynchocoelomic diverticula as Amphiporus stannii, the stylet is unknown; in both species these structures are restricted to the nephridial region, and in other characteristics they are very much alike too: they have no eyes, both possess small cerebral organs without a sac, behind or partly behind the brains, both have lateral nerve-cords (not ventral as in Drepanophorus), both have a layer of glands in the head that fails in all Polystilifera and is present in Amphiporus, both have brains that are quite different from all Polystilifera, with a very small dorsal and larger, perfectly separated ventral ganglia, in both the vascular system differs from that of Polystilifera by the presence of a dorsal loop over the brain, &c., &c. Bürger says in his monograph of the Valdivia expedition (**9, p. 174**): 'Leider ist aber der Rüssel nicht vorhanden, und die Organisation weist einige Züge auf, die mehr auf Amphiporus als auf Drepanophorus hindeuten; indessen ist dieses Stück dem Genus Drepanophorus zuzurechnen, weil sein Rhynchocölom, wenigstens im vordersten Abschnitt, laterale, einander gegenüber entspringende seitliche Aussackungen besitzt, die bisher nur von Drepanophorus bekannt sind.' He forgets, however, that he himself described them in 1895 in Amphiporus stannii in the monograph of the Nemerteans of Naples, p. 571, and Pl. xvii, figs. 5, 13, and 14. A comparison of these figures with those of the Valdivia (Pl. xxxi) gives the striking resemblance of the above-discussed species, which certainly belong to one genus, which I might mention Valdivianemertes. The presence of the only stylet in Valdivianemertes stannii (Grube) makes it certain that both V. stannii and V. val-

Pelagica no traces of a reduction in the proboscidian system are to be found, as emphatically stated by Brinkmann, we must consider this tribe as the more primitive one in the Polystilifera.

The structure of the muscular wall of the rhynchocoelomic cavity seems to prove this. In 1914 I tried to demonstrate (**18**) that the proboscis and its sheath together are an invagination of the body-wall, and that all parts of the body-

Text-fig. 16.



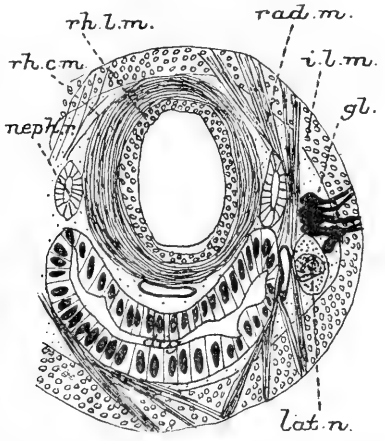
Section through the proboscis of Amphiporus pulcher after Bürger (**6**, Pl. xxiii, fig. 3).

wall are to be found in situ, either in the proboscis or in the wall of the sheath. In the Anopla this seems quite clear, but in the Enopla several difficulties arise. The presence of the third or inner muscular layer of the body-wall, which in Palaeonemerteans is characteristic, the inner circular muscle-coat (Text-fig. 1, *a*), has never been demonstrated, though in Drepanophorus, as I know now, its presence is quite clear in the stomodaeal region. Also the dorsoventral muscles show the same peculiarities as in the Anopla, where they are derivates of this musculature. So it was not certain whether we had a right to look for this layer in the proboscidian system of the

diviae (Bürger) belong to the Monostilifera. Through this conclusion we have excluded Drepanophorus valdiviae from the Polystilifera, in which it might cause much trouble by the different structure of almost every organ.

armed Nemerteans. The other difficulty was that the spot where delamination took place seemed to be different in the Enopla. For in the unarmed Nemertini the inner longitudinal muscle-layer was split into two parts that enclose the rhynchocoelomic cavity, which is lined by an endothelium. However, in the Hoplonemertini a circular muscle-layer lies between the longitudinal fibres and the endothelial lining of the proboscis (Text-fig. 16). I then suggested that these circular

Text-fig. 17.

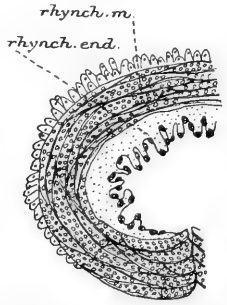


Section through Emplectonema gracile after Bürger (**6**, Pl. xv, fig. 27).

fibres are a new acquisition and do not belong to the primary proboscis, as the proboscis sheath itself is built like that of the Anopla (Text-fig. 17). Chuniella, one of the most primitive Polystilifera, seems to prove this supposition, as the proboscis has no circular muscles beneath the endothelium, and in Monostilifera the genus Zygonemertes (**19**) shows the same feature, as sections from South African species reveal. We knew nothing then about Polystilifera with the exception of the genus Drepanophorus, in which the wall of the rhynchocoelomic cavity consists of longitudinal and circular muscle-fibres, that are interwoven (Text-fig. 18). In many Pelagica

this is the case too, as in Siboganemertes and all Reptantia ; but in Chuniella, Nectonemertes, Natonemertes, Para-, Pro-, and Balaenanemertes no traces of interlacing of these fibres are found, and the longitudinal layer lies next to the endothelium exactly as in the Monostilifera (Text-fig. 17). Brinkmann considers this kind of rhynchocoelomic musculature not as primitive, because the layers show another arrangement at the place of insertion of the proboscis. We know, however, from the Anopla that exactly in this part of the proboscidian

TEXT-FIG. 18.

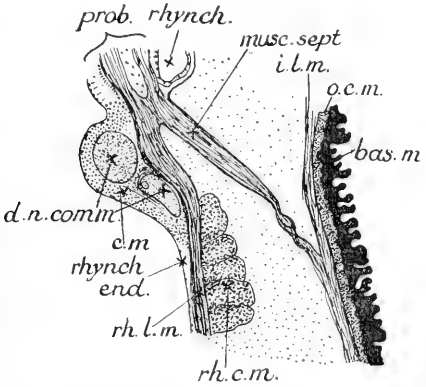


Section through the rhynchocoelomic wall of Drepanophorus after Bürger (**6**, Pl. xxiii, fig. 37).

system the first traces of the new outer longitudinal layer appear, when the middle part holds the older structure ; that here other layers disappear first, that in other species fail absolutely and in more primitive genera are present in all parts ; that at this spot new constrictors and retractors can develop, that in most species are unknown, to be short, that all changes start in this part of the proboscidian system. When we remember, moreover, that this spot is the place where originally the invagination of the whole system took place, that by the development of the precerebral region, as will be discussed later, the continuity with the body musculature was broken, ' Muskelseptum, Rüsselfixatoren ' originated, ' Seitenstammmuskeln ' developed ; that the inner circular muscle-layer is and must be present in the rhynchocoelomic wall, but almost disappeared in the body-wall ; that originally here the central

nervous system was found embedded in the longitudinal musculature, as seems still to be the case in some species (Brinkmann, **4**), and that its place changed in the different genera, then we understand that we cannot look for primary conditions in this part of the proboscidian system. That the longitudinal musculature of proboscis and sheath are in contact with each other at the place of insertion is quite natural (Text-fig. 19), while they both are part of originally one layer and from one place take their origin.

Text-fig. 19.



Dorsal part of a longitudinal section through the anterior region of Balaenanemertes musculocaudatus (Brinkmann) with protruded proboscis (**4**, Pl. 15, fig. 10).

Brinkmann's statement that the brains of Pendonemertes and Balaenanemertes are situated in the middle of the musculature of the proboscidian system is of the greatest importance; for we know from Drepanophorus that the longitudinal musculature is in contact with the same parts of the body-wall by a muscular septum, which separates the precerebral or head-region from the brain and the body and always expands just before the ganglia. In the Pelagica this septum as a rule is broken up into several muscles which Brinkmann calls 'Rüsselfixatoren' and that as a rule lie outside and before the nerve-ring. The brain lies at the same

place as the nerves of all Enopla, on the inner side of the inner longitudinal musculature. So we have to look for the inner circular muscles of these genera behind the brain, and not before it. If we ask where the brain lies exactly in Pendonemertes and Balaenanemertes, Pl. v, fig. 1, Pl. xiv, fig. 19, Pl. xv, figs. 3 and 4, Pl. xvi, figs. 17 and 18, of the monograph (**4**) show us that it really is found between two layers of longitudinal musculature. Whether the outer layer consists of strands that go to the body-wall (' Rüsselfixatoren ') or of the inner parts of them that still have to join the wall of the cavity, is not clear. But in any case it is certain that we can expect the inner circular muscle-layer only behind the brain and not before it. Wherever Brinkmann describes the exact relations of the muscle-layers in these parts, it invariably is mentioned that the first traces of the outer circular musculature of the proboscis sheath are found behind the brain. This cannot always be so, for I know cases in the Reptantia where the remains of the circular musculature of the body-wall are found around the hinder parts of the brain, and in this case it is evident that the contact with the rhynchocoelomic part of this layer must be looked for before the brain. In such cases we must expect the outer circular musculature of the sheath to be in the nerve-ring. In others I noted the same beginning of this layer as Brinkmann, i.e. behind the nerve-ring.

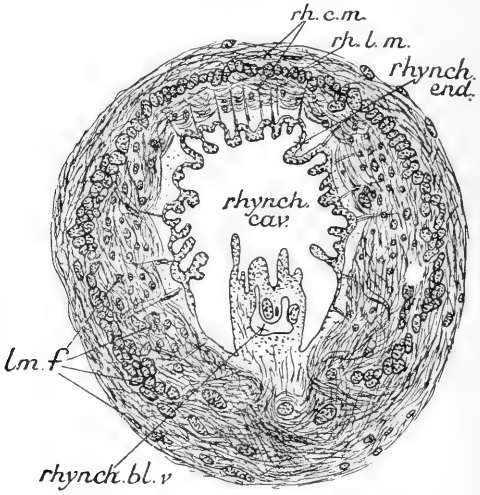
The wall of the cavity before the brain is built differently in different cases. It is interesting to note that in one Malayan species all circular muscle-fibres are absent in front of the brain, in another all longitudinal, and always the interlacing begins behind the nerve-ring. Brinkmann describes in all his genera of Pelagica the presence of an inner circular muscle-layer (as the direct continuation of the new proboscidian layer) before the brain and outside of it a longitudinal layer which, he says, passes through the circular musculature behind the brain and so comes to lie inside (Text-fig. 19). If, however, this really was a passing through we should find an interlacing of fibres at this place. Though Brinkmann is very exact in his statements he never mentions this, and his figures show

everywhere a very distinct border of the musculature (Text-fig. 19). Pro- and Parabalaenanemertes, Balaenanemertes, Nato-, Nectonemertes, and Chuniella show us this type of rhynchocoelom, as found in all Monostilifera too, and so does the greater part of the sheath of Pelagonemertes. The development of a third layer, which might be indicated where the proboscis is affixed, never took place, as I will now demonstrate.

The interlacing of circular and longitudinal muscle-fibres, which in Pelagonemertes took place in the hinder part of the rhynchocoelomic wall, is found in the genera Protopelagonemertes, Plotonemertes, Pendonemertes, Mergonemertes, Paradino-, Phallo-, Crasso-, and Planktonemertes, in all Reptantia and in Siboganemertes weberi. Whether the new circular muscle-coat of Brinkmann is present or not we cannot decide in these genera; in Pelagonemertes it certainly is absent, and in the Malayan species referred to above the interlacing took place between the two original layers, as I will describe in Siboganemertes. The proboscis has a thin outer circular muscle-coat and a thick longitudinal coat, but the new layer is absent. The precerebral septum which connects the longitudinal musculature of the body with the rhynchocoelomic wall and proboscis lies exactly in front of the brain. Inner circular muscle-fibres between this septum and the endothelial lining of the cavity of the sheath fail absolutely; a great quantity of fine mesenchymatic fibres, which stain quite differently and are found at many places in the body parenchyma too, lies inside the endothelium and between these the first longitudinal fibres are embedded. Outside of these the first circular fibres appear behind the nerve-ring and they are very few. The whole muscular wall is thin and, in the ventral part, disappears but for a few longitudinal fibres. It is, however, quite clear that in the proximal part an interlacing of fibres takes place, and here certainly the new circular layer has not developed. It is absent in the proboscis too. From the results obtained in Siboganemertes, and from similar facts in some Drepanophoridae and in Pelagonemertes, I might conclude that the development of

the wall of the sheath in Pelagica took place in the same way. Primary are the stages with two muscle-layers, as Chuniella, &c. The first stages of interlacing are given in Armaueria and Dinonemertes (Text-fig. 20). In these genera started the penetration of the circular muscle-coat by the longitudinal fibres, or vice versa ; but the longitudinal musculature has not yet reached the outside. We see an interlaced inner wall, a longitudinal middle coat, and outside of it a circular layer.

Text-fig. 20.



Transverse section through the rhynchocoelomic cavity of Dinonemertes alberti (Brinkmann) (**4**, Pl. vii, fig. 13).

As Dinonemertes seems to be connected with Mergo-, Phallo-, and Paradinonemertes and Armaueria with Pendonemertes, I might rather solve the problem of these genera as the beginning of the interlacing, which is completed in Protopelago-, Ploto-, Pendo-, Crasso-, Plankto-, Mergo-, Phallo-, and Paradinonemertes.

Pelagonemertes kept the more primitive stage in the proximal part of the sheath, which fact seems to point to the hinder part of the cavity as the place of origin of the interlacing. Bürgeriella gives still a higher development that confirms these

views, as it is the most specialized genus of the Pelagica in many respects. The distal part acquired an inner circular layer, as the originally outer circular coat after the stage of interlacing, shown still in the proximal part, lost its longitudinal fibres which all lie outside of it. This is in accordance with the other anatomical facts, that make us look for the nearest relations of this aberrant genus among species with a basket-like structure of the sheath; proximally it is basket-like in Bürgeriella too; the distal part has an inner circular and an outer longitudinal layer. Compared with Text-fig. 20 of Dinonemertes the position of the fibres is this, that the whole circular muscle-coat traversed the longitudinal one (in Dinonemertes only the inner parts) and so came to lie inside. Other traces of an inner circular muscle-coat fail, and as Bürgeriella evidently is a highly specialized genus it would be rather incomprehensible why it should be the only one that had beheld this primitive feature on Brinkmann's explanation of facts.

The other support of Brinkmann's theory of a third muscle-layer of the wall of the sheath is Protopelagonemertes, in which genus the interlacing of fibres is already found in the nerve-ring. However, if we suppose, as I do, that the interlacing starts in the hinder part of the cavity and goes on from behind forwards, as shown in Bürgeriella and Pelagonemertes, every reason fails why the interlacing should stop with the brain as the nerve-ring lies in the muscular septum. Protopelagonemertes bears its name quite undeserved, as Pelagonemertes seems not to be related to it and is also to a certain extent more primitive.

The result of this discussion is that the genus Chuniella, which after Brinkmann's description must be one of the most primitive genera if not the most primitive genus of the Polystilifera, perhaps even of the Hoplonemertini, has a proboscis with exactly the same muscular layers as all primitive Anopla, Malacobdella, and some Monostilifera, and as Siboganemertes weberi, the most primitive genus of the Reptantia; that also the wall of the sheath in this genus is

built like that of all Anopla and of all Monostilifera, and that this wall in the Polystilifera is found in the pelagic genera Nato- and Nectonemertes and the family Balaenanemertidae as well. That the interlacing of these two original muscle-coats, which is characteristic of all Polystilifera Reptantia and elsewhere is unknown, is also found in many Pelagica ; that the process of interlacing seems to start in the hinder part of the rhynchocoelomic cavity and proceeds proximally, as shown in Pelagonemertes. That we see the penetration of the two layers go on in Dinonemertes and Armaueria, and that the interlacing is completed in all other genera of Pelagica and in the Reptantia. That in one genus this process resulted in the inversion of the original layers, i.e. the aberrant genus Bürgeriella, where the proximal part of the sheath has the basket-like structure characteristic of Polystilifera, and the distal part, as in Pelagonemertes, shows the result of this process.

If we look at the digestive tract three remarkable points are to be distinguished. The position of the mouth under the brain was stated to be very primitive in armed Nemerteans and even in Polystilifera to be quite unusual. As to the oesophagus we have the statement of Brinkmann that this part of the stomodaeum is absent in all Pelagica with the only exception perhaps of Planktonemertes. His fig. 23, Pl. xiii (**4**), gives no right to compare this small oesophagus with that of Siboganemertes (Text-fig. 11) ; after his description on p. 24, however, we can hardly speak of an oesophagus, and truly can say in Pelagica the oesophagus is absent, as in the unarmed genera. But in the Reptantia a well-developed oesophagus is always, in the Monostilifera, as a rule present. We know its absence in Amphiporus marmoratus (Bürger) (**6,** Pl. xvi, fig. 1), though in Amphiporus marmoratus (Joubin) it is well developed as in most other species (**12,** p. 564, fig. 4). This figure interests us still more because the different parts of the stomodaeum with the exception of the oesophagus show about the same features as Siboganemertes. The pyloric tube of Joubin's species is much wider than in our specimen, but it opens into the gastric cavity at the same

place. What is the continuation of the oesophagus in A. marmoratus (Joubin) is the ventral unpaired diverticulum of Siboganemertes, and the true gastric cavity lies enclosed between the pyloric tube and the ventral diverticulum. All parts of the stomodaeum are unpaired in Siboganemertes but the intestinal blind-gut shows at the side of ventral unpaired pouches paired lateral diverticula that are longer than the blind-gut itself, as in A. marmoratus (Joubin). Nothing of this kind was ever found in Polystilifera, though some very interesting features are known from Brinkmann's studies on the bathypelagic species. Not only do they show the absence of an oesophagus, but the whole stomodaeum is very short and much less differentiated. In almost all his figures the pylorus is already shown beneath the brain, and as a rule the blind-gut extends till here. In fig. 9, Pl. xv, a longitudinal section shows the short and narrow gastric cavity of Balaenanemertes musculocaudatus; fig. 13, Pl. xii (Text-fig. 26), gives the same features in Nectonemertes primitiva, and Brinkmann states that in N. minima the epithelium of the gastric cavity is unfolded, the cavity still narrower and shorter. Brinkmann takes these forms as the most reduced ones. However, how can we explain these differences in the same structure within a monophyletic group, as the Polystilifera certainly are, the highly differentiated gastric cavity of Siboganemertes, the quite differently but not less highly developed structures of the Drepanophoridae and the more or less simple stomodaeum of the Pelagica, if we do not suppose these to be primitive ?

The stomodaeum in armed Nemerteans is a structure different from that in the Anopla, as is shown by its development and by the presence of an entodermal blind-gut in the first. We know it to be a simple structure, a mere narrow tube in Ototyphlonemertes, in Zygonemertes it is not much more ; we know that the oesophagus is absent in Geonemertes, in Stichostemma. Why then must the Pelagica, that have the same peculiarities, have developed from highly differentiated forms as Drepanophorus ? On the contrary we see here how the simple

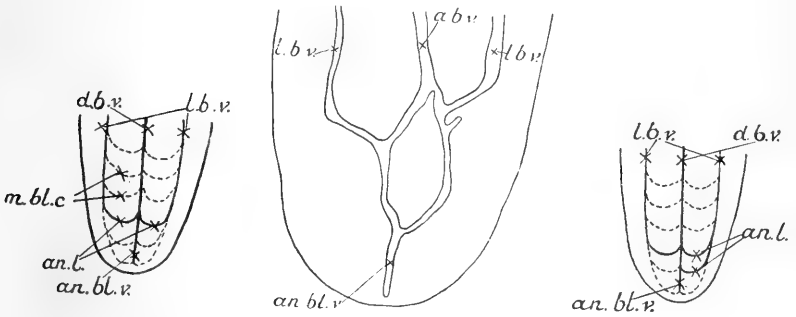
invaginating stomodaeum with a few glandular cells only develops into a gastric tube that opens only a short distance behind the beginning of the enteron, as in Balaenanemertes musculocaudatus or Nectonemertes. With the greater development of the stomodaeum the different parts become better differentiated, in the first place gastric cavity and pylorus, and this can be obtained in different ways, as shown by Siboganemertes (Text-fig. 11) and Drepanophorus (Text-fig. 8), or by A. marmoratus (Joubin) and A. marmoratus (Bürger); the Pelagica provide us with a whole series of stages in this development. Thirdly an oesophagus is differentiated, which all Pelagica lack and often even other groups. So Siboganemertes with its diverging structure of the digestive system is not as primitive as the pelagic forms; but it cannot be included in the Drepanophoridae either, as the development goes in a different direction.

The vascular system shows a very primitive type, as in Siboganemertes there are no anastomozing vessels with the exception of the cephalic loops. The cephalic vessels bend into the nervous ring of the brain in the ordinary way and a dorsal vessel is present, as far as sections were made; but it is not in contact with the rhynchocoelomic cavity. The absence of metamerically situated vascular loops is known in Uniporus, in Reptantia, and in all Pelagica. The occasional presence of a double anal loop in an abnormal individual of Pendonemertes levinseni (Text-fig. 21, *b*) and the existence of a blind dorsal median vessel in the tail arising from the anal loop, makes it at first sight rather plausible that the reduction series as given by Brinkmann on p. 163 of his monograph (**4**) gives a true account of the facts. But when we know that in primitive Anopla the anastomozing vessels are absent, that they fail in Uniporus (in which genus even the anal loop should be absent) (**3**), and that they fail in Siboganemertes, we become sceptical to the explanation of their absence in Pelagica. Moreover the reconstruction of the tail of Pendonemertes on p. 20 (Text-fig. 21, *b*) and the scheme on p. 163 (Text-fig. 21, *c*) are rather different, and it seems not at all certain that the hinder vessel

is the continuation of the lateral vessel; the course of the blood-vessels seems too irregular to decide anything from this single abnormality.

Another interesting fact in Siboganemertes is the absence of any connexion between the dorsal vessel and the rhynchocoelomic cavity. This is known from one form of Polystilifera, Armaueria fusca. The Monostilifera exhibit the same feature in some Prostoma species (P. amphiporoides, duboisi, antarcticum, gulliveri (Bürger)), and it is found in Malacobdella. Wherever a dorsal blood-vessel exists

Text-fig. 21.



Blood-vascular system in the tail of Pendonemertes levinseni. *a*, Schema of normal individual after Brinkmann (**4**, p. 163, Text-fig. 29, II); *b*, abnormal individual (**4**, p. 20, Text-fig. 4); *c*, schema of this abnormality (**4**, p. 163, Text-fig. 29, III).

in the unarmed forms, it is in connexion with the rhynchocoelomic cavity (Text-fig. 20), though other special rhynchocoelomic vessels may be present. In most Palaeonemerteans, however, the dorsal vessel is quite absent. This is a rare case in Hoplonemertini, and, as far as I know, it has been described in Pelagonemertes moseleyi, Balaenanemertes chuni, and Carcinonemertes carcinophila. Brinkmann, guided by the opinion that the Pelagica are reduced Drepanophoridae, considers these forms

as the most advanced ones; I, however, believe that the formation of a dorsal blood-vessel takes place in the Pelagica; that it has been formed in two ways, either in relation to the rhynchocoelomic cavity, or quite separately. This last way is represented in three aberrant genera, Armaueria in the Pelagica, Malacobdella, the Bdellonemertean, and Siboganemertes, the representative of a new group of Reptantia. Perhaps other genera passed this stage to acquire a rhyncho coelomic vessel afterwards, as might be plausible in Prostoma. Some genera, however, seem to have got this rhynchocoelomic vessel directly, as is shown in Pelagonemertes rollestoni and the nearly related genus Natonemertes with a short, blind-ending proboscidian blood-vessel, or in the family of the Balaenanemertidae, where a dorsal vessel is absent in B. chuni, and in other species of the same genus a blind rhynchocoelomic vessel is present as well as in Probalaenanemertes and Parabalaenanemertes. Another fact of interest in the blood-vascular system, and which seems to demonstrate how the organisms of this group try to obtain a certain result in different ways, is the development of what Brinkmann calls 'Ovarialschlingen'. He demonstrates in Dinonemertes investigatoris that the lateral blood-vessels in the gonadial region make large, irregular loops between the ovaries and the entodermal sacs. These loops that convey the nutrition from the sacs to the ovaries are absent in all other forms; but I found them also in Siboganemertes. It is supposed that the vascular loops between the dorsal and lateral vessels of other Nemerteans have the same purpose, and Brinkmann remarks that this fact states Dollo's law of irreversibility, as the regular loops that once disappeared did not return, but another structure took on the same function. What we find in Dinonemertes and Siboganemertes can perhaps just as well be the beginning of what results in vascular loops between the vessels. So everywhere I reach the same result; the Pelagica show the development of every organ, from the primitive stages known in Palaeonemerteans to the specialized features of Drepanophoridae and Monostilifera; the

development supposed by Brinkmann seems to have taken place in the reverse direction; what he calls highly reduced, I call primitive, and vice versa.

This disagreement does not extend to the nephridial system; for this is present in all Nemerteans without exception that do not belong to the Pelagica. That it has not yet been found in Prosadenoporus must partly be due to the highly developed head-glands that extend into the nephridial region, partly to the smallness of the canals or the preservation. As all Platyhelminths possess a well-developed nephridial system, we are obliged to explain its absence in the Pelagica by reduction. In Siboganemertes nephridia are present, but of a type that differs from that of the Reptantia. A large efferent duct is present at each side, extends behind the real nephridium, and has a more caudal, lateral mouth. The nephridia lie at the side of the dorsal brain-lobes and the cerebral organs, and just behind these obtain their greatest development. The ducts open laterally behind the end of the pyloric tube. This type is known from primitive Anopla, a well-localized system of canals with a long efferent duct, quite different from the other types of nephridia that extend through the whole body in the same way and have one or more short ducts. In the Reptantia also it is much less circumscribed, extends as a rule from the end of the brain along the stomodaeal tract, and has one efferent duct that can take its origin in any part of the system and opens directly to the exterior. Our knowledge of Monostilifera is as yet too incomplete to understand the value of these facts.

The gonads, however, seem to be much more interesting. The only individual of Siboganemertes happened to be a male with well-developed testes, a fact of the greatest importance, as the Pelagica exhibit an extraordinary feature in the position of these glands that is characteristic of the group.

As a rule the gonads lie, be they ♀ or ♂, in the intestinal region in armed and unarmed Nemerteans. The only exception are the testes of the Pelagica that never are developed in this region, and always lie before it, directly behind, at the side of,

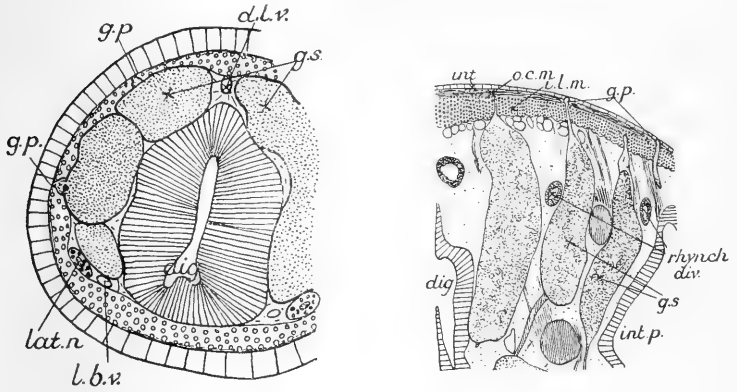
or even before the brain. The ovaries are developed in the usual way. These facts can easily be understood as we know the testes of Platyhelminths to be developed all over the body. The arrangement of the gonads in primitive Anopla without intestinal pouches is absolutely irregular, as shown by Bürger in Tubulanus (**8,** Pl. iv, fig. 2). Two interesting facts are to be mentioned: the gonads are placed in several irregular rows, and the gonopores lie on the dorsal surface. In the Anopla we can follow the development from this stage without intestinal diverticula to the pseudo-metameric arrangement in Lineus and Cerebratulus, where always one gonadial sac lies between two intestinal pouches, opening to the exterior by one row of dorsal gonopores.

In Enopla the Bdellomorpha display the same irregular position, as for instance Tubulanus polymorphus, and have dorsal gonopores. The Hoplonemertini show a great variety of arrangement. First we have to look at the Monostilifera, of which Geonemertes, Nemertopsis (Text-fig. 22, *a*), Prosadenoporus, Prosorochmus have a number of gonads between two following intestinal pouches, the first stage of arrangement that follows on the above-described displacement of Tubulanus polymorphus in the Anopla. All these worms are more rounded than the flat Malacobdella and the Tubulanidae. In consequence the gonopores partly lie more laterally, but always above the nerve-cords. The next stage is the reduction of the number of gonads per pseudomere to one on each side, as in Prostoma coronatum (**8,** Pl. ii, fig. 3) and Amphiporus species. At last we get a still greater reduction of this number as in A. pulcher (**8,** Pl. xiii, fig. 6).

In the Polystilifera we know two genera of Reptantia, Uniporus and Drepanophorus. Uniporus (Text-fig. 22, *b*) has in each pseudomere two to five gonadial sacs with dorsal pores and exhibits in consequence a very primitive feature. In Drepanophorus we know the great regularity in which intestinal pouches and gonads alternate, one sac between two pouches. But the gonopores lie laterally (Text-fig. 23, *a*) as in D. willeyanus, cerinus, indicus, or ventrally

(Text-fig. 24, D. albolineatus). Moreover, these sacs have, as Punnett (**14, 15**) showed, a peculiar form. In more rounded species, as the first, they are V-shaped (Text-fig. 23, *a*) with the two legs above and beneath the intestine and the lateral gonoduct at the place of junction of the legs. In Siboganemertes this is found too, but the sperm is not developed in this large sac alone. Peripherally small sacs are found

TEXT-FIG. 22.



a. *b.*

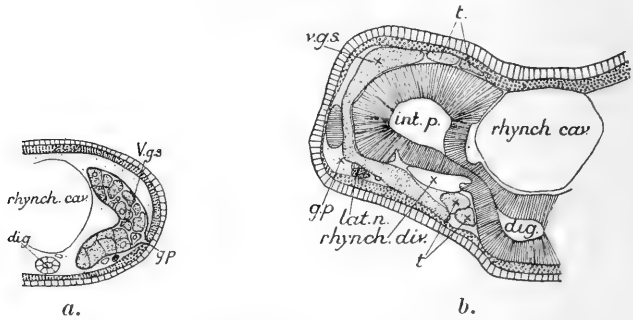
Sections through gonadial region: *a*, of Nemertopsis peronea after Bürger (**6,** Pl. xv, fig. 5); *b*, of Uniporus hyalinus after Brinkmann (**3,** Pl. 1, fig. 5).

(Text-fig. 9) which open into the central V-shaped sac (Text-fig. 23, *b*). Bürger found the same development of ovaries in Drepanophorus albolineatus (Text-fig. 24), one egg in each sac; but in other species the central sac itself is filled with eggs or sperm (Text-fig. 23, *a*). It seems to me that the small sacs are comparable with the gonadial sacs of Uniporus, Geonemertes, Tubulanus, Malacobdella, and that the central V-shaped pouch is a new growth in the Reptantia and Siboganemertes. The epithelium of the central pouch, whether it is simply a new gonoduct or the invaginated ectoderm with the original gonopores, acquires later the function of the gonadial epithelium and the original gonads disappear. In

each case we see the tendency of the gonads, whether testes or ovaries, to arrange themselves metamerically and become reduced in number.

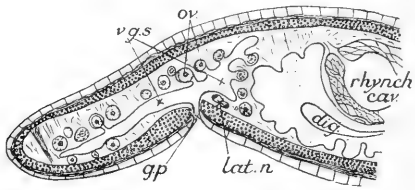
In the Pelagica the differences of sexes are greater. The

TEXT-FIG. 23.



Sections through the gonadial region: *a*, of Drepanophorus willeyanus (Punnett) (**14,** Pl. lix, fig. 20); *b*, of Sibogaenemertes weberi.

TEXT-FIG. 24.



Section through Drepanophorus albolineatus with an ovary after Bürger (**6,** Pl. xxvii, fig. 52).

ovaries are metamerically developed, never more than one between two intestinal pouches, but the gonopores lie on the ventral side. This may be the case in Drepanophorus too (Text-fig. 24). In broader and flattened forms the body becomes thin with flat edges, outgrowths of the body-wall and parenchyma, that in certain species contain no organs at all, in others rhynchocoelomic diverticula only. In some of these species it is quite obvious that the outgrowth took place above the

lateral gonopores, that in this way became ventral (Drepanophoridae-Siboga expedition) ; the V-shaped gonadial sac still is quite clear. In D. albolineatus (Text-fig. 24) the gonad acquired another, third part, that lies in this ' Seitenrippe ', and the ventral primary leg was somewhat reduced ; but all three parts are present. In the Pelagica the development of the ventral ovaries cannot have taken place in this way, as all traces of the V-shaped sac or of many ovaries are absent, and real dorsal gonopores are unknown in both sexes. The only cases, as far as our present knowledge goes, in which the gonopores are not ventral but lateral seem to be the testes of Armaueria and Parabalaenanemertes, and even in these the testes open partly ventrolaterally. As to the ovaries they are rather uniformly built, and there is a reduction of the number of eggs, which grow very large and contain much yolk. The ovaries are so small that it seems unnatural to derive them from the large sacs of true Reptantia ; it is more justifiable to compare these gonads with the smaller sacs of Uniporus and other Hoplonemertini that are reduced to one pair per pseudomere, or even to less as in Pelagonemertes and other genera. That the ovaries secondarily migrate into a more central position is shown by Brinkmann in one case ; the young ovaries lie outside the peripheral lateral nerves and when they become older grow inside and become a tube. This tube may bend over the nerves to the inside of them ; but always the origin of the sacs seems to be at the outside of the nerves. The medioventral gonopores of Balaenanemertes, Probalaenanemertes, Pelagonemertes, and Armaueria may take their origin from the inner leg of such forms, though it may just as well be possible that in Pelagica the more central position of the nerve-cords, as compared with those of other Hoplonemertini for the first time make the displacement of the gonopores to the middle line possible.

The development of the testes proceeds in two distinct ways. Instead of being present in the middle and hinder parts of the body like the ovaries, they are found only in the anterior part from the brain to the enteron, and in some cases even

before the brain. Brinkmann showed in his monograph that this characteristic seems to be a most important fact in the propagation of the species, coinciding with the development of copulatory organs. In this part of the body the pseudomeric arrangement is not so well developed or is even absent as it is in the stomodaeal part, and only the entodermic blind-gut can show metamerically arranged diverticula. In the genera in which testes are known these are arranged in two ways : (1) with a tendency to metamerical arrangement (only behind the brain) in Plotonemertes, Paradinonemertes, Phallonemertes, Chuniella and Dinonemertes, Bürgeriella. Chuniella, with its long irregular rows of testes, seems to be the most primitive ; the influence of the diverticula of the blind-gut is seen here as well as in Bürgeriella, where they lie more irregularly but are less in number. Plotonemertes represents the next stage, and the regularity seems to be perfect in Phallonemertes, Paradinonemertes, and perhaps in Dinonemertes alberti. (2) In the other group the irregularly placed testes show a tendency to discharge the sperm as near to the head as possible and to form clusters. The effect of this arrangement is shown by Brinkmann. In Nectonemertes with its tentacles the testes lie in two irregular rows behind and at the side of the brain, but long gonoducts have developed (Text-fig. 25) that all point to the head ; they are still more forward and irregular in Armaueria ; in Natonemertes a pair of irregular clusters lies just beneath the brain, and in Para- and Balaenanemertes the clusters lie at the side of and before the brain and have their gonopores all directed to the proximal edge of the body. Pelagonemertes shows the same features as Balaenanemertes. So the gonads of the Pelagica developed quite differently from those of all other Nemerteans.

The result of the examination of the anatomical features of the Pelagica, the Reptantia, and Siboganemertes in these pages is :

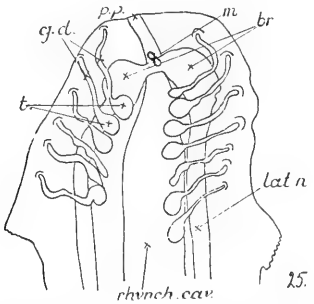
1. That the division of the Enopla into Polystilifera and Monostilifera is well founded, as not only the differences in the armature of the proboscis exist, but also the way in which the

connexion between proboscis pore and mouth can be formed differs in these sub-orders.

2. That the Polystilifera exhibit the more primitive features, as in most genera the proboscidian and digestive systems are quite separate from each other, and the mouth even can be found underneath the brain as in Siboga- and Paradinonemertes.

3. That in the Polystilifera the Pelagica are the more primitive, though a specialized tribe.

Text-fig. 25.



Position of testes and ejaculatory ducts on the ventral side of Nectonemertes minima (Brinkmann) (**4**, p. 104, Text-fig. 23).

4. That the absence of cerebral organs, of a highly differentiated brain, of a long much-developed stomodaeum with oesophagus, of rhynchocoelomic diverticula, of metamerically arranged vascular loops, are primitive features, and that reduction did not cause them.

5. That the development of the musculature of the proboscis and its sheath in all Hoplonemerteans is in perfect accordance with our knowledge of the anatomy of the Anopla and of their embryology, and that every stage of this development from the Anopla stage to the interlacing of Drepanophorus is found in the different genera of Pelagica.

6. That the blood-vascular system in the different genera of Pelagica shows the development of the dorsal blood-vessel from a short blind rhynchocoelomic vessel, that in some species

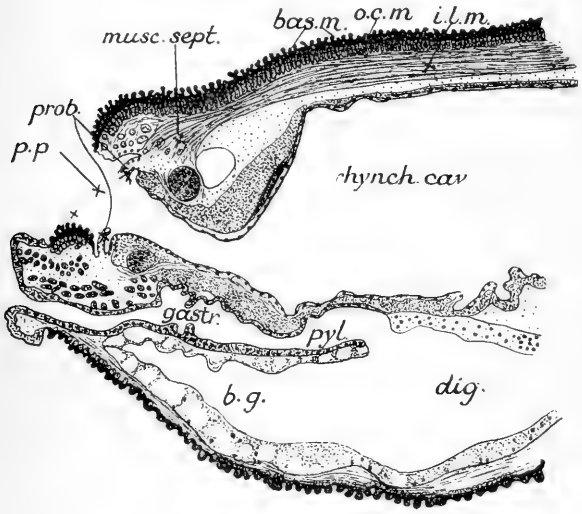
still is absent, to a large vessel, that communicates in the tail with the lateral vessels.

7. That the reproductive system shows quite a number of characteristic features that are unknown in all other Nemerteans and cannot have developed from stages known in Monostilifera and Anopla, nor from those in the Reptantia ; that the influence of the pelagic habits, which caused reduction of the number of eggs as these grew larger, and copulation, cannot account for all these facts, though the ventral position of the gonopores may be due to it.

Here I might call attention to another fact of importance. If we look at the figures of the pelagic Nemerteans it must be evident to everybody, especially on comparison with illustrations that give the anatomy of the whole animals, that the region we call the head in other Nemerteans, or the precerebral region, is absolutely absent. As already stated above, the insertion of the proboscis and the muscular septum before the brain mark the place where originally the invagination of the proboscidian system took place. This we see actually in the pelagic forms ; the rhynchodaeum is only very short and there is no true head region (Text-fig. 26). Brinkmann states several times that the rhynchodaeum may be extremely short, and only in this way can we understand a dorsal migration of the proboscis pore that comes to lie above the brain as in Armaueria or Parabalaenanemertes. In the primitive genus Siboganemertes the precerebral region is extremely short too, and the broad line which demarcates the proximal end of the animal reminds one of the same feature in the headless Pelagica. The brain lies directly behind the septum, i. e. quite terminally, as seen in all the illustrations of Brinkmann. A comparison of Text-figs. 25 and 26 with Text-figs. 2, 3, 6, and 8, shows this very clearly. In the armed Nemerteans a displacement of the mouth goes hand in hand with the development of the head, and in consequence of this the development of the stomodaeum and oesophagus. If we understand the head region of Nemerteans in this way the difference in the structure of the body-wall before and behind the brain at once becomes

clear, and the presence of muscle-strands in the snout can be understood also. The newly developed region could only get some small outer layer of the musculature as the bulk of the muscular coat is used by the formation of the proboscidian system. So we find the greater part of the longitudinal musculature as the septum before the brain or as proboscidian muscles,

TEXT-FIG. 26.



Longitudinal section through the anterior region of Nectonemertes primitiva (Brinkmann) (4, Pl. 12, fig. 13) to show the total absence of a head and rhynchodaeum. The proboscis is lost and the stomodaeum protrudes through the mouth. The tip of the snout is indicated by a cross.

and only a very thin layer of longitudinal fibres is seen to accompany the epithelium and the few circular fibres underneath. That in this process of division of the musculature some longitudinal fibres are found in the parenchyma of the snout that connect the musculature of body-wall, rhynchodaeum, and septum seems to be quite natural. The aberrant structure of the head of all Nemerteans as far as concerns the musculature can only be understood in this way.

Even in this feature the Pelagica are very primitive in the absence of a true snout.

With regard to Siboganemertes we have to state the following facts :

The presence of cerebral organs, rhynchocoelomic diverticula, an oesophagus, nephridia, metamerically arranged male gonads brings it in near relation to the Reptantia.

However :

1. The position of the mouth under the brain is more primitive than in any of these genera.
2. The absence of a snout, present in other Reptantia, recalls this characteristic of the Pelagica.
3. The proboscis has but two muscular layers, and not three as in Reptantia and Monostilifera.
4. The rhynchocoelomic diverticula lie on the inside of the entodermal diverticula and never peripherally as in the other Reptantia.
5. The brain is most primitive, as in Pelagica, without large, free, dorsal lobes.
6. The cerebral organs are the most primitive we know in Hoplonemertini, consisting of free independent parts, without a bifurcation of the canal characteristic of the Drepanophoridae.
7. The digestive tract has a short bulb-like oesophagus.
8. The stomodaeal parts are much more highly developed than in the other Reptantia, displaying the same features as in certain Amphiporus species, and all parts are distinctly and sharply separated from each other, as is never the case in other known Polystilifera.
9. The entodermal blind-gut has unpaired diverticula.
10. The nephridia are different from those of the Reptantia.
11. The lack of metameric blood-vessels is a primitive feature in common with all Pelagica and Uniporus.
12. The dorsal blood-vessel never lies in the rhynchocoelomic cavity, a rare feature known in Armaueria in the Pelagica, in Malacobdella and in some Prostomas in the Monostilifera, but unknown in Reptantia.

13. The testes consist of many small peripheral sacs that open into a large V-shaped sac as known in the Drepanophoridae only, and representing probably a more primitive stage than that of most Drepanophorus species.

Every organ of Siboganemertes is either more primitive than in the other Reptantia or quite differently developed (rhynchocoelomic diverticula, digestive system, nephridia, dorsal blood-vessel). We must include it in the well-defined group of Reptantia as given by Brinkmann. On the other hand we cannot include this genus in his family Drepanophoridae, nor in the Uniporidae or any other family of the Siboga material. The real relationship between the known Drepanophoridae and Siboganemertes we can only indicate by dividing the tribus Reptantia (Brinkmann) into two subtribus, the Archireptantia and the Eureptantia, of which the first contains the family Siboganemertidae and the other the different groups of Drepanophoridae as yet known.

The diagnoses of the different systematic divisions of Enopla are as follows :

Sub-classis Enopla (Max Schultze).

The body-wall consists of a one-layered epithelium, a basement membrane, a circular muscle-layer, and an inner longitudinal muscle-coat. The nervous system is embedded in the body parenchyma. Cerebral organs, where present, separated from the brain. Proboscidian and digestive system show a tendency to acquire a common mouth. Blood-vascular system without lacunae.

Ordo I. Bdellomorpha (Verrill).

Parasitic Nemerteans with a sucker. The proboscis is inserted in the wall of the digestive system ; without armature. Digestive tract a more or less winding tube without diverticula and blind-gut. Blood-vessels highly branched.

Ordo II. Hoplonemertini (Hubrecht).

Proboscis armed. Digestive system with blind-gut and paired diverticula ; straight. Vascular system without tree-

like branching ; as a rule with metamerically arranged loops between the three longitudinal vessels.

Sub-ordo I. Polystilifera (Brinkmann).

Hoplonemertini with many stylets on a crescent-shaped base. Proboscis pore and mouth are separate or open separately in a common atrium. The muscle-coats of the rhynchocoelomic cavity interlace and become complicated as a rule.

Sub-ordo II. Monostilifera (Brinkmann).

Hoplonemertini with one stylet on a handle-shaped base. The mouth opens into the rhynchodaeum. The rhynchocoelomic wall never shows interlacing, and consists of an inner longitudinal and an outer circular muscle-coat.

The sub-ordo Polystilifera contains the following groups :

Tribus I. Pelagica (Brinkmann).

Pelagic Polystilifera without a distinct snout. Cerebral organs, nephridia, rhynchocoelomic diverticula, metameric blood-vessels, and oesophagus absent. Testes only in stomodaeal region. Gonopores ventral.

Tribus II. Reptantia (Brinkmann).

Polystilifera with cerebral organs, rhynchocoelomic diverticula, nephridia, and oesophagus, and with metamerically situated gonads in the intestinal region.

Sub-tribus I. Archireptantia.

Without a snout. Central rhynchocoelomic diverticula. Small dorsal ganglia and a primitive cerebral organ. Different parts of stomodaeum sharply separated and well developed. Nephridia with a large, distal efferent duct. Without metamerical vascular loops.

Sub-tribus II. Eureptantia.

With a snout. Peripheral rhynchocoelomic diverticula. Cerebral organs with a sac. Large, free dorsal ganglia. Different parts of stomodaeum continued into each other. Nephridia with, as a rule, proximal efferent ducts. With metamerical vascular loops.

LEYDEN,
March 1, 1923.

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THE ASSOCIATION WAS FOUNDED 'TO ESTABLISH AND MAINTAIN LABORATORIES ON THE COAST OF THE UNITED KINGDOM, WHERE ACCURATE RESEARCHES MAY BE CARRIED ON, LEADING TO THE IMPROVEMENT OF ZOOLOGICAL AND BOTANICAL SCIENCE, AND TO AN INCREASE OF OUR KNOWLEDGE AS REGARDS THE FOOD, LIFE CONDITIONS, AND HABITS OF BRITISH FOOD-FISHES AND MOLLUSCS'.

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