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On the Anatomy and Systematic Position of Incisura (Scissurella) lytteltonensis.

By

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With Plates 1—5.

When Mr. Geoffrey W. Smith was in Tasmania in 1907–08 I asked him to collect for me any rare or remarkable specimens of gastropod molluscs and preserve them in a form suitable for anatomical and histological examination. Among other forms Mr. Smith obtained for me, through the kind offices of Mr. C. Hedley, of the Australian Museum, Sidney, a number of specimens of the little gastropod which is the subject of the present memoir. They were preserved in Perenyi's fluid, which of course dissolved the shells, but except for the difficulty of staining always resulting from a prolonged immersion in this reagent, the histological condition of the specimens leaves little to be desired.

Scissurella lytteltonensis was described in 1893 by E. A. Smith (**16**), who noted certain differences between the shell of this and other species of the genus Scissurella, but evidently did not consider them of generic importance. In 1904 C. Hedley (**8**) recalled attention to these differences, and founded the new genus Incisura for the reception of the species which, he maintained, is marked off from all other Scissurellidæ as also from all Pleurotomariidæ by the brevity of the slit in the shell, by the absence of raised rims or keels on either side of the slit, by the subterminal apex,

by the absence of spiral sculpture, and by the remarkable solidity of the shell. He further asserted that his new genus cannot, because of the above-mentioned differences, be included among the Scissurellidæ, and suggested that it is a member of the Fissurellidæ in which development has been arrested, so that the larval characters of the shell have persisted in adult life. Hedley was evidently unacquainted with Pelseneer's (12) memoir, containing an account of the anatomy of this very species and of Scissurella costata, which, brief as it is, leaves no doubt that the New Zealand and the Mediterranean species are members of the same family, but at the same time discloses so many anatomical as well as conchological differences that they may well be placed in different genera. After some consideration I am of the opinion that Hedley's genus should stand, because the New Zealand species, in addition to the conchological characters enumerated above, differs from the Mediterranean species in the following particulars: (1) In the shape of the radular teeth. (2) In the shape of the foot, which is long and narrow in S. costata and S. crispata, but short and broad in Incisura lytteltonensis. (3) In the absence of cirrhi below the epipodial tentacles in Incisura. (4) The greater development of the right columellar muscle, and the more symmetrical disposition of the mantle in Incisura. In its general anatomical features Incisura bears much the same relation to Scissurella as Septaria bears to Paranerita among the Neritidæ. The systematic position of the Scissurellidæ will more conveniently be discussed at the end of this paper.

Scissurella is placed by most authors among the Pleurotomariidæ, though a few recognise the Scissurellidæ as a separate but closely allied family. A full description of its anatomy is therefore much to be desired, but the accounts that have hitherto been published are insufficient. Vayssière (18) has given a short and, as far as it goes, a good account of the external features of S. costata var. lævigata, and has figured and described the radula and jaws of this species.

Pelseneer (12), in his well-known memoir on the morphology of primitive mollusca, gives seven figures of sections of S. costata and two of Incisura lytteltonensis in addition to three figures of the external features of the latter species. The description he gives in the text is concise, and furnishes a good general idea of the anatomy of the family; but he does not give sufficient detail to enable one to make a critical examination of its systematic position. Hence, having sufficient material at my disposal, I have thought it worth while to make a thorough study of the anatomy of Incisura lytteltonensis.

Incisura, as Mr. Hedley states in a letter accompanying the specimens, is found on the seaweed Cystophora in rock-pools in Lyttleton Harbour, where it is associated with Rissoina, Cantharides, and Gibbula. It may be inferred from its shape and structure that it is semi-sessile in habit, but it is not attached to one spot like a limpet. On the contrary, it is fairly active, and one of the specimens was observed to crawl for a distance of nearly half an inch in the space of a quarter of an hour. When alive it is of a pink colour, and this tinge is sometimes preserved in the shell. The length of the animal, when contracted in spirit, is about 1 mm.

External features.—These have been correctly if somewhat diagrammatically figured by Pelseneer. A three-quarter ventral view of the animal is given in fig. 1. Attention may be called to the following points: The visceral spire is attenuated and much reduced, its coiled apex containing only some lobes of the liver and, in some specimens, a portion of the gonad. The last whorl is greatly expanded laterally, compressed dorso-ventrally, and contains all the important organs of the body. The snout is moderately long, terminating in a trumpet-shaped expansion, on the ventral side of which is the mouth. The mantle is large, and in contracted specimens completely covers the head and the greater part of the snout. The mantle slit, corresponding to the labral incision in the shell, is short, and situated nearly opposite the right eye; its margins are furnished with short digitiform

processes bearing projecting sense-papillæ, such as have been described by Vayssière in Scissurella costata. The cephalic tentacles are moderately long, reaching in their contracted state as far forward as the end of the snout. They are fringed with a large number of small, conical sense-papillæ, which, in Incisura, are not scattered all over the surface of the tentacles as figured by Vayssière for S. costata, but are arranged in two multiple rows on the inner and outer margins of each tentacle (fig. 27), somewhat like the pinnules on the tentacles of an Alcyonarian polyp. The structure of these sense-papillæ will be described further on. The eyes, which are closed and provided with a cornea and lens, are situated on prominences at the outer sides of the bases of the tentacles. Just below and behind the tentacle of each side is a short sub-ocular tentacle which does not bear sense-papillæ like the cephalic tentacles, but is richly ciliated and glandular in structure. In the single male specimen of which I have cut sections, the sub-ocular tentacle of the right side is somewhat enlarged, spatulate in form, and more abundantly provided with gland-cells than in the females. In all the females I have examined the sub-ocular tentacle of both sides is digitiform.

The foot, as is shown in fig. 1, is rather short and triangular in shape, the apex of the triangle being posterior. In shape and in the size of the broad, creeping sole it differs considerably from the narrower elongated foot of S. costata and S. crispata. The epipodium begins as a low ridge in about the middle third of the foot, and increases in size posteriorly. As described by previous authors it bears three moderately long epipodial tentacles on each side of the body towards the posterior end of its course. These tentacles bear lateral rows of sense-papillæ exactly like those of the cephalic tentacles, but there are no ventral cirrhi in connection with them as in S. costata. The epipodial folds meet posteriorly above the posterior end of the foot, and just dorsal to and in front of their union is a muscular opercular lobe bearing the operculum. The last-named structure is small, horny, and

multispiral, as in other Scissurellidæ. It must be regarded as vestigial since, as is the case in Pleurotomaria, it cannot be of any use in closing the aperture of the shell. There are two columellar or shell-muscles (fig. 2) symmetrically disposed right and left of the middle of the body, the right muscle being slightly larger and extending rather further back than the left.

As it is almost impossible to make dissections of an animal scarcely exceeding 1 mm. in length, the following account of the anatomy of Incisura is mainly founded on reconstructions from sections, but I succeeded in making some satisfactory whole preparations of the ctenidia, and have checked the results of my reconstructions as far as possible by the study of whole specimens cleared in various ways. Fig. 2 is a camera drawing of a specimen stained in picro-carmine and mounted in oil of cloves; it shows as much of the general anatomy as can be made out by this method. Figs. 3, 4, and 5 are reconstructions from sections showing respectively the anatomical relations of the alimentary tract, the kidneys and pericardium, and the nervous system. Figs. 6 to 12 are camera drawings of some of the sections from which the reconstructions were made.

Organs of the pallial complex.—Incisura is typically zygobranchiate, and the position and general characters of the ctenidia, hypobranchial glands, left kidney, and pericardium have been correctly described by Pelseneer.

The ctenidia.—Both right and left ctenidia take their origin from the roof of the mantle-cavity, close to the anterior end of the columellar muscle of their respective sides of the body. The left ctenidium lies almost transversely across the neck of the animal, its anterior extremity reaching nearly as far as the base of the right tentacle (fig. 2), and it is closely compressed between the body-wall and the roof of the mantle. The right ctenidium, on the other hand, lies for the most part in front of the right columellar muscle, and the bulk of it hangs vertically downwards in the space enclosed between the mantle and the outer side of the foot (fig. 7). Pelseneer

has described the right ctenidium as mono-pectinate, but, as may be seen in fig. 7, it is really bi-pectinate; the external lamellæ, however, are few in number, and in some specimens are so feebly developed that they might easily be overlooked. It is at first rather difficult to make out the details of the structure of the ctenidia and to institute an exact comparison between them and those of closely allied Aspidobranchs, but a careful study of sections and whole preparations shows that they are constructed on the familiar pattern. Each ctenidium consists of an axis, the posterior part of which is fused to the roof of the mantle-cavity and extends back in the angle of that side of the mantle-cavity to which it belongs, lying just above the columellar muscle. The anterior end of the axis is free, and the large osphradial ganglion, as is always the case in Aspidobranchia, is situated at the point where the axis becomes free from the mantle. This point, in Incisura, corresponds with the anterior end of the columellar muscle. In the case of the left ctenidium that part of the axis which is fused to the mantle bears no filaments, but, as will be described further on, this statement does not hold good for the right ctenidium. Taking the left ctenidium for the purpose of description: its free apex projects into the mantle-cavity in front of the columellar muscle as a thin, triangular lamina, which, as already explained, is bent over to the right, and also is twisted about its own axis from right to left in such wise that the morphologically outer row of filaments become posterior in position, the morphologically inner row anterior. The efferent branchial vessel runs, as is always the case, along the dorsal, here the posterior margin, and the afferent vessel along the ventral, here the anterior margin of the axis. The inner and now anterior filaments borne on the free portion of the axis are short and not more than four or five in number, and are folded backwards over the upper (morphologically ventral) side of the axis, apparently as a result of the latter being twisted from right to left in a narrow space. The morphologically dorsal edges of the anterior filaments are consequently maintained in a dorsal

position. But in the case of the posterior filaments, which are eight in number and much longer than the anterior filaments, the twisting of the axis has brought the ventral surfaces into a dorsal position. Fig. 16 represents a section through the anterior and fig. 17 a section through the posterior row of filaments. Each is more or less quadrangular in outline, its lateral walls formed of long columnar cells bearing long and fine cilia, which in contracted specimens appear to interlock like the cilia of the ciliated discs of filibranch Lamellibranchia. I do not think, however, that their function is to hold the filaments together, but simply to create currents over the surfaces of the filaments. Their interlocking is simply due to their becoming matted together in consequence of the contraction of the gill in spirit. On the ventral surface of each filament is a band of very short cilia. The dorsal edge of the filament bears no cilia externally, but, as shown in the figures, is produced to form a peculiar bolster-shaped swelling, which, as far as I am aware, has no analogue in the gills of any other mollusc. This dorsal glandular ridge, as I will call it, takes its origin from near the free distal end of the filament, and extending along the dorsal face of the latter is closely fused to it for the greater part of its length, but on approaching the proximal end of the filament the glandular ridge becomes free and ends in a small rounded projection. The ridge is traversed throughout its length by a small ciliated canal, which makes no communication with the blood channel of the filament, but opens into the mantle-cavity in the angle between the free proximal extremity of the ridge and the filament. This communication with the mantle-cavity, as seen in section, is shown in the central filament in fig. 17. In the filament on the right hand in the same figure the section passes through the middle of the glandular ridge, and the ciliated canal is seen to be closed in on all sides and to be situated near the ventral, i.e. the filamentary side of the ridge. The same features are shown in the ridge attached to the right-hand filament in fig. 18, but in the case of the left-hand filament in this figure the

section passes through the more distal part of the ridge, and the ciliated canal is seen to be smaller and situated near the dorsal side of the ridge. A little further on it ends blindly. As the figures show, the ridge is made up of a sheath or cortex of elongated, fusiform cells, which pass nearly transversely round the periphery of the ridge, and a medulla of large, closely packed ovoid or fusiform cells having large nuclei and granular cell contents. The cells abutting on the lumen of the ciliated canal are usually larger and more granular than those more peripherally situated, and their histological characters leave little doubt that they are secretory. It is noticeable that there are very few if any glandular cells interspersed among the columnar ciliated cells of the filament, and the glandular ridge appears to have taken over the secretory functions, and to replace the secretory cells scattered over the surface of gill-filaments of other Mollusca. The extreme specialisation exhibited by the formation of a closed canal into which the secretory cells discharge their products is certainly a remarkable feature in Incisura.

The central blood-channels of the filaments, as may clearly be seen in the figures, are elongate-oval in shape, and their walls are strengthened, for about half their extent, by flattened, chitinous, skeletal bars, which, as in other molluscs, may be traced to the proximal end of each filament, where they diverge from one another, and curve round to run up in the walls of the blood-spaces of the adjacent filaments (fig. 19). As M. F. Woodward (**19**) has shown that in Pleurotomaria these skeletal bars run along the dorsal edges of the gill-filaments, whereas in Nucula they run along the ventral edges, it is of some interest to determine the position of these bars in Incisura, which is usually reckoned as belonging with the genera Scissurella and Schismope to the Pleurotomariidæ. It is clear from an inspection of fig. 17, representing a transverse section through the posterior gill-filaments of the left ctenidium, which, as explained above, are turned upside down, that the skeletal bars lie on the dorsal sides of the filaments, and the same thing can be

seen still more clearly by inspection of fig. 7, in which the relations of the gill-filaments to the axis are obvious. In the anterior gill-filaments of the left ctenidium the skeletal bars appear to be ventral in position, but this is because these filaments are reflected backwards and their natural surfaces are reversed. Incisura, then, agrees with Pleurotomaria, and also with Trochus (*fide* Fleure and Gettings) and Fissurella, and differs from Nucula. But it must be observed that Woodward went further than the facts warranted when he asserted that the position of the gill-bars indicated a more remote affinity between Pleurotomaria and the primitive Lamellibranchia than is generally supposed. As a matter of fact the skeletal bars differ considerably in position in some not remotely related mollusca. In Solenomya, for instance, they are shifted to a more dorsal position than in Nucula, and in the Filibranchia they are actually dorsal. The fact is, as Woodward himself pointed out, these skeletal bars have a physiological rather than a morphological significance, and are always developed in close relation to the tracts of cells bearing specially long or functionally important cilia. Hence, in Filibranchia we find them related to the ciliated discs, which are near the dorsal edges of the filaments.

In so small an object as Incisura it is very difficult to make sure of the presence or absence of a septum dividing the blood-channel into an afferent and an efferent moiety, but I am tolerably certain that such a septum exists, as shown in fig. 18. But it is not always placed transversely, but may be oblique or even nearly longitudinal.

The attached portion of the axis of the right ctenidium extends far back in the extreme right-hand corner of the mantle cavity, lying close above the columellar muscle of that side, and gives off some three or four short filaments before reaching the level of the osphradial ganglion. At this spot there is a break in the continuity of the filaments, none being formed in the immediate proximity of the ganglion, but in front of it the ctenidial axis becomes free, and drops vertically down in front of the columellar muscle to hang in

the space between the foot and mantle, as shown in fig. 7. The basal portion of the axis is also enlarged at this point, and gill-filaments are given off from both sides, both of the free apex and of the broad basal portion. These filaments are not simply digitiform like those of the left ctenidium, but are plate-like, with the glandular ridge running along their dorsal margins, as shown in fig. 7. As the skeletal bars and glandular ridges are on the inner side of the filaments of the inner row, the free axis must have been rotated through 45° to bring the dorsal surface inwards. The plate-like filaments springing from the expanded base of the free part of the axis spread out on, and are attached to, the adjacent parts of the mantle; the filaments, or as they more appropriately might be called, the "gill-lamellæ" of the inner row extending dorsally along the inner surface of the mantle, while those of the outer row, two or three in number, pass round the front edge of the columellar muscle and run back for some distance below it as ridges projecting inwards from the dependent margin of the mantle (fig. 8, *m. br.*) The blood supply to the ctenidia will be described in connection with the heart.

The rectum runs diagonally from left to right in the roof of the mantle-cavity, and the anus opens opposite the slit in the mantle edge. In much contracted specimens, such as that from which fig. 2 was drawn, the anus is situated some distance from the slit, but in other less contracted specimens it is close to it.

The hypobranchial glands lie in the roof of the mantle on either side of the rectum, between it and the ctenidia. Both consist of a more or less extensive modified glandular patch of the internal epithelium of the mantle. The gland-cells are very large relatively to the size of the animal, and are of two kinds: large ovoid cells filled with large granules which stain deeply in hæmatoxylin and green in picro-indigo-carmine; these are therefore mucigenous cells. The other gland-cells are of nearly the same size and shape, but have clear or minutely granular contents. The left hypobranchial

gland is much the smaller of the two (figs. 7 and 8); posteriorly it is a narrow strip of glandular epithelium lying between the terminal part of the rectum; anteriorly in front of the anus it becomes broader and extends about as far forward as the level of the mantle slit, but stops far short of the anterior border of the mantle. In this pre-anal region the right and left hypobranchial glands are very closely approximated in the middle line. The right hypobranchial gland has approximately the same anterior extension as the left, but runs backwards on the right side of the rectum nearly to the posterior end of the mantle-cavity. Comparing the arrangement with that described by Woodward for Pleurotomaria, it is obvious that the pre-anal portions of the two glands of Incisura correspond to the large anterior hypobranchial gland, "partially divided by a median longitudinal furrow into two halves," of Pleurotomaria, and the posterior portions of the two glands of Incisura correspond to the two "additional mucous glands" lying on either side of the rectum of Pleurotomaria. But whereas in the latter genus the left additional gland is conspicuously the larger, in Incisura it is the right posterior portion of the gland which preponderates in size, the left gland being small, no doubt because of the relatively large size of the left kidney, for the hypobranchial gland does not extend beneath this organ.

The pericardium, as in all Rhipidoglossa except the Helicinidæ, is traversed by the rectum. It is relatively of large size, and can always be distinguished in whole specimens as a clear space surrounding the first bend of the rectum on the left side of the body behind the columellar muscle. At this point it lies close to the surface of the body, and its outer wall is very thin and transparent (fig. 11). The exact limits of its extension to the right are very hard to make out, because the left kidney projects into it from above, and its cavity is largely blocked by the auricles. Its extent, as far as I am able to determine it by reconstruction from sections, is represented by the thick black line in fig. 4. The large transverse extension of the pericardial space, as compared

with its narrow limits in Pleurotomaria, Haliotis, or Trochus, is correlated with the tendency towards a secondary bilateral symmetry, the development of two columellar muscles, and the position of the ctenidia wide apart from one another on the right and left sides of the body. The necessary result is an increased breadth of the body, and the blood returning to the heart by the efferent branchial vessels has to traverse a considerable distance before reaching the ventricle. In other words, the auricles are considerably elongated, and the pericardium has to be extended to receive them. Very similar relations are seen in Fissurella.

The heart and circulatory system.—The ventricle is placed rather far forward on the rectum; no further forward than in Fissurella, but much further forward than in either Pleurotomaria or Haliotis. The walls of the ventricle are so thin and feebly muscular that they are difficult to recognise, even with the highest powers of the microscope. The auricles also have very thin walls but are more easily recognisable. The left auricle is relatively very large (fig. 10), and its anterior border gives off a number of short and wide sinuses, which penetrate the folds of the wall of the left kidney and vascularise this organ. The right auricle is of smaller size. The course of the blood-vessels, as far as I was able to determine it, is of the usual diotocardiate type, and is diagrammatically represented in fig. 4, which is fully lettered and needs no further description. I was unable to trace the course of the aorta, but the blood, after passing to the foot and the various viscera, is evidently collected in a large sinus lying below the pedal ganglia, and is returned to the afferent branchial vessels by sinuses running over the dorsal side of the great mass of muscle-fibres which diverge on each side of the foot to form the columellar muscles.

The kidneys.—The left kidney (figs. 8, 9, and 13) is of comparatively large size, but its structure and histological characters leave no doubt that it corresponds physiologically to the papillary sac of the Pleurotomariidæ, Haliotidæ, and Turbonidæ, for it is unquestionably phagocytic and not depu-

ratory. It is a triangular sac lying close alongside of the rectum and projecting largely into the pericardium. It opens into the mantle-cavity by a simple slit-shaped aperture with somewhat tumid lips (fig. 9). The majority of the specimens of which I cut sections were females, and in all of them the cavity of the sac was large and but slightly broken up by ridges or papillæ projecting into it. In all the specimens the epithelium lining the cavity of the sac and covering the papillæ had the characters shown in fig. 14. The cells are large and pale, with pale nuclei, and most of them are stuffed with rod-shaped masses which stain very deeply with iron hæmatoxylin. Whatever may be the nature of these rods, which, as shown in the figure, have rhomboid outlines and are apparently crystalline, they have clearly been taken up by the amœboid cells of the left kidney from the adjoining blood-spaces, for these latter are also filled with similar rods, which, however, are smaller, more transparent, and stain less deeply in hæmatoxylin. The left kidney differs considerably in appearance according to its functional activity. In some specimens no rod-shaped bodies can be detected in the cells, and the walls of the kidney sac then appear pale and thin. In other specimens, again, no rod-shaped bodies can be seen in the blood-sinuses, but the kidney-cells are stuffed so full of them that their outlines are no longer distinguishable. In other specimens, again, the rod-shaped bodies are abundant in the blood-sinuses and more or fewer are present in the kidney-cells. A portion of the epithelium of a specimen in the last condition is represented in fig. 14. The fact that the histological character of the left kidney or papillary sac in Haliotis and Trochus is different from that of the right kidney was established by Rémy Perrier in his careful studies on the kidneys of prosobranch Gastropoda, and Pelseneer (11) afterwards showed that the amœbocytes of the papillary sac take up solid particles, such as carmine or Indian ink, injected into the blood-sinuses, whereas the secretory cells of the right kidney eliminate sulphindigotate of soda injected in solution into the blood. Both kidneys of Patella are depuratory, that

is to say, they take up sulphindigotate of carmine from the blood, but there is still some doubt as to the very rudimentary left kidney of Fissurella. Rémy Perrier (**14**) describes its histological structure as identical with that of the right kidney, and consequently it has been generally assumed that, like the left kidney of Patella, it is depuratory in function, but this is not certain and the subject requires renewed investigation. All observers agree in describing the left kidney of the Fissurellidæ as being in a rudimentary condition, and it is possibly nearly if not quite functionless. It may even be absent in some species of Fissurella, for I have been unable to find a trace of it in transverse and longitudinal sections of F. græca.

In the single male specimen of Incisura of which I have sections the left kidney is larger than in any of the females; the papillæ projecting into its cavity are more numerous, are covered with a much more definite layer of epithelial cells, and I could not find any trace of phagocytosis in the latter. Whether this is a constant sexual difference I cannot say, as I was unable to find another male. A section through this kidney is represented in fig. 13, which also shows the left reno-pericardial canal. The last-named structure is found in the same position in both male and female. It opens into the kidney close to the external aperture of the latter, and runs towards the left as a very fine canal which traverses the floor of the kidney and opens into the left-hand corner of the pericardium, as indicated in the figure. The cells lining the nephric end of the canal appear to bear very fine cilia, but I am unable to speak with certainty on this point. The right kidney of Scissurella and Incisura has been very briefly described by Pelseneer (**12**), who figures it as a very small tube lying below the rectum in S. costata and to the right of the rectum in Incisura. He describes it as being rather narrow in its anterior portion and says further: "Il s'etend partiellement sous le rectum, comme chez Trochus, et pénètre dans la masse viscerale, au côté droit de ce corps, sur et entre les convolutions de l'intestin."

I may amplify this account by saying that the right kidney of Incisura is a structure of considerable size and importance which may be described as consisting of three lobes. The most anterior lobe varies considerably in size: it lies in the roof of the mantle-cavity to the right of the rectum (figs. 4 and 10) and somewhat posterior to the left kidney. It opens by a simple slit-shaped aperture (fig. 10, *k.r.o.*) into the mantle-cavity, and a few sections further back than the one figured it extends over to the right, forming a considerable projection into the posterior part of the mantle-cavity. Posteriorly it gives off two lobes. That on the right runs nearly vertically downwards close to the right side of the vertical loop of the intestine and passes inward among the viscera, curving round the floor of the middle part of the stomach and eventually coming in contact with the gonad, but it does not effect any communication with this organ. The left posterior lobe passes below the rectum and overlies the anterior cæcal end of the stomach.

The excretory cells of the depuratory kidney of Gastropods are notoriously difficult to preserve, and in my specimens were too much macerated to admit of a satisfactory study of their structure. For the same reason I have been unable to satisfy myself completely as to the relations of the right reno-pericardial canal. For some time I was uncertain whether any communication existed between the right kidney and the pericardium, but the series of sections represented in figs. 22 to 26 demonstrate that this connection does exist, and that, as in Trochus, there is an intimate connection between the right reno-pericardial canal and the gonaduct. In fig. 22 the oviduct (*od.*) is seen lying close to the right side of the anterior lobe of the kidney, and from it a narrow canal leads upwards and inwards. The histological features of this canal are not well preserved in any of my specimens, but its walls appear to be formed by cubical epithelial cells containing small, deeply staining nuclei, whose characters as shown in figs. 22 and 23, suggest that they bear cilia and form a ciliated funnel opening into the pericardium. The connection between the canal

and the pericardium is clearly shown in fig. 24, and figs. 23 to 25 show that the lower end of the canal is, in fact, continuous with the gonaduct, and opens along with it into the kidney, close to the external orifice of the latter. It should be noted as a peculiar feature in Incisura that there is no distinct duct to the right kidney; its simple slit-like opening into the mantle-cavity is a Pectinibranch character.

The gonad, in both sexes, is a simple tubular structure lying to the left side of the stomach, and in the case of the ovary partly embracing this organ. The anterior end of the gonad extends as far forward as the posterior limit of the mantle-cavity and ends blindly below the first bend of the rectum. The cavity of the ovary, in all my specimens, is filled with ova in all stages of development, the ripe ova being very large relatively to the size of the animal, and abundantly supplied with yolk-granules. The testis, in the single male I have been able to examine, is very small, and I think the individual must have been a spent one, as the cavity of the testis only contained a few free spermatozoa and I could find no trace of spermatogenesis.

The course of the gonaduct and its connection with the right kidney has been correctly but all too briefly described and insufficiently figured by Pelseneer. He only says of it: "La glande genitale est unique et occupe le sommet de la masse viscerale. Elle n'a pas d'orifice exterieure; son conduit arrive au rein droit." But it would be difficult for anybody to guess the course of the gonaduct before its arrival at the right kidney by an inspection of his fig. 115, perfectly correct as it is. As shown in fig. 4 the ovary, which in the more anterior and broader part of the visceral mass is on the left side of the stomach, extends into the narrow commencement of the terminal whorl of the spire, and here its posterior end is produced from left to right into a fairly spacious thin-walled sac which lies between the upper and lower of the two posterior lobes of the liver extending into the spire. The walls of this sac are not lined by a germinal epithelium but its cavity often contains a ripe ovum.

It is the commencement of the oviduct. Rapidly narrowing in diameter it passes forward to the right of the posterior end of the stomach and the liver lobes originating from it, and maintaining a position close below the external body-wall, it passes as a very much flattened and very thin-walled duct along the right side of the visceral mass, gradually mounting from a more ventral to a more dorsal position till it arrives above the right-hand loop of the intestine. All this while it has laid close to the outer body-wall, and it is extremely difficult to follow its course, owing to its being flattened between the liver and the external integuments. It turns inward just above and in front of the right visceral ganglion and runs in the roof of the posterior end of the mantle-cavity towards the right kidney (fig. 11). Here its walls become thicker and are lined by a distinct cubical epithelium. The duct does not at once enter the kidney but runs along its outer wall and opens into it in close proximity to the renal orifice. As stated above the gonaduct opens into the kidney coincidently with a reno-pericardial canal, the relations being very similar to those in Trochus. The vas deferens takes the same course as the oviduct.

The alimentary tract.—The buccal bulb is relatively of enormous size. There are two large odontophoral cartilages on either side whose shape, as seen in section, is very similar to that of the cartilages of Fissurella as figured by Boutan. As shown in figs. 6, 7, and 8, the anterior and dorsal cartilages are the larger, and support the radula; the posterior cartilages lie ventrad of the hinder ends of the anterior cartilages and have concave upper surfaces, with which the hinder ends of the latter articulate. A similar arrangement obtains in Trochus, and has been well described by Randles (**15**). The musculature of the buccal bulb is powerful, but I have not attempted to follow it out in detail. It is noticeable, however, that the cross-striation, both of the intrinsic and extrinsic muscles of the odontophore, is very well marked. Though it is well known that these muscles are cross-striped in Gastropods, I am not aware that the character of the stria-

tions has been carefully studied, and I take this opportunity of giving a drawing (fig. 15) of three fibres of the extrinsic muscles attaching the anterior end of the odontophore to the integuments of the snout. These were specially well-stained, and it is obvious that the ends of the fibres nearest the snout are in a state of contraction, while their odontophoral ends—the lower ends in the figure—are relaxed. The fibres are not round but elongate oval in cross-section. That on the right has been cut through its long axis; in the two fibres on the left the section passes through the shorter axis, near the edge of the fibres. It can be seen that each fibre is a single metamorphosed cell, with a single nucleus situated near its broader end. The central portion of the cell, in which lies the nucleus, is composed of but little-altered cytoplasm, exhibiting an alveolar or reticular structure, differing from the normal only in the fact that the meshes of the reticulum are very regularly disposed in rectangular fashion. This cytoplasmic core of the fibre is invested by a sheath of contractile substance, which is thickest at the two ends of the long axis of the oval, and therefore appears as two bands in the right-hand fibre in the figure, while in the two left-hand fibres only the contractile substance is cut through. The whole is invested by a delicate sarcolemma. The most interesting thing about these fibres is that the reticular arrangement of the cytoplasmic core corresponds exactly with the striations of the contractile substance in the upper part of the fibre on the right side of the figure, and in the left-hand fibre the cross-striations are very obvious and close together in the uppermost contracted part of the fibre, but lower down as the fibre becomes more relaxed, the dark transverse lines become progressively broader and fainter, and each may be seen to be made up of a number of dark longitudinal striæ, which may well be interpreted as nodal thickenings of a reticulum. It is, of course, possible that the difference between the two ends of the fibres is due, not to a difference in the state of contraction, but to a greater specialisation of the broader end. Whichever interpretation is correct, the

appearances lend support to the reticular theory of the constitution of striped muscle-fibre, and are inconsistent with the opposing theory of sarcomeres.

The mandibles occupy the usual position at the sides of the mouth, and are composed of a number of plates or "tesseræ" as described by Vayssière for Scissurella costata. Randles has shown that each tessera is the product of a single epithelial cell in Trochus, and the same is evidently the case in Incisura. The radular sac occupies the usual position. Lying at first between the upper horns of the odontophoral cartilages it maintains a median position to the posterior end of the buccal bulb, and then curves to the right between the right œsophageal pouch and the pedal ganglia and soon terminates in a swollen bilobed extremity lying on the right side of the hæmocœle. The radular teeth are represented in fig. 20. The centrals are squarish, with an expanded basal plate; their anterior margins decurved, and furnished with five very distinct and sharp-pointed denticulations. The next three teeth (medio-laterals) are oblong, with decurved denticulate margins; they decrease somewhat in size from within outwards. The next tooth is much smaller, has a somewhat sigmoid curvature, a thickened base, a narrow neck, and a single recurved marginal denticulation. The next tooth is very large, shaped somewhat like a rake with a crooked handle, its expanded margin decurved and bearing about a dozen denticulations. Then follow the marginals or uncini, which are numerous, curved, slender, with expanded and recurved denticulate margins. The radular formula may be written :

$$\infty \; 1 \; (4 + 1 + 4) \; 1 \; \infty$$

Vayssière has given a good figure of the radula of Scissurella costata, which is similar to but differs in small details from that of Incisura. The radula of the Scissurellidæ is usually described as resembling that of Trochus, but it is much more nearly like that of the Fissurellidæ. A reference to Thiele's figures in the concluding chapter of Trösche1's 'Gebiss der Schnecken' shows that the radula of Incisura

very closely resembles that of Subemarginula picta, the shape and relative size of the outer medio-lateral tooth being almost identical, as also the characters of the centrals and uncini. The large and specialised outer lateral tooth, though it differs widely in detail in different species, is characteristic of the Fissurellidæ. A close resemblance also exists between the radula of Incisura and that of Emarginula pileolus, and a less clearly marked resemblance can be seen in the radulæ of various species of Fissurella. On the other hand, no comparison with the radula of Pleurotomaria is possible. A general view of the alimentary tract, as determined by reconstruction from sections, is given in fig. 3, which so far explains itself that little description is necessary. The œsophagus is enormously dilated in the anterior part of its course, forming in addition to the wide lateral diverticula or œsophageal pouches (figs. 9 and 10, *œ. p.*) a spacious ventral pocket or "jabot." These are all lined by a soft-looking glandular epithelium. Behind the level of the pedal ganglia the posterior section of the œsophagus leaves the jabot as a narrow tube with thick, longitudinally ridged walls formed by a long ciliated columnar epithelium. It runs back below the stomach and opens into the latter near its posterior end. Near the œsophageal opening numerous liver cæca open into the posterior end of the stomach. There is no spiral cæcum connected with the entry of the liver-ducts as in Pleurotomaria, Haliotis, and Trochus, but there is a deep ciliated ventral groove, the lips of which are bordered by specially long ciliated columnar cells, extending along the floor of the stomach from the œsophageal opening to the pylorus. A precisely similar groove occurs in the stomach of Fissurella, and has been well described and figured by Boutan (2).

Randles has shown that in Trochus a cæcal groove, bounded by two conspicuous folds, extends into the spiral cæcum from the œsophageal opening, and that the larger of the two bile-ducts opens into this groove. Though the spiral cæcum is absent there can be little doubt that the ventral groove of the Fissurellidæ and Incisura corresponds in function to the cæcal

groove of the Trochidæ, and it has the same relation to the liver-ducts. It should be noted in this place that Incisura, in the possession of numerous biliary apertures, resembles Fissurella and differs from Trochus, which has two, and Pleurotomaria, which has only one bile-duct. The intestine leaves the stomach on the ventral side of the anterior third of the stomach in Incisura. Beyond it the stomach narrows rather abruptly, and is continued forward as a small cæcal diverticulum, the front end of which is inserted in the loop formed by the left-hand bend of the rectum. The walls of the blind end of this diverticulum are covered internally by a thick chitinoid layer, and thrown into complicated folds and ridges, but the cæcum is not spirally coiled, and situated as it is at the end of the stomach furthest from the bile-ducts, it cannot be homologised with the spiral cæcum of Pleurotomaria, Haliotis, or Trochus. It must, however, be the cæcum referred to by Pelseneer (**12**). The walls of the intestinal end of the stomach of Incisura have the columnar cells with striated borders and thick cuticle so fully described by Randles for Trochus.

The intestine is provided throughout its length with a single longitudinal ridge or typhlosole. On leaving the stomach it makes a sharp bend from left to right, passes vertically upwards to above the level of the stomach, thence turns sharply to the right, describes a wide loop on the right hand, as shown in fig. 3, and bending sharply again to the left, passes nearly straight across the body till it reaches the left-hand corner of the pericardium, when it turns upward and to the right in the mantle roof, and becoming rectum, traverses the pericardium in its diagonal passage across the roof of the mantle-cavity to end in the anus opposite the mantle-slit.

The liver cæca, as may be seen in figs. 3 and 11, are few in number, of relatively large size, with large lumina bordered by large secretory cells. As far as I could determine they do not branch, but have somewhat convoluted courses, and open independently into the œsophageal end of the stomach.

A few details may be added relative to the structures connected with the buccal cavity and œsophagus.

In the mid-dorsal line the roof of the buccal cavity is deeply folded to form a median ridge containing a narrow lumen T-shaped in transverse section. This lumen of course communicates freely below with the buccal cavity. This median fold or ridge is deepest anteriorly over the mouth, and extends backwards for about two thirds of the length of the buccal bulb, gradually shallowing posteriorly till it dies out altogether. The walls of this ridge are composed of simple columnar cells, the internal ends of which have a striated border, and bear short cilia. On either side of the anterior part of this mid-dorsal ciliated groove is a somewhat shallower but still conspicuous groove appearing on the dorsal surface as a pair of folds lying close and parallel to the median ridge. These may be called the salivary grooves, for the small, simple, tubular buccal or anterior salivary glands open into them near their anterior extremities (fig. 21, *sg.* and *s. d.*). These anterior salivary glands are simple short cæca lined by an epithelium, consisting mainly of large finely granular secretory cells with a few columnar supporting cells between them. The salivary grooves die out posteriorly at the point where the œsophagus leaves the buccal cavity, and at this level a second or posterior pair of salivary glands opens into the roof of the buccal cavity, just to the outside of the salivary grooves. These posterior salivary glands are very small tubular structures with minute lateral diverticula. They correspond in position to, but are much smaller than, and not so much branched as the second pair of salivary glands in Fissurella. Otherwise the structures just described are identical in the two genera. As soon as the œsophagus is separated from the buccal cavity its right and left walls are produced into the broad and flattened œsophageal pouches, but from the first the right-hand pouch is considerably larger than the left. The T-shaped lumen of the dorsal ciliated groove may be traced for some way along the roof of the œsophagus, but presently it dies out, and is replaced by a

band of ciliated cells which diverges towards the left, and eventually passes completely over to the left side and passes into the narrow posterior part of the œsophagus. Ventrally, to the right side of the narrow œsophageal tube, the floor of the spacious anterior œsophageal cavity is produced into a capacious pouch or "jabot," which runs back for some distance alongside of the narrow œsophageal tube (fig. 11, *j*), and eventually ends blindly. The deviation of the œsophagus to the left and the preponderant size of the right œsophageal pouch have been noted by Boutan in Fissurella, and it is indeed a common feature in the Rhipidoglossa, indicative, as Amandrut has pointed out, of the larval torsion which brings about the asymmetry of the adult Gastropod.

The nervous system.—Fig. 5 is a diagram of the principal ganglia and nerve-trunks, as reconstructed from sections. Pelseneer's description of this system in Scissurella costata and Incisura lytteltonesis is as follows: "Dans les deux espêces, les cordons pédieux sont dans la masse musculaire du pied, et s'étendent jusqu'à la partie postérieure. À leur extrémité tout à fait antérieure se trouvent des ganglions pleuraux bien distincts. La commissure viscerale naït de ces derniers; elle est croiseé et porte un ganglion supra-intestinal presque accollé au ganglion branchial ou osphradial gauche, comme dans Trochus. Tout ce système nerveux ressemble done beaucoup plus à celui de Trochus qu'aux parties correspondantes connues de Pleurotomaria, telles que les ont décrites Bouvier et Fischer." Since this was written we have had the more complete account of the anatomy of Pleurotomaria by M. F. Woodward, and the difference between the nervous system of this genus and that of the Scissurellidæ is even more apparent than before.

As may be seen from the diagram, the nervous system of Incisura is at once typically Rhipidoglossate and specialised. As the nervous systems of various Rhipidoglossa have been described in great detail by sundry authors, it will only be necessary here to mention the more important and peculiar features.

The cerebral commissure is long and situated far forward in front of the anterior pair of salivary glands. It is a true nerve, not ensheathed by a layer of ganglion cells, differing in this from Pleurotomaria. The cerebral ganglia are of large size, sub-triangular in transverse section, and produced into prominent lobes at the origins of the more important nerves. The tentacular and optic nerves have separate origins from the cerebral ganglia, Incisura agreeing in this point with Trochus and Fissurella but differing from Pleurotomaria. The labial lobe is very large, and forms a long, conical, tapering, antero-ventral process of the cerebral ganglion, which curves inward below the odontophore on either side, maintaining its thickness for about two thirds of its course towards the middle line. Then it tapers abruptly to form a thin labial nerve, which passes between the muscles of the lower lip, and as far as I can determine is connected by an extremely fine prolongation with its fellow of the opposite side, thus completing the labial commissure. The buccal commissure is given off from the labial lobe about half way between the cerebral ganglion and the mid-ventral line. It passes inwards among the muscles of the odontophore and at once turns abruptly upwards to run between the extrinsic and intrinsic muscles to the top of the buccal bulb. Here it enlarges to form a ganglion of considerable size, lying close to the inside of the cerebral ganglion, and from this a stout nerve—a true nerve without a sheath of ganglion cells—passes inwards and backwards over the top of the odontophore and enlarges below the origin of the œsophagus into a small ganglion, which is connected by a very short commissure with its closely adjacent fellow of the opposite side. Bouvier (3) has figured and described two swellings at the ends of each of the elongated buccal ganglia of Turbo setosus, but I infer from his description that they are not separate ganglia, but merely swellings at the ends of a long and ill-defined ganglion. I find precisely the same arrangement in Fissurella græca, but Boutan figures four clearly defined ganglia in F. reticulata. The sub-division of this elongated ganglion into two distinct

ganglia is an indication of specialisation and a peculiar feature in Incisura. For the rest the characters of the cerebral ganglia, the size of their labial lobes, and the relations of the buccal ganglia are very similar in Turbo, Fissurella, and Incisura.

The cerebro-pleural connective, as is commonly the case, is larger than the cerebro-pedal; both are true nerves, devoid of any sheath or local accumulations of ganglion cells. The pleural ganglia are distinct and that of the right side is relatively large, but both are fused to the dorsal surfaces of the pedal ganglia. The visceral commissure is typically streptoneurous, and for the same reason that the osphradial ganglia are situated far forward, the whole commissure is contracted antero-posteriorly as in Patella; on the other hand, it is considerably extended right and left. The sub-intestinal ganglion is distinct, but elongated and rather ill-defined; as Pelseneer remarks it is connected by a very short nerve with the large left osphradial ganglion. The left symmetrical pallial nerve passes straight out from the left pleural ganglion almost immediately below the supra-intestinal ganglion, and traverses the posterior fibres of the left columellar muscle, turning nearly vertically downwards to enter the thickened border of the mantle. Before turning downwards it gives off a very fine branch, which makes connection with the short nerve uniting the supra-intestinal with the osphradial ganglion, thus establishing a left-hand dialyneury very similar to that of Trochus.

The subintestinal nerve is very stout, and crosses over the dorsal surface of the hinder part of the pedal ganglia almost at right angles to the long axis of the body. The sub-intestinal ganglion is fairly large and distinctly indicated by an accumulation of nerve-ganglion cells. It is triangular in shape, and from its right-hand lower corner the visceral nerve, and from its right-hand upper corner the osphradial nerve is given off. The latter is a very slender nerve, which passes into the substance of the columellar muscle, and turns vertically downward and then forward along the dependent edge of the mantle, running in this part of its course at the base

of the gill-filaments, which, as has been explained above, run back along this region of the mantle. At the anterior edge of the columellar muscle the nerve expands to form the large right osphradial ganglion. The right symmetrical pallial nerve takes its origin from the ventral side of the right pleural ganglion, just where the latter becomes fused to the pedal ganglion. It runs outward, traverses the columellar muscle some way in front of the osphradial nerve, and takes a direct course to the right osphradial ganglion, which it crosses dorsally, and in so doing enlarges and makes an intimate connection with it. Just in front of the osphradial ganglion the pallial nerve divides into two branches. The posterior branch, which is slender, runs back along the thickened border of the posterior part of the mantle. The anterior branch runs forward to the mantle-slit, where it expands to form a small ganglion, indicated by a distinct accumulation of nerve-ganglion cells, and is here joined by a slender nerve from the anterior end of the osphradial ganglion. This little ganglion at the hinder border of the mantle-slit gives off an external branch supplying the posterior sense-papillæ of the mantle-slit, and a stout anterior branch which passes round the mantle-slit and is continued forward as the peripheral pallial nerve, meeting and uniting with its fellow of the opposite side on the anterior border of the mantle. There is thus a very intimate dialyneury on the right side. These relations are very hard to make out, and require careful study with high powers of the microscope, but I can vouch for the correctness of the account here given of them. The relations in Fissurella are somewhat similar, but the proportions of the lengths of the nerves differ greatly, and apparently differ in different species, for in my sections of F. græca the sub-intestinal is close to the right osphradial ganglion, whereas in F. reticulata Boutan figures them as far apart and connected by a long slender nerve, as in Incisura. The origin of the right symmetrical pallial nerve from the upper surface of the pedal ganglion rather than from the right pleural ganglion is identical in Incisura and Fissurella.

The visceral loop bears three distinct accumulations of ganglion cells, forming as many ganglia. The right ganglion lies close below the gonaduct and gives off a slender nerve to that organ. The pedal ganglia, as may be seen in fig. 5, are very much concentrated. Anteriorly they are rather flat, but in about the middle of their length they increase considerably in thickness, this increase being due to the addition of a considerable ventral thickening to each ganglion. In this region, in fact, each pedal ganglion consists of a dorsal and a ventral moiety, as is the case in all Rhipidoglossa (fig. 9). Here also the whole of the pedal ganglia lies in the hæmocœle, as is the case with the more elongated pedal cords of Fissurella. But in Incisura the dorsal moieties of the pedal ganglia have very little posterior extension. The ventral moieties, on the other hand, extend back behind the dorsal moieties, and, narrowing in diameter, plunge into the muscular substance of the foot (fig. 10). There they are continued backwards for a short distance, giving off nerves from their outer edges, and diminishing rapidly in diameter, partly because of fibrils given off to the different nerves, but also largely because of the thinning out and eventual disappearance of their coating of nerve ganglion cells. Posteriorly the cords become simple nerves, and end some distance in front of the posterior end of the foot. Pelseneer states of Scissurella costata and Incisura lytteltonensis: "Dans les deux espèces, les cordons pedieux sont dans la masse musculaire du pied, et s'étendent jusqu'à la partie posterieure." This is certainly not the case in Incisura; the left pedal cord, or rather nerve, dies out at a distance of 125 μ from the posterior end of the foot in two specimens in which I calculated its extent, and remembering that the animal is only 1 mm. long this is a considerable distance. In short, one can hardly speak of pedal cords. The pedal centres, particularly the dorsal portions of them, have become concentrated into two clearly defined pedal ganglia, and it is only the ventral portions that are continued backwards to represent in some measure the elongated pedal centres of other Rhipidoglossa. In addition

to the thick anterior commissure connecting the dorsal portions of the ganglia, there is a single anterior thin commissure connecting the ventral portions, but this is the only trace of the usually numerous cross commissures of other lowly organised Gastropoda. Such a concentration of the pedal centres is very unusual if not unique among Aspidobranchia, and indicates that Incisura, and, if one may judge from the similar relations indicated in Pelseneer's figures of S. costata, the Scissurellidæ in general are highly specialised. Much has been written about the significance of the dorsal and ventral moieties of the pedal cords of archaic Gastropods. The French authors hold that the upper moiety is pleural, or, as they say, pallial, the lower moiety pedal in character. Pelseneer and most English and German authors hold that both moieties represent pedal centres. The facts in Incisura seem to uphold the latter view. I have no wish to re-enter upon a controversy which has become almost wearisome by repetition, but may state that in Incisura the cerebro-pedal connectives certainly join the dorsal moieties of the ganglia; that the epipodial nerves are certainly given off from the dorsal moieties, and that whereas the left symmetrical pallial nerve is undoubtedly given off from the left pleural ganglion, the right symmetrical pallial nerve certainly appears to be given off from the dorsal moiety of the right pedal ganglion and not from the right pleural, both in Incisura and Fissurella. Advocates of the French view will take this last fact as evidence in support of their theory. The nervous system of Incisura certainly bears no resemblance to that of Pleurotomaria. On the whole it most nearly resembles that of the Fissurellidæ, in which family the pedal cords, though still elongate and ganglionic, and provided with several cross-commissures, have undergone a considerable reduction in length as with those of other Rhipidoglossa.

The sense organs.—The eyes, as already stated, are closed and provided with a distinct lens. Their structure resembles that of the eyes of the Fissurellidæ, and differs from the eyes of the Pleurotomariidæ and Trochidæ, which are open.

The otocysts occupy the usual position on the dorsal surfaces of the pedal ganglia and present no unusual features (fig. 9).

The osphradia are strips of modified epithelium running for some little distance along the lower side of the gill-axes in front of the osphradial ganglia and just ventral to the osphradial or branchial nerve (fig. 16). They are very similar in structure and position to the osphradia of Fissurella græca.

Sense-papillæ occur not only on the cephalic tentacles but also on the epipodial tentacles, all round the margins of the mantle and on the cirrhi bordering the mantle-slit. Those on the cephalic tentacles are by far the largest, those on the margins of the mantle are very minute, but all have essentially the same structure. Fig. 28 represents a longitudinal section through three of the papillæ of the cephalic tentacles. Each papilla is a conical projection of the integument of the tentacle and is composed of a number of elongated cells of two kinds, closely packed together like the cells in a taste-bud from the human tongue. The larger cells with larger, pale nuclei are evidently supporting cells, their characters being similar to the adjoining epithelial cells. The more slender, finely granular cells with smaller, deeply staining nuclei are the sense-cells, and each ends in a short stiff cilium projecting from a small cup-shaped depression at the end of the cone. According to Vayssière these cilia are in constant movement in the living animal. The tentacles of Fissurella are clothed with a vast number of minute papillæ giving a velvety texture to the surface. These papillæ, though not so highly specialised, have each a single apical sense-bulb, the structure of which is similar to that of the sense-papillæ of Incisura.

Finally, mention may be made of the pedal glands. The anterior pedal gland consists of a mass of unicellular glands lying in the hæmocœle below the buccal bulb (fig. 7, *p. gl.*). It extends back nearly as far as the pedal ganglia. Anteriorly these glands become more deeply seated and pass into the muscular mass of the foot, where they debouch into a median ciliated duct (fig. 6) which runs forward and opens

on the anterior face of the foot in the groove between it and the lower surface of the snout. The posterior pedal glands are a mass of unicellular glands lying above the epithelial cells of the sole of the whole posterior surface of the foot. Each unicellular gland has its own duct, which runs between the epithelial cells to open on the surface.

The genera Scissurella, Schizotrochus, Incisura and Schismope, which have been grouped as a separate family Scissurellidæ by some few authors, are generally placed in the family Pleurotomariidæ because they are zygobranchiate Rhipidoglossæ, with a labral incision of variable length and position in the shell. There is no frontal veil between the cephalic tentacles, an epipodial ridge is present, and there is a corneous multispiral operculum. Fischer (5) writes: "Quelques auteurs distinguent deux familles, Scissurellidæ et Pleurotomariidæ, mais les differences qui existent entre ces deux types n'ont pas plus d'importance que celles qu'on constate entre les divers groupes de Trochidæ. Je les considere comme des sous-familles." Pelseneer (13), who had studied their anatomy, retains these forms in the family Pleurotomariidæ. Yet it is obvious, from what precedes, that the Scissurellidæ cannot possibly be retained in this position. The differences in the radula alone are sufficient to distinguish the two types. But in addition to this the Scissurellidæ differ from the Pleurotomariidæ in a number of characters, which may be summarised as follows:

(1) The Scissurellidæ have two columellar muscles; Pleurotomaria has only one.

(2) The eyes of Scissurellidæ are closed; thoso of Pleurotomaria are open.

(3) The subocular tentacles of the Scissurellidæ are absent in Pleurotomaria.

(4) The epipodium of Pleurotomaria is destitute of tentacles, cirrhi, or lappets.

(5) The wide distance apart of the ctenidia, the large size of the pericardial cavity, the forward position of the ventricle of the heart, and the more distinct shifting of the organs of

the pallial complex into a median position in the roof of the mantle-cavity are all points in which the Scissurellidæ differ from Pleurotomaria.

(6) In Pleurotomaria the right kidney has a distinct duct, with thickened glandular walls in the female; in the Scissurellidæ there is no such duct.

(7) There is no spiral cæcum to the stomach in the Scissurellidæ, and the form of the stomach differs largely from that of Pleurotomaria.

(8) The hepatic orifices are numerous in Scissurellidæ, whereas there is only a single orifice in Pleurotomaria.

(9) The nervous system of the Scissurellidæ differs in detail in almost every point from that of Pleurotomaria, particularly in the concentration of the cerebral ganglia; the extreme fineness of the labial commissure; the presence of distinct pleural ganglia; the well-developed symmetrical pallial nerves establishing a right and left dialyneury; the presence of distinct supra- and sub-intestinal ganglia; the shortness of the visceral loop; the concentration and abbreviation of the pedal centres.

Not only are the Scissurellidæ distinct from the Pleurotomariidæ, but they are clearly less closely related to them than the Haliotidæ or even than the Trochidæ and Turbonidæ, for the last-named families, though they have lost the labral incision in the shell, as also the right ctenidium and the structures correlated to it, have retained many anatomical features which find their counterpart in Pleurotomaria.

Where, then, shall we find the nearest relatives of the Scissurellidæ? Though Mr. Hedley was clearly in error in removing Incisura from the Scissurellidæ, I think he came very near the truth in suggesting the affinity of this genus with the Fissurellidæ. His comparison of the adult Incisura with the post-larval stage of Fissurella is a just one. Almost all the differential external features which serve to distinguish the adults disappear on comparison of the adult of the one type with the post-larval stage of the other. In the young Fissurella we see a coiled shell with spiral sculpture, a labral incision of considerable length to the right of the middle line.

There is a pair of ciliated post-ocular tentacles on either side of the head (I find vestiges of these structures in the adult of F. græca), a well-developed pair of ciliated epipodial tentacles in the vicinity of the opercular lobe, and a corneous multispiral operculum. Even the gills, if one may judge from Boutan's figure (Pl. xlii, fig. 8), have a close resemblance to those of a Scissurellid. If the animal were sexually mature one would not hesitate to place it among the Scissurellidæ. In the next or Rimuliform stage the epipodial tentacles are multiplied; Boutan figures six in addition to the sub-ocular tentacles in F. reticulata and two in F. gibba. The labral incision has been converted into a foramen by the approximation of its edges at the labrum, but a suture still connects the foramen with the margin of the shell. This condition is exactly paralleled by the Scissurellid genus Schismope. Subsequent development leads to the assumption of Fissurellid characters. The visceral spire, and with it the spiral coils of the shell, become obsolete. The foramen in shell and mantle become situated at the summit of the Patelliform shell, the post-ocular and epipodial tentacles (which obviously belong to the same series) degenerate, the operculum is cast off, and the opercular lobe disappears. In short, the Fissurellid develops along lines which remove it further and further from the Scissurellid condition of the larva.

But, as must be apparent from the preceding pages, there is a considerable number of anatomical features in which the adult Scissurellid more nearly resembles the adult Fissurellid than any other family of the Rhipidoglossa. These features may be shortly recapitulated, Incisura being taken as a type of Scissurellid structure.

The jaws of Incisura in position and structure very closely resemble those of a Fissurella. The radula of Incisura lytteltonensis finds its nearest counterpart in the radula of Subemarginula picta, and in general is distinctly Fissurellid in character. In the alimentary tract the characters of the salivary glands and œsophageal pouches, the absence of a spiral cæcum in the stomach, the presence of an œso-

phageo-intestinal groove in the capacious stomach, the existence of numerous hepatic ducts, are all points in which Incisura agrees with Fissurella, and differs, to a greater or less degree, from the Pleurotomariidæ, Haliotidæ, Trochidæ, and Turbonidæ. The presence of a right and left columellar muscle in the Scissurellidæ is evidently an antecedent stage of the horse-shoe shaped columellar muscle of the Fissurellidæ.

The eyes, which are open in Pleurotomariidæ, Haliotidæ, and Trochidæ, are closed in both the Scissurellidæ and the Fissurellidæ.

The subocular and posterior epipodial tentacles of the Scissurellidæ are paralleled by the similar larval organs in the Fissurellidæ.

In both the Scissurellidæ and Fissurellidæ the increased size of the last whorl of the shell and the diminution of the visceral spire has led to a broadening of the dorsal part of the body, in consequence of which the bases of the ctenidia are widely separated on the right and left sides of the body, the pericardium is transversely elongated, and the heart and kidneys are shifted towards the mid-dorsal line in the roof of the mantle-cavity. In these respects Incisura is intermediate between Fissurella and the other families of Rhipidoglossa enumerated above.

The nervous system of Incisura, though much specialised, shows more resemblance to that of the Fissurellidæ than to that of any other Rhipidoglossa, as has been explained in detail in the descriptive part of this paper. The correspondence in the labial commissure, the buccal ganglia, and the visceral commissure is very exact. The pedal centres of the Scissurellidæ have undergone great concentration, but this is foreshadowed in the pedal cords of the Fissurellidæ, which are much shortened in comparison with the elongated scalariform pedal centres of such families as the Pleurotomariidæ, Haliotidæ, and Trochidæ.

There can be little doubt, then, as to the affinity of the Scissurellidæ with the Fissurellidæ, but the exact relationship of the two families remains to be considered. In my opinion

it is not exact to say, as Hedley has, that Incisura represents an arrested stage of development of a Fissurellid. It is a more reasonable inference from the facts that the two families have descended from a common stock, and have diverged in different directions. There are several arguments in favour of this inference. One which in my opinion has great weight is derived from the condition of the left kidney in the two families. In the Scissurellidæ, as I have shown, the left kidney is relatively of large size, and is a true "papillary sac," phagocytic in function like the left kidney of the Pleurotomariidæ, Haliotidæ, and Trochidæ. In the Fissurellidæ this organ is reduced to a mere rudiment, and may, I believe, disappear altogether in some species, for I have failed to find a trace of it in transverse and horizontal sections of F. græca.

Remy Perrier (**14**) has stated that the epithelium of the left kidney of Fissurella is identical with that of the right kidney, but there is some doubt about this, and a renewed investigation of the left kidney of several species of the Fissurellidæ is much to be desired. But there is no doubt that it is a vestigial organ, and that in this respect the Fissurellidæ have been specialised along a different line to the Scissurellidæ, which have retained the left kidney in a fully functional state. Per contra, while the Fissurellidæ retain to a large extent the primitive scalariform character of the pedal centres, the Scissurellidæ have in this respect surpassed them in specialisation, for their pedal centres are concentrated to a degree elsewhere unknown among the Rhipidoglossa. The divergence of the two types is obvious, and one may conclude that both have been derived from a stock very nearly represented by the so-called Emarginuliform larva of Fissurella, which had a spirally coiled shell with a large umbilicus, spiral sculpture and a considerable labral incision. A corneous multi-spiral operculum and a well-developed epipodial ridge bearing sub-ocular as well as posterior epipodial tentacles were present. The left kidney was a well-developed papillary sac, and the pedal centres were

elongate and scalariform. Such an ancestral form would not be far removed from a Pleurotomaria, but would differ from it in the development of a double columellar muscle and in the tendency to acquire a secondary symmetry always correlated with the doubling of this muscle. The Scissurellidæ have retained most of the features of this parent form, but have undergone considerable specialisation in the nervous system. The Fissurellid branch must early have acquired a "sessile" habit, and have been much modified in connection with it, but its members have largely retained the primitive condition of the pedal centres. The Scissurellidæ, though for the most part constant to the primitive type, are also undergoing modification in the same direction as the Fissurellidæ. In Incisura the visceral spire is reduced, the shell is becoming thick and solid, the spiral sculpture is absent, the margins of the aperture are in one plane, the foot is becoming short and broad, and its whole organisation is indicative of a semi-sessile habit. Further specialisation along these lines would give it Fissurelliform or rather Emarginuliform characters. It is interesting to note that another member of the family, Schismope, while retaining its spiral coil and widely open umbilicus, has undergone specialisation in another direction, for the labral slit has been converted into a foramen by the approximation of its edges, so that although distant from the margin it is connected with it by a suture. In this respect it closely resembles Semperia, a sub-genus of Emarginula. Semperia leads on to Rimula, and as we have seen there are Emarginuliform and Rimuliform stages in the development of Fissurella. This is an undoubted example of the developmental stages of one form resembling the adult stages of other forms, a phenomenon the occurrence of which some persons are inclined to deny nowadays, though the evidence in favour of it is very large.

The parallel stages of evolution among the Scissurellidæ and Fissurellidæ afford interesting examples of the phenomenon of convergence, and illustrate a principle which, I think, has not been sufficiently attended to in drawing inferences as to

the affinities of animals from morphological evidence, namely, that a similar environment and similar habits of life reacting on a similar organisation may often produce very similar structural results. Not, however, identical, for however similar the results may appear at first sight in all cases of convergence a close analysis will always disclose differences which exclude the idea of direct descent of the animals in question. This instance is particularly instructive; the Haliotidæ, Scissurellidæ and Fissurellidæ have all inherited the same structure from a presumably Pleurotomariid ancestor, viz. the slit in the mantle and the corresponding labral incision in the shell. It has been variously modified, and similar modifications are displayed independently by different groups, the similarity of the evolutionary series being, as far as one can judge, correlated with the adoption of similar habits.

Addendum.

It is long since I first read the short but profound essay of Sir Ray Lankester (9) "On the Use of the term Homology in Modern Zoology, and the Distinction between Homogenetic and Homoplastic Agreements." On referring again to this essay, I find that the conclusions arrived at in the foregoing paragraph, as also similar conclusions arrived at after a detailed study of various members of the Neritidæ (1), are unconsciously expressed in nearly the same words that he used forty years ago. I have to beg Sir Ray Lankester's pardon for not making specific reference to his essay in my former paper. But I find a certain satisfaction in not having had the form of his argument clearly in my mind while I was working to the same conclusion from evidence gathered from the study of the probable lines of descent of animals belonging to a different class to that which he used to illustrate his original thesis. Had I consciously set out to prove, or even to disprove, his contention, I could not have avoided a certain amount of bias. To have arrived unconsciously—or subconsciously, for the idea of homoplasy inculcated by him was

always present to my mind—at an identical conclusion is to give unequivocal support to the validity of the arguments by which it was sustained. In the essay in question Lankester showed that the term homology, which really belonged to the platonic school of the natural philosophers of the end of the eighteenth and the beginning of the nineteenth century, acquired a new connotation after the publication of the 'Origin of Species.' But this new connotation was indefinite. On the one hand structures were said to be homologous which "are genetically related, in so far as they have a single representative in a common ancestor." For this kind of homology Lankester proposed to substitute the term "homogeny." On the other hand, various organs were described as homologous which could not possibly be included under the idea of homogeny, because, over and above general resemblances such as might be referred to inheritance from a common ancestor, they exhibited a number of detailed resemblances such as could not possibly be supposed to have been represented, in like detail, in a generalised ancestral form. Therefore, Lankester pointed out, there must be a second quantity covered by the term homology, and he described it in the following words: "When identical or nearly similar forces or environments act on two or more parts of an organism which are exactly or nearly alike, the resulting modifications of the various parts will be exactly or nearly alike. Further, if, instead of similar parts in the same organism, we suppose the same forces to act on parts in two organisms, which parts are exactly or nearly alike and sometimes homogenetic, the resulting correspondences called forth in the several parts of the two organisms will be nearly or exactly alike. . . . I propose to call this kind of agreement homoplasis or homoplasy. . . . What exactly is to be ascribed to homogeny and what to homoplasy in the relations of a series of structures is a matter for careful consideration." Somewhat further on in the essay homoplasy is defined as "depending on a common action of evoking causes or moulding environment on homogenous (= homogenetic)

parts, or on parts which for other reasons offer a likeness of material to begin with."

The term "homoplasy" has passed into current use, and the principle expressed by it has been freely used to explain numerous large and general resemblances which have obviously been evolved independently, such as the general resemblances between different kinds of patelliform gastropod shells, e. g. between Patella, Fissurella, Septaria, Capulus, and Siphonaria, or the general resemblances of external morphology of fishes and cetacea. But the term homogeny has not been so generally accepted, and many, if not most, zoologists have preferred to retain the old word homology, and in so doing it is clear that many of them have failed to distinguish between the two quantities contained within the single term, of which the differences were so clearly pointed out in Lankester's essay. For it must be evident to anybody who is well acquainted with the morphological literature of the last thirty years that, so far from attempting to distinguish between homogenetic and homoplastic resemblances, a large number of authors have shown a vast amount of ingenuity in referring the most minute resemblances in the organs of animals, which are certainly not very closely related to one another, to homology. The most extreme instances of this tendency to ascribe every resemblance, however detailed, to inheritance, ignoring the possibility that similar structural changes may be induced by the incidence of similar forces, are to be found in the works of those authors who attempt to derive the lower members of one phylum of the animal kingdom from highly differentiated members of another phylum.

It is, of course, true that several of the most thoughtful and best informed among contemporary zoologists have been fully aware of the error lurking in the indiscriminate use of the term "homology," notably Gegenbauer and Fürbringer in Germany; Cope, W. B. Scott, E. B. Wilson, and Osborn in America. It is not my present intention to enter upon a long discussion of this subject, which I hope to return to on a future

occasion. But I take the opportunity of dealing with an interesting and suggestive essay by Osborn (**10**), in which Gegenbauer's admirable analysis of the different forms of resemblances obtaining among animal structures is largely quoted.

In the first place Osborn makes it evident that I, in common with others, have fallen into an error in using the term "convergence" to denote the parallel stages of evolution among the Fissurellidæ and Scissurellidæ. In the common meaning of the word, convergence might appropriately be used to signify that apparent approximation of structural characteristics which not infrequently leads to two forms being classified together in the absence of sufficiently complete information as to their internal anatomy. But it has acquired a special meaning, defined by Osborn as the "independent similar development of unrelated animals, bringing them apparently closer together." As it has been the purpose of my paper to show that the families of Molluscs treated of are related, and closely related, the term convergence is not applicable to resemblances recurring in those families. But when I come to consider whether other resemblances between various mollusca should be described as due to "parallelism" or "homoplasy" I find myself in a difficulty. Parallelism is defined as the "independent similar development of related animals, plants, or organs"; homoplasy as the "independent similar development of homologous organs or regions giving rise to new parts." It is added that homoplasy always involves homology, while parallelism and convergence may or may not involve homology.

In Incisura the reduction of the visceral spine, the obliteration of spiral sculpture, the levelling of the margins of the aperture, the alteration in the shape of the foot are changes parallel to those observed in the ontogeny of a Fissurellid, and they involve homogenetic organs; the parallelism in this case involves homology and should be called homoplasy. In Schismope the conversion of the labral slit into a foramen is a change parallel to that observed in the

ontogeny of a Fissurellid and it involves a homogenetic character, therefore it also is due to homoplasy. On the same reasoning the resemblances in the shell, foot, and mantle of more distantly related forms, the Patellidæ, Septaria, the Capulidæ, and Siphonariidæ are homoplastic. But should the pallial branchiæ of a Patella and the gill of a Siphonaria, be attributed to parallelism or homoplasy? They are certainly not genetically derived from the typical molluscan ctenidium, and to this extent are deficient in the element of homology which Osborn says should always be associated with homology. On the other hand they are vascular outgrowths of the mantle, which is assuredly a homogenetic structure in all the forms in question, and therefore there is an element, though a more remote element, of homology. In this case it is simply a question of the importance attached to the degree of homology whether these structures should be ascribed to parallel or homoplastic development. But Lankester's term, homoplasy, as originally defined, covers all the cases. It appears to me that, while there is a contrast between homoplasy and convergence, there is no such contrast between homoplasy and parallelism, and that for the sake of clarity the last term should be abandoned, homoplasy being retained in the sense originally defined by Lankester. It has the priority over Fürbringer's term homomorphy, which, as Osborn points out, has the same connotation; and it has the advantage of indicating a resemblance due to the moulding influence of environment, whereas homomorphy only calls attention to similarity of form.

In the latter half of his essay Osborn raises a most interesting question, which has presented itself with various degrees of insistence to workers in various groups of the animal kingdom. Drawing his evidence from palæontological as well as recent types, he points out that the accessory cusps in the molar teeth of Mammalia arise in the same order and with the same relations to the primary cusps in groups which can be proved to have diverged widely from one another before any complication of the tooth pattern arose. Here, then, are

examples of detailed resemblances which cannot be due to inheritance nor yet can they be due to external forces acting upon homogenetic parts, for the teeth are formed below the gum and the cusps are in place before any mechanical forces are brought to bear on them. The characters of the teeth are clearly congenital, and the resemblances between the patterns which have arisen independently in different groups cannot be accounted for by the preservation of fortuitous variations by natural selection, for palæontological evidence shows that variation has in each case proceeded along one line and not along several lines, one of which has been selected.

Calling to mind Lankester's suggestion of the "common action of evoking causes . . . on parts which for other reasons (than homogeny) offer a likeness of material to begin with," Osborn pleads for the recognition of a latent or potential homology, by which term I understand him to mean a tendency or capacity to produce a definite structure, which capacity must have been present in the ancestors of the existing orders of Mammalia, but has only manifested itself in such groups as possessed or were subject to the co-operating factors necessary for evoking the latent capacity, and thus producing the structure in question.

The objections to a principle of this kind are that, in the first place, as Osborn himself admits, it leads us on the dangerous ground of teleological speculation; and, in the second place, that it might, if loosely applied, be used to explain anything or everything by a phrase.

Nevertheless, I think that some such principle may be admitted, with due caution, in explanation of a large number of difficulties which present themselves, with increasing insistence, to every class of zoological workers. In a recent paper on the Neritidæ I alluded to the great difficulty of finding a satisfactory theory to account for the distribution of the fresh-water Neritids, described as species of the sub-genera Paranerita and Septaria, in remote oceanic islands. As their general anatomical and conchological characters

differ in a very small degree from those of the marine species of the genus Nerita, abounding in the seas in which the oceanic islands inhabited by the fresh-water Neritids are placed, it did not seem an unwarrantable assumption that in each locality the marine species had ascended from estuaries into rivers (just as prawns do in so many parts of the tropics), and had been similarly modified as a result of the fresh-water environment. But when I found that the accessory generative organs of the fresh-water species from different localities were always alike, and differed in the same direction and to the same degree from the accessory generative organs of the marine species from the same localities, particularly in the fact that the female gonaducts of the freshwater species are always triaulic, whereas those of the marine species are diaulic, I was no longer able to sustain the opinion that I had first formed as to the possibility of the independent but similar modification of the marine species in different parts of the world. It seemed to me impossible that the triaulic condition should have been evolved several times over. The problem, however, is of the same kind as, though of less magnitude than, that presented by the cusps of mammalian molar teeth. If we can conceive the presence in the germ-plasm of Neritidæ of factors competent to produce the triaulic condition of the genital ducts, but that the activity of these factors is only excited by the co-operating action of other factors—in this case by reduction of the salinity of the water—the detailed resemblances between structures existing in animals living so far apart but under similar conditions are susceptible of explanation.

A few years ago such an explanation would have been inadmissible. But since Mendelian experiments have shown that definite changes affecting parts of the organism in a similar manner may require the co-operation of two or more factors, and cannot be produced unless those factors are brought together; and since such experiments as those of Stockard on Fundulus have shown that a relatively slight change in the salts dissolved in water may induce profound

changes in certain organs of developing embryos, it is no longer possible to reject such suppositions as fanciful and incredible.

Those who have given unprejudiced consideration to the objections raised against the all-sufficiency of natural selection, must have felt that a term is wanting somewhere in the current forms of argument used to explain resemblances between structures which are only doubtfully homogenetic. The missing term may possibly be found when we have a more exact knowledge of the kinds of factors whose co-operation is necessary to produce specific structure. Some of these factors must be germinal, but evidence is accumulating that germinal factors are not simple but compound, and may be split into subordinate factors which, taken alone, do not produce the specific result. There is further evidence that germinal factors react differently to different external factors, and if this be so many kinds of resemblances and differences may be accounted for by laws of interaction of which we are as yet only dimly aware.

The evidence on these matters is insufficient to enable us to arrive at definite conclusions, but it is at any rate sufficient to earn respect for a suggestion supported by such a large number of positive facts as that of Osborn.

I believe that in the future morphologists, in conjunction with systematists, will be largely occupied in attempting to discriminate between the different kinds of resemblances among animal structures, between similarities due to the "common action of evoking action or moulding environment," and similarities due to direct descent, and I venture to think that such morphological studies, carried out with scrupulous attention to detail, are not useless, but will give precision to, and perhaps modify our views on, the causation of modification of animal structure.

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EXPLANATION OF PLATES 1—5,

Illustrating Mr. Gilbert C. Bourne's paper "On the Anatomy and Systematic Position of Incisura (Scissurella) lytteltonensis."

Lettering for all the Figures.

An. Anus. *a. g. f.* Anterior gill-filaments. *au. l.* Left auricle. *au. r.* Right auricle. *B.* Buccal ganglia. *b. b.* buccal bulb. *b. c.* Buccal cavity. *br. n.* Branchial nerve. *b. sk.* Branchial skeleton. *b. v.* Blood-vessel. *C.* Cerebral ganglia. *car.* buccal cartilage. *c. c.* cerebral commissure. *cil.* Cilia. *cil. c.* Ciliated canal of dorsal ridge of gill-filament. *cil. l.* Lateral ciliated cells. *c. pd.* Cerebro-pedal connective. *c. pl.* Cerebro-pleural connective. *di. l.* Left dialyneurous connection. *d. g. r.* Dorsal glandular ridge of gill-filament. *e.* Eye. *ep.* Epipodium. *ep. n.* Epipodial nerve. *ep. t.* Epipodial tentacle. *F.* Foot. *f. c.* Frontal cilia. *g.* Ganglion behind mantle-slit. *g. f.* Gill-filaments. *g. n.* Genital nerve. *hy. g. l.* Left hypobranchial gland. *hy. g. r.* Right hypobranchial gland. *i.* Intestine. *i. v.* Vertical loop of intestine. *j.* Jabot. *jw.* Jaws. *k. l.* Left kidney. *k. r.* Right kidney. *l. b.* Left branchial ganglion. *lb. l.* Labial lobe. *l. c.* Labial commissure. *l. c. m.* Left columellar muscle. *l. ct.* Left ctenidium. *li.* Liver. *li. d.* Liver-ducts. *l. œ. p.* Left œsophageal pouch. *lt.* Lateral tooth of radula. *m.* Mouth. *m. c.* Mantle-cavity. *md. t.* Medio-lateral teeth of radula. *m. f.* Median dorsal fold of buccal cavity. *m. s.* Mantle-slit. *mt.* Mantle. *n.* Nucleus. *od.* Oviduct. *od. o.* Opening of oviduct into right kidney. *œ.* Œsophagus. *os.* Osphradium. *ot.* Otocysts. *ov.* Ovary. *P.* Pedal ganglia. *pa.* Pallial nerve. *pc.* Pericardium. *p. g. f.* Posterior gill-filaments. *p. gl.* Pedal gland. *phg.* Phagocytic cells of left kidney. *pl. l.* Left pleural ganglion. *pl. r.* Right pleural ganglion. *p. n.* Pedal nerves. *p. v.* Pallial blood-vessels. *R.* Rectum. *r. b.* Right branchial ganglion. *r. c. m.* Right columellar muscle. *r. ct.* Right ctenidium. *rd.* Radula sac. *r. œ. p.* Right œsophageal pouch. *rp. d.* Reno-pericardial duct. *sb. i.* Sub-intestinal ganglion. *sg.*[1] Anterior salivary glands. *sg.*[2] Posterior salivary glands. *sn.* Snout. *s. o. t.* Sub-ocular tentacle. *sp. i.* Supra-intestinal ganglion. *sp. l.* Left symmetrical pallial nerve. *sp. n.* Supra-intestinal nerve. *sp. r.* Right symmetrical pallial nerve. *st.* Stomach.

sy. p. Sensory papillæ. *T.* Cephalic tentacle. *tn.* Tentacular nerve. *un.* Uncini. *V.* Ventricle of heart. *v. aff.* Afferent branchial vessel. *v. eff.* Efferent branchial vessel. *v. g. l.* Left visceral ganglion. *v. g. r.* Right visceral ganglion. *v. n.* Visceral nerve.

[All the figures are of Incisura lytteltonensis.]

Fig. 1.—A specimen viewed from the left side and below to show the size and shape of the foot, the operculum, the epipodial tentacles, etc. × about 40.

Fig. 2.—Dorsal view of a female specimen which has been stained and mounted as a transparent object. × 80.

Fig. 3.—A reconstruction of the alimentary tract; semi-diagrammatic. × 80.

Fig. 4.—A diagram showing the relations of the right and left kidneys, the heart, pericardium, ovary and oviduct. The extent of the pericardial cavity is indicated by a thick black line. × 80.

Fig. 5.—The nervous system as determined by reconstructions from sections. × 80.

Fig. 6.—♂. A transverse section through the posterior part of the head, including both eyes. × 135.

Fig. 7.—♂. A transverse section taken just behind the mantle-slit, showing the bi-pectinate character of the right ctenidium. × 135.

Fig. 8.—♂. A transverse section through the anterior ends of the pedal ganglia. × 135.

Fig. 9.—♀. A transverse section through the hinder ends of the pedal ganglia. Note the position of the left kidney, *k. l.*, and its opening into the mantle-cavity; the size and extent of the right and left œsophageal pouches, *r. œ. p.* and *l. œ. p.*; the size and position of the right and left pleural ganglia, *pl. r.* and *pl. l.*, and the supra-intestinal ganglion, *sp. i.*; the pedal ganglia, *P.*, are clearly seen to be composed of a dorsal and a ventral moiety. × 135.

Fig. 10.—♀. A transverse section passing through the posterior end of the mantle-cavity, showing the large size of the left auricle, *au. l.*; the orifice of the left kidney, *k. r. o.*; the pedal nerves, *p. n.*, which are the posterior continuations of the ventral moieties of the pedal ganglia shown in fig. 9. × 135.

Fig. 11.—♀. A transverse section passing through the posterior end of the foot. Note that the large pedal nerves shown in the previous figure do not extend into the hind part of the foot. × 135.

Fig 12.—♀. A transverse section taken near the terminal part of

the visceral spire, showing the opening of the oviduct, *od.*, into the hinder end of the ovary, *ov.* × 135.

Fig. 13.—♂. A section through the left kidney showing the reno-pericardial duct, *rp. d.* Note the band of ciliated cells, *cil.*, on the floor of the mantle-cavity opposite the opening of the left kidney. × 225.

Fig. 14.—♀. A portion of a section through the left kidney showing the rounded phagocytic cells, *phg.*, which have taken up solid rod-shaped bodies from the subjacent blood-vessel, *b. v.* × 1000.

Fig. 15.—Striped muscle-fibres attaching the anterior end of the buccal bulb to the integument. × 1000.

Fig. 16.—A transverse section through the anterior filaments of the left ctenidium. Note the osphradium, *os.*, lying under the branchial nerve, *b. n.* × 535.

Fig. 17.—A transverse section somewhat posterior to that drawn in fig. 16, passing through the posterior filaments of the left ctenidium. In this and the previous figure note, *d. g. r.*, the dorsal glandular ridges of the gill-filaments. × 535.

Fig. 18.—A transverse section through two gill-filaments of the right ctenidium; *cil. c.*, the ciliated canal traversing the dorsal glandular ridges of the filaments. × 1000.

Fig. 19.—The left ctenidium stained and viewed from above as a transparent object. × 225.

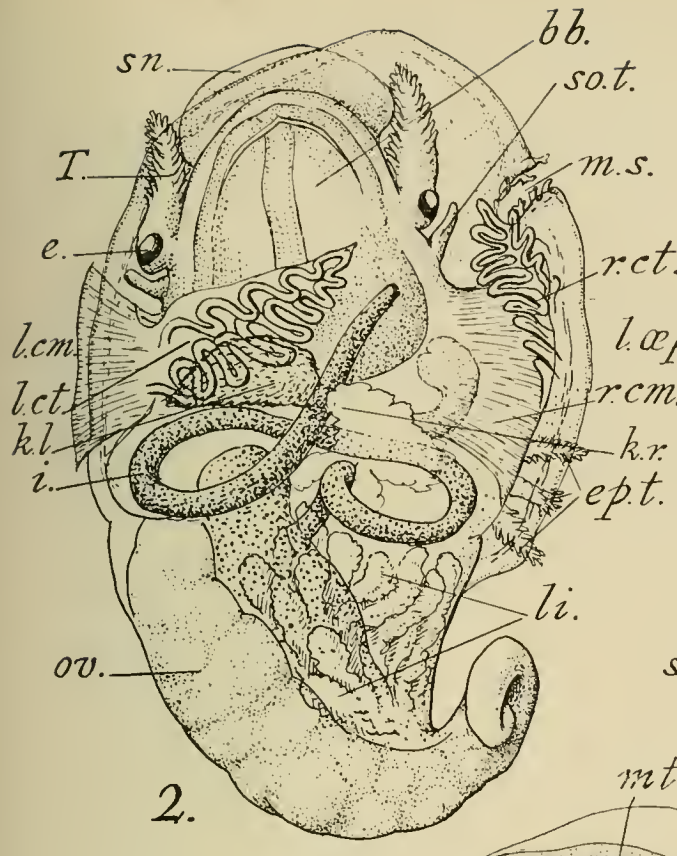
Fig. 20.—A portion of the radula. × 800.

Fig. 21.—Part of a transverse section passing through the anterior end of the buccal bulb to show, *m.f.*, the median dorsal fold of the buccal cavity and, *s. g.*^{1}, the anterior salivary glands and their ducts. × 535.

Figs. 22–26.—A series of transverse sections through the right-hand posterior corner of the mantle-cavity showing the relations of the oviduct and the right reno-pericardial duct to the right kidney and the pericardium. × 225. (These figures are drawn as seen reversed under the microscope.)

Fig. 27.—A cephalic tentacle showing the two multiple rows of sensory papillæ. × 225.

Fig. 28.—A longitudinal section through three sensory papillæ of a cephalic tentacle. n^{1}, pale nucleus of a supporting cell; n^{2}, deeply stained nucleus of a sense-cell; *cil.*, cilia borne at the ends of the sense-cells. × 1000.



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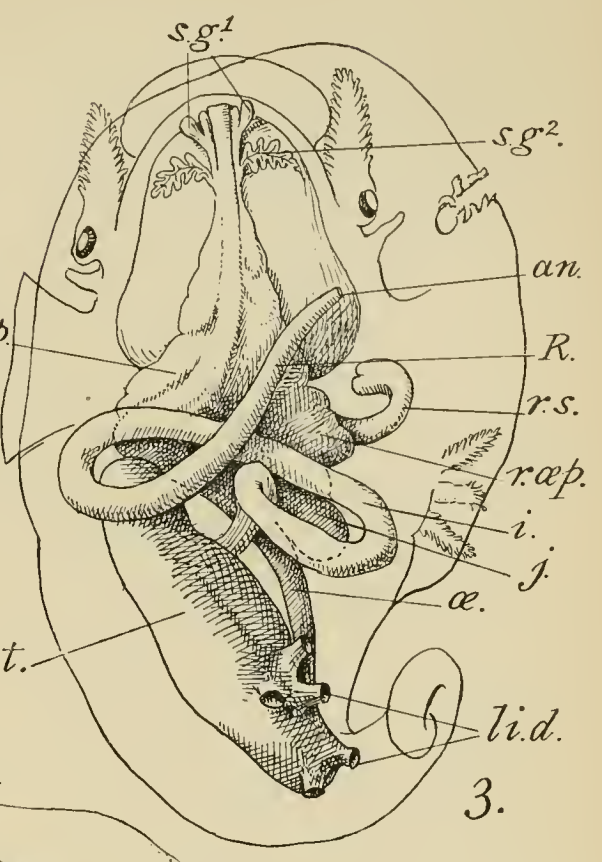

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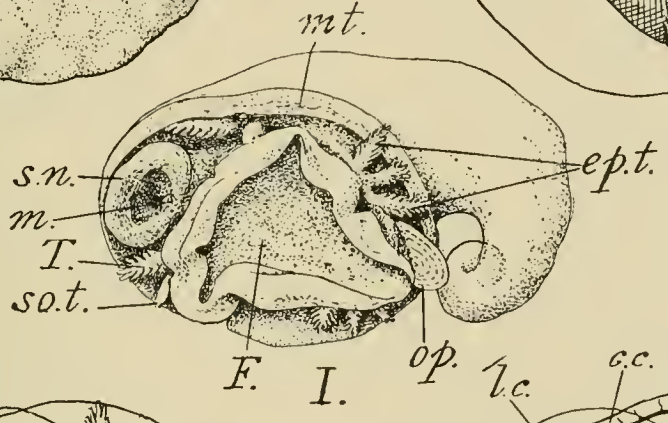

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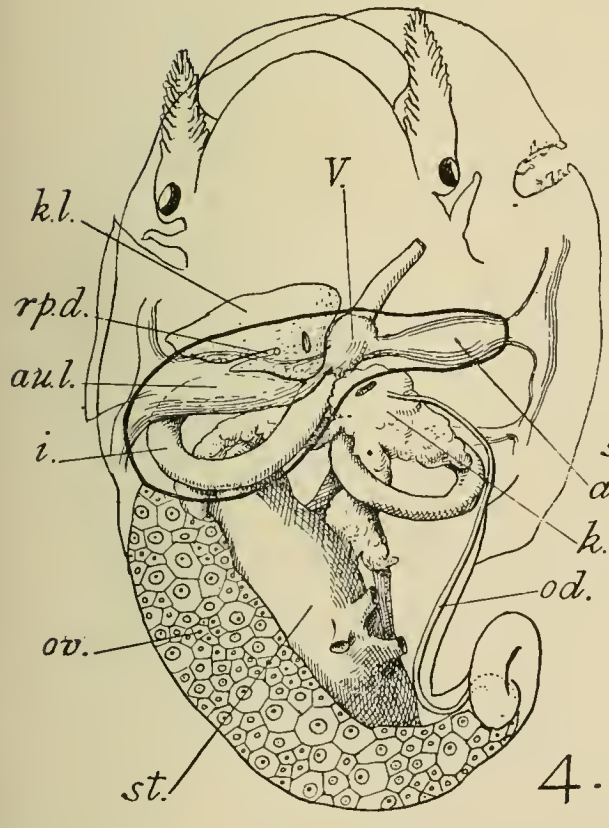

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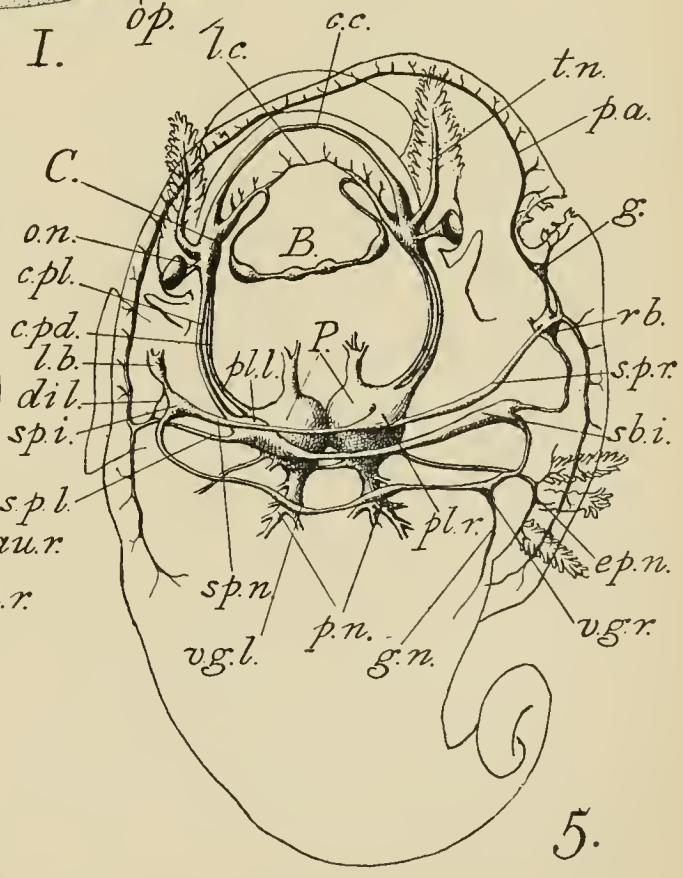

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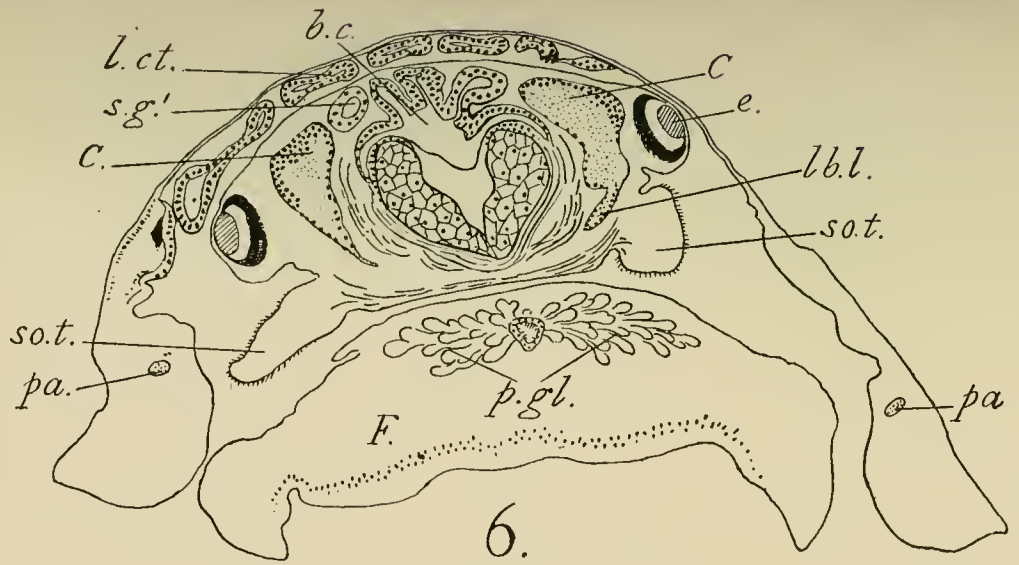
ANATOMY OF INCISURA.



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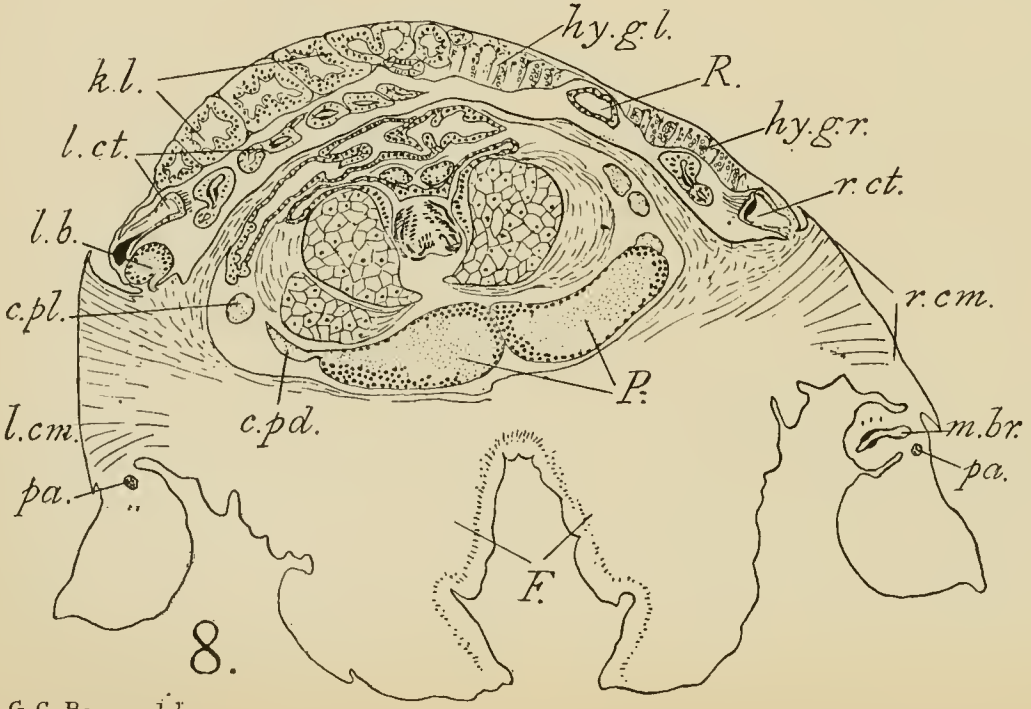

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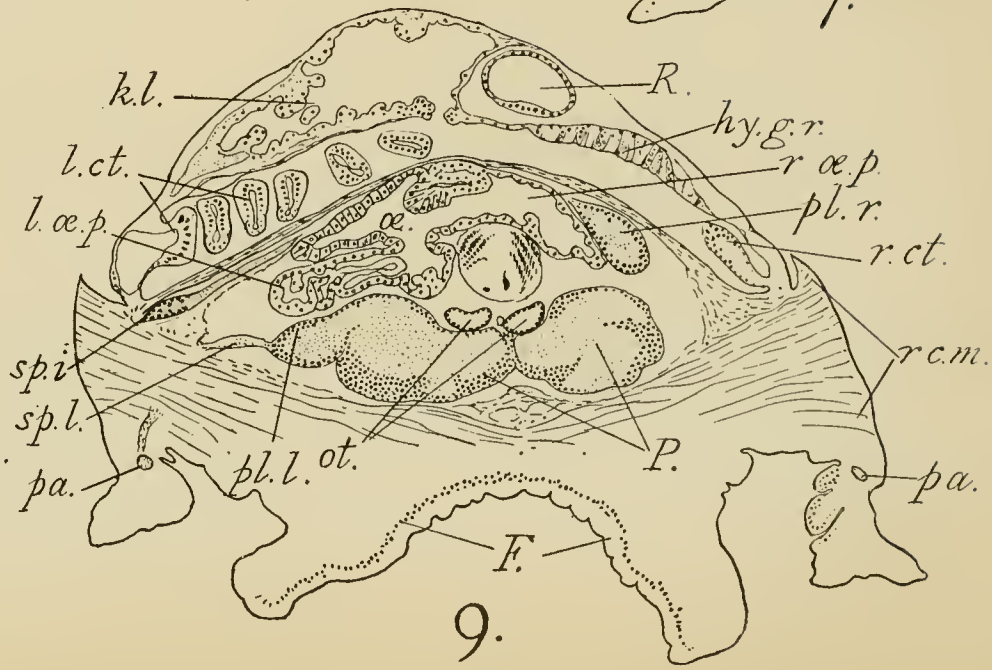

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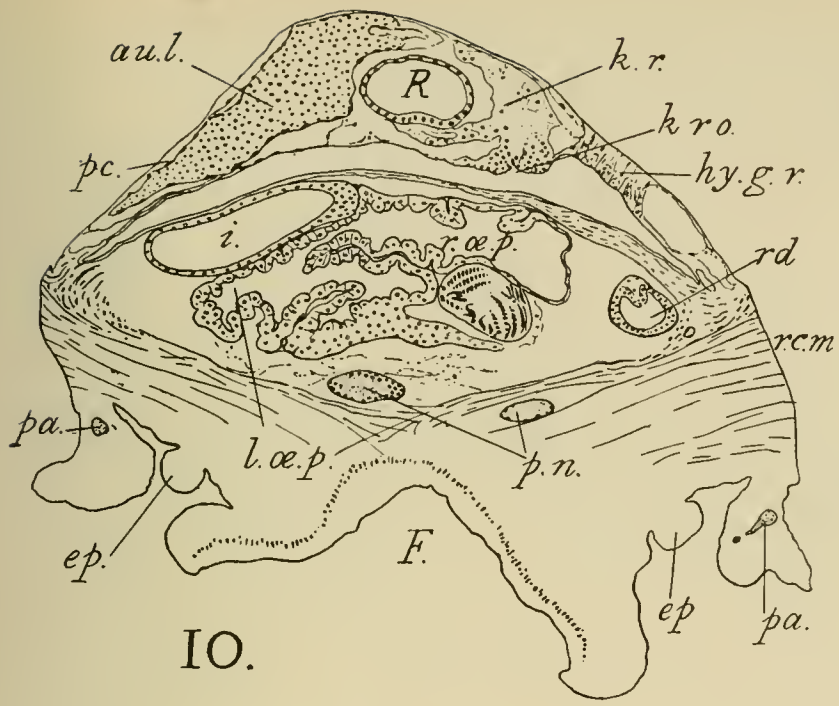

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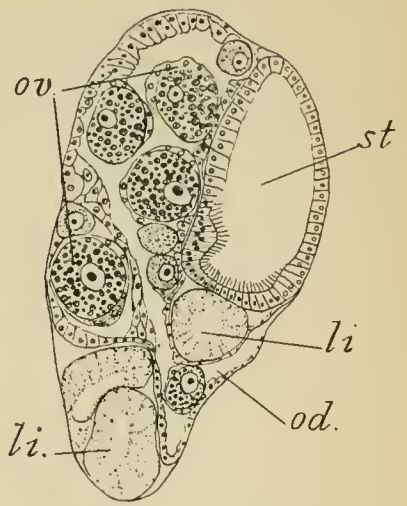

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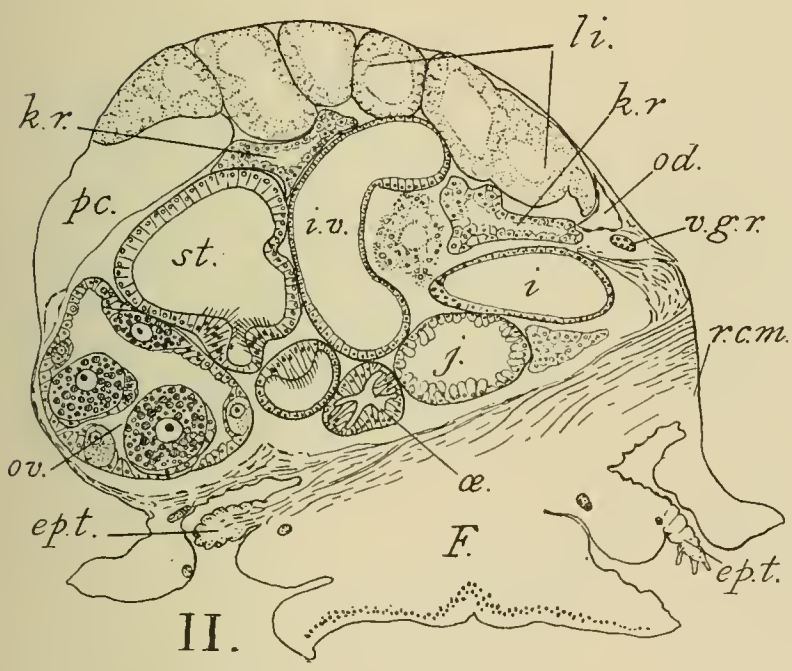

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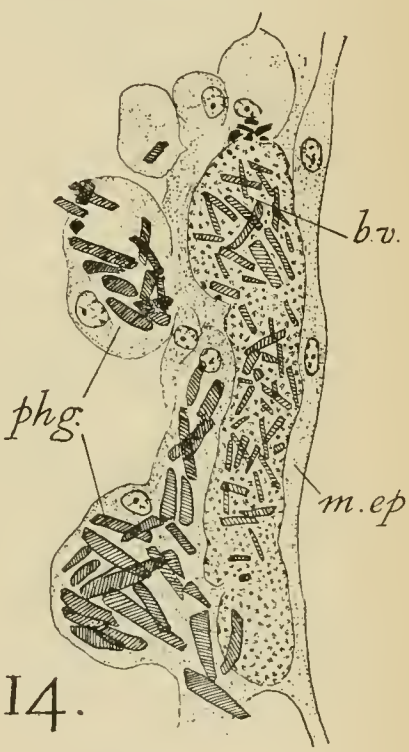

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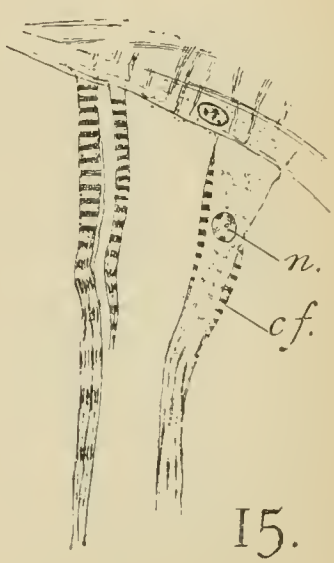

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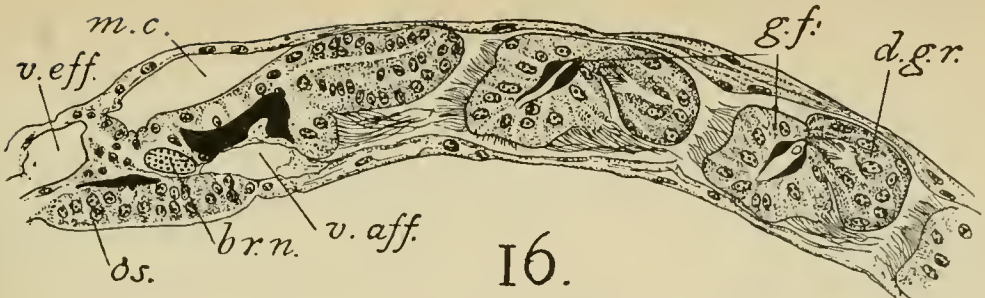

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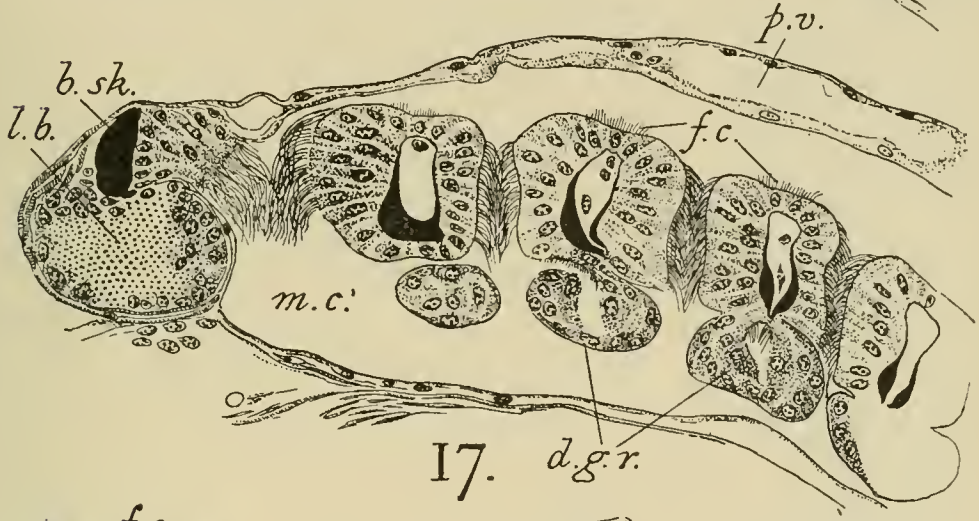

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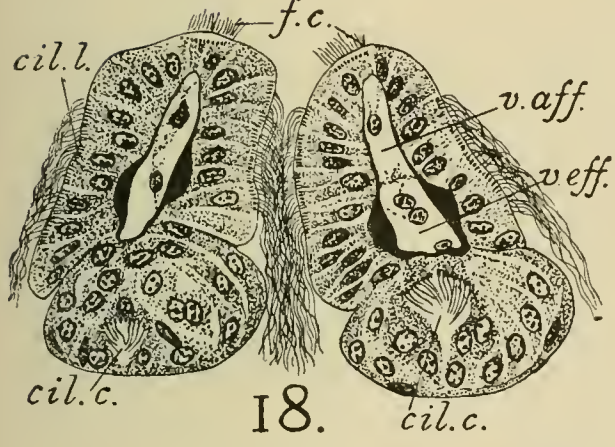

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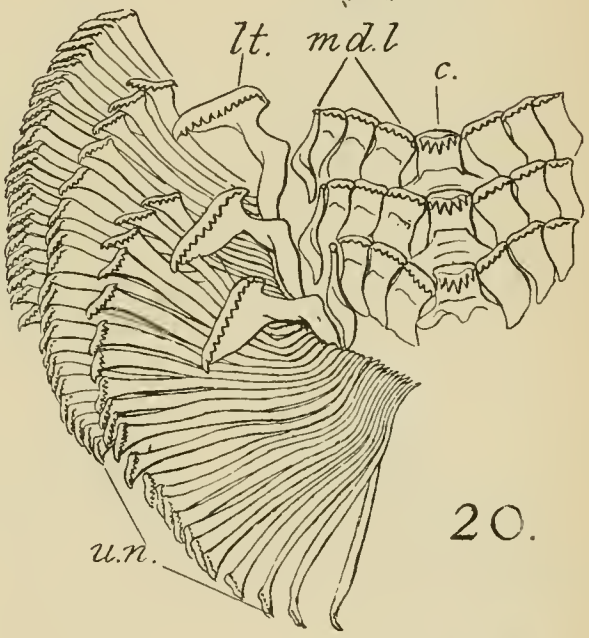

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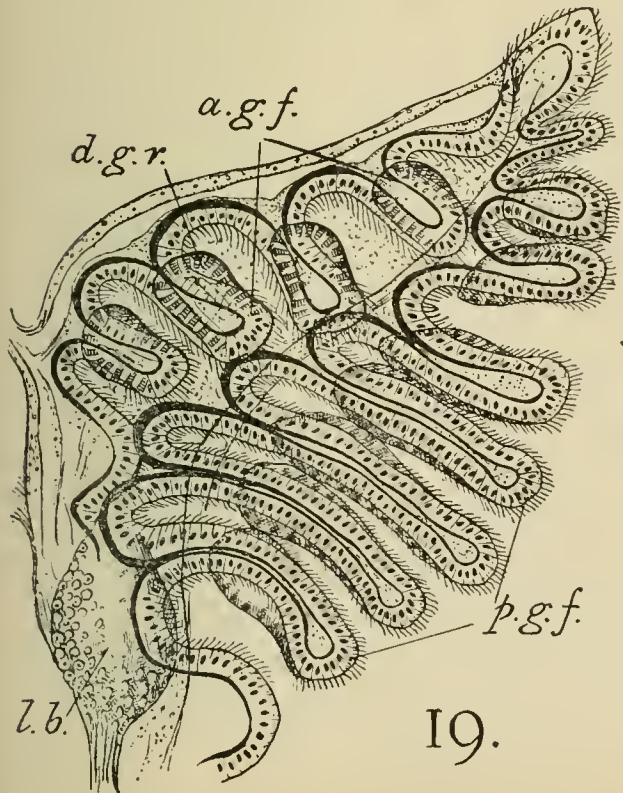

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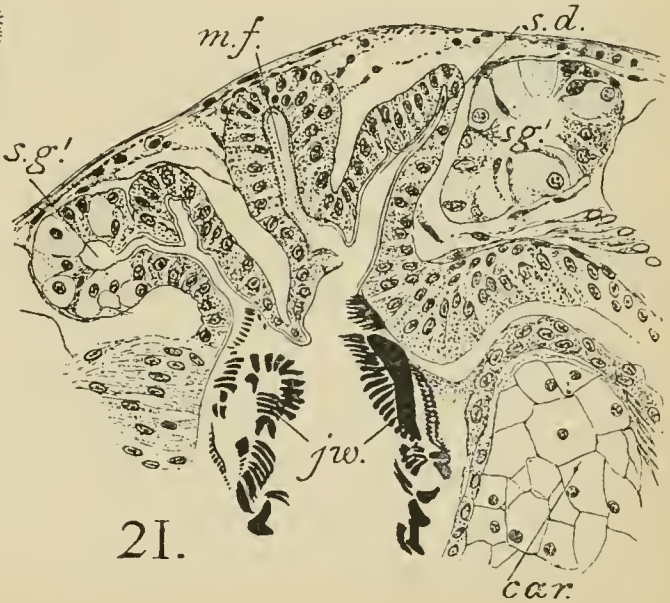

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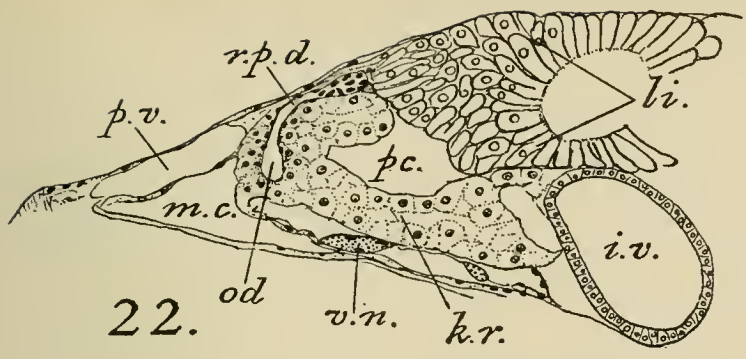

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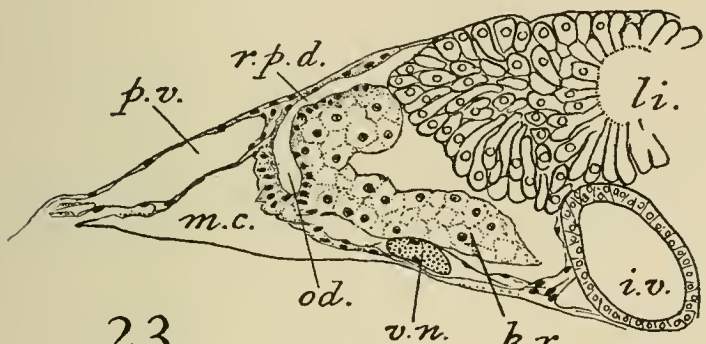

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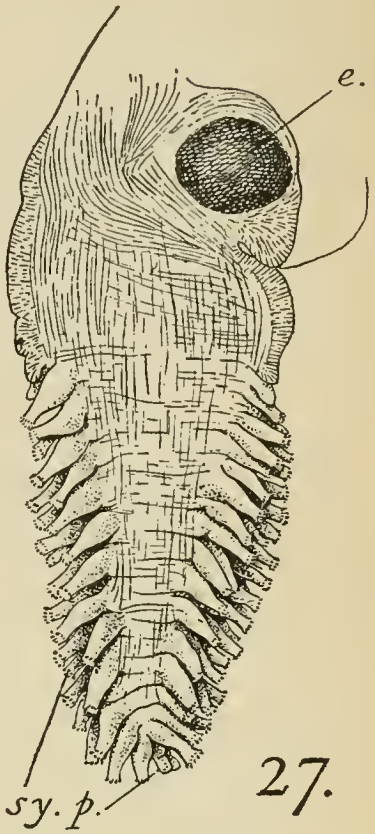

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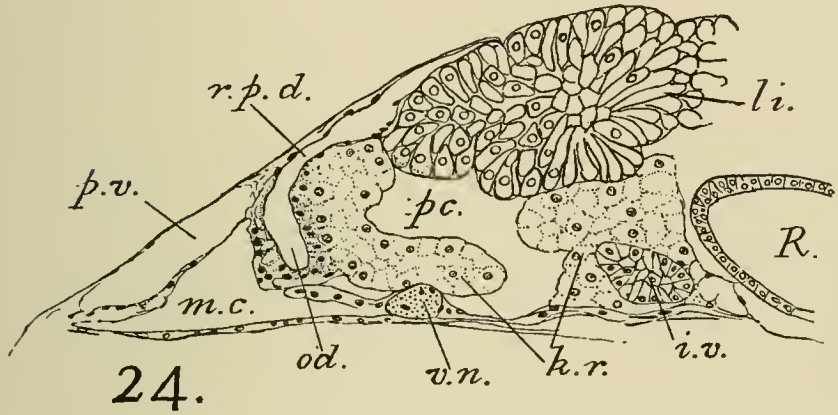

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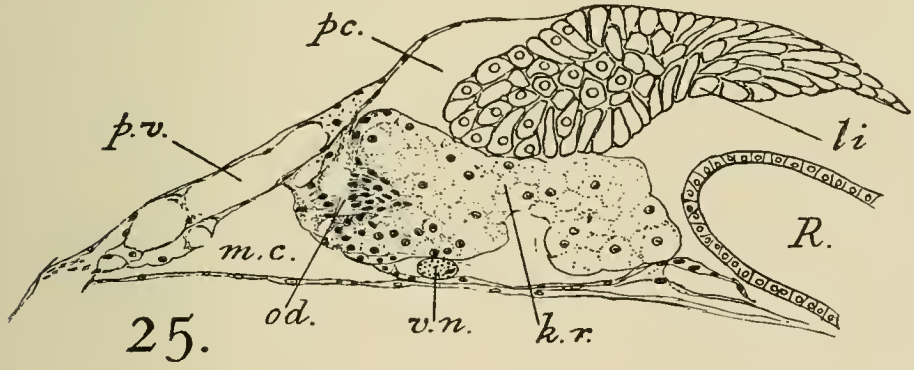

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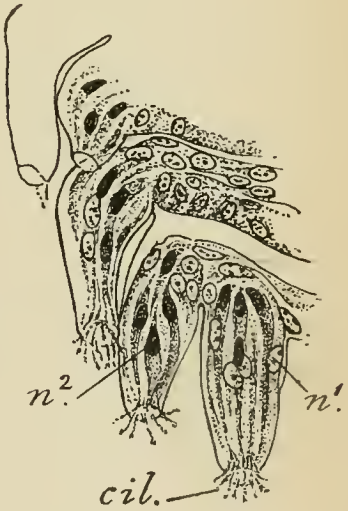

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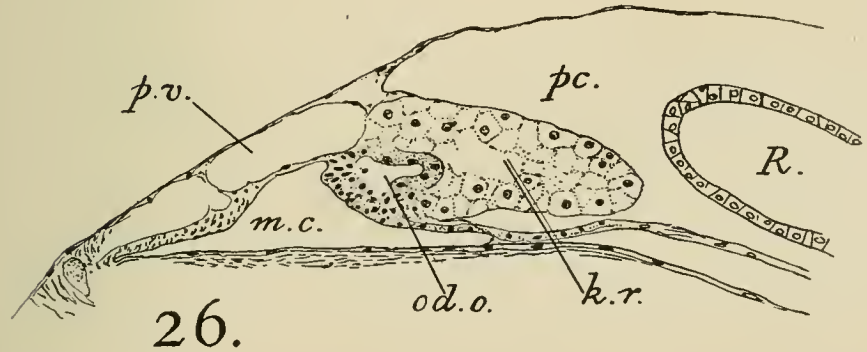

26.

G.C. Bourne del

ANATOMY OF INCISURA.

The Eye of Pecten.

By

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With Plates 6 and 7, and 2 Text-figs.

The first reference to the eyes of Pecten that I have been able to find is that of Poli in 1795. Since that date more than a score of investigators have studied these small organs and treated in more or less greater detail the histology. Each has made new discoveries, which have in very many cases been refuted by their immediate successors, to such an extent, in fact, that it was almost impossible to determine from the literature on the subject the truth in regard to certain parts. One of the last and most reliable papers was that of Hesse, published in 1900 (**34**). He pointed out that some points were still unsolved (though adding one or two discoveries himself), and that the success of the methylene-blue method, if attained, would possibly elucidate all.

In 1904 a paper appeared by Miss Hyde (**39**), embodying the results of a successful employment (according to the author) of the methylene-blue methods for nerve-endings in the retina, but these results were certainly not those expected by Hesse nor probably by other authors, for they stand in striking opposition to the views previously held. Whilst working at a memoir on Pecten in 1907, I came to the conclusion that this, the latest investigation of the Pecten eye, differed greatly from the preceding ones, and that only one

more confusing series of results had been added to the already existing multiplicity.

I determined therefore to make a complete study of the histology of the eye. The privilege of occupying the British Association Table at Naples enabled me to carry out this investigation on a species previously examined by most writers on the eye—Pecten jacobæus—and this was completed by a considerable stay at the Port Erin Biological Station. The results have been the discovery of several new points, the confirmation and refutation of many discoveries of different workers, and I hope the complete elucidation of the structure of the retina. It has been due to the frequency of occurrence of artefacts and the difficult histological work required for such complicated organs that the structure of these eyes has remained so long a puzzle.

By the use, however, of numerous methods it has been possible to eliminate to the greatest extent the artefacts, and incidentally the trial of so many fixatives, etc., has enabled me to obtain practically all the appearances seen and figured by the various investigators.

The account of the structure will be given at some length, since a comparison of the various views is necessary, and, with the exception of Hickson's and Patten's papers very little has appeared in English. I am indebted to the British Association for permission to use their table at the Zoological Station of Naples, and also to the staff of that well-known institution. My thanks are also due to Professor Herdman and to the Curator of the Port Erin Biological Station for the trouble taken in supplying me with material and apparatus for carrying out detailed work at the latter place, and to Professor Drew, of Maine, for specimens of P. tenuicostatus.

History.

Only the history of the references to the Pecten eye before and including the fundamental paper of Hensen will be given in this section, since the other works will be discussed more

fully when describing the structures involved, and it will avoid repetition if they be omitted here. In 1795 Poli, in his large work on the Mollusca (1), gave figures illustrating the general anatomy of Pecten, in which the eyes are depicted, and also a view of the mantle-edge showing more clearly the tentacles and eyes, but no details of structure are given whatever except the external pigmented ring bounding the cornea and the pigment stripe on the tentacles.

He recognised a likeness to the human eye, and as usual applied some of the names given to parts of the latter, a feature followed by his successors, who naturally recognised at once the resemblance to the vertebrate eye, which is such a striking character of the eyes of Pecten. These organs were mentioned, though left practically undescribed by succeeding naturalists. Cuvier refers to them as "globules verdâtres," and Lamarck as "tubercules oculiformes."

The next description is to be found in Robert Grant's 'Comparative Anatomy' (2), where reference is made to the "smooth cornea," the "iridescent choroidea," and a "small crystalline lens." Another English writer, Robert Garner (3), 1837, continued the work. He states that Pecten, Spondylus, and Ostrea (probably Pecten jacobæus, Ostrea jacobæus of Poli) possess "small, brilliant, emerald-like ocelli, which, from their structure, having each a minute nerve, a pupil, a pigmentum, a striated body, and a lens, and from their situation at the edge of the mantle, where alone such organs could be useful, and also placed, as in Gasteropoda, with the tentacles, must be organs of vision." There are no figures illustrating his short account. Almost simultaneously Krohn (5) and Grube (4) published descriptions of the eye. Grube described the position and number of the eyes in P. jacobæus, P. varius, and P. opercularis. Krohn gave a much more detailed account. He stated that the eye was a closed spherical vesicle containing two transparent bodies separated by a septum (he was therefore the first observer to see this structure). The hinder of these bodies he described as being of fibrous texture. Krohn was the first investigator to notice

that the nerve in the eye-stalk divided into two branches, one of which ran up to the optic vesicle, where he lost it, whilst the other passed up the side and entered the vesicle, lying on the septum.

Will (6) noticed the cellular structure of the lens, and Keferstein (12) recognised the retina in the hinder transparent body of Krohn. This brings us to Hensen's paper (13) published in 1865, which is the first account of the histology of the retina. Hensen divided this part of the eye into five layers:

1. First cell layer.
2. Second cell layer.
3. Rods.
4. Tapetum.
5. Pigment layer.

The cells of the first layer, which may be arranged in a single or double row, are spindle-shaped. The second layer is made up of cylindrical cells (the rod-cells), the third layer is that of the rods, and then follow two others—the tapetum (first demonstrated by Krohn), and the pigment layer.

The innervation is described as follows: The proximal branch of the optic nerve does not bore through the optic vesicle below, as Keferstein had assumed, but splits into a number of small branches which enclose the lower part of the optic vesicle, and these branches of the nerve form a plexus in the peripheral region of the retina. Apparently Hensen assumed that they were connected with his second cell-layer (the rod-cells)—"Der Zellenausläufer geht so continuirlich in den Nerven über, dass man nicht sagen kann, wo der eine anfängt und der andere aufhört."

The other nerve-branch penetrates the septum, and the fibres become connected to the cells of the first layer. Hensen, it will be seen, discovered the different groups of cells in the retina, described the nerve innervation correctly (though since he did not recognise two types of cells in the outer layer and in the rod-cell layer, this was probably more accidental than otherwise), and saw the axial fibre in the

rods—truly a marked advance in the knowledge of the eye-structure.

Technique.

This investigation of the eye has been carried out by the study of sections (paraffin and paraffin-celloidin), by maceration preparations and by the teasing of fixed material.

It is impossible to over-estimate the value of macerations in conjunction with section work, and the true shape of many cells could not have been determined without this method. For both fixation and maceration it was found that different reagents were necessary according to the cells to be studied. In the retina alone the various elements reacted very differently to fixatives and macerating fluids, and it was surprising to notice how different the preservation of the different cells might be after treatment with the same fixative.

The fixatives giving the best general fixation of all parts were Zenker's fluid and Carnoy's mixture. Zenker was used as follows: Fixation lasted for about twelve to twenty-four hours, and was followed by washing first with water and then in alcohol of gradually rising strength. Sections were made after paraffin embedding, the usual thickness being that of the rod-cells, namely 6 μ, but others were only 2 μ, and some were 10 μ thick. The stains used after Zenker were Mallory (connective-tissue stain), iron hæmatoxylin (Heidenhain), a modified Weigert, and picric acid—säurefuchsin.

Mallory's connective-tissue stain.—The sections, on slides, were stained in an aqueous solution of säurefuchsin, 0·05 per cent., for ten minutes, then rinsed quickly in water and placed in 1 per cent. solution of phosphormolybdic acid for three to five minutes. After washing in several changes of water for five to ten minutes the sections were stained in the following solution for eight to fifteen minutes:

Aqueous aniline blue (Grübler)	0·5 gr.
Orange G.	2·0 gr.
Oxalic acid	2·0 gr.
Water	100·0 c.c.

This was followed by a rapid washing out in water, dehydration in 90 per cent. alcohol to absolute, and mounting after xylol or origanum oil in balsam.

Iron hæmatoxylin.—The sections were mordanted for twenty-four hours in a 4 per cent. solution of iron alum washed in water, and stained in a 0·5 per cent. to 1 per cent. solution (aqueous) of hæmatoxylin for twelve to twenty-four hours. This was followed by differentiation under microscopic observation with 2 per cent. iron alum solution. Tap-water, alcohol dehydration, etc., as usual.

The modified Weigert method was only used after Zenker fixation. It was partly like that used by Schreiner (**30**), but modified in combination with Zenker.

Schreiner used a 10 per cent. alcoholic solution of hæmatoxylin (P. Mayer says that the "10 per cent." must be a misprint).

I used a 5 per cent. solution, but did not investigate the effects of a stronger nor of a 1 per cent. solution, which Mayer believes to be the one intended by Schreiner.

The sections (on slides) were placed in a 3 per cent. solution of potassium bichromate for twenty-four hours, then rinsed in water and alcohol, and placed in a 5 per cent. solution of hæmatoxylin (alcoholic) for a time varying from ten minutes to an hour. The sections must be black, and this takes place much quicker after the hæmatoxylin solution has been used once or twice and is oxidised by contamination with bichromate. After staining, the sections were rinsed in water and placed in a saturated aqueous solution of copper acetate, which turns them a steel-blue colour. Differentiation was carried out (under microscopic observation) in the following solution:

Borax	2·0 gr.
Pot. ferricyanide	2·5 gr.
Distilled water	100·0 c.c.

The sections were then washed in tap water and mounted in the usual way, after alcohols and xylol, in Canada-balsam.

Picric acid—säurefuchsin (van Gieson).—The sections

were stained in Delafield's hæmatoxylin and washed well in tap water. This was followed by staining for five minutes in a mixture of—

1 per cent. solution (aqueous) säurefuchsin .	5·0
Saturated solution of picric acid in water .	100·0

The stained sections were washed in tap-water and taken up to balsam as usual.

Carnoy's fixative was used in the following strength:

Chloroform	10·0
Acetic acid	30·0
Absolute alcohol	60·0

This is the best fixative for the retina. Iron hæmatoxylin and Bethe's toluidin blue were the stains used on material so fixed.

Bouin's fluid ('Lee,' edit. vi., p. 76) gave excellent results for rod-cells and rods, especially when followed by Mallory's stain. The axial fibre of the rods was stained better by the säurefuchsin in this method than by any other except the modified Weigert. Zenker's fluid, Mann's fluid,[1] and a mixture of equal parts of corrosive sublimate saturated aqueous solution, and Hermann's platinum-osmic fluid were useful for the lens, especially the latter.

Other fixatives used were 4 per cent. formol, corrosive sublimate (aqueous solution and solution in salt water), Mayer's picronitric mixture ('Lee and Mayer,' ed. vi, p. 68), Flemming, Von Rath's picro-platinum-osmic mixture, and treatment with pyroligneous acid. The latter did not give particularly good results. There were also special fixing and other processes connected with the following methods—Golgi's silver process (Cajal's modification), Bielschowsky-Paton silver method for neurofibrillæ (**41**), Apathy's nachvergoldung and hæmatein IA methods, Nabias' gold method, Lists' eosin method, and methylene blue processes. The latter were failures, though injection methods, staining in aqueous solutions, solutions in Pecten serum, and dusting powder over the eye were all tried. The results given by the other and more

[1] Mann, 'Physiological Histology,' p. 96 (solution d).

ordinary methods were more complete than by the complicated ones, and there was usually a far greater freedom from artefacts. There remains finally the maceration methods to be referred to. The lens-cells, with all their peculiar processes, were easily isolated after immersion of the eyes directly in a 3 per cent. solution of chloral hydrate in sea-water for about four hours. The same solution was used for the retinal cells, and the eyes were placed, as above, directly into this medium. After two hours the retina was dissected out from the eye, placed in a drop of water on a slide, and a cover-glass supported by wax feet placed above it. Gentle tapping on the cover-glass separated the elements. Chromic acid solutions in sea-water of $\frac{1}{50}$ per cent. strength gave very good results for macerations of the rod-cells and rods.

This was also used as advised by Patten after fixation of the eyes in $\frac{1}{5}$ per cent. chromic acid for five minutes.

The maceration preparations were examined unstained, and stained with picro-carmine.

The chief species examined have been Pecten maximus and P. jacobæus, with the following others: Pecten opercularis, P. varius, P. tigrinus, and P. tenuicostatus.

Position and Number of Eyes.

The eyes of Pecten occur on the mantle-edges of both valves. The mantle-edge can be said to be divisible into three folds, the periostracal fold, the ophthalmic fold, and the velum (Pl. 7, fig. 2, *V.*). All three possess tentacles, those situated on the first two being long and mobile sensory structures, well provided with sense-cells for the perception of tactile and olfactory stimuli, whilst those on the velum are short and rather immobile.

The eyes are situated on the median fold, between the periostracal groove and the base of the velum (Pl. 7, fig. 2, *Eye*), and amongst the long tentacles. Poli in 1795 noticed a certain resemblance of the eye-stalks to the tentacles, and considered them as modifications of the latter.

The number of eyes present varies considerably for the

different species, and there is, further, considerable variation among the individual members of any species.

Carrière (**21**) stated that those species with large eyes possessed fewer than those with small eyes; that there were always more on the upper mantle-lobe than on the lower; and that in general, large specimens had more eyes than smaller ones of the same species.

This latter sentence was an important assertion, since it implied growth and development of new eyes during life, and certainly it appeared supported by the fact that large and small eyes exist side by side.

Patten (**22**) also pointed out that there were more eyes present on the left valve than on the right, and that they were larger, but he disagreed with Carrière, stating that no new eyes develop after a size of 2 centimetres has been attained. Rawitz (**25**) found similarly more eyes on the left mantle-lobe than on the right, and agreed with Patten on the development. Schreiner (**30**) agrees also with reference to the number of eyes on the two mantle-lobes, but states that those of the right are not smaller than those of the left (Patten). Had Schreiner examined P. jacobæus, the chief species investigated by Patten, he would not have made this assertion. The eyes are always more numerous on the left mantle-lobe than on the right, as all observers have found. The exact relations, however, vary in different species. The eyes are situated in three groups, on each mantle-fold, one group on the anterior auricular area (two to seven eyes close together), another on the posterior auricular area, close up against the hinge-line, and the third and largest group along the ventral margin of the mantle. Spaces without eyes separate these three regions. In each series the eyes vary considerably in size. Patten (**22**) asserted, in fact, that a regular arrangement of small and large eyes existed, and Rawitz (**25**), though denying the existence of Patten's arrangement, stated that a large eye was always followed by a small one. I have examined all the species referred to by Patten and Rawitz and find no such arrange-

ment. There is a quite irregular series, and a small eye may be followed by another small one or by two large ones, or a group of large eyes may exist together. The eyes on the left mantle-lobe exceed in number those on the right, in particular in species with the most inequivalve shells (as far as the species I have examined are concerned), that is, in P. maximus and P. jacobæus, and this difference in numbers is greatest in P. jacobæus.

The eyes in this species are far less numerous on the right lobe, and are also very much smaller (Pl. 7, fig. 2) (contra Schreiner).

I believe, however, that the greater number of eyes on the left mantle-lobe is due primarily to the fact that this valve is always uppermost, and not to its shape; and if a Pecten is turned over on to the left valve, it very soon rights itself by a peculiar turning movement. Patten (**22**) connected this numerical superiority of eyes on the left valve with its position but was puzzled to see how this could be an advantage to the animal, since the eyes on the lower mantle-fold received the light direct from above, and the eyes on the upper one were apparently directed downwards.

Schreiner (**30**) also figures them as lying pointed to the ground and at an angle of 45° to the valve. If a Pecten be watched as it opens the valves, it will be seen that the eyes of the left mantle-lobe project just outside the shell, and their field of view is practically as much above the animal as that of the eyes in the right valve. The upper valve is also a little shorter than the lower one, and lies inside it when the shell is closed; the mantle lining the lower valve is retracted accordingly to a greater extent when the shell is closing. The valves of the almost equivalve species meet, however, ventrally, and the conditions appear either more favourable to the eyes of the right mantle-lobe than in P. jacobæus, or else, as will be referred to again, this form is an older and more specialised one, and the eyes have begun to degenerate in the lower valve. Some figures are appended which will give an idea of the number of eyes in the three

groups on the mantle-edge of P. opercularis and also of the individual variation in this species (the specimens were from the Irish Sea).

Length of ant.-post. diameter. of shell in cm.	Left mantle-lobe.			Right mantle-lobe.		
	Total No.	No. on ant. ear.	No. on post. ear.	Total No.	No. on ant. ear.	No. on post. ear.
7·5	59	6	4	42	0	3
6·4	50	4	6	41	0	4
5·8	48	4	4	39	0	3
3·8	37	4	5	31	2	5
6·4	54	2	5	40	0	4
5·8	50	4	4	39	1	4
4·25	44	4	5	41	2	4
5·1	52	3	6	42	2	4
5·1	52	4	5	50	2	6
5·25	45	3	4	39	2	3
5·15	54	5	5	35	1	3
5·15	53	5	6	48	3	3
5·85	55	5	5	49	3	4
7·25	58	7	5	47	2	5
4·10	59	4	5	52	1	5
5·0	55	4	3	50	2	4
7·4	51	2	4	38	1	5
5·25	61	7	7	47	4	5
4·75	55	5	6	43	1	4
4·70	54	5	5	42	1	4
5·3	62	6	9	39	1	4
4·4	51	6	5	41	1	5
4·9	57	5	5	43	1	4

It will be seen from these figures that there is no relation between the size of the animal and the number of eyes, though if the first five only had been taken the reverse would have appeared to be the case. Possibly Carrière only examined a few and chanced to get an accidental series. No one appears to have examined the very small eyes occurring with the large ones. I sectioned some of those taken from the right mantle-lobe of Pecten jacobæus and found that they agreed in every respect with the large eyes of the left lobe, all parts being represented and in the normal positions. The

only difference was in the number of cells present; they were apparently as large as usual but fewer in number. These eyes, in fact, appeared to be young ones, or rather, they had been arrested in development and had remained with the small number of component cells characteristic of young eyes, though they were just as old as the large ones.

In examining hundreds of eyes one meets some strange abnormalities, though the latter are of rather rare occurrence. In a specimen of P. opercularis two eyes were fused together so that the pupil was oval with a slight constriction indicating the boundary of the separate organs. Often the eyes appeared with very little black pigment—that is, all the eyes of a specimen, even the "iris" cells being almost unpigmented.

I never found any of the eyes completely covered with pigment as stated by Patten, nor has this feature been met with by any of his successors.

General Structure of Eye-Stalk.

The eyes are situated at the ends of short stalks (Pl. 6, fig. 1), which, as already pointed out, were considered by Poli as modified tentacles. This eye-stalk is made up of connective tissue, which is a direct continuation of that of the mantle-edge and is clothed by an epithelial layer, also a direct continuation of the pallial epithelium.

The connective tissue is more homogeneous or hyaline in appearance than that of the tentacles, and is not broken up so much by crossing muscle-fibres, which, as might be expected, are a prominent feature of the retractile tentacles. This homogeneous tissue extends also below the eye-stalk for some distance, and the transverse muscle-fibres which raise the velum are absent under the eyes, being arranged in bundles situated between these sense-organs. Large blood-spaces occur irregularly scattered in the stalk, communicating with one another and usually containing blood-corpuscles (Pl. 6, fig. 1, *Lac.*). There is, however, scarcely such a

defined space as a "Hauptader des Augenstieles" to which these lacunæ belong (Rawitz [**25**], p. 105). Neither do they always surround the nerve (Schreiner, p. 11).

Whilst the long sensory tentacles are, in the living animal, continually in motion, being retracted and again extended, and moved from side to side, the eyes are practically motionless and point fixedly in one direction only. They contract and may move away from a point of stimulation, this being rendered possible by means of muscle-fibres, which lie longitudinally arranged, near the epithelium (Pl. 6, fig. 1, *Mus.*). The latter are narrow fibres, and are not striated, as figured by Patten. Striated muscles do occur, though elsewhere, in the mantle-edge of Pecten (**45**). The muscles occur on all sides of the eye-stalk. They terminate, according to Rawitz, always at the proximal end of the optic vesicle and are never to be found higher ([**25**], p. 105). Rawitz has presumably taken the finer muscle-fibres, which do extend up to the cornea, for connective-tissue fibres. Schreiner found practically no muscles in small eyes ([**30**], p. 11), and states that in P. islandicus, where they were exceptionally well developed on the shell side, they could be traced to the entrance of the distal branch of the optic nerve. I have traced them to this point in P. maximus, but more delicate fibrils (Pl. 6, fig. 1, *M.f.*), staining quite differently from connective-tissue fibres, extend under the epithelium as far as the edge of the cornea, and are, moreover, present between the cornea and the lens (Pl. 6, fig. 1, *N. Lf.*; Pl. 7, fig. 7, *Lf.*). These are evidently the "fine smooth fibres" mentioned by Patten in contradistinction to his "long striated muscle-cells" of the lower part of the eye-stalk. These fibrils do not, however, enter into any connection with the epithelial cells bounding the cornea, and Patten's "ciliaris" does not exist. They will be referred to again when discussing the fibres situated between the lens and cornea.

Ganglion cells do not occur scattered in the connective tissue of the eye-stalk, a fact already noted by Patten's successors, who criticised his observations on this, as on other

details, somewhat severely. The epithelium covering the eye-stalk is a direct continuation of the pallial epithelium, but is modified in various regions of the eye-stalk and becomes a transparent cornea over the free pole. Below the optic vesicle the cells are small and cubical, or rather deeper than wide (Pl. 6, fig. 1). They contain no pigment here, and the nucleus is situated near the base. A distinct cuticle is present. Some little distance below the optic vesicle these cells increase in depth and at the same time begin to contain pigment. This pigment extends further down that side of the eye which is uppermost (see fig. 1, Pl. 6; the right-hand side is the shell side of the eye and also the uppermost, since it is an eye from the left valve). At the level of the middle of the optic vesicle, that is, about the plane of the septum, the epithelial cells have attained their greatest depth and are almost filled with dark pigment, occurring in the form of fine granules. The external portion of the cells is usually less thickly crowded, and if the sections are stained to bring out the nuclei it will be seen that these have moved, with the acquisition of pigment, so that they reside near the surface instead of at the basal end. The statements of Rawitz and Schreiner in regard to the colour of this pigment in the different species appear to me to be of little importance, and in any case I can hardly confirm them. The colour of the granules in Pecten jacobæus, P. maximus, and P. opercularis is dark brown, and the exact shade varies in any one species and according to fixation and preservation; moreover, the cells are completely filled in P. jacobæus, or at least those of the upper side of the eye-stalk.

Another point that may be noted here is that the increase in height of the epithelial cells opposite the optic vesicle is common to all the species I have examined, though Rawitz states that in P. jacobæus the epithelium is everywhere the same in height and figures it as such ([**25**], p. 106). Pecten abyssorum possesses (Schreiner) no pigment in the cells of the mantle-edge or of the eye-stalk. Patten appears to be the only one who has noticed that there is more pigment

present on the upper side of the eye-stalk, and there is really a longitudinal band present, exactly similar (though not so definite) to the one on the corresponding side of the tentacles. The pigmented area bounding the cornea was termed the "iris" by Patten. Since, however, as described above, these pigmented cells extend far down the eye-stalk on both sides, it is difficult to make any division into regions or to define a boundary. If, moreover, the physiological action of the iris were considered solely to be that of a diaphragm, keeping out oblique rays, the name might perhaps be applied, but, as Rawitz pointed out, there is no proof whatever of this area being capable of contraction with diminution of the "pupil," and since this region is not to be homologised with the vertebrate structure of the same name it is better to use the term pigment-mantle (Pl. 6, fig. 1, *P. man.*) if a special one is necessary. Patten considered that the "pupil" could be diminished to almost half its previous diameter (p. 571), but I have been unable to find any trace of this under natural conditions, nor do any other authors appear to have been more fortunate. The same writer states that on the shell side even in fully formed eyes the pigment may sometimes be absent so that a colourless fissure is left—termed by him the "choroid fissure" (p. 578). I have not seen this in any eye examined, and fail to find any references confirming the statement of its existence.

The pigmented epithelial cells pass suddenly into the transparent cells of the cornea (Pl. 6, fig. 1, *Co.*), through which is seen in the living specimen the silvery glance of the subretinal structures. In P. maximus the depth of the tall epithelial cells may decrease slightly in one or two cells, and then the next is much lower and completely free from pigment. Sometimes, however, the decrease in height takes place after the pigment becomes absent.

The nuclei take up again a central position or a position nearer the base in the corneal cells, but there are certain exceptions which will be considered later. The cells are hexagonal in surface view and are much flatter than those of

the pigment-mantle. They are usually constricted in the middle, so that they appear hour-glass-shaped in section, an intercellular space being left between them (Pl. 7, figs. 4 and 10). Externally there is a very distinct striated cuticle (Pl. 7, fig. 10, *Cut.*) which forms a hexagonal plate over the cell, and if the cornea is carefully focussed down upon from above these hexagonal plates are seen with their edges in close contact forming a definite mosaic (Pl. 7, fig. 3). If the corneal cells are now brought into focus at about the level of the nucleus, they appear still hexagonal in section though rather irregular, and the cell-walls do not touch. The spaces left between the cells on each side are crossed by numerous intercellular bridges (Pl. 7, fig. 4). I have no doubt that these are what Patten took to be interlocking processes of the cells. Carrière (**26**) was the first to discover their true nature, but asserted that Patten could not have seen them at all, since they were finer than his interlocking processes. Schreiner (**30**) stated that the intercellular spaces were filled with a prominent cement substance which, through shrinkage during fixation, caused the appearance seen by Patten, and does not mention any intercellular bridges whatever. Rawitz was also of the same opinion and does not refer to Carrière's statement (Rawitz [**25**], p. 109). I have seen them quite distinctly in the pigmented cells of the pigment-mantle as well as in the cornea, and they have the same structure in both places. There is another detail to be mentioned here which illustrates the difficulties caused by artefacts. Patten stated that the corneal cells had basal processes like the lateral ones, but which were longer and penetrated the underlying connective tissue, reaching the lens. This has been denied by all investigators since, and I had seen no traces of any such structures in hundreds of sections examined. After using the Bielschowsky-Paton silver method, however, the result figured (Pl. 7, fig. 10) was obtained. The tissues were fixed in 4 per cent. formol and lay in 1 per cent. silver nitrate solution for three weeks, which one might say was a likely method for artefacts. On the other hand, the structures

appeared well preserved and very little contraction had taken place. The processes were very definite, and had I found them by other confirming methods I should not have hesitated to describe them as actual cell processes. I have figured, however, the preparation, and prefer to leave the question of their true nature open. The type of cornea just described is that of Pecten jacobæus, P. maximus, and P. opercularis.

Rawitz ([**25**], p. 108) divides the types of cornea into three classes: (1) Cells of cornea considerably smaller than those of the pigment-mantle, ex. P. flexuosus, P. glaber, and P. opercularis; (2) cells of cornea, smaller at periphery against the pigment-mantle, but rapidly increase towards the centre, where they equal the pigment-cells in height, ex. P. jacobæus and P. varius; (3) corneal cells are as high as cells of the pigment-mantle at periphery, but increase rapidly in height towards the centre, the nucleus lying near the base, ex. P. pusio. I hardly think it advisable to make such a division, since, in the first place, the appearance often varies with the size of the eye, and it is difficult to fix a boundary between the two first groups. The corneal cells of P. jacobæus are, moreover, not equal in height to those of the pigment-mantle, though they are much higher in comparison with the same cells in P. maximus. There is, however, a well-marked division in which Pecten pusio and also P. tigrinus can be placed. The latter is figured (Pl. 7, fig. 12). In this species the corneal cells are very different from those of P. maximus. Those next to the pigment-mantle are of similar size, or smaller than the adjoining pigment-holding cells, but towards the centre the cells increase in height very considerably until they are deeper than the pigment-cells, the height of the corneal cells being double that of the latter. The cell-boundaries are not very distinct, and intercellular bridges are not to be seen. I have been unable to make out any reason for the peculiar difference in these two forms.

The connective tissue of the eye-stalk has already been

referred to; it is continued around the optic vesicle (Pl. 6, fig. 1, *Con.*) forming the inner wall of this (the outer being formed by the epithelium), and finally persists much diminished in thickness as a thin, transparent, and practically structureless layer underlying the cornea and separating this from the lens (Pl. 6, fig. 1, *Co. S.*). This is the "pseudo-cornea" of Patten, and the "innere Pellucidaschicht" of Rawitz. Nuclei are on rare occasions to be seen in it, but generally it is free from the connective-tissue fibrils and muscle-fibrillæ, which appear in that part just outside the corneal area, under the pigment-mantle (Pl. 6, fig. 1, *M.f.*). This more hyaline character is in all probability due to the fact that light rays have to pass through this layer before entering the optic vesicle.

The Lens.—The lens (Pl. 6, fig. 1, *L.*) is one of the structures that gave much trouble to the early investigators, but has lately been considered, entirely understood, and passed over somewhat lightly. Hesse made out some new and highly interesting structures, which I have been able to confirm. I find, however, that the shape of the lens-cells has been quite misunderstood, and the cells are certainly of a very peculiar nature.

The early authors could not determine the correct shape of the lens itself. Keferstein believed it to be spherical; Hensen was uncertain, but believed it to be bi-convex ([13], p. 222); Hickson considered it, however, as elliptical ([18], p. 447).

The confusion was again due to artefacts. It may be taken as definitely proved that the lens is bi-convex. The distal surface is, however, almost flat, whilst the proximal is very convex, and may appear dome-shaped. The actual degree of convexity depends largely on the contraction which has taken place in the eye during fixation, and the lens, dissected free from its limiting elements in a living specimen, probably alters in shape considerably, since it is not of very firm consistency. The lens is suspended from the subcorneal connective tissue (Pl. 6, fig. 1, *Co. S.*), against which its lesser convex surface is fastened. In surface view this face is circular and

not elliptical. Its diameter is a little greater than the cornea, since its periphery extends under the pigment-mantle for a short distance (Pl. 6, fig. 1).

The space in which the lens is suspended is bounded by the connective-tissue wall of the optic vesicle, the subcorneal extension of the same, and by the septum (Pl. 6, fig. 1, *Sep.*), a membrane separating the dioptric part of the eye from the retina. This space was regarded by Patten as a blood-space. Carrière (**21**) first saw the blood-corpuscles in this part of the eye, and Patten, though also finding them, was at a loss to account for their presence, since the retina seemed to shut off all communication with the blood-lacunæ of the eye-stalk. Rawitz appears to have found a definite vessel running on the outer surface of the optic vesicle and entering the distal part of the eye ([**25**], p. 113). Schreiner considers these corpuscles due to pathological conditions, and remarks that the three other authors named above considered them as normal ([**30**], p. 17). This is not strictly correct, since Patten stated that they might be forced into the cavity artificially by the contraction of the connective tissue through the action of reagents.

I have only found blood-corpuscles present in this space on extremely few occasions, and on one of these, when there were many, I could trace quite easily a series of spaces in the connective tissue, connecting up the lacunæ of the eye-stalk with the lens-cavity. This may of course have been an abnormal condition, and the lacunæ may have been produced artificially. These corpuscles had been forced in on the inner side of the eye, and I find no traces of Rawitz's blood-vessel on the outer side.

The blood plays an important part in the extension of the tentacles, and if a small living Pecten is watched under the microscope, the corpuscles can be traced running rapidly along the cavities of the tentacles as they are extended and back in the reverse direction as they contract. I believe their presence in the eye is due to contraction, and that they are forced there from the lacunæ of the eye-stalk.

There is no membrane covering the lens and helping it to retain its shape. Hensen and Hickson could not find such, but Patten described a "suspensory ligament," and also stated that the lens was attached to the septum by a connective-tissue ligament (*P. varius*). None of Patten's successors could find any suspending capsule, neither does the connective-tissue ligament exist. The lens may touch the septum (it very often appears so in sections), but this depends on the contraction during fixation, and usually the retina leaves the posterior wall of the optic vesicle and lies across the middle, coming naturally against the proximal end of the lens. Patten's connective tissue was in all probability the sheath of the distal nerve-branch (Pl. 6, fig. 1, *Op. Ds.*), which would be touching the lens and lying between this and the septum if the retina had been forced up. Patten's theories of accommodation as expressed at some length on p. 571 I cannot confirm, and they are somewhat irrational. They have not been referred to at all by his successors. He believed that the contraction of certain muscles supposed to be attached to the suspensory ligament would cause a movement of the lens towards the retina. This meant an inward movement of the septal membrane to which the lens (according to Patten) was attached. The elevation of the lens was to be brought about "by the tendency of the elastic septal membrane to return to its natural position, after the contraction of its peripheral circular fibres has relaxed the tension upon the central portion."

There is, however, no suspensory ligament nor attached muscles, and the lens is not attached to the septum. The septum, moreover, cannot move forward without taking the whole of the retina with it, and if this was the case (rather an absurdity) the recipient elements would always be the same distance behind the lens, whether it had been elevated or otherwise. Accommodation will be referred to later when discussing Hesse's theory.

The lens cells had received little attention until Hesse described them (34). Hensen stated that the lens consisted

of polygonal cells with thick walls. Patten described them as irregular with excentric nuclei, which appear in many cases to have disappeared from the cells near the inner surface. Rawitz described them as polygonal and membraneless with small nuclei, and Schreiner terms them "pretty large" vesicular cells, the peripheral ones flattened, with a large nucleus and no cell-membrane. The latter writer noticed that in sections of the lens some cells appeared to be without a nucleus (see Pl. 6, fig. 1), but went no further into the question.

Hesse says (**34**) the lens "besteht wie schon lange bekannt, aus zahlreichen, dicht neben einander gepackten Zellen, deren Körper sich an einander abplatten und bisweilen eigenthümliche Formen auf den Durchschnitten zeigen."

Later he adds (p. 395) ". . . da man ferner aus einem Durchschnitt auf die Gesammtgestalt der Zellen nicht schliessen kann, so ist es nicht möglich hier einen Zusammenhang zwischen Lage des Centralkörperchens und Gestalt der Zelle festzustellen." Hesse, however, did not adopt any maceration methods to solve the difficulty presented by sections. In sections through the lens, which is well preserved in formol-fixed specimens or Hermann-sublimate, the cells only rarely possess a polyhedral shape, in fact it is only here and there that they appear sharply angular. The cell contours are very distinct and appear rounded, so that there are irregular oval, pear-shaped and long band-shaped cells (Pl. 7, figs. 5 and 6). The size, too, varies considerably, and a very small, apparently non-nucleated cell may adjoin a large one. If, however, this small cell be followed through several sections, it will be found to be merely the continuation of a cell which is elongated to an extraordinary degree. The true shape of the cells was found after macerating the eye in $2\frac{1}{2}$–3 per cent. chloral hydrate solution in sea-water for four to six hours. This medium preserves admirably the delicate processes of the cells, and the preparation gives the lens-cells, separated, uncontracted, and with all details of structure undamaged.

The cells vary considerably in shape. Those near the surface of the lens, particularly the proximal surface, are flattened and are strap-shaped (Pl. 7, fig. 6, *c*.), or are constricted in the middle and have two bulging ends. The length may be very considerable. The common appearance is that depicted in fig. 5 (Pl. 7). The cells are pyriform, with the cell-body drawn out into extraordinary long tapering processes many times the length of the swollen part. In addition to this, processes are often given off very abruptly from the broad end. Other cells are more rectangular, yet also with rounded contours and the same abrupt fine processes. These extensions are wedged between adjacent cells (Pl. 7, fig. 5), which fit close together, and the result is a mass of great compactness, whose components, though having the most varied shape, fit together without intercellular spaces being left between them.

It is often quite difficult to separate some of the cells in macerations. It is now quite obvious why there appears to be no nucleus in many cells in sections, for it may be at one end and the cell be so long that many sections may cut through the latter without touching the nucleus.

The cells have a very distinct membrane, and it is difficult to imagine how this could have been missed by Rawitz and Schreiner, especially after Carrière had asserted its presence. It is easier now to understand why there is no need of a lens-capsule or supporting ligament, for the soft protoplasmic cells are tied together by their processes and the superficial cells are practically converted into fibres or straps. The contour of the lens is, in fact, as even as if formed by a connective-tissue sheath or a layer of pavement epithelial cells. The cell contents are finely granular, with a slight trace of pigment, and stain intensely with eosin. The nuclei are similar in size to those of the epithelial cells, and since the lens-cells are usually somewhat larger than the latter the nuclei can hardly be termed pretty large (Schreiner), though such terms are purely arbitrary. Hesse (**34**) discovered in the lens-cells of P. jacobæus, which had been fixed in sublimate

and stained in Heidenhain's iron hæmatoxylin, a remarkable structure. In addition to the nucleus there was present a dark staining body from which delicate but very distinct fibrils radiated out to the periphery and became attached to the cell-wall. Most of them were straight, some were bent, but all went out from the one point and all could be followed to the cell membrane if their whole length lay in the section. I have found the same structures (Pl. 7, fig. 6, *b.*), not only in material fixed and stained as above but also after the following treatment:

After fixation in Hermann-sublimate mixture and staining in iron hæmatoxylin, the shape of the cells is well preserved, the contents are homogeneous or very finely granular and stain grey, the nucleus is black, and radiating fibrillæ appear distinctly in many cells though not in all. After Zenker fixation and Mallory's stain the cell contents are very granular in appearance and stained deep red, the nuclei being yellow-red, and there is just a slight trace of the fibrillæ. They are also to be made out, though not distinctly, after Bouin fixation. Von Rath's treatment caused the cell contents to appear very granular and vesicular (Pl. 7, fig. 6, *d.*) the radiating fibrillæ were often very distinct, but the central dark staining body did not look exactly like the normal centrosome of dividing cells.

This permanent centrosome (Pl. 7, fig. 6, *Cent.*), if it be such, does not appear to have any definite position, but since it cannot be made out in macerations it is almost impossible to determine its true position, for sections cut the cells in all directions. In addition to the species enumerated by Hesse I have found these structures in P. tennicostatus, and probably they are present in all species. Hesse naturally compared these with the centrosome and astral rays which appear in cells undergoing mitotic division. Such structures have been demonstrated as persisting in the resting stages of certain cells, in pigment-cells of fishes, and more particularly in leucocytes. It has not been possible for Hesse or myself to determine any connection with cell-division. The astral

rays are very fine and remarkably definite. There are three explanations of these structures that may be given. The first and most unlikely is that they are artificial productions; the second, that they are modified astral rays and centrosome kept permanently for another function; the third, that they are entirely different from those functioning in the cell division, but have arisen in a similar way and are purely supporting fibrillæ. The appearance of the structures and their presence after such varied treatment is against the first view. It would only be possible to demonstrate which of the latter were correct if the origin of the aster had been observed. I believe they are supporting fibrillæ whatever be their mode of origin, and this is Hesse's view, he considering they are for the purpose of increasing the elasticity of the cells. This is put forward in an interesting theory of accommodation, and the fibrillæ are considered to form the antagonistic apparatus to another, to be referred to presently, which alters the shape of the lens. Between the sub-corneal connective tissue and the lens is a layer of peculiar fibres, first seen, though incorrectly described, by Patten. He made out two layers, a series of radiating fibres extending from the centre of the distal surface of the lens to the periphery, superimposed on a layer of strong circular fibres concentrically arranged (p. 581). As such do no fibres exist. Rawitz saw none here whatever, and regarded Patten's structures as artefacts ([25], p. 113). Hesse discovered the true conditions, which I can confirm with some slight additional features. There is one layer of fibres only (Pl. 7, fig. 8), and these have a kind of spiral arrangement, so that towards the centre of the lens surface they are running at almost right angles to their previous course. Near the periphery they run more or less concentrically (Pl. 7, fig. 8). They do not terminate at the centre of this surface, but continue across for some distance, and there results a series of fibres crossing one another in all directions.

In thin sections cut parallel with the plane of the cornea it is possible to see a number of nuclei here, with very deli-

cate cell-outlines enclosing them (Pl. 7, figs. 7 and 9). These cells have their ends drawn out into the long fibres seen in macerations so easily, and which are many times the length of the cell-body (Pl. 7, fig. 2). In some cases, as Hesse pointed out, a number of fine parallel fibrils appear to pass out of and through the cells (Pl. 7, fig. 7). He regards the fibres as muscle-fibres, and the cell-body as containing the remaining myosarc and nucleus. This view is based on the reaction to picric acid—säurefuchsin, which stains muscle yellow and connective-tissue red. I was not sure that they were not connective tissue cells, and in fact believed them to be such. For this reason Mallory's connective-tissue stain was used as recorded on p. 53. The fibres and cells were stained by this process an intense red, against the blue subcorneal tissue above (Pl. 7, fig. 7). They stain therefore as muscle-fibres. Hesse says ([**34**], p. 397) that these fibres extend to the edge of the lens but not further.

The same fibres, however, are to be found in the connective tissue extending down the sides of the optic vesicle (Pl. 6, fig. 1, *M.f.*) and often quite near or even on the inner surface of the same. I believe they have a far wider distribution than Hesse supposed. This is the apparatus that, aided by the lens-cells, is (according to Hesse) concerned with accommodation. Through the contraction of these fibres the outer surface of the lens becomes reduced in extent, the lens-cells are compressed together here, and, being plastic, change their shape, the contents swelling towards the inner surface where there is less tension. The result is an alteration in the shape of the lens and hence of the focus. If the muscles are relaxed the elastic cells (aided by the fibrillæ) return to their previous shape and the focus is adapted for more distant objects. No physiological proof has yet been brought to support this theory, and, as far as experiments go, I could find no evidence of accommodation (see p. 102).

Hesse has built up his theory simply to account for the fibres on the lens and the persistent astral rays in the cells. The function of the latter may be simply to give greater

rigidity to the lens, and if the former were accommodation muscles one would expect a more definite and efficient arrangement. The same red-staining fibres can be traced, however, down the sides of the optic vesicle in the connective tissue, and those present between the lens and cornea may be simply for the purpose of tying the lens to the subcorneal layer. Before leaving the lens it will be advisable to refer to another condition seen in some of the lens-cells. This is a peculiar condition of the nucleus (perhaps pathological) observed in one or two cells in preparations fixed in von Rath's fluid and also in Hermann-sublimate mixture (preparations stained with Heidenhain's iron hæmatoxylin). The latter specimen was an eye from a small P. opercularis or P. varius. The nucleus (Pl. 7, fig. 6, *a*, *nuc.*) is perfectly spherical and much larger than the normal ones. The size of the normal nuclei was 5·3 μ by 4 μ (they are oval in shape), whereas the spherical ones attained a diameter of 10·6 μ. These nuclei were homogeneous, not staining deep black as the normal ones, but rather grey, slightly darker than the cytoplasm. A very delicate nuclear membrane appeared to be present with the remains of deeply stained chromatin substance attached to it. The cells containing these nuclei do not look distorted nor vacuolated by fixatives and the nucleus appears perfectly natural; no other stages could be found connecting these with the normal nuclei.

The Retina.

The retina, being the recipient region of the eye, is of great interest, and this is increased by the wonderful complexity for an invertebrate and by the numerous conflicting views that have been published as to its histological structure.

I agree with Rawitz when he said that to Patten must be given the credit of solving much of this structure. He was the first to reduce chaos to order, and though he was unfortunately carried a little too far by his imagination, he published a very creditable work, especially since very little

was known previously about this part of the eye. I believe, also, that most of Patten's good work was due to the great use of maceration preparations, though perhaps owing to the more primitive methods of section work he did not check his results as much as he possibly could by this means. It is a great pity, therefore, that his description should have been couched in terms which, accentuated by his theories, did much to bring the whole paper into some disrepute.

The retina covers almost exactly half of the interior of the optic vesicle, and since it is of considerable thickness compared with the size of the eye there is not much space left in the proximal hemisphere. The retina and underlying layers will be considered together. They are separated from that part of the eye previously considered by a membrane, the septum, first discovered by Krohn (**5**).

This septum is a homogeneous sheet of connective tissue which is slightly thicker in the middle than at the sides, and at the periphery it appears to become continuous with the inner wall of the proximal half of the optic vesicle, that part termed the "sclerotica" by Patten (Pl. 6, fig. 1, *Sc.*). This author described it as cellular, but no traces of cells or nuclei are to be seen, though the corresponding structure in the eye of Spondylus is formed of distinct cells. Patten also stated that it was double. This has not been alluded to by other observers, but I thought I had detected this double nature (**44**). I have since found out my error and I believe also the cause of Patten's mistake. He writes that the distal branch of the optic nerve, which lies across the septum, has no sheath, since the latter terminates where the nerve enters the optic vesicle. The nerve, however, has a distinct sheath, and this accompanies it to the middle of the retinal surface, where just as the nerve branches (Pl. 7, fig. 18) and spreads out over the centre, the nerve-sheath spreads out too, covering all the diverging nerve-fibres which lie therefore between two sheets of connective tissue, the nerve-sheath above and the septum below (Pl. 6, fig. 1). This nerve-sheath fuses with the septum, and I think the two sheets of tissue were

regarded by Patten as both belonging to the septum. In preparations stained by Mallory's method the blue connective tissue is brought out very distinctly against the retina, whose elements are stained red, and hence both septum and nerve-sheath can be easily followed. In some sections there appears to be a delicate concentric striation in the septum, but this is all the structure to be made out. The distal branch of the optic nerve penetrates the septum, the fibres boring through separately.

The retina has been divided into several layers by previous writers, but anatomically as well as for purposes of description it will be better to consider it as made up of two layers only:

(1) The outer layer of distal sense-cells with their interstitial supporting cells (Pl. 6, fig. 1, *D. S.*; Pl. 7, fig. 13, *O. I. c.*).

(2) The inner layer of rod-cells and their continuations the rods, together with interstitial supporting cells (Pl. 6, fig. 1; Pl. 7, fig. 13, *R. C.* and *I. I. c.*).

A table is appended (p. 77) giving the synonyms that have been used, which shows also the gradual changes that have taken place in our knowledge of these structures.

Hensen (13), as will be seen from the table, placed all the cells present in the retina distally to the rod-cells and rods in one category, called this stratum the "first cell layer," and said it was composed of one or two layers of spindle-formed cells, whose contours were not very distinct. The layer of rod-cells was called the "second cell layer" and the nuclei of the inner interstitial cells considered to be their nuclei.

Patten found that the outer cells of Hensen were not all of the same shape. He supposes, however, that physiologically they are alike and calls them all outer ganglionic cells. Of these he described three types, one of which had broad ends bearing many fibrous processes which penetrated the septal membrane and became continuous with the nerve-fibres of the distal branch of the optic nerve.

One of his most important discoveries was the finding of the interstitial cells of the rod-cell layer, which he termed "inner ganglionic cells" (Pl. 7, fig. 13, *I. I. c.*). Only the

Terms used in Descriptions of the Retina.

Hensen, 1865.	Carrière, 1885.	Patten, 1886.	Rawitz, 1888.	Schreiner, 1896.	Hesse, 1900.	Present paper.
Erste Zellenschicht	Schicht der spindelförmigen zellen	Outer ganglionic layer	Ganglienzellenschicht	Äussere Ganglienzellenschicht	Distale zellen Zwischenzellen	Distal sense-cells. External interstitial supporting cells.
—	—	Inner ganglionic layer	Secundäre ganglienzellen	Innere ganglienzellenschicht	Zwischenzellen	Inner interstitial supporting cells.
Zweite zellenschicht	Stäbchenzellen	Retinophoræ.	Stäbchenzellen	Stäbchenzellen	Stäbchenzellen	Rod-cells.
—	Siebmembran	Terminal membrane[1]	Grenze von Stäbchen und Stäbchenzellen	Äussere Siebmembran	Siebmembran	—
Stäbchen	Stäbchen	Rods	Stäbchen	Stäbchen	Stäbchen	Rods.
—	Substanz zwischen Stäbchen	Outer part of rods	Mantel des Stäbchens	Mantel des Stäbchens	Zwischensubstanz	Rod-matrix.
—	—	Vitreous network	—	Innere Siebmembran	Deckmembran	Basement membrane.
Tapetum Pigment stratum	Tapetum Pigmentschicht	Argentea Tapetum	Tapetum Pigmenthaut	Tapetum Pigmentschicht	Tapetum Pigmentschicht	Tapetum. Pigment layer.
—	—	Sclerotica	—	—	—	Outer modified wall of optic vesicle.

[1] This supposed structure is not exactly equivalent to the "Siebmembran" (see text).

nuclei of these cells had been seen before and they were thought to lie inside the rod-cells.

Rawitz agreed with Patten in almost all respects, but made a retrograde step in asserting that a division of the outer cells into three types was unnecessary because "die gesamten Zellen dieser Schicht vollständig einander gleichen, abgesehen natürlich von den nebensächlichen Differenzen im äusseren Habitus, und weil sie, vielfach miteinander in direkter Kommunikation stehend, eine physiologische Einheit repräsentieren." Schreiner also refers to the two layers of ganglionic cells (the outer being a mixed layer, see table, and the inner one the non-nervous inner interstitial cells), and states that the outer layer is four or five cells deep in the middle of the retina. He noticed, however, that the cells of the outermost row (Patten's first type) differed from the others, though considers that all are of the same physiological nature. Hesse in 1901 (**34**) was the first to upset the prevalent ideas of these cells. He stated that there was only a single layer of cells, and that the fibres of the distal nerve were not connected with them. Hesse had forgotten, however, that the previous observers would also have considered the outer ganglionic layer to be of but one layer of cells if they had only meant it to include the cells of Patten's first type. The other cells Hesse alludes to as being pushed in between those of the outer row, which he states are of epithelial-like nature. In any case, to Hesse belongs the credit of having separated off the outer interstitial cells from those of the most distal layer, and breaking up the idea that all were ganglion-cells and alike in function.

In addition to the difference in shape and the fact that the outer cells bear cilia-like processes (Pl. 7, fig. 13, *D. S.*), he also noticed that the nuclei of the outer cells were somewhat different from those of the others, now termed "Zwischenzellen." This difference has often been very apparent to me, and it is strange that the earlier writers missed this point unless fixation and staining of these cells had been rather indifferent.

Hesse finally noticed the resemblance of these outer interstitial cells to the inner ganglionic cells of Patten, Rawitz and Schreiner, and called all of them "Zwischenzellen," stating at the same time that they did not bear exactly the appearance of nerve-cells, but his preparations showed that the fibres of the distal nerve arose from them. He did not regard them as ganglion-cells but considered them to be optic sense-cells. The function of the outer cells is not stated, but they are not supposed to be connected with the distal branch of the optic nerve.

The next mention of these cells occurs in Schneider's 'Text-book of Histology' (**38**). Schneider finds no connection existing between the "Zwischenzellen" and the distal branch of the optic nerve, nor any junction of the latter with the outer layer of cells, but finds that the nerve-fibres penetrate between them and cannot be traced further. He also describes how at the edges of the retina the cells of the outer layer at various places surround, collar-like, branches of the nerve. I believe this (see his illustrations, p. 560) must have been caused by artefacts. The interstitial cells are not considered to be sense-cells.

In 1904 appeared Hyde's remarkable account of the nerve-endings in the retina, which really caused my attention to be drawn to the Pecten eye. Hesse had previously stated that methylene-blue methods had failed him, but that the problems of the retina would in all probability be solved by the attainment of success with this stain. According to Hyde, methylene-blue methods were perfectly successful and solved all, the result being a description of the retina which stands in striking opposition to all previous work. Hyde finds that the inner interstitial cells are the nerve-cells connected with the axial fibre of the rods, and only mentions one row of outer cells which are supposed to be connected to the fibres of the optic nerve.

So much for the outer cells; I shall have occasion to make further reference to Hyde's work later. In 1908 Hesse refers again to the Pecten eye (**43**), and now finds a connection

existing between the distal cells and the distal branch of the optic nerve, so that these are also included as sense-cells, but his views of the interstitial cells remain unaltered. He had apparently neither seen nor heard of Hyde's paper, which has remained, therefore, uncriticised. Such is the mass of conflicting evidence at present existing. There is no doubt that the relation of the distal nerve to the outer distal cells and interstitial cells is the most difficult histological problem of the retina. It is extremely difficult to trace the endings of the nerve-fibres in sections, and impossible to make out the shape of the interstitial cells. I have been able to make out, however, the shape of the latter from macerations, and to trace the extent of their branches, which can be confirmed by sections. A schematic figure has been built up from macerations and sections which shows the relation of the cells to one another (Pl. 7, fig. 13).

The structures are as follows: The distal surface of the retina is bounded by a single layer of cells (Pl. 7, fig. 13, *D. S.*), the distal cells of Hesse, and the first type of Patten's outer ganglionic cells. They are somewhat regularly placed so that an epithelial-like layer is formed. The outer ends of these cells, which are directed towards the septum, are broad and bear cilia-like processes, so that a space exists between septum and cell-layer, which is crossed by the nerve-fibres from the distal nerve and filled by the processes of the distal cells, which for the most part do not reach the septum (this may be caused, however, by breakage of the fine processes during fixation). The cells are cylindrical, transverse sections cut in the plane of the retina, being perfectly circular (Pl. 7, fig. 16, *D. S.*). Their lower ends are rounded, and in some cases appear to terminate in a short pointed process. This, however, could not be followed far, and I have only seen it in some maceration preparations.

The cell contents are finely granular. Dark-staining granules (basal granules) are present at the bases of the cilia-like processes (Pl. 7, fig. 13), and these sometimes produce the appearance of a dark-staining edge. There are also

delicate longitudinal fibrillæ in the protoplasm of the distal ends of the cells, running to the bases of the processes (Pl. 7, fig. 13, *D. S.*). Like Hesse I have found no motion of the processes in living cells. Between the cells pass branches of the distal nerve, which can be traced quite easily through the septum, but with great difficulty in the retina, where it has been uncertain whether they entered into connection with the outer cells, interstitial cells, or ended free.

I think it is certain that they terminate, however, in the distal cell-layer and become connected with the cells, not by the cilia-like processes, but to their sides (Pl. 7, fig. 13). It is easy to see in sections the nerve-fibre passing to the side or apparently one corner of the distal cell, and in macerations each distal cell can be seen to possess a long, thicker process which appears to arise at the edge of the distal end, but can often be traced some distance down the side wall. This is unfortunately very difficult to make out, but is confirmed, I think, by the character of the distal cells, which are those of sense-cells, and by sections of young eyes, where the interstitial cells are only slightly or not at all developed (as noticed by Hesse).

The nucleus requires special consideration since it differs from that of the interstitial cells. Fig. 16 (Pl. 7) illustrates a transverse section through distal and interstitial cells stained with Mallory. The nucleus of the first-named (fig. 16, *D. S. n.*) is large, perfectly round, and contains a number of small chromatin granules, which stain orange red (orange G. and säurefuchsin) in addition to the distinct nucleolus which is always present and stains more distinctly orange (there may be two nucleoli present). The cytoplasm is stained red. These nuclei are very similar in appearance to those of the rod-cells to be considered below and to the nuclei of nerve-cells from the various ganglia. The character of the outer interstitial cells (fig. 13, *O. I. c.*) is very different, and I have termed them "supporting cells." They bear no resemblance to sense- or nerve-cells, and no connection between them and the inner interstitial cells or the fibres

of the distal nerve could be found. The isolated cells obtained by macerating the retina in chloral hydrate solution are illustrated in fig. 15 (Pl. 7), but these were only obtained on a few occasions and after a long search, for it is most difficult to separate them from the distal cells.

The cell-body is very small and there is but little cytoplasm left surrounding the nucleus, but from this extend long branched processes. The nucleus retains the blue stain after Mallory when it has been taken from the nuclei of the distal sense-cells, and generally it may be said that the interstitial cell-nuclei stain darker and are more homogeneous, it being more difficult to resolve the granules. They are furthermore flattened and are only about half the size of the sense-cell nuclei. The processes lie in close contact with the distal sense-cells, there being often two clasping them and extending between them towards the septum (Pl. 7, fig. 15, *a*.).

From the proximal end of the cell may arise one or more irregular processes which branch and penetrate some distance between the rod-cells. It is quite easy to understand how these long processes, which in my opinion tie and support the sense-cells, have been for a long time considered as nerve-endings, either of nerve-cells or of the fibres from the distal nerve. In many ways the interstitial cells resemble in shape and staining the neuroglia cells found clasping the nerve-cells in the various ganglia of Pecten and other lamellibranchs. The outer ganglionic layer of Patten is composed, therefore, of two types of cells—sensory cells forming an outer layer and connected with the distal nerve, and supporting non-sensory cells interpolated between them. Miss Hyde did not recognise the latter at all. I have had no success with methylene-blue methods, but I do not think they would be of much advantage unless fixation was very good (a thing not by any means easy to attain with many special methods), for it would be almost impossible to check the results and to determine whether, in the confusing mass of fibres, nervous or both these and non-nervous processes had taken the stain.

We have now to consider the second sensory part of this

remarkable retina, innervated by the proximal branch of the optic nerve. This region is most obvious in sections and is composed of a row of pillar-like rod-cells, bearing rods, with a series of interstitial cells lying between the former and once supposed to be their nuclei.

The rod-cells (retinophoræ of Patten) (Pl. 7, fig. 13, *R. C.*) occupy a very large part of the retina. In very young eyes, however, the distal cells are more prominent and occupy a proportionately much larger part. They are extremely long cells, especially those situated in the centre of the retina. The outer ends, to be found at the periphery of the retina, are attenuated and pass gradually into the nerve-fibres of the proximal branch of the optic nerve (Pl. 6, fig. 1, *Op. P.''*), so that it is impossible to say where one ends and the other begins. From this point they increase in thickness, the first third of their length or more lying almost horizontally under the outer layers of cells, embraced by the processes of the supporting cells. Some little distance from the periphery, not very different for cells from different parts of the retina, each swells rather suddenly round its nucleus (Pl. 7, fig. 13, *R. C.n.*), and from this point the thickness remains practically the same to the basal end, though there is a slightly more constricted part below the nucleus. All the rod-cell nuclei are situated in a scattered cluster not far from the edge of the retina, so that the nucleus is nearer the proximal end in rod-cells belonging to the centre of the retina, whilst in the middle or slightly nearer the base of rod-cells from the peripheral regions.

The distal cylindrical portions of the rod-cells lie parallel with one another, perpendicular to the plane of the retina, and terminate at the same level, forming a well-defined line between them and the layer of rods. This line (Pl. 7, fig. 13, *S.m.*) has been described as the section of a membrane (see table), which extended across the retina and was pierced by the rods (Pl. 7, fig. 13, *Rod*). These are direct continuations of the rod-cells, and rod and rod-cell form together one entity—the product of one cell. Patten described a delicate

membrane supposed to separate the protoplasm of these two parts, but there is no trace of one, and the cell contents of both are continuous.

The appearance of two definite structures separated by a membrane is due to an external flange or projection existing on the wall of the rod-cells at their junction with the rods, by means of which adjoining rod-cells are connected. This produces in sections the effect of a "sieve-membrane" with circular holes through which the rod-cells and rods protrude.

It is a rather difficult point to decide. Hensen, so far back as 1865, said that by reason of the rod-cells ending at the same level a sharp bounding line was formed, which could easily be mistaken for a membrane, but this was not present. Patten did not see it either, but, as stated above, believed there was a delicate membrane, the "terminal membrane," in each rod-cell. Rawitz found no membrane either inside or external to the cells, but Schreiner and Carrière both affirmed its presence. Hesse (**34**) refers to a sieve-membrane, and on p. 409 he remarks that in some specimens of P. jacobæus and P. maximus the inner interstitial cells can be followed up to the sieve-membrane, which is possibly a product of these cells.

In my opinion the sieve-membrane is, as above stated, due to the extended walls of the rod-cells, and has no part from the interstitial cells. This line is usually well marked in the marginal regions of the retina, where there are no rods borne by the rod-cells (Pl. 6, fig. 1, *M. ret.*). Where necessary, the well-marked line above referred to will be called a "pseudo-sieve-membrane" for convenience in description. In macerations of the retina, in $\frac{1}{50}$ per cent. chromic acid (the preparations being stained with picro-carmine and examined with the oil-immersion) a series of very delicate parallel fibres could be seen running longitudinally on the surface of the rod-cells (Pl. 7, fig. 13, *Cells A*). It was not possible to follow them proximally to the nucleus. At the junction of rod-cell and rod they bear thickenings (Pl. 7, fig. 13, *S. m.*),

which stain more distinctly, and it is probably these only which form the "flange" and the attachment of rod-cells to each other.

The fibres are supporting fibrillæ, and in preparations where the rods had broken off (Pl. 7, fig. 13*a*) the tube of fibrils could be distinctly seen. Where the rods remained attached to the rod-cells the fibres were continued below the thickenings, but had left the surface of the rod, enclosing the latter in a kind of sheath (Pl. 7, fig. 13).

Whether they lie on the rod-wall in the normal condition or in the interstitial substance to be presently considered I cannot say. Another point concerning the shape of the rod-cells remains to be referred to. Above the nucleus the rod-cell does not become gradually less in diameter, but after a constriction there often occurs one or more irregular swellings, which give the attenuated end of the rod-cell a more or less varicose appearance.

Patten saw one of these and described it as a delicate oblong vesicle containing a second faintly staining and often invisible nucleus. Rawitz would not consider the presence of a nucleus, but saw the enlargement and said it might be artificial. Schreiner also figures it. It is most easily seen in isolated rod-cells, in a maceration. I find that there may be one or more, and that they are simply due to the rod-cell being flattened in places by the pressure of adjacent cells; the flattened part appears as an enlargement if not seen in edge view.

The rods are cone-shaped with the apices rounded. The base has the same diameter as the rod-cell, that is, where they are continuous, and from here the diameter gradually decreases towards the lower end, though at first very gradually. They are separated and surrounded by a homogeneous substance (Pl. 7, fig. 13, *R. mat.*), which fills up all the cavities that would otherwise have remained between them, and also forms a layer below them. This substance is stained black by iron hæmatoxylin, it is blackened by osmic acid, and is stained blue by Mallory's connective-tissue stain. I believe

it is a semi-fluid substance of connective-tissue-like nature, which contains some oil or fatty body, and I have called it the rod-matrix (Pl. 7, fig. 17, *R. mat.*).

Patten described the rods, which are very difficult to preserve, as consisting of a "hyaline refractive sheath surrounding a pyramidal axial core filled with a watery non-refractive fluid, and a short distance from the inner ends of the rods, terminating in a rounded apex" ([22], p. 585). This axial core is, in my opinion, the true rod, and what he described as the sheath is the surrounding rod-matrix. Carrière (21), had noticed this before Patten, and described the rods as being immersed in a fatty substance. Patten, however, adds that this was due to the fusion of the sheaths of the poorly preserved rods. Rawitz agreed with Patten about this sheath, though he differed slightly in regard to its optical properties, and Schreiner also does not accept Carrière's view. Hesse's view is, however, the same as mine, and he has emphasised the error of Patten, Rawitz, and Schreiner, whose peculiar idea of the rod was due to the fact that they believed an outer sheath to be necessary. The rod structure differs from that of the rod-cell in the fact that there is much less stainable protoplasm, and this is usually aggregated round an axial fibre (Pl. 7, fig. 13, *Ax. f.*). It will be unnecessary here to go into further comparisons of the previous views on these structures. The rod-cells have been described almost correctly, though with deficiencies by most observers, with the great exception of Hyde, whose account I am leaving until later.

In sections of well-preserved rod-cells and rods, such as those fixed in Bouin or Zenker and stained in Mallory's stain, an axial fibril will be easily seen running through the rod. It is with reference to this structure that most of the confusion has arisen. Patten stated that each rod-cell contained an axial nerve-fibre which entered the attenuated end, passed through the first vesicle-like swelling, passed the large nucleus, and went on down to the lower end of the rod, whence it issued, and divided into two main branches which

became connected with the axial fibres of neighbouring cells (see Patten's fig. 140, Taf. 32). Furthermore, he describes how towards the lower ends of the rod-cells the axial nerve-fibre begins to give off radiating fibrillæ, which are so numerous in the rods as to constitute the greater part of their substance. Hensen was the first to see the axial fibre in the rod. Patten figured it as being equally distinct and of the same diameter in rod-cell and rod. Rawitz found, however, that there was a fine canal running through the former in which lay the fibre, which, he adds, is the continuation of a nerve-fibre from the proximal branch of the optic nerve. This central canal and fibre was supposed to be present in the rod but terminated without the complicated connections of Patten. Carrière, in his second paper (an answer to Patten's criticisms of his first) (**26**), could not bring the existence of a nerve-fibre inside a cell into line with histological teaching, and hence said that what was present was simply a differentiation of the cell-substance. Schreiner came to the conclusion that a detailed examination was necessary owing to the diverging opinions of previous authors, and found after making sections and teased preparations that there was no axial fibre at all in the rod-cells, and what had been seen there was only one of the contours of a rod-cell produced by pressure causing these normally cylindrical cells to be angular. He found it very distinctly stained, however, in the rods, and it ran straight to the end where it terminated in a point. He adds that it differs somewhat in staining qualities from nervous tissue and is too thick for a nerve fibre (p. 72).

Hesse found after all this research that it was necessary to go back to the earlier views, for he made out the axial fibril running through both rod-cell and rod.

He states, however, that it is far more easily seen in the rods, and even there it varies in the same preparation.

It is less distinct in the rod-cells because thinner (except in P. aratus), and in some cases Hesse saw more than one present. This bring us to Hyde's views (**39**) regarding rod-

cell, rod, and axial fibre, which are based on methylene-blue methods. It appears somewhat difficult for me to understand how the material presumably stained could remain in good condition for four years until taken up for completion.

We are told that a rod consists of a nerve-cell whose small anterior end (upper end ?) projects slightly beyond the median limiting membrane, and whose much elongated posterior portion is tubular and bluntly terminated. This portion is encased in a hyaline sheath, with the end capped by a homogeneous cuticular substance, which in methylene-blue preparations appears like the matrix separating the rods. A small nucleus lies in the anterior end of the rods and from this an axial fibre extends to the posterior (lower end).

There is another series of important elements in the retina —"bipolar cells." These extend from the median limiting membrane (presumably the same as the line dividing rod-cells from rods) outwards towards the margin of the retina. "Their large granular elliptical nuclei may be seen in longitudinal sections extending in a row, a short distance from the median limiting membrane. The whole cell with its afferent and efferent axon is encased in a hyaline sheath, under which are scattered blue granules of various sizes." The rest of Hyde's conclusions are difficult to understand, but putting figures and descriptions together, one gathers that the rod-cells of all previous writers are the same as certain "supporting cells of the median layer" of Hyde. The bipolar nerve-cells above referred to are the inner interstitial cells (Pl. 7, fig. 13, *I. I. c.*) or inner ganglionic cells of other authors, and from them arise two fibres, one of which runs to the edge of the retina and the other to the pseudo "sieve-membrane," following the course of the median supporting cells of Hyde and lying between them. These are the afferent and efferent axons. Distally the afferent axon has a dendritic termination, which comes into relation with the upper end of the axial fibre of the rod. Proximally the efferent axon terminates with other efferent axons in a common large ganglionic cell. These marginal ganglionic

cells, besides connecting up various axons of bipolar cells, give off fibres which make up the proximal branch of the optic nerve. This means in short that the sensory structures (the rod-cells) of all other writers are merely median supporting cells, the inner ganglionic cells of Patten and Rawitz (the interstitial supporting cells) are bipolar nerve-cells, and the marginal ganglionic cells of Hyde have not been seen by any other investigators. Patten and others must have mistaken, adds Hyde, the axons of the bipolar cells for axial fibres in the rod-cells!

I took some little trouble to see if it were possible for any of these results to be correct, though from à priori reasons, assuming a little of the previous work to be satisfactory, it appeared very doubtful.

In the first place Patten and his successors could not have seen the bipolar cell axon inside a rod-cell, since they all described it as being outside and possible of separation in teased preparations.

In the second place, the bipolar cell of Hyde has always been described as multipolar, and hence though two long afferent and efferent axons might have been missed, her predecessors had a better idea of its true shape. Finally, since rod-cell and rod are in direct continuation it is impossible for the axial fibre of the latter to become connected with the process of a cell lying between the former. The results are, in fact, impossible. The rod-cell in its general features I have found to be exactly as described by most other writers. The "bipolar cell" is the interstitial supporting cell to be subsequently described, and the rod contains no nucleus at all. The marginal ganglionic cells as described by Hyde do not exist. I must now refer to the axial fibre and the internal structure of the rods. The first idea striking an observer is that the true condition of things is like that described by Schreiner, viz. an axial fibre is present in the rods, but not in the rod-cells. After staining with iron hæmatoxylin, but especially after using Mallory's stain, with Bouin's fluid as fixative, traces of a much thinner fibre or fibres are to be seen

in the rod-cells (Pl. 7, fig. 13, *cells B.*). In a memoir on Pecten (**44**) I made the statement that this was probably the true condition, and I find that Schreiner in his text-book on histology (**38**) has done the same. The latter author refers to the axial fibre as a neurofibril, a structure which has risen in importance since Apathy's work in 1897 and about which very much has been written, chiefly on the continent, in the last few years. I believed that the thick neurofibril easily seen in the rods divided into numerous delicate, more elementary fibrillæ in the rod-cells, a view rendered more probable by the fact that whilst the contents of the latter are uniformly distributed, filling the cell, the protoplasm of the rods is usually aggregated in the middle. I could not at that time, however, find proof of this in Pecten, although Hesse stated that sometimes he had seen more than one fibre present.

Usually the axial fibre is thickest and stains most darkly in the upper half of the rod, though sometimes the whole length in the rod is much the same in appearance.

It begins to disappear a little below the line of junction with the rod-cells, but again sometimes extends quite as distinctly a little above this. This disappearance, or partial disappearance, is due to the separation into delicate branches which extend right through the rod-cell (Pl. 7, fig. 13, *B., R.C.*).

The separation is irregular, and sometimes one fibril is left much thicker and may be followed easily through the rod-cell: presumably this feature gave rise to Patten's view.

The point of separation of the axial fibril of the rods into finer fibrillæ varies even in the same section, and in rod-cells situated near the margin of the retina (young rod-cells) the axial fibre may often be seen as thick and distinct as in the rods. In macerations in chloral hydrate solution or chromic acid and also in teased fresh material the axial fibril is seen as distinctly as in stained sections.

It is rather thick and quite stiff like a bristle in these preparations, never having normally the snaky course

ascribed to it by Hesse. Often the more delicate rod is broken up in maceration and the axial fibre is then left sticking out from the protoplasmic remains of the cell (Pl. 7, fig. 14, *Ax. f.*).

After seeing these preparations one is rather inclined to believe that this is also a supporting structure.

In sections, however, the appearances are more favourable to the nervous view. The separation of the components of the axial fibre is similar to that often taking place in neurofibrillæ, and the fibre occurs in a sense-cell and stains always like the nerve-fibres in the same preparation. In the rods the axial fibre differs somewhat in appearance from a typical neurofibril in thickness and distinctness. These structures considered as the conducting elements of the nervous system were unknown to the earlier writers on the Pecten eye.

There are two views, then, that may be taken of the function of these fibrillæ. We may regard the axial fibril in the rod as a true neurofibril, a "primitive fibril" formed by the apposition of several elementary fibrillæ which pass through the rod-cell, the apposition occurring normally or through fixation. These neurofibrillæ have, then, the function assigned to them by Apathy and Bethe—the conduction of nerve impulses. On the other hand we may consider the whole to have only the function of a system of supporting fibres. The latter view would resemble that put forward by Nansen and accepted by several investigators, who consider the neurofibrillæ to be the supporting, and not the conducting elements of the nerve-cells. It is also conceivable, of course, that the structures are not homologous with the neurofibrillæ of nerve-cells at all. There is at present, to my mind, much confusion existing in reference to fibrous structures in nerve-cells, especially since Holmgren has shown (**37**) that processes of the neuroglia actually penetrate into ganglion cells and act as supporting fibres.

An axial fibril of the same type as that occurring in the Pecten eye is a feature of the rod-cells of many other invertebrate eyes. For example, in the Lamellibranchiata it is

present in Arca, Lima, Spondylus (**34**), and Cardium (**42**); in the Cephalopoda it is probably of general occurrence. It is very definite in the rods of the Alciopiden, and has been found in the Polychætes Nereis and Lysidice by Hesse (**33**). In Gastropods a definite bundle of neurofibrillæ has been found in the visual cells of Limax (Smith [**40**]). In other forms there occur, instead of one thick axial fibril, a number of fibrillæ which terminate in a comb-like margin ("Stiftchensaum" of Hesse). This is a feature of the distal cells of the Pecten eye, and according to Hesse is practically universal, the fibrillæ occurring also in the rods and cones of vertebrates.

The rods or analogous structures are also of widespread occurrence in optic sense-organs, though it would be difficult to homologise many of the rod-like structures with one another. Hensen, and later Grenacher, looked upon all the rods as cuticular structures, but I doubt now if any rod can be shown to be cuticular, not even the rhabdome of the Arthropods, a differentiated part of the reticular cells. Hesse regards the neurofibrillæ then as the universal actual recipient elements of the visual cell and the plasmatic part of the rod as a support for the fibrils. Experimentally it is impossible to determine whether the neurofibrillæ are the recipient elements or not, but from the constancy of their presence I believe they play a great part in this process. I have shown how in macerations the rod-cell may break up, leaving the axial fibre (Pl. 7, fig. 14). It does not appear from this as if the rod could give much support to the latter, but the true state of things in the living eye may possibly be different. I am rather inclined to believe, however, that the plasmatic portion of the rod acts conjointly as a recipient organ, and that the stimulus is passed on to the neurofibrillæ which conduct the nerve impulse wider.

I consider Hesse's estimation of the number of rods in a retina to be rather low for the large eyes of P. jacobæus or P. maximus. In the latter species there were about ten thousand in the retina of one specimen examined, and the number of rod-cells therefore exceeded this number, since the marginal ones do not bear rods.

Below the rod-matrix which underlies the rods is a limiting membrane, the basement membrane (Pl. 7, fig. 13, *B. m.*), which extends completely across the eye. It corresponds to Schreiner's "Innere Siebmembran," but is a perfectly continuous thin sheet. It is stained by hæmatoxylin similarly to the matrix but darker, and since the rods terminate a little distance above it it is obvious that they cannot pass through it. It occupies a similar position to Patten's "vitreous network," but his description also refers to a thin layer of hyaline substance perforated by large holes into which the inner ends of the rods fit, and Schreiner states that the points of the rods come to lie against the tapetum. No traces of any cell-structure have been made out in this bounding membrane, which, as noted above, is not perforated by the rods.

Reference has already been made to the marginal area of the retina (Pl. 6, fig. 1, *M. Ret.*). This is best studied from specimens fixed in Carnoy's fluid. The rods remain practically similar in size until about the tenth from the margin of the rod-bearing region, and then follows a rapid decrease in size, leading to the apparently fibrous lateral parts where no rods are present. Careful examination will reveal the fact that the so-called outer sieve-membrane can be traced to the very edge of the retina, but the space between it and the basement-membrane is exceedingly small. This corresponds, however, to the space occupied by the rods in the middle part of the retina. The axial fibre or neurofibril can be seen more distinctly in these marginal rod-cells, which for a little distance are similar in diameter to the much longer ones in the centre of the retina. They next become much less in diameter until finally the boundaries become difficult to detect, and the axial fibril is the most distinct part of the cell. It can also be seen extending below the line of the pseudo sieve-membrane, though without any rod. Between these modified rod-cells are more supporting cells.

The marginal region differs, therefore, from the central part of the retina in being composed of rod-cells which are far shorter than those of the latter region, whose diameter is

reduced, and which bear practically no rods, though the axial fibril, which is very distinct, appears to extend a little way below the pseudo sieve-membrane. I believe that this region is occupied by young rod-cells and rods, and it can be seen how the rod is a gradual product of the rod-cells, as the appearance of the former in other parts of the retina naturally suggests. The gradual increase in size of the rods at the junction of the marginal and the central rod-bearing region is well marked. Probably the former region does not play any active part in vision at all.

Hensen called this area the "Retinawülste," because of the folded appearance in sections, and Hickson's figures also show the retina in this form. I have found the same condition after several fixatives, including Von Rath's fluid and Bethe's fixative for methylene blue. It is due to contraction, and is not normal.

The inner interstitial supporting cells (Pl. 7, fig. 13, *I. I. c.*) have already been referred to several times. They lie in close contact with the rod-cells, between which they send their processes, and they are situated not far from the pseudo-membrane (Pl. 7, fig. 13, *S. m.*). Patten was the first to recognise that the nuclei of these cells really belonged to cells lying between the rod-cells; they had been considered the nuclei of the latter by his predecessors. He figured them correctly as multipolar cells, but fell into error in regard to the nucleus, just as he and most of his successors considered that all the cells between the rod-cell layer and the septum had the same type of nucleus and were physiologically alike. It is quite easy to see in preparations stained with Mallory or iron hæmatoxylin that these nuclei resemble exactly those of the outer interstitial cells. There is a considerable difference between them and the large nuclei with distinct nucleolus and chromatin granules, which are present in both the distal sense-cells and the rod-cells (Pl. 7, figs. 13 and 16, *R. C. n.* and *D. S. n.*).

The shape of the cells can be best seen in isolated retinas after macerating in $\frac{1}{50}$ per cent. chromic acid for several days

and staining in picro-carmine. There is very little protoplasm round the nucleus, and the processes are so irregular that beyond the fact that the cells are multipolar no definite shape can be ascribed to them. They are, on the whole, slightly larger than most of the outer interstitial cells. The processes wrap round the rod-cells, and may even extend through the basal pseudo-membrane between the rods. It has been said by Hesse that the inner interstitial cells are so rare in the centre of the retina that there is only one to four or five rod-cells. They are just as numerous here as elsewhere, except, perhaps, the peripheral modified region.

Patten and Rawitz considered these cells to be ganglion-cells. Schreiner figured their shape incorrectly (as did I myself in a previous memoir) and found them to be connected with the distal nerve. Hesse also believed these cells to be nervous, for he states that the connection with the distal nerve is sometimes very distinct. In his last paper, however, he has altered his views of the relations between the distal cells and the nerve, and the question of the interstitial cells is therefore left open. Hyde, as already noted, regarded them as bi-polar nerve-cells connected with the axial fibre of the rods. Everything, however, points to the conclusion that the inner interstitial cells, like the outer, are simply supporting cells, their structure being quite unlike that of nerve- or sense-cells, and no connection with nerves having been found.

Sub-retinal Layers.

Below the retina there is generally a space, a split between it and the next layer, which may be of considerable size. All writers have figured this, but it is impossible, in most cases, to discover whether they regarded it as normal or not, since only Hesse refers to it, and he regarded it as due to shrinkage. I have figured it as it usually occurs in sections (Pl. 6, fig. 1), but it must be remembered that this space is simply due to fixation, etc. In some cases, for example, the next layer, the tapetum (Pl. 7, fig. 1, *Ta.*), will be found for some distance

attached to the retina, and then will occur a stretch where it has evidently been torn off, and remains attached to the underlying pigment-layer (Pl. 6, fig. 1, *Py.*). This layer is also very often pulled away from the wall of the optic vesicle, and, whilst remaining attached to the tapetum, leaves fragments adhering to the wall, indicating where it once has been. In the normal eye, retina, tapetum, and pigment-layer are all in contact with one another, and no space occurs between the latter and the wall of the eye.

The tapetum.—This layer is very conspicuous both in the living eye and in sections, and was very early discovered by Krohn (5). Hensen stated that it consisted of polyhedral cells. Patten called it "the argentea" (a name which I previously employed, but since "tapetum" is more correct by order of priority I have gone back to it). It is unfortunate that the term "tapetum" has been used to designate two different layers.

Hickson and Carrière believed the structure was formed of a number of fine fibres crossing at right angles. Patten considered it to be a modification of two layers of cells into refractive laminated membranes composed of minute square plates. Hesse found the tapetum to contain always a single nucleus surrounded by some residual protoplasm and therefore derives this layer from a single large cell.

The tapetum is made up of several layers of minute square plates (Pl. 7, fig. 19), which are yellow by transmitted light and reflect the light like silvery plates.

This gives the diamond-like lustre to the living eye, and I have even a series of transverse sections, mounted in canada-balsam, which retain the same property. The layer is thickest in the centre and shades off gradually to a very thin peripheral region, which can be traced between the retina and the pigment-layer to the wall of the optic vesicle.

I have been unable to trace Hesse's nucleus, and in adult eyes it is impossible to detect any remains of cells. I believe rather that this layer is formed by the underlying pigment containing cells or by other cells which disappear, but more

probably by the former, since some of the granules contained in these cells may resemble the substance of the tapetum.

The pigment layer was also an early discovery because of its conspicuous appearance, and it is often possible to see the red pigment through the substance of the eye-stalk if there is little pigment in the epithelium of the latter. This layer was Patten's tapetum. Hickson had regarded it as a fluid with no cellular elements at all. Carrière thought it was a continuation of the septum, and Rawitz describes it as being differently coloured in the various species. Schreiner explains Hickson's view on the grounds that in P. maximus, which he examined, the pigment was really a fluid mass containing large and small granules, but adds that in other species this layer is a single or double row of rather large polygonal cells.

I have investigated several species and find that this layer is cellular in all, though the boundaries of the cells may be difficult to see in the adult. In young specimens of Pecten, only a few millimetres in diameter, the pigment-layer appears to be composed of a single layer of epithelial-like cells with little or no pigment present.

As the eye grows the pigment increases, the cells become filled and usually very irregular in shape, so that in large eyes of P. maximus the epithelial arrangement persists often only in the marginal part, and in the middle the layer may be irregularly two cells thick.

The actual colour of the pigment is of little importance, since it varies in specimens of the same species and often in cells of the same eye. It is some shade of red-brown, and generally the cells are filled with a finely granular dark brown pigment, but with here and there frequently large, more darkly coloured bodies, like round concretions (Pl. 6, fig. 1, *Ta. c.*). There are large and small bodies of this nature, and sometimes also iridescent granules resembling in appearance the substance of the tapetum. The nuclei are best seen in iron hæmatoxylin preparations. In P. maximus

they are round and contain a conspicuous nucleolus together with scattered chromatin granules.

The cells of the pigment-layer appear to be continuous with the retinal cells at the periphery of the retina. Patten considered this layer, in fact, to be homologous with his outer ganglionic layer. I am unable to say whether it should be considered as a modified continuation of the distal sense-cells or of the outer interstitial cells. The nuclei are much more like those of the former, but the development of the Pecten eye still requires elucidation. This completes the account of the structures enclosed in the optic vesicle. A reference must be made here to the inner wall of the proximal hemisphere of the latter. It is formed of connective tissue, and Patten called the surface layer the "sclerotica" (Pl. 6, fig. 1, *Sc.*). He described it as a two-layered, tough, hyaline, connective-tissue membrane continuous with the septum.

Rawitz disagreed entirely with this and objected to the term "sclerotica," because of its inappropriateness, considering the use of this term in the nomenclature of the vertebrate eye. This membrane of Patten is, however, well marked in longitudinal sections of the eye, though it is simply the limiting or surface layer of the connective tissue of the eye-stalk and directly continuous with it. In sections stained with Mallory's fluid it is very conspicuous (Pl. 6, fig. 1, *Sc.*), and stains a deep blue against the light blue of the ground tissue of the eye-stalk. It also differs from the latter in being hyaline and containing neither fibrous elements nor nuclei. The connective tissue forming the wall of the distal part of the optic vesicle lacks this differentiated surface layer entirely. In reactions to several stains it resembles the septum, and it also appears to be continuous with this membrane. The layer is thus obvious, but is not to be considered as a separate structure in Patten's sense, and the term "sclerotica" is certainly inapplicable.

I have called it simply "the modified connective tissue-wall of the optic vesicle." It must be remembered that the terms "cornea," "sclerotica," "iris," etc., used by Patten and others

cannot be compared directly with those designations in the vertebrate eye, for the structures bearing these names are not homologous, and in fact the whole structure of the eye is not to be homologised with that of the vertebrate optic organ. The resemblances are pure cases of homoplasy, and there is absolutely no proof of a genetic community of origin.

Innervation and General Conclusions.

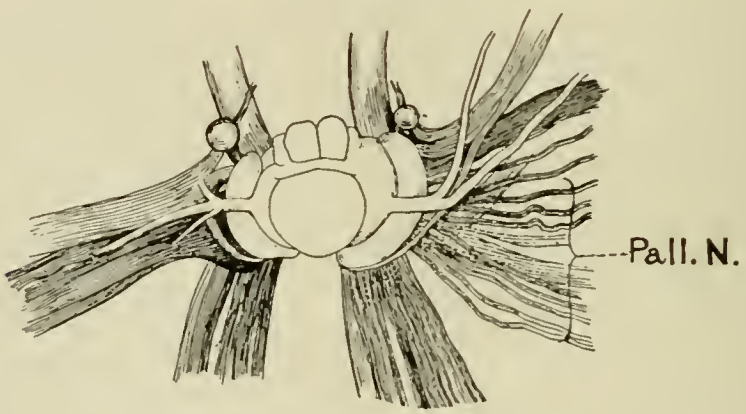
It has already been pointed out that the retina is innervated by two branches of an optic nerve which passes down the centre of the eye-stalk (Pl. 6, fig. 1; Pl. 7, fig. 2, *Op. N.*).

This nerve has been considered as an offshoot from the circumpallial nerve. In sections which cut the optic nerve obliquely, so that only a small part appears in a section, this may very easily appear to be the case, but if a section cuts the mantle exactly in the plane of the optic nerve, so that a long stretch appears in one section, it will be seen that the real state of things is somewhat different. At irregular intervals nerves pass radially through the mantle-lobes (between the radial pallial muscles) from the visceral ganglion to the circumpallial nerve (Pl. 7, fig. 2, *Circ. N.*). Some of the fibres of these nerves pass into the latter, but at certain places (below the eye-stalks) the bulk of the fibres pass round the circumpallial nerve (on the shell side of it, Pl. 7, fig. 2), touching it, but not entering it, and these innervate the eye. Some fibres appear also to leave the circumpallial nerve and to enter this optic nerve, but it will be evident that most of the nerve-fibres come directly from the visceral ganglion.

Now the visceral ganglion of Pecten is extremely complicated in build and I think unique among the Lamellibranchiata. No details will be given here, since a paper is being prepared on this subject, but it will be seen from the figure (text-fig. 1) that there are several lobes, of which two lateral ones are very conspicuous. From these radiate out on either side the pallial nerves (*Pall. N.*). The ganglion is asymmetrical, the left lateral lobe being larger than the right, and

it is from these lobes that the nerves arise which innervate the eyes. It is interesting, therefore, to observe how the development of the eyes has affected the ganglion, for in P. jacobæus and P. maximus, where the number of eyes on the left mantle-lobe exceeds that on the right, the left lateral lobe of the visceral ganglion is considerably larger than the right, especially in the former species, whereas in P. opercularis, where the number of eyes is more equal on both sides, the left lobe is but slightly larger than the right. Probably the presence of both lateral lobes is due in the first instance to the great development of pallial structures.

Text-fig. 1.



1.

The retina of Pecten is of the inverted type, that is (like the vertebrate eye), the recipient bodies, the rods, are directed towards the tapetum, and away from the source of light (text-fig. 2). In addition to this feature we have a complexity only paralleled in a few cases in the invertebrata (and even then without the inversion), for there are two series of recipient cells. Inversion occurs in the Platyhelmia, though the eyes are much simpler than the Pecten eye. In the Lamellibranchiata the eyes are either absent or much more simple as a rule than the eye of Pecten, but we have as a matter of fact the two eyes most like the one we are considering in this group, namely, the pallial eyes of Spondylus, which are practically the same as Pecten, and the eyes

(siphonal) of Cardium. In both cases there are two series of recipient cells and the retinas are inverted.

There are some interesting analogies; thus, for example, the ocelli of Agrion (a dragon fly) possess a retina which has also two series of recipient cells very like the rod-cells with rods and the distal cells of Pecten, but there is no inversion.

TEXT-FIG. 2.

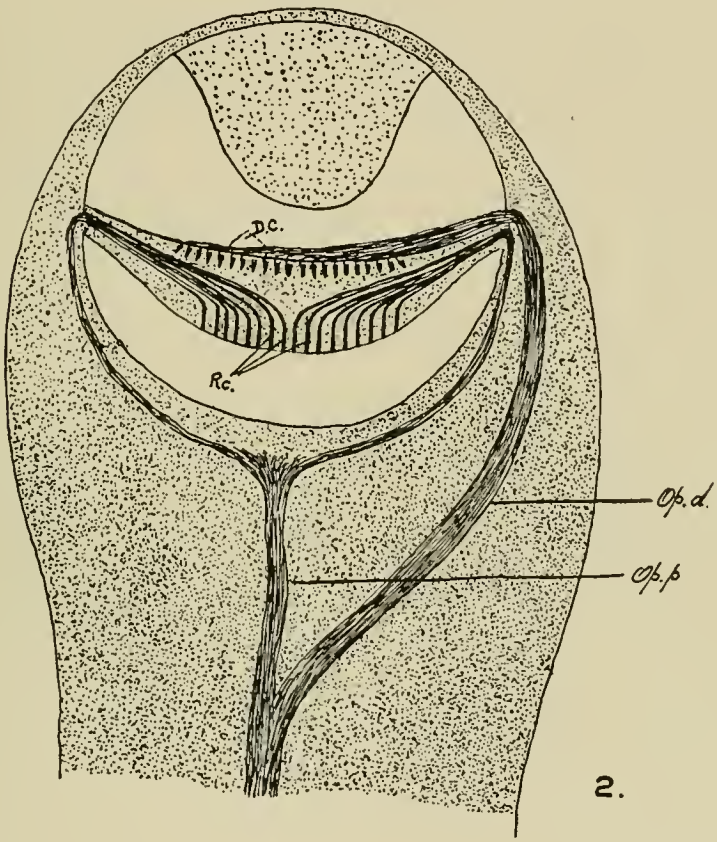


Diagram showing distribution of Nerve elements in Eye
Op.p. Optic Nerve, proximal branch. Op.d. Distal branch of Optic Nerve
D.C. Distal Sense Cells. Rc. Rod cells with Rods.

We are also familiar in the vertebrate eye with two kinds of recipient structures—the rods and cones—though these bodies are situated in practically the same layer (Bernard [**36**] has, however, stated that in Amphibia the cones are earlier stages in the development of new rods).

When all things are taken into consideration the eye of Pecten and also of Spondylus appears a very remarkable

development, especially for a Lamellibranch, and the complexity of structure, together with the large number of eyes, has been a difficulty felt by most writers who have sought for an explanation of these organs. Patten put forward an extraordinary theory, calling the eyes "heliophags." It is hardly necessary to go into this here, since a criticism appeared in the 'Quarterly Journal of Microscopical Science,' vol. 27, which may be referred to.

The eyes have shown no evidence of being phosphorescent organs, though I have observed and stimulated them at night and in the dark. A shadow thrown on to the eyes of an open Pecten causes a closure of the valves, and this reaction usually takes place very rapidly, though very often the perception of light stimuli does not appear to be any better than by Arca with very simple eyes or others with pigment spots. If, however, the shadow thrown on to a Pecten does not extend over a number of eyes there appears to be no reaction, and, just as Rawitz observed some time ago, a small object quite near produces no effect unless its shadow falls on a large number of eyes in quick succession. No evidence of accommodation could be obtained experimentally. Furthermore, it is hardly possible to correlate the presence of these structures with the active habits of the animal, e.g. swimming, for Lima swims just as well as Pecten, but has extremely simple eyes. Again, Spondylus has eyes practically identical with those of Pecten, but does not swim, and the same thing applies to the only other Lamellibranch with an eye approaching that of Pecten in structure, namely Cardium. In the latter case the eyes are confined to the tentacles of the siphons. It would be interesting to determine by biometric methods whether these organs were still being kept up, or were degenerating, especially in forms like P. jacobæus and P. maximus, where there exist very small eyes side by side with the large ones.

These may be growing, or they may be eyes which have retained their young form, have not grown, and will not grow. They agree with young eyes in structure. The

variation, however, in specimens of the same size renders the examination of a large number a necessity, and I have been unable to obtain a fraction of that number. It is possible that P. jacobæus and P. maximus are more highly developed forms than P. opercularis and P. varius, for they possess no byssus, though the gland is present and they have passed through a byssus stage, and the retractor muscles of the foot, of which one is left in P. opercularis, are even more vestigial in P. maximus. If these two forms are considered older we find that there has been a reduction in the number of the eyes, for they are more numerous in P. opercularis, P. tigrinus, and other smaller forms, and this reduction has then taken place to a greater extent on the under convex valve than on the upper flat one. The increase in convexity and difference between the two valves, reaching a maximum in P. jacobæus, has been accompanied by a reduction of the eyes on the convex mantle-lobe both in number and size. These are, however, only hypotheses. The large number of eyes present is probably to be accounted for by the reason put forward by Rawitz, namely, that the actual recipient area in each eye is small, that oblique rays are cut off, and that in life the eye-stalks remain still; a large field of view is therefore only possible with numerous eyes.

The presence of two series of recipient elements has not been explained by previous writers and has in fact been usually passed over. No experiments have enabled me to state anything definitely about this, except that, as already mentioned, there appears little evidence of accommodation. It might be advisable to point out here that the removal of an animal like Pecten from the dim regions at the bottom to the daylight and shallow water of the aquarium has possibly an injurious effect, and probably it would be a delicate complicated structure like the eye that would suffer most. Hence it may be that our aquarium experiments are almost useless in this respect.

The presence of the distal layer of sense-cells as well as

that of the rod-cells and rods may be a device for increasing the area of the recipient elements without increasing to any extent the size of the retina, but more probable is perhaps the following view. There has not yet been definitely proved to exist any special apparatus for accommodation in the eye (though Hesse's theory has not been disproved). Now it may be that the two layers of recipient cells are for the reception of images of objects situated at different distances from the eye, which are focussed at different distances from the lens. Thus the image of near objects would be focussed on the rods and that of distant objects on the outer distal cells. A similar condition would apply to the ocelli of Agrion, and, in fact, Hesse describes such (35), but adds, "Ich kenne nirgends eine ähnliche Einrichtung." In the Heteropod eye there also appears to be a device for the reception of rays from objects at different distances from the eye. There is, however, only one series of cells, but the free ends bearing the comb-like margins are turned so that they are at right angles to the plane of the retina, and some are nearer the lens than others.

The development of the Pecten eye still remains incompletely known, and Patten's observations need confirmation. The derivation of the various layers will certainly throw much light on the structure of the adult eye and the inversion of the retina. Unfortunately the material for such a research is somewhat difficult to acquire as all the elements are formed in extremely young specimens, and I have been unable therefore, so far, to follow out this line of inquiry.

It will be perhaps useful if the most interesting features in the general structure of the Pecten eye are summarised here and a few comparisons made with other eyes, which may bear some resemblance to the former. The eye is a closed vesicle; there is a cellular cornea continuous with the surface epithelium, and below this a cellular lens. The retina is made up of two series of recipient cells innervated by two branches of an optic nerve. The cells of the distal layer have each a comb-like margin, and the proximal visual cells bear rods

with an axial neurofibril. The retina is of the inverted type. The eyes are not cephalic, but occur on the mantle-lobes.

There is no ground whatever for placing the Pecten eye in the same class as the vertebrate eye, for the resemblance is very superficial, and though the retina is inverted in both cases this has been produced in very different ways. If we consider Bütschli's observations as correct the retina of the Pecten eye has been formed from an invagination of the ectoderm, which forms a closed vesicle cut off from the surface. The distal wall of this gives rise to the retina, and the proximal to the pigment layer.

Amongst invertebrate eyes that of Spondylus is the only one that can be safely homologised with the Pecten eye. The structure of these organs is identical but for one point, a layer of cells in Spondylus takes the place of the non-cellular septum of the Pecten eye. The eye of Cardium can also be homologised, though with less certainty. There is a cellular lens, an inverted retina with two series of recipient cells, and also layers corresponding in position to the tapetum and pigment layer of Pecten. There is, however, another layer (the choroid) interpolated between the retina and tapetum, which may be taken as equivalent to the interstitial cells of the Pecten eye.

These are, so far as I am aware, the only vesicular eyes occurring in the Lamellibranchiata.

In the highly organised cephalopod eye we do not meet any resemblance to the Pecten eye, except that the visual cells bear rods with an axial neurofibril like these recipient structures in the latter. There is a single layer of recipient cells directed towards the light, and the lens is not cellular and arises quite differently from the lens of the arthropod eyes.

Amongst the Polychæta there are some highly organised visual organs, in particular those of the Alciopina, ex. Alciopa and Vanadis, and the large and complex organs of these forms have been studied in detail by Greeff and Hesse. The eye takes the form of a closed vesicle as in Pecten, the free pole being formed by a cellular cornea, a continuation of the

general epithelium of the body-wall. The inner wall of this optic vesicle is, however, also made up of a layer of cells, which though forming a complete hollow sphere, are differentiated in three regions, in structure and function. Those cells immediately under the cornea just spoken of are low and form a second and inner cornea. The cells lining the proximal half of the optic vesicle are the retinal cells, and between this area and the inner cornea the cells are again different and contain pigment.

There is only one series of recipient cells in the retina, and they bear rods which resemble those of the Pecten eye and contain a very distinct axial neurofibril. They are, however, directed towards the lens, that is, not inverted. The lens is spherical and non-cellular, and another difference from the eye of Pecten is produced by the presence of a vitreous body between lens and retina.

There are several interesting arthropod eyes that may be briefly referred to. The ocelli of Cloëon (one of the May-flies) are distinctly peculiar and are superficially rather like the Pecten eye, but this resemblance is due to the dioptric part of the eye, and not to the retina. We have again a closed vesicle. The cuticle extends over the cornea, but remains thin and does not form a corneal lens. The hypodermis forms a cornea similar to that of Pecten. Under this cornea and lying in the optic vesicle is a cellular lens strikingly like that of Pecten and altogether unlike other arthropod eyes. The retina is made up of two layers of cells, but the distal ones are not visual and the proximal ones forming the retina proper are not inverted.

Another interesting arthropod eye is the ocellus of Agrion. This bears some resemblance to the Pecten eye in the fact that there are two series of recipient cells in the retina. They are, however, not inverted. The distal part of the optic vesicle is quite different, and the chitinous exoskeleton or cuticle is thickened over the free surface, forming a corneal lens. This is a monomeniscous arthropod eye therefore, and the arrangement of the retinal cells is interesting.

The distal layer of sense-cells lie touching the lens, almost like the outer cells of Pecten touch the septum.

A striking difference from the Pecten retina is, however, present which lends at the same time support to the view of Leydig, upheld by Lankester in 1883, namely that the compound eye is formed by the segregation of the elements of a simple eye, and this is the segregation of the retinal cells. The visual cells do not remain, as in the Pecten eye, altogether independent with their recipient ends directed towards or away from the lens, but bear a comb-like margin of neurofibril endings laterally and are collected in groups of threes, each group being a retinula. Thus we have a monomeniscous eye with a retinulate retina, the whole being very different from the Pecten retina except in the one point —the presence of visual cells arranged in two layers.

The central eyes of the Scorpions may finally be mentioned here. These are also monomeniscous and present a far greater resemblance to the Pecten eye than appears at first sight. They are vesicular, though the cavity of the vesicle has disappeared and the retina is inverted, though, owing to a secondary reversion during development, this is not obvious.

The eyes are developed from an involution of the hypodermis or ectoderm, which, however, does not lie vertical to the surface. The outer wall becomes thickened and forms the retina; the inner wall remains thin and represents the post-retinal layer of ectoderm cells in the adult. This is strikingly like the process in the Pecten eye where the inner wall becomes the pigment layer. The retinal cells are of course inverted. The nerve-fibres are attached to the outer ends of these cells in the embryo, but, owing to reversion in the course of development, become connected to the inner ends in the adult eye. In the course of these changes the optic nerve must penetrate the post-retinal layer, and this has been shown by Ray Lankester and Bourne (**46**) to be the condition actually prevailing in the adult. Beyond this remarkable similarity in development the eyes

are very different: there is a retinulate retina of one layer of recipient cells which are segregated in groups of fives, and the dioptric part is again represented by a corneal lens.

It will be seen, therefore, that no eye outside the Lamellibranch group presents anything but isolated features of resemblance, and the only common structures appear to be the general occurrence of rods with axial neurofibrillæ or visual cells with a margin of cilia-like processes arranged like the teeth of a very fine comb, and these margins may form rhabdomes.

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EXPLANATION OF PLATES 6 AND 7,

Illustrating Mr. W. J. Dakin's paper on "The Eye of Pecten."

List of Reference Letters.

Ax. f. Axial fibril of rods. *B. m.* Basement-membrane. *Circ. n.* Circumpallial nerve. *Cent.* Centrosome of lens-cells. *Co.* Cornea. *Co. S.* Sub-corneal connective tissue. *Con.* Connective tissue of eye-stalk. *Cut.* Cuticle. *D. S.* Distal sense-cells. *D. Sn.* Nuclei of distal sense-cells. *Eye.* Eye. *I. I. C.* Inner interstitial cells. *L.* Lens. *L. C.* Lens-cells. *Lac.* Blood lacunæ of eye-stalk. *L. f.* Muscle-fibres on distal surface of lens. *M.* Mantle. *M. f.* Muscle-fibres of connective tissue of optic vesicle. *M. lf.* Muscle-fibres of lens surface. *Mus.*

Muscles of eye-stalk. *M. Ret.* Marginal area of retina. *N. Lf.* Nuclei of muscle-fibres of lens surface. *nuc.* Nucleus. *O. I. c.* Outer interstitial cells. *Op. D.* Distal branch optic nerve. *Op. Ds.* Sheath of distal nerve. *Op. N.* Optic nerve. *Op. P.* Proximal branch optic nerve. *Op. P*^{II}. Fibres (separated) of proximal branch of optic nerve. *P. man.* Pigment-mantle. *Pg.* Pigment-layer. *R. C.* Rod-cells. *R. C. n.* Nuclei of rod-cells. *R. mat.* Rod-matrix. *Rod.* Rod. *S. m.* Pseudo sieve-membrane (see text). *Sc.* Modified connective-tissue wall of optic vesicle. *Sep.* Septum. *Ta.* Tapetum. *ta. c.* Pigment layer concretion. *V.* Velum.

PLATE 6.

Fig. 1.—Section through eye-stalk and eye, P. maximus, in a plane at right angles to that of the mantle surface; the right side of the figure represents the shell side of the eye. The various parts, lens, retina, etc., have been drawn with the camera lucida, but from different preparations, each showing best the part drawn. × 270.

PLATE 7.

Fig. 2.—Diagrammatic section through both mantle-lobes of P. jacobæus, illustrating the course of the nerves and difference in size of the eyes. The left mantle-lobe is to the left in the figure.

Fig. 3.—Upper surface of cornea, P. maximus. × 1000.

Fig. 4.—Transverse section of corneal cells at about the middle of their height. P. maximus. × 1000.

Fig. 5.—Isolated cells from the lens. P. maximus, maceration in chloral hydrate solution. × 570.

Fig. 6.—Lens-cells as seen in sections. *b.* Normal cells from Hermann-sublimate fixed specimen, P. varius. *a.* Cell from same specimen with large nucleus. Stain iron hæmatoxylin. *d.* Cell from lens fixed in von Rath's fluid. × 660.

Fig. 7.—Transverse section cutting layer of fibres between lens and subcorneal tissue. The fibres and cells are stained red with Mallory's connective-tissue stain, the subcorneal tissue blue. P. tenuicostatus. × 310.

Fig. 8.—Fibres between lens and subcorneal tissue; attached to the latter in a maceration preparation (chromic acid). P. jacobæus. × 300.

Fig. 9.—Cells and nuclei between lens and subcorneal tissue, as seen through the cornea, which has been teased from an eye fixed in Zenker's fluid. Iron hæmatoxylin. P. maximus. × 330.

Fig. 10.—Transverse section of cornea and subcorneal tissue of P. jacobæus (Bielschowsky-Paton method). × 650.

Fig. 11.—Isolated cells from distal surface of lens. P. maximus, chromic acid maceration. × 330.

Fig. 12.—Transverse section of cornea and pigment-mantle of P. tigrinus. Fixed Zenker, stained Mallory. × 300.

Fig. 13.—Schematic view of retinal elements, reconstructed from sections and macerations. The two left-hand rod-cells are shown in external view, from macerations, and the two right-hand ones in section. × about 920.

Fig. 13*a*.—Distal ends of two rod-cells (chromic acid maceration).

Fig. 14.—Rod-cells with partly broken-up rods, showing the bristle-like appearance of axial fibre. P. maximus (chromic acid maceration). × 900.

Fig. 15.—Isolated interstitial supporting cells from retina; *a* and *b* are two outer interstitial cells. P. maximus. Chromic acid and chloral hydrate macerations. × 900.

Fig. 16.—Transverse section of distal sense-cells and outer interstitial cells. Mallory's connective-tissue stain. P. maximus. × 940.

Fig. 17.—Transverse section of rods and rod-matrix. P. jacobæus. Fixed Zenker, stained by modified Weigert method. × 800.

Fig. 18.—Distal branch of optic nerve, breaking up into branches on surface of septum. P. jacobæus. From teased preparations. × 250.

Fig. 19.—Tapetum in surface view. From sections. The large circle shows relative size of a rod-cell in section. × 1600.


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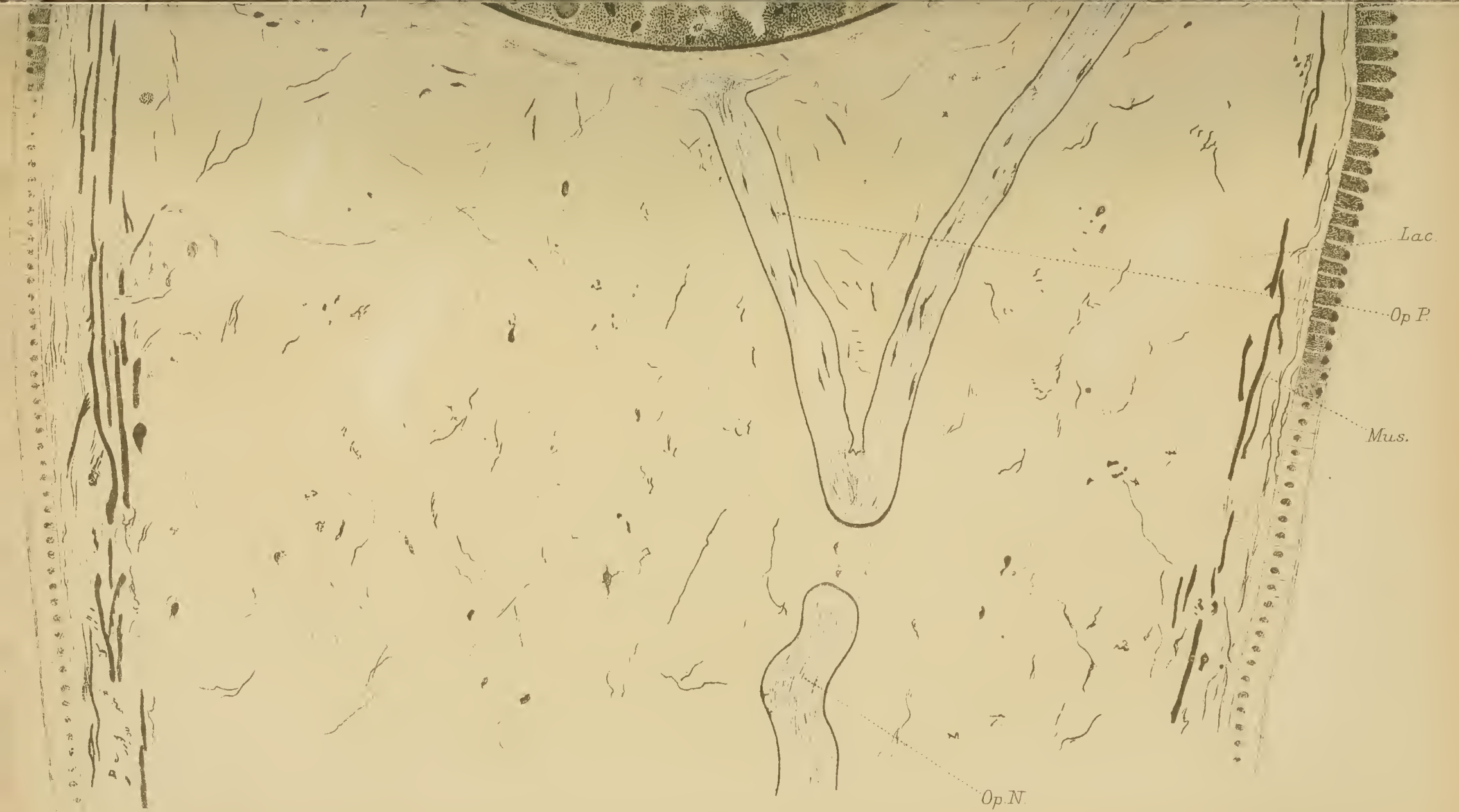

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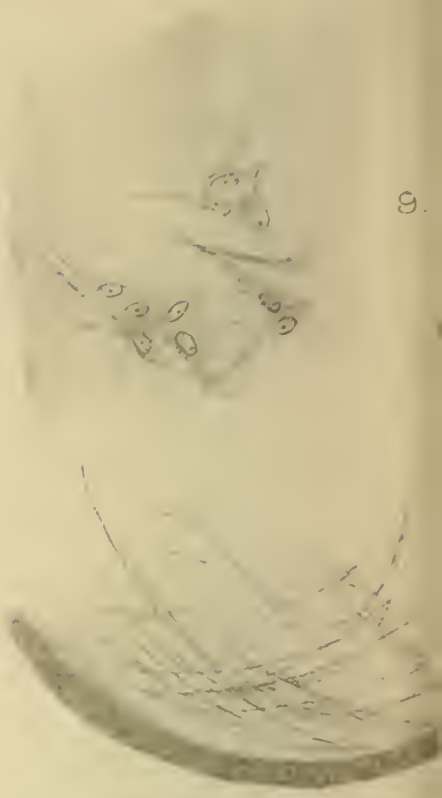
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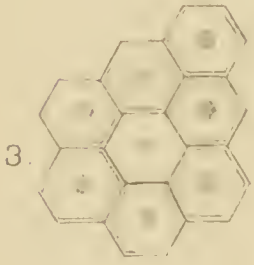
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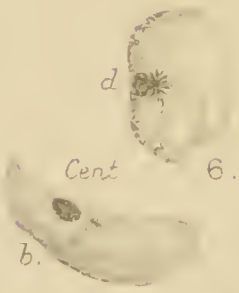
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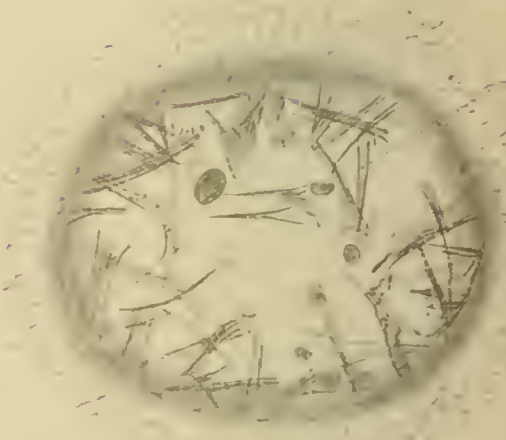
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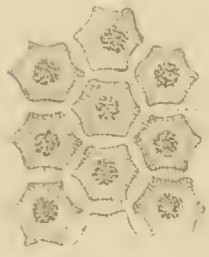
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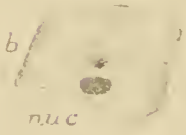
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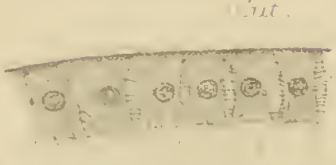
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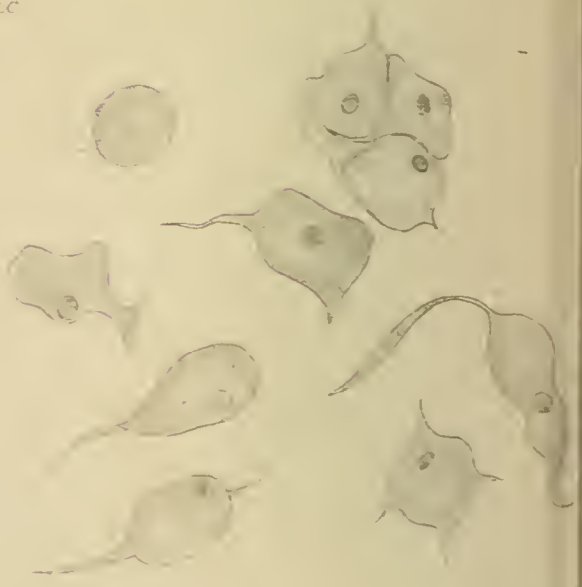
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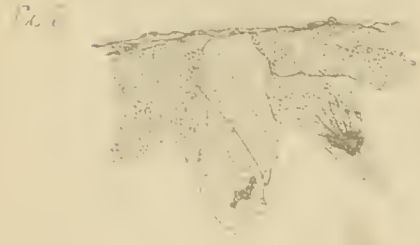
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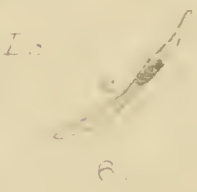
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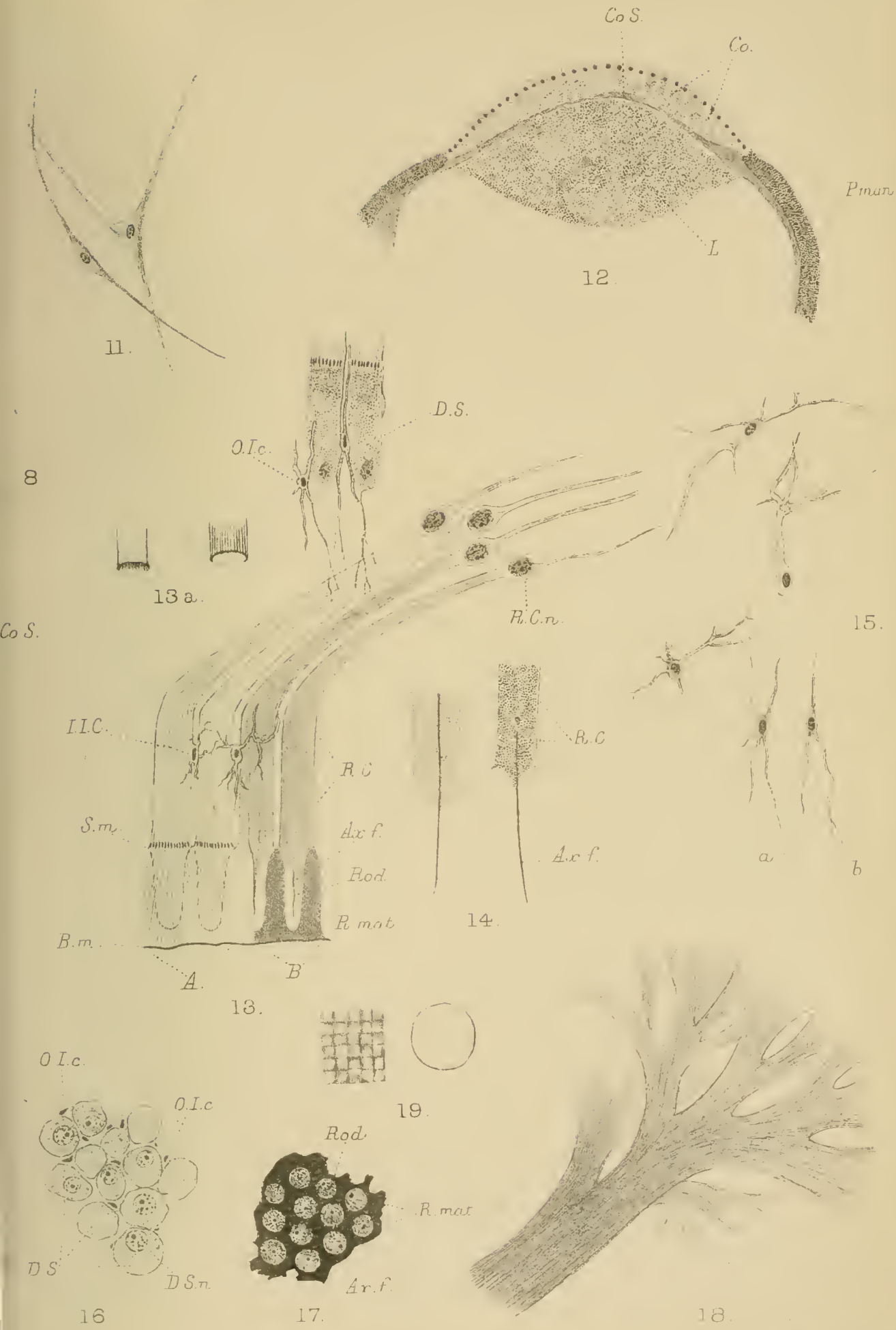
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Observations on certain Blood-parasites of Fishes occurring at Rovigno.

By

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With Plates 8–10.

The objects of this paper are twofold. The first is the description of a hæmogregarine and a trypanosome occurring in a species of Trigla (T. lineata), which is a common gurnard in the Northern Adriatic. We are unaware of any published observations of blood-parasites in this fish, and we regard the hæmogregarine as a new species, for which we propose the name Hæmogregarina rovignensis. Neumann, in his recent account of hæmatozoa of marine fishes at Naples (**15**), mentions the occurrence of a trypanosome which he names T. triglæ in another species of Trigla, namely, T. corax. It is not at all improbable that the trypanosome we have found is the same species as that described by Neumann; we therefore do not propose to give it a new specific name.

The second object that we have in view is the comparison of the minute structure of the nucleus of the above-named hæmogregarine with that of the trophonucleus of a trypanosome. For this purpose, as the trypanosome of the Trigla is very rare in our material, a trypanosome which occurs in much greater abundance in skates (Raia, sp.) in the neighbourhood of Rovigno has been selected. This form is most probably T. raiæ Lav. and Mesn.

With regard to the technique, permanent preparations were made according to both the principal methods in use. Smears

on slides were fixed in osmic vapour[1] for half a minute and then placed in absolute alcohol for ten minutes or so. They were stained with Giemsa's solution in the ordinary manner for two and a half to three hours and afterwards differentiated with orange-tannin and acetone. Smears on cover-slips, on the other hand, were at once dropped face downwards into sublimate-acetic mixture or Schaudinn's fluid for fixation, after which they were brought up to 90 per cent. alcohol. These smears were stained either with iron-hæmatoxylin or with Twort's stain and finally mounted in balsam. Fuller details regarding this mode of procedure will be found in Minchin (**11**), the method there described being that followed in the present case. The figures of the hæmogregarine are all drawn to a magnification of 3000, those of the trypanosomes to a magnification of 2000. We are indebted to Miss Rhodes for drawing the greater number of the figures and for colouring nearly all, and take this opportunity of expressing our thanks to her.

I. Hæmogregarina rovignensis, n. sp.

(Figs. 1–28, 39–50, and 58–62.)

This parasite was found on three occasions in the early spring. In the three gurnards in which it was seen it was not at all infrequent in the blood, as we have since learnt by the study of stained preparations. The parasite, chiefly in its small intra-cellular form, was observed in the living condition in cover-slip preparations of fresh drops of blood, but only with difficulty, since it was not readily distinguishable from the cytoplasm of the blood-corpuscle. When made out with certainty it was seen as a small oval area, somewhat clearer and paler than the surrounding cytoplasm. No prominent refractile granules were noticed, nor could the nucleus be made out satisfactorily. We did not observe any alteration

[1] A 4 per cent. solution of osmic acid was used, to which a drop or two of acetic acid had been added.

or movement in these intra-cellular forms so long as they were kept under notice, but in a few of the living preparations one or two small forms were seen free. These in all probability corresponded to the small intra-cellular parasites. They appeared as sausage-shaped bodies very slightly curved. One third or so of the body was granular in character; this probably was the region of the nucleus (cf. description of fixed and stained parasites below). They underwent no active displacement, and it was difficult to feel sure whether they really moved or altered in shape at all or not. Now and again the concavity would appear to lie first on one side of the body and then on the other, and now and then the body would stand on one end, appearing in transverse optical section as a round globule; but these changes were extremely slow, and may have been due to passive rotation of the vermicule, caused by very delicate currents in the blood-plasma at that spot, not sufficient to disturb the corpuscles. As we did not see any intra-cellular forms actively liberating themselves from the corpuscle, it may possibly be that these free individuals were accidentally liberated by the rupture of the corpuscle during the process of manipulation, which would explain their apparent immobility. In the stained smears only one such free form has been found (fig. 3).

In most of the permanent preparations made only a little searching with the oil-immersion lens is required to find a hæmogregarine. The parasite does not appear to be in quite the same period of its development in all three gurnards. In gurnards 1 and 3 the hæmogregarine is present chiefly or almost entirely in the one phase—the small type. Large forms (see below) do occur, but they are evidently very rare. Two or three individuals of the latter type have been found both in the general circulation (fishes 1 and 3) and in a smear from the kidney (fish 1), but they have not been observed in smears from the liver or spleen, and no other phases have been seen in any of the organs. In fish 2, on the other hand, in which the parasites are rather more numerous, besides the small forms many large forms occur

in the general circulation, and these belong to two distinct types. Most unfortunately, as it has turned out, no smears from the organs were made in the case of this fish.

Considering first the relation of the parasites to their host-cells, we find that nearly always a single individual is present in a blood-corpuscle. Instances of a double infection occur, but they are very rare; we have only noticed two. In these cases two small forms lie side by side in one half of the corpuscle, the nucleus being displaced to the opposite end (fig. 27). The only effect which the parasites appear to have on the corpuscles is a mechanical one. In no case does the hæmogregarine attack the nucleus or cause its hypertrophy or other degenerative effect.[1] In corpuscles infected with the small forms the nucleus is generally displaced to one side (cf. figs. 1, 2, 16, 17). In the case of the large forms, the nucleus of the host-cell is often displaced quite to one side, lying at the periphery of the corpuscle (figs. 18–26). It may be slightly flattened or compressed (figs. 18–20), but shows no other sign of alteration.

In a few cases indications of the presence of a delicate membrane or envelope around the intra-cellular parasite appear to be furnished by the stained preparations in a rather interesting manner. Usually no signs of such a structure can be detected; even where, as occasionally happens, the parasite has shrunk slightly from the enclosing corpuscle, leaving a clear space around itself, it cannot be said, as a rule, that a definite sheath is apparent. But now and again the stain is deposited in a marked manner in the protoplasm of the corpuscle immediately around the parasite, which points to the existence of a layer of somewhat altered character, acting as a hindrance to the further penetration of the stain. This is especially well seen in those cases where the cytoplasm of the parasite happens to have partly or entirely shrunk (as in figs. 6, 39), leaving a deeply stained line bordering the outer edge of the clear space. We have noticed this appearance occasionally around both the small

[1] Hence this parasite does not belong to the genus Karyolysus.

forms and the large ones of the wide type (fig. 11), but it is not shown clearly in the case of the large forms of the narrow kind, perhaps because the deeply stained cytoplasm of these individuals renders it less apparent. We regard this sheath as probably in the nature of a cytocyst, i. e. an altered layer of the blood-corpuscle.[1]

In describing the structure of the parasites we may begin with the small forms. These show, on the whole, great uniformity in size and appearance. The body is nearly always oval in shape, either a fairly regular oval (figs. 39–42) or slightly pyriform, the half in which the nucleus lies being in this case somewhat narrower than the other half, and the end less broadly rounded (figs. 2, 8, 16). The average size of these small individuals—the mean being taken of several measurements—is 4·8 μ in length by 2·3 μ in greatest width on "wet" smears (sublimate-acetic mixtures, iron-hæmatoxylin), and 4·9 μ in length by 2·4 μ in width on "dry" smears (osmic, absolute alcohol, Giemsa). We regard the above figures as representing, as nearly as possible, the true or correct average size of this phase. The largest "normal" dimensions noticed are 5·2 μ by 2·6 μ, and the smallest 4·4 μ by 2·2 μ. We add "normal" because it is interesting and instructive to note one or two instances which well illustrate the considerable variation in this respect, which may be caused solely by the technique. One of them[2] is afforded by an extremely thin, "dry" smear in which both corpuscles and parasites are greatly flattened out and consequently enlarged. On this smear the apparent average size of the small form is 5·6 μ by 3 μ, and some individuals measure as much as 6·2 μ by 3·3 μ. On the other hand, in a particular "wet" smear (Schaudinn, Twort) both corpuscles and parasites are uniformly smaller than the normal average on other films made

[1] We have observed nothing so well marked as the capsule, with lines of rupture, described by Sambon and Seligmann, for instance, around certain hæmogregarines of snakes (**21**).

[2] It is, perhaps, scarcely necessary to mention that these cases are not included in the above "standard" average.

at the same time. Here the average is only 4·2 μ by 2·2 μ (cf. figs. 58, 60, 61). We are rather at a loss to account for this case, but it seems evident that shrinkage must have taken place sometime during the manipulation.

In the small type of the parasite the nucleus is relatively large. As already mentioned, it is generally situated entirely in one half of the body and near one end, but now and then it occupies a more median position (fig. 5). In Giemsa-stained preparations the nucleus appears to take up quite half the body, or even more, and to occupy its entire width or even to bulge out slightly at the sides (figs. 1, 2, 7). From preparations stained with iron-hæmatoxylin, however, it is apparent (cf. figs. 39–45) that in the former smears there is a certain amount of artificial enlargement, due to the characteristic overloading with the stain which so detracts from the value of the Romanowsky method. We reserve a detailed description of the structure of the nucleus until later, when we compare it with that of a trypanosome. The general cytoplasm appears fairly homogeneous in character, and with the exceptions to be mentioned, is usually devoid of granules. A conspicuous vacuole is frequently present; this lies about the middle of the body, generally close to the nucleus. In Giemsa smears it is sharply defined, because the cytoplasm, as a rule, is distinctly stained, either purplish or bluish in tint (figs. 1, 8, 16, 17); but in iron-hæmatoxylin preparations it is often difficult to make out, probably owing to the fact that the cytoplasm of the parasites in these smears is itself very pale and scarcely stained at all; sometimes, however, it is well seen (fig. 40). Rarely the cytoplasm contains two or three vacuole-like areas, with less sharply defined limits (cf. figs. 2, 41).

The most striking feature in the morphology of the parasites, as brought out by iron-hæmatoxylin, is afforded by one or two large granules, which take up this stain with intense avidity (cf. figs. 39, 40, 43, 46). They are often present, but not always (figs. 41, 42). When they occur these granules are by far the most prominent objects in the body, appearing

always larger and much blacker than the chromatic grains of the nucleus. The granules are of about the same size whether two are present or one. They are always distinctly outside the nucleus, lying usually close to it, however, about the middle of the body. They were first noticed in the iron-hæmatoxylin preparations, and we surmised at first that they might correspond to part or all of the conspicuous vacuolar region seen in Giemsa smears, but have found since that this is not the case. In some individuals both grains and vacuole are seen to be present (figs. 1, 13, 16, 40). Sometimes one of the grains appears to lie in the vacuole, but we think that, in such a case, the grain is really outside the vacuole, lying above or below it instead of at the side (cf. especially fig. 43, where one grain is at the side, the other apparently in the vacuole). Although the grains (or single grain) usually lie close to the vacuole, this is not always so; for instance, in the parasite drawn in fig. 46 the grain is well removed from the nuclear zone, while in fig. 39 it is on the opposite side of the nucleus, near the other end of the body.

Turning next to Giemsa smears for indications of these grains, we have found that there is often considerable difficulty in recognising them with certainty. This is chiefly because none of the individuals show any signs of bodies which have taken up the Giemsa stain in the same intense manner in which the above-described granules stain with iron-hæmatoxylin. In individuals which do show grannles that can be reasonably identified with those, the granules are markedly smaller in size. Hence care has to be taken not to be misled by stray, reddish-staining grains of the ordinary chromatoid character, of which occasionally one or two are present in the cytoplasm. Making all possible allowance for such, we do nevertheless find in some parasites one or two definite grannles, situated close to the cytoplasmic vacuole, in a position similar to that often occupied by the granules in the iron-hæmatoxylin smears, which there can be little doubt actually correspond to those bodies. They stain dark-reddish in most smears, about the same colour as the chromatin-masses of

the nucleus, whereas isolated chromatoid grains are a fainter red. Figs. 16, 17 show instances of which we feel fairly sure, as also figs. 4, 25 of a large form. In the latter the two granules contrast distinctly with the mass of chromatoid substance, of which we have more to say below. Their position in this case, some distance from the nucleus, agrees closely, it will be noticed, with that of the one grain in the iron-hæmatoxylin individual drawn in fig. 49. On the specially thin smear, already alluded to, which was only lightly stained, most of the parasites show no signs of these granules; only in one or two individuals is a round, faintly pink-staining body present, which probably represents one (figs. 8, 9).

There is another point to mention in this connection. In Giemsa-stained smears, parasites which show these granules clearly are much scarcer than in iron-hæmatoxylin smears. The explanation is probably as follows: To judge from the iron-hæmatoxylin smears, in certain of the parasites the grains (or grain) are more or less separated from the nucleus (cf. figs. 44, 46, 49), but in others they are close to it and may be in contact with it. Hence in the Giemsa films, where the nucleus is so obviously enlarged by being overloaded with stain, the granules may be swamped, as it were, and not distinguishable from the nuclear mass. We may expect, therefore, to be able to recognise them only where they are well out of the nuclear zone.

Since the granules appear distinctly smaller in Giemsa-stained preparations than they do in iron-hæmatoxylin ones, it is most likely that the former depict more nearly the true size, and that in the latter there is some overloading, due to the strong affinity the granules have for the iron-hæmatoxylin stain. This is rather important in considering the significance to be attached to these bodies. For if they were of chromatin in the ordinary sense—like the grains and masses in the nucleus—we should expect to find them apparently quite as large in the Giemsa smears as in the iron-hæmatoxylin ones; since, as is well known, the Romanowsky stain is deposited most heavily in and around chromatic structures, with result-

ing enlargement of size (cf., for instance, the kinetonucleus of a trypanosome). The fact that this is obviously not the case here suggests that the granules are not chromatic structures.

In this connection certain of our Twort preparations are very instructive. As has been shown by Minchin (l.c.) the Twort stain is in one respect superior even to iron-hæmatoxylin, in that chromatic elements can be distinguished from others by the fact that they alone stain red, everything else being green. These particular Twort smears were examined soon after being made to see if they showed granules corresponding to the ones we had already found in iron-hæmatoxylin films. In several individuals one or two granules were seen, which we regarded as those for which we sought. They were small, however, and not particularly conspicuous. They were very faintly stained red, nothing like so deeply or sharply as the grains in the nucleus. Some of the individuals showing these granules were noted and sketched at the time, and then the smears were put aside to work out on our return to England. On finding the same parasites again recently, in order to draw them, we could no longer see the red granules in any of the individuals marked. All signs of them have vanished, although the red of the nucleus has not faded at all. In one or two cases, however, in about the position which was occupied by the red granules (according to our sketches), small rounded areas, somewhat diffuse in outline, can be made out, staining a rather deeper green than the surrounding cytoplasm (cf. fig. 59). It may be that these greener areas mark the position of the structures which stain so intensely with iron-hæmatoxylin. In the great majority of the individuals stained with Twort, however, the body is uniformly pale green in colour, and cannot be said to show any indications of the granules.

To sum up, we regard the above-described characteristic bodies as composed, at any rate, chiefly of achromatic material. Our opinion is based on the one hand upon a comparison of their staining reactions to iron-hæmatoxylin and to Giemsa, and

on the other hand upon the fact that they show very little, if any, real affinity for the neutral red of Twort's stain. It may be pointed out that, besides the fact of their being frequently double, these grains differ from kinetonuclei in their staining reactions. After Giemsa, they appear only reddish and have not the characteristic dark purple or almost black colour associated with true kinetonuclear elements; compare, for instance, the intensely staining chromatic grain described by Woodcock (23) in the case of a Halteridium of the chaffinch, which is quite distinct from the ordinary nucleus. Again, their appearance after Twort's stain shows no resemblance at all to that of kinetonuclei. With regard to the staining of these grains by Twort and iron-hæmatoxylin, one of us (E. A. M.) has found an interesting parallel in the case of the blepharoplast (basal granule) of the flagellum of Trypanosoma lewisi. In the multiplying (not the adult) forms of this parasite the blepharoplast appears after Twort's stain as a diffuse green patch, but after iron-hæmatoxylin it is seen as a definite black granule. This comparison suggests that the grains seen in H. rovignensis are centrosomic in nature, but apart from the above facts we have no further evidence to bring forward in support of this conclusion.[1]

We pass now to the consideration of the large forms of the parasite. These are, as already mentioned, of two distinct types: (1) Long, comparatively slender individuals, often slightly curved or bow-shaped, which possess a small nucleus (figs. 18–21); and (2) broad forms, oval or bean-shaped, which have a much larger nucleus (figs. 22–26). In the wide forms both ends of the hæmogregarine are generally similar

[1] We have noticed one or two references to the occurrence of bodies in other hæmogregarines, which may perhaps relate to a similar organella. Thus Miss Robertson (18) describes and figures two large oval "red bodies," staining red with Giemsa, in H. vittatæ: and again (19) she mentions the occurrence of an eosinophile body or vacuole, beside which is a sharply staining grain, in a hæmogregarine from Pleuronectids. Whether, on the other hand, the "Plastinkerne" described and figured by Prowazek (16) in H. platydactyli also represent a corresponding body appears more doubtful.

and more or less bluntly rounded. In the slender forms, on the other hand, one half of the body is usually narrower than the other and tapers towards its extremity, the opposite end being bluntly rounded. The more pointed end may be curved or partially bent up on itself (figs. 18, 21), this being most likely due to limitations of space; the drawn-out corpuscle is apparently incapable of being stretched to the full extent of the length of these forms. (We have not seen any phases in this parasite, it should be said, indicating the development of a definite U-form such as occurs in many hæmogregarines.) The average size of the slender type is 12 μ by 2·1 μ; and individuals up to 14 μ long have been observed, the breadth in this case being 2·2 μ. The stout forms average 10·6 μ by 3·4 μ, and their extreme size is about 11 μ by 3·5 μ. The size of the nucleus differs greatly in the two cases, and this is a constant feature. In Giemsa smears the nucleus of the slender type averages 2·6 μ by 1·7 μ and that of the wide type 4·8 μ by 2·9 μ; in iron-hæmatoxylin preparations the former is 2·5 μ by 1·4 μ and the latter 4 μ by 2·2 μ. In the slender forms the nucleus is always in the narrower, tapering half.

The structure of the nucleus appears practically the same in both the stout and slender individuals; it agrees closely also with the structure of the nucleus in the small forms. The cytoplasm of these large forms, however, differs greatly in character from that of the small parasites to judge from the effect produced by the Giemsa stain. The difference is especially noticeable in the case of the slender forms. Here the broader half of the body (not that in which the nucleus is situated) is nearly always more or less completely filled with some substance which stains red, and which may indeed appear at times almost as deeply and intensely stained as the nucleus (cf. figs. 19, 21). Often the colour increases in depth regularly towards the broad end, as if the substance which attracts the stain were most concentrated in that region of the body (fig. 18). From the effect produced by the Giemsa stain it would seem as if the mass were of a finely granular character and consisted of chromatoid material. Some of the

wide individuals show little or no indications of this substance (figs. 22, 23), but in others the condition is present to a greater or less degree (figs. 24, 25). It is never so prominent as it frequently is in the slender type of form. It is somewhat remarkable that neither iron-hæmatoxylin nor Twort show anything at all corresponding to this appearance so far as can be seen. After Twort's stain the body of the large form either appears finely granular and faintly tinted green (fig. 62) or else it is very pale, scarcely, if at all, stained (fig. 61).[1] Yet it is quite evident that there must be something more than merely the ordinary cytoplasm present to account for the appearance seen after Giemsa.

We are inclined to doubt whether the characteristic granules above described occur so frequently in these large forms as they do in the small ones. They are not visible, for instance, in either fig. 50, of a slender individual, or in fig. 48 of a broad one. One granule is seen in the stout form of fig. 49, however, and a couple in that drawn in fig. 25. Also in the slender parasite in fig. 4 there are a couple, but this is the only full-sized individual of this type in which we have made them out with certainty. Fig. 45 represents a slender form of intermediate size, and this shows a single prominent granule.

Nearly all the individuals of the slender and broad types appear to be full grown. We have been able to find, however, two or three examples of what are undoubtedly young, growing individuals of these forms. They occur in slides of the series containing many of the large forms. Two young parasites of the slender type are seen in figs. 12, 13; and a young individual which would probably have developed into a stout form is drawn in fig. 14. One of the slender forms shows very conspicuously the two characteristic granules; and in the small broad individual we think it not at all

[1] The shading, often irregular, of the iron-hæmatoxylin parasites in figs. 45 and 49, for instance, is meant to indicate the slightly varying thickness of the densely stained cytoplasm of the corpuscle lying over the parasite.

unlikely that one or both of the grains at the edge of the nucleus, on its inner side, may represent the same bodies. Individuals which are somewhat larger, but still distinctly intermediate in size, are seen in figs. 45 and 15, the former being of the slender type, the latter of the broad type.

We have not found any other phases of the hæmogregarine, besides those above described, in our preparations. In the gurnards from which smears of the internal organs were made the parasites happened to be nearly all small forms, and large forms are very scarce. In these cases, although preparations from the liver, kidney and spleen have been searched, no signs of schizogony have been noticed.[1] Perhaps if smears had been taken from these organs in the case of the gurnard in which the large types of form are frequent, multiplication phases of a particular kind might have been found.

It is important to note that we have never found any individuals of either of the large types free from the blood-corpuscle. Further, in only a solitary instance has one of the small forms been noticed free (fig. 3). This occurs on a smear from the kidney. The parasite is not very vermicule-like, and resembles the small intra-cellular type of form. This scarcity of free forms quite agrees with our observations of the parasites in the living condition (cf. above, p. 115).

General Considerations.

We have now to consider what is the significance of the different phases observed, in what relation do they stand to one another, and how do they compare with the known forms of other hæmogregarines ?

The ordinary small forms doubtless represent an early

[1] The only possible indication of commencing schizogony which we have noticed is found in two or three of the small forms in a preparation from the infected gurnard examined. The nucleus of the parasite shows a constriction about the middle, which causes it to appear somewhat dumbbell-shaped (fig. 28). This may, perhaps, signify commencing nuclear division, prior to fission of the parasite, but we do not feel at all certain upon the point.

stage of the infection, and may be regarded as derived from sporozoites which have penetrated the blood-corpuscle. They are for the most part very uniform in size and appearance and probably destined to become schizonts.

The large forms are, we consider, of two distinct types, and not directly connected with one another—that is to say, an individual of one kind, e.g. a broad form of parasite, does not pass into one of the other kind, the slender type, by a process of elongation and simultaneous bending-up, such as is described by Börner (4) in H. stepanowi; nor, on the other hand, is the broad form to be derived from the slender type by a process of change comparable to that frequently described among Reptilian hæmogregarines, where a U-shaped form gives rise, by the fusion of its two arms, to an oval or bean-shaped form (cf., for example, H. tunisensis of Bufo mauritanicus, Billet [2], H. bagensis of Emys leprosa, Billet [3], etc.). Our reasons for regarding the two large forms of H. rovignensis as independent are three: In the first place, we have noticed no transitional forms indicating such a connection as just mentioned; secondly, there is always a well-marked difference between the nucleus of forms belonging to these two types; and lastly, young individuals of each type are clearly distinct. We suggest that these large forms show sexual differentiation, the slender type with the small nucleus being of male character, and the stout form with the large nucleus being of female sex.

With regard to their origin, it is very probable that they have been developed from two forms of merozoites, smaller and larger, for the young individuals of the slender or male type in figs. 12, 13 are manifestly different from the other small forms, and not likely to have arisen from them. There is one point, however, in this connection which at first was not at all clear. In the second gurnard examined (figs. 12–14, 16–26, 44–50) the number of the young parasites found belonging to the type which we regard as male is very small indeed as compared with the number of the ordinary small forms present, whereas about equal numbers of the full-grown

parasites of both types occur, and these are fairly plentiful. We think the most probable explanation is that we have to deal here with more than one infection. The great majority of the small forms must be regarded as the early stages of a new or recent infection; the forms showing sexual differentiation, on the other hand, belong, we think, to an older infection. Most of them occur as adult large forms, and only a few are to be met with as young or intermediate-sized individuals. The young parasites of the female type (belonging to the older infection) are probably not very different in appearance from the numerous small forms of ordinary type (of the recent infection), and thus are only distinguishable where they are beginning to grow into the stout type (fig. 14). The two forms of merozoites, which give rise in time to the large individuals, have doubtless been developed by a schizogonous process,[1] probably occurring in the internal organs.

The large forms themselves must represent one of two phases, schizonts or gametocytes respectively. If, according to the first of these interpretations, we have here micro- and macro-schizonts which will give rise again to a fresh series of micro- and macro-merozoites, it is evident that sexual dimorphism is apparent throughout the schizogonous series of generations (as is known to occur among certain Coccidia, e.g. Adelea, Cyclospora). If, on the other hand, our two types are micro- and macro-gametocytes respectively, we have no indications with regard to the nature of the ordinary schizogony (or fission) in this parasite, which may be very likely all of one kind, that is to say, "indifferent" in character, with no sexual dimorphism manifest (as in many Coccidia and all known Hæmosporidia). In this case it will only be in the last generation produced by schizogony that sexual dimorphism appears, in the formation of what are really the young micro- and macro-gametocytes (cf. above).

Unfortunately, from our own preparations alone, we cannot

[1] The process is, perhaps, comparable to the formation of merozoites of two sizes in cysts of Karyolysus (cf. Labbé [5]), both as regards the development and the significance of the small elements formed.

pronounce definitely between these two views. When, however, we compare the facts which we have learnt concerning H. rovignensis with what is known in the case of other piscine hæmogregarines, we are strongly inclined to consider the latter view the correct one, and that the two distinct types of form are micro- and macro-gametocytes. Fission or schizogony is now known to occur in many piscine hæmogregarines, and in most of the instances described it appears to be of one kind, no indications of sexual dimorphism being mentioned (cf. H. bigemina, Laveran & Mesnil [6], H. quadrigemina, Brumpt & Leb. [9, fig. 3, p. 382], H. simondi, Lav. & Mesn. [6], etc.). Neumann, in his account of piscine hæmogregarines (15), regards the schizogony in these instances as resulting in the formation of "gametes," but does not attempt to explain why only one kind is described and figured. Further, in his account of H. polypartita from Gobius pagenellus, he regards similar crescentic forms, four of which are developed in a blood-corpuscle, also as "gametes." He endeavours to show that these forms exhibit sexual differentiation, but such distinctions as are apparent in his figures seem to us to be due merely to slight differences in size (or age) and in tint of colour (otherwise degree of staining). We do not find anything approaching the pronounced and constant differences, both in form and in the size of the nucleus, which are shown by the large types of H. rovignensis. We certainly consider the curved forms of H. polypartita—equally with those of Neumann's other new species, H. clavata—as "merozoites," probably really "indifferent" in character,[1] and quite comparable with those described in the above-mentioned parasites.

[1] It is quite possible, of course, that in some hæmogregarines the schizogonic forms exhibit sexual differentiation. Up to the present, however, we do not consider this has been shown to be the case. Wenyon (22) has figured "barillets" both of micro- and mega-merozoites in connection with H. gracilis from the liver of Mabuia. Certain of Wenyon's figures suggest strongly the schizogony of a Coccidian, and we think this explanation is not at all unlikely, both in

There is another point which is of considerable importance. In all the above instances these long, slightly curved forms (adult merozoites) were readily observed free, as "vermicules," in smears as well as in the living condition. This is quite natural if, as we consider, these vermicules are ready to penetrate a fresh host-cell, probably in an internal organ, and there give rise by schizogony to the sexually differentiated forms. We think it will be useful to distinguish these free individuals as schizokinetes, meaning thereby a temporarily motile schizont.

In striking contrast to the above cases we have the entire absence, so far as we are aware, of free individuals of either of the large types of H. rovignensis, although the great majority of them seem to be full-grown and mature. We have no grounds whatever for thinking that these individuals become free from the corpuscle while still in the fish.

This difference in behaviour also points to these large forms of the Trigla-parasite being gametocytes and not schizokinetes. On the supposition, which is most probable, that the sexual process of these hæmogregarines takes place in a leech, these is no reason why we should expect to find gametocytes liberated in the blood of the fish, because they are awaiting the stimulus of the invertebrate host before being set free from the corpuscles. We may compare in this respect other intra-cellular blood-parasites, for instance, Halteridium, Proteosoma, or Leucocytozoon of birds, with which we have had much to do. If a drop of blood containing these parasites is taken and smeared quickly, scarcely any of the ripe gametocytes present have ruptured the host-cell and become rounded off. It is only when the blood is allowed to cool for a short time in the living condition that the sexual forms become free, as indeed is well known. It may be said that if the large forms of H. rovignensis are gametocytes, we ought to have seen some of them at any rate become free in the living preparations examined. This does not follow at

view of the situation and also on account of the nuclear structure (cf. also below, p. 149, footnote).

all, for the particular stimulus which effects the liberation of these elements in the above cases is here lacking, namely, the fall in temperature. Lastly, it is not out of place, perhaps, to refer in this connection to Miller's account of "Hepatozoon" in rats (10). In this case the sexual forms (which apparently show little or no differentiation) are encysted in lymphocytes. Miller found that when blood containing the parasites was mixed with the expressed juices of the mite (Lelaps), it was ten to thirty minutes before the host-cells were dissolved and thirty minutes or more in addition before the gametocytes were liberated from their capsules and became motile vermicules.

From all these facts we conclude that where large free vermicules of a hæmogregarine are found in the circulating blood, at least in fishes, they are schizokinetes, which have yet to give rise to the true sexual forms, and not themselves the gametocytes ("gametes" according to Neumann). It is very doubtful whether the full-grown gametocytes of hæmogregarines ever become free until the blood is drawn from the body.[1]

Characteristics of H. rovignensis nobis.—The principal characters of this species from Trigla lineata, so far as we have been able to ascertain them, are as follows: A very small parasite, one of the smallest piscine hæmogregarines yet described. Schizonts usually oval in form. Average size (full grown?), before fission has commenced, 4·8 μ by 2·5 μ. Gametocytes large and well differentiated. Female forms wide, ovoid or bean-shaped; average size 10·6 μ by 3·4 μ. They possess a large nucleus. Male forms fairly slender, with one end somewhat club-shaped, the other end usually slightly recurved; average size 12 μ by 2·1 μ. These possess a small nucleus. Individuals of all types may show one or two characteristic granules, extra-nuclear in position, and most probably achromatic in nature; they are particularly promi-

[1] From the published descriptions and figures of reptilian hæmogregarines which we have seen we consider it most probable that a similar state of affairs obtains in their case also.

nent in parasites stained with iron-hæmatoxylin, which stain they take up with great avidity.

II. Trypanosoma sp. (cf. triglæ) in Trigla lineata.

(Figs. 29–31, 51, and 65.)

In the three gurnards which were infected with Hæmogregarina rovignensis a trypanosome was also found. This parasite was always very rare, and never more than two individuals could be seen in a cover-slip preparation of living blood. Neumann (**15**) gives only a very brief notice of T. triglæ, from a single Trigla corax, and does not remark upon the strength of the infection in this particular case, but he states at the beginning of his paper that as a rule trypanosomes appear to be very rare in the blood of infected fish.

Observed in the living condition, the trypanosomes performed very active movements of contortion, but did not actually displace themselves much in the blood-fluid. The movements were of the wriggling and twisting kind so characteristic of fish trypanosomes. The body would be coiled into a spiral or S-shape and then unbent again only to become twisted in the reverse sense with the greatest rapidity. Occasionally the parasite would burrow into a mass of corpuscles and pass through them. Particular parasites, whose position was noted, were found to remain alive from five to eight hours in the drawn blood or cover-slip preparations. Their movements had become extremely sluggish by the end of this time, but no alteration in form was noticed. Only in a single case was a parasite seen alive after a longer interval (twenty-four hours); the body of the trypanosome was then bent up and motionless but the flagellum waved feebly, showing that the parasite was still alive. The parasites did not live any better in preparations in which the blood had been mixed with a drop of salt-citrate solution or of sea-water. In remaining alive such a short time in drawn blood this trypanosome

differs markedly from T. raiæ, as will be seen from our statements below.

Owing to the scarcity of this parasite very few individuals are present on our stained preparations. At the most two occur on a film; more usually only one has been found, and on some smears there appear to be none. All the parasites seen belong to one type and show no pronounced variation in size. Individuals on "wet" smears are generally a trifle smaller than those on "dry" ones; we think the former are slightly contracted. The average size of the trypanosome, as seen on Giemsa-stained smears, is 59 μ in total length (i.e. inclusive of the flagellum) by 4·5 μ in total width (inclusive of the membrane). The free flagellum averages 8 μ in length. Fig. 29 shows a typical example of the parasite, with dimensions almost as given. The longest parasite observed (fig. 31) has a length of 62 μ and a breadth of 4·7 μ. The length may be really 2 or 3 μ longer, as the flagellum is very faintly stained, and its free portion probably continues a little farther than can be made out. On the other hand the parasite from an iron-hæmatoxylin film drawn in fig. 51 has a total length of only 54 μ and a width of only 4 μ, inclusive of the membrane; but here also the free part of the flagellum is so faintly stained that we cannot be certain its entire length is represented.

All the trypanosomes on our slides belong to this long, slender type. The flagellar extremity is narrow and finely tapering (figs. 29–31), a feature which is more usual in these parasites of marine fishes than in those of fresh-water ones. The distance of the kinetonucleus from this end of the body is generally about 6 μ, and may be as much as 7 μ. The free part of the flagellum at the opposite end is comparatively short, and varies from 6 to 10 μ. The trophonucleus is generally in the flagellar half of the body (figs. 29–31), but may be occasionally more centrally placed (fig. 30). The undulating membrane is well developed. It appears under two aspects. In the first, which we think represents the more natural condition, it shows well-marked folds and pleats, of

which there are six to eight, of varying prominence and depth (figs. 29, 31). In other cases the membrane appears as an extensive flap or fin, with a slightly wavy border, and widest about the middle of the body (fig. 30). We think the difference between these two aspects is more apparent than real, due to a large extent to the flattening out of the parasite on the slide, in the second case. On the other hand, in the few individuals we have observed on "wet" films the membrane appears narrow and inconspicuous (cf. figs. 51, 65), and we should certainly be inclined to say it had undergone some shrinkage here.

One or two remarks which we have to make upon the nuclear structure may be deferred until we consider the trypanosome-nucleus generally in the second part of this paper. The only detail with regard to the general cytoplasm that requires mention is the occurrence in certain cases of numerous granules. These granules are only noticeable in parasites on Giemsa-stained smears. In some individuals (fig. 31) the cytoplasm is quite free from them; in others there are a certain number of small dark granules, chiefly in that part of the body lying between the two nuclei. The flattened-out individuals, however, are rendered conspicuous by the presence of numbers of large granules, apparently occupying most of the body (fig. 30). Moreover, many are seen lying apparently in the membrane. These granules stain a purple colour of a somewhat deeper tint than the lilac of the cytoplasm.

The trypanosome from Trigla corax, to which Neumann has given (l. c.) the specific name of triglæ, is described very briefly and without any figures. According to Neumann's account the parasite is of a different type of form from that which we have above described. Its total length is about the same, namely, 60 μ, but the free flagellum is rather longer, being 15 μ. The chief difference, however, is in the breadth, which appears to be much greater. Neumann gives the width of the body as 8 μ and that of the membrane as 4 μ, the entire width being thus 12 μ as compared with an average

of 4·5 μ in the case of our form.[1] Further, the aflagellar end of the body is short and somewhat blunt, as indeed is often the case in these "stumpy" types of trypanosome, and the membrane does not show well-developed folds.

At first sight these two trypanosomes, from different species of Trigla, might be considered to belong without doubt to different species; and probably many authors would not hesitate to give both parasites a distinct name. In our opinion this would be decidedly premature, for we think it quite likely that both are merely different forms of one and the same species. Polymorphism is now known to be of common occurrence among trypanosomes. To give only one or two instances, Minchin (**11**) has recently shown clearly, by photographs, the marked (sexual) polymorphism in T. gambiense, while one of us (H. M. W.) has been struck by the polymorphism, of a character quite similar to that implied in the case before us, which occurs in an Avian trypanosome. We do not intend here to ascribe any special sexual significance to the "stout" (wide) form of the Trigla trypanosome; we suggest, however, that Neumann's form is a particular type of the parasite we have described above, the latter being the one which we are inclined to regard as the more "ordinary" type. Hence our reasons for including the trypanosome from Trigla in the species T. triglæ.

In connection with this point, it may be remarked, it is especially among piscine trypanosomes, where the parasites attain to such large dimensions that marked variations in form and appearance may be expected to occur, due either to young forms or to different types of the parasite. It seems to us that there has been too much tendency to ascribe hard and fast limits to the size of a specific trypanosome. Many authors, in describing new species, appear to have overlooked

[1] It is quite possible that the real width is not so much as these figures indicate; for we have found that it is especially in such stout or stumpy types that flattening-out in dry smears may be most appreciable and most liable to give an incorrect idea of the true width of the parasite.

the possibility of considerable variation in size and appearance, and as a result have given fresh names to parasites which are in all probability only phases of trypanosomes already known from the same or closely allied hosts. We have little doubt that Neumann has made such a mistake in distinguishing his "Trypanosoma variabile" from T. raiæ of skates.

III. T. raiæ from Raia sp.

(Figs. 32–38, 52–57, and 66–68.)

As this parasite has been described already by several workers, we need not give a general account of it here. One or two points of interest have been observed, however, which may be mentioned. In the fish examined the trypanosomes were exceedingly abundant, but no dividing forms were seen, nor were any hæmogregarines found.

With regard to the behaviour of the trypanosomes in freshly taken blood under a cover-slip, we found that they remained alive and active for a much longer period than did T. triglæ kept in a quite similar condition. Many were seen quite unaltered, but undergoing less active moments, after twenty-four hours. Also after fifty-four hours several were seen, their movements being now sluggish. At the end of seventy-two hours only four were found, two of them being individuals which had been noted and marked after twenty-four hours. They showed no alteration, but their movements were very slow and feeble, being confined to little jerks of the flagellum and a very slight twisting of the body. Three of these individuals were seen again on the fourth day, after ninety-six hours, when they appeared in much the same condition. Lastly, on the fifth day one trypanosome only was still seen living, extremely feeble and moving very slightly at intervals. The remarkable point is that none of these parasites showed any alteration in form; nor during the earlier periods, when several individuals were still alive and fairly

active, did we notice anything comparable to the rounded-off phases described by Miss Robertson (17). It is probable that the percentage of individuals which undergo this alteration on the slide is very small compared with the number that do so when the parasites pass into the leech.

The trypanosomes in our permanent preparations show considerable differences in size. On Giemsa-stained smears the largest individual observed has a total length of 72 μ and a breadth of 5·6 μ, including the membrane. The corresponding dimensions of the smallest form seen are only 55 μ by 4 μ (fig. 33). These two extremes are connected by intermediate forms of varying size (c f. figs. 34, 35). The average size works out at about 67 μ by 5·2 μ. The free flagellum varies from about 10 μ to 15 μ, with an average length of 13 μ. The length of the flagellum does not seem to stand in any very close relation to the size of the parasite, and now and again is shorter in a large individual than in one of intermediate size. The largest trypanosome noticed on "wet" films stained with iron-hæmatoxylin is drawn in fig. 53; it is a comparatively wide, plump individual, which would probably have seemed even wider on a Giemsa-stained smear. It measures 65 μ by 6·6 μ. It is probably somewhat longer in reality, for the free flagellum, which is unusually short in the drawing, comes into contact, at the point where it apparently ends, with a corpuscle which is stained deep black; although it probably runs across this for some distance, its course cannot be followed. Other parasites on iron-hæmatoxylin-stained films are seen in figs. 52–56. They are mostly a little shorter than the parasites on "dry" films; this difference is most noticeable in comparing the body-protoplasm, for the free flagellum itself is in most cases actually longer and averages 14·8 μ against 13 μ on the dry smears. We are inclined to think this is due to the contraction of the general cytoplasm to a greater extent than the entire flagellum (i. e. the flagellar border + free flagellum) in iron-hæmatoxylin films.

There is another rather interesting point brought out by a comparison of the trypanosomes fixed and stained by the

two methods, which, incidentally, may also help to explain this difference in apparent length. The great majority of the individuals on the "wet," iron-hæmatoxylin-stained films are in a different position from those on the "dry," Giemsa-stained smears. In the former they are usually found in a twisted S or corkscrew-like position (figs. 52–56), while in the latter the parasites are nearly always simply rolled or coiled up to a greater or less extent (figs. 32–37). Now, in life the trypanosomes are generally observed in a twisted or S-shaped condition, and only rarely, and as it were transiently, in the form of a simple C. We may conclude, also, that death and fixation are at least as instantaneous in the case where the parasites actually come into contact with sublimate and acetic as in the case where the slide is placed in a tube containing osmic vapour. Hence we consider that the position of the parasites on the wet films approximates most nearly, as a rule, to that in which they were the instant before death. What is the cause, then, of the parasites assuming the very different rolled or coiled-up form on "dry" films?

The manner of attachment of the undulating membrane to the body has an important bearing upon this question. Figs. 52, 53, and 56, from iron-hæmatoxylin slides, show very clearly that in these individuals the undulating membrane was wound spirally round the body at the instant of death. The flagellar border runs now under, now over the general cytoplasm, and in fig. 56 it is seen to run twice under. Are we to regard the membrane as actually attached spirally to the body (when the latter is in a "passive" condition), or as being merely twisted round it at the time by the voluntary contortion of the protoplasm? We think the latter view affords the true explanation. The appearance of the parasites on Giemsa-stained films gives probably a fairly correct representation, from a morphological point of view, of the manner of attachment of the membrane—that is to say, it lies along one side of the body, more or less in one plane. The membrane itself, especially on its outer side, is longer than that part of the body to which it is attached. In life the

membrane is usually twisted in a spiral fashion round the body by the voluntary contortion of the protoplasm, this being in all probability effected by the contraction of myonemes. Minchin (13) has recently published figures clearly showing myonemes in T. percæ and T. granulosum, and we have no doubt they are present in other fish-trypanosomes, though we have not had the good fortune to see them in T. raiæ. In wet films the parasites have retained their twisted position. In "dry" smears, on the other hand, the body becomes untwisted, and, at the same time, passively or mechanically coiled up in one plane, by the mere fact of the attached membrane being longer than the body is.

We do not think this different behaviour on "dry" smears is to be explained by a flattening-out process due to actual drying. In the first place, in our procedure, the slides are removed from the osmic-acid tube and placed in absolute alcohol before the moisture dries off from the greater part, at all events, of the slide; it is only along the edges that drying sometimes occurs. And after the smear has been hardened in alcohol little or no alteration, we consider, takes place in the form of the parasites, even though the smear is allowed to dry off ultimately. In fact, as Minchin has already shown in his account of the technique in connection with T. lewisi (14), this method is probably the best for the general form and size of the parasites. Secondly, now and again where the body of the parasite really appears to be somewhat flattened out due to an actual drying at first, this C-form is not shown (cf. fig. 30 of T. triglæ). Indeed, this process of untwisting and coiling would seem to require the presence of a film of moisture for its accomplishment. The following explanation appears to us the most probable. In fixation by the "wet" method, both death and fixation are practically instantaneous. In fixation by osmic vapour, on the other hand, death probably occurs appreciably before fixation. In the twisted condition, during life, the flagellar border of the membrane is probably to a certain extent in

a state of tension, from which it relaxes, in virtue of its elasticity, on the death of the body; in so doing it automatically unwinds the body, at the same time causing it to become more or less C-like, before actual fixation occurs. In this connection it should be pointed out that Danilewsky (4A), who studied trypanosomes carefully in the living condition, frequently figured them with the undulating membrane spirally wound round the body, but in some cases he shows it attached along one side of the body.

Lastly, if, as we have been led to consider, the parasites on wet films are generally in a spirally twisted condition, we might expect to find a slight shortening in length; this, together with a certain amount of contraction due to shrinkage, would be sufficient to explain the difference in average length between the parasites on wet films and those on dry ones.

In many of the parasites on Giemsa-stained smears numbers of small bodies occur, which appear to be prominent granules (fig. 37). They are deep black at the middle focus, but are bright and glistening at the upper focus. They are not comparable to ordinary chromatoid granules, which stain more or less red in colour. Moreover they are most abundant in the aflagellar part of the body, especially between that extremity and the kinetonucleus, a region which is generally free from chromatoid grains. They are also scattered throughout the body, and some, which cannot be distinguished by appearance from the others, lie occasionally in or on the undulating membrane. In wet films, stained either with iron-hæmatoxylin or with Twort, the same bodies, if present, are not nearly so conspicuous. In the body generally no sign of them is to be seen; but near the aflagellar end, which is often slightly vacuolated in character, a certain number of granules can be seen, not stained very differently from the cytoplasm (figs. 54–56). We are not sure, however, if these granules are the same.

Returning to the parasites on dry smears, we have recently noticed the peculiar fact that, since the individual of fig. 37 was drawn, all the black granules have vanished, leaving only

small, clear areas, like spaces, in the position in which they were. In fig. 34 is another parasite which showed originally a very similar condition as regards the granules; this has been drawn since they disappeared. Two or three of the granules are still seen, and the small vacuoles indicate the position originally occupied by many others. It seems most probable that these black "granules" are really minute globules of oily or fatty substance, which are blackened by the osmic acid used in fixation, and are liable to be dissolved away by the frequent washings with xylol given to the slide, of course after immersion-oil has been upon it.

We entertain no doubt that this trypanosome belongs to the species T. raiæ, Lav. and Mesn. These authors, in describing this species, gave its size as from 75 μ to 80 μ in total length, by about 6 μ in width (inclusive of the membrane). Apparently, as has been so often the case, the species was characterised solely from the full-grown individuals of the ordinary type which were encountered, and no reference is made to young forms or to variations in type. Further, Laveran and Mesnil found trypanosomes which they regarded as belonging to the same species in four species of Raia, namely R. punctata, R. mosaica, R. clavata, and R. macrorhynchus. We do not know the name of the species of Raia in which we found the parasite. The dimensions of the largest individuals we have observed, however, are only very slightly less than those above mentioned, and the general appearance of the parasites, as shown in our figures, agrees so closely with that of the individual figured in Laveran and Mesnil's original account (7) that there is every probability that the trypansome is the same in the two cases. Neumann (15) has given the name T. variabile to a trypanosome from R. punctata, principally or solely because he has found variations in size and form in the parasite which are not referred to by Laveran and Mesnil; though he states more than once that his parasite resembles T. raiæ closely and in its largest form agrees with that trypanosome. As we have described above, we have found forms of T. raiæ very much

smaller than the full-sized ones; and many of Neumann's figures of T. variabile resemble strongly these smaller forms. In fact, neither from Neumann's description nor from his figures is there any reason to suppose that T. variabile is not a synonym of T. raiæ.

Comparison of the Nuclear Structure of a Hæmogregarine with that of a Trypanosome.

A most interesting and important result of our study on the above-mentioned blood-parasites of fishes is afforded by the evidence it has given us of the essential difference between the nucleus of a hæmogregarine and the trophonucleus of a trypanosome. This difference is brought out forcibly by all the three methods of technique employed, though, of course, one method may show a certain feature or detail better than another.

Considering first the case of Hæmogregarina rovignensis in Trigla sp., the conclusion arrived at by comparing and combining the impressions given by different stains is that the nucleus in this parasite consists of a regular or irregular meshwork or reticulum, itself chromatic or impregnated with chromatin, on which are suspended chromatin grains and masses of varying size and form. The reticular ground-work is best seen in iron-hæmatoxylin or Twort preparations (figs. 39–50, 59–64); in the latter it is always distinctly red (chromatic) in colour. The limit or border of the nucleus appears to be itself part and parcel of the reticulum, the peripheral segments of the latter being usually arranged so as to give a fairly regular oval contour or "membrane." This structure is well shown in figs. 48 and 49 of large forms. Hence one cannot speak here of a true nuclear membrane as something distinct and separate from the general nuclear substance. This "membrane" also has numerous chromatic granules strung upon it; these are generally smaller than those occurring in the more central parts of the reticulum. The chief chromatic aggregations

sometimes tend to run together, or to lie in short streaks. In no case have we observed any signs whatever of a definite central body or karyosome in the nucleus.

In individuals stained very faintly and sharply on the particular Giemsa-stained smear to which allusion has been made several times, the above-described characters of the nucleus can be made out quite well (cf. figs. 5–10). In such cases the picture represents fairly accurately the true condition. In other individuals, however, slightly more deeply stained, the nucleus appears more granular and already somewhat "blotchy"; this is due to the enlargement of the chromatic grains and to the deposition of the red stain in the nuclear sap, more or less occluding and obliterating the reticulum. This leads on naturally to the appearance generally seen in deeply stained Giemsa smears of a dense mass, staining red or purple, in which bodies and streaks still more darkly coloured can be made out, representing the chromatic grains.

We may add that we have been struck by the considerable resemblance between the nucleus of the parasite and that of its host-cell; this will, indeed, be apparent from many of the figures (fig. 14).

Turning now to the trophonucleus of a trypanosome, we find a remarkable contrast. T. *raiæ* being a very large trypanosome and possessing a correspondingly large nucleus is a most advantageous form to study for this purpose, since the various nuclear details—particularly of the karyosome—can be made out more readily than in the case of a comparatively small parasite, such as T. *lewisi*, for example.

Our description is based upon the appearances yielded after iron-hæmatoxylin and Twort, for in this case—far more so than when considering the hæmogregarine nucleus—it would be difficult, if not impossible, to arrive at what we regard as the correct interpretation of the nuclear structure by studying the Giemsa appearance alone. Having obtained a fairly accurate idea of the nuclear constitution from iron-hæmatoxylin and Twort preparations, we can then interpret the widely different picture seen after Giemsa. Miss Robertson

(17) has recently described the nuclear structure in certain developmental forms of a trypanosome occurring in Pontobdella, which trypanosome she regards (and we think correctly) as T. raiæ. Our own observations quite agree with her account; we are able, perhaps, to add a few more details with regard to the karyosomatic mass. (Our main purpose, as we have already said, is to emphasise the contrast between this type of nucleus and that of a hæmogregarine.)

The nucleus is very generally oval in shape and always possesses a well-defined, regular contour. Its size varies not inconsiderably (cf. figs. 52–56), and, as might be expected, is in accordance with the size of the parasite, small- or intermediate-sized individuals having a smaller nucleus than the large ones. The size may be as small as 2·4 μ by 1·7 μ, or as large as 3·2 μ by 2·6 μ. The greater part of the nucleus is occupied by a prominent deep-staining body—the karyosomatic mass. Around this appears a practically clear space, which is bordered or limited by a sharply marked line, the nuclear membrane. Any space or halo surrounding this on the outside again, as sometimes occurs (cf. figs. 52 and 54), is most likely a shrinkage-space. Delicate rays, sometimes four or five in number, sometimes more, proceed from the central mass to the membrane; these are usually very faintly stained, but can be made out with a good illumination, especially in iron-hæmatoxylin preparations. Both membrane and rays are always green after Twort (figs. 66, 67, 68*a*). Hence we may regard them as achromatic in structure. The rays are probably comparable to a linin framework for the support of the karyosome. The membrane is a much more definite structure than in the case of the hæmogregarine-nucleus. In this parasite both membrane and rays appear to be, as a whole, remarkably free from chromatin, very different in this respect from the chromatic reticulum of the hæmogregarine. The only possible indications of chromatin are furnished by small dots or condensations at the junctions of the rays with the membrane; they are best seen in iron-hæmatoxylin preparations (fig. 54). We can get no evidence of a red colour

at these points after Twort, however, and so do not feel at all certain that they are chromatin.

A correct interpretation of the characteristic central body in the nucleus is best gained from Twort films. Iron-hæmatoxylin films must be very well extracted, and then the same or a similar condition is revealed. But in those iron-hæmatoxylin smears where the whole karyosomatic mass is stained almost uniformly black (as in figs. 53 and 56 for example), it is safe to say that an excess of stain still prevents the details from being apparent. The true structure appears to be as follows: In the centre is a fairly large, clear region, oval or rounded, which is stained grey in iron-hæmatoxylin films and a pale green (distinctly paler than the rays) in Twort preparations. This is the basis or ground-work of the karyosome and is probably of a plastin-like nature. The chromatin is located at the surface, or at any rate in the peripheral region of this plastinoid basis. In the smaller nuclei the chromatin is mostly in the form of granules or small masses of varying number (usually three to five) and size, and more or less separate from each other (figs. 55, 57*a*, 68*a* and *b*); but in the large nuclei we frequently find the chromatin forming a complete zone or ring around the paler area, with thickenings or bulgings here and there (figs. 57, *f* and *g*, 68, *c*, *d*) corresponding to the small masses in the other case.

One important detail remains to be mentioned, namely, the presence of a small, distinct granule in the centre of the plastinoid area, which is probably of constant occurrence. It is readily made out in Twort preparations (cf. fig. 67, 68, *a–f*); sometimes it is green in colour, but in other cases the colour appears to be a mixture of both the red and the green; it is, however, never of the same sharp red colour that the chromatic masses are stained. This granule can be distinguished also in the individuals on iron-hæmatoxylin smears, but not so easily.

Comparing, now, the appearance of the nucleus in Giemsa smears, a condition is generally found which at first sight seems to be diametrically opposite to that shown by iron-

hæmatoxylin films—that is to say, centrally or excentrically is a comparatively clear, faintly stained area, while all the rest of the nucleus is stained red more or less deeply (figs. 34, 35, 38, *a–d*), the periphery, in the neighbourhood of the membrane, being perhaps darkest. The clear area corresponds without doubt to the central part of the karyosome, i. e. to the plastinoid basis free from chromatin. Rather curiously, the central granule, referred to above as occurring in the plastinoid part of the karyosome, is often very conspicuous, probably because it is to a certain extent artificially enlarged by the stain. The remarkable feature about these Giemsa-stained nuclei, and the one which creates such a false impression, is that the nuclear sap is often so loaded with stain that not only the rays but also the chromatic zone or ring immediately surrounding the central area is indistinguishable as such. Occasionally, in more favourable pictures, the chromatic zone is more deeply stained than the nuclear sap and can be distinguished somewhat better (fig. 37); and now and then coarse indications of the rays proceeding to the periphery can also be made out (fig. 38, *b*, *c*). Hence we have little doubt that here also the structure of the nucleus agrees really with that above described.

Owing to the scarcity of Trypanosoma triglæ in our preparations the few individuals present on wet films do not show the nuclear structure very satisfactorily. Extraction had to be carried on quite in the dark, as it were, and neither in the individual drawn in fig. 51 from a film stained with iron-hæmatoxylin, nor in that of fig. 65, from a preparation stained with Twort, has the extraction been carried far enough. From these two examples, however, it is quite obvious that the nucleus is of the same karyosomatic type, and fig. 51 affords indications that the structure of the karyosome itself is similar to that above described.

We regard the above instances as indicative of the typical character, speaking broadly, of the nucleus of a hæmogregarine and the trophonucleus of a trypanosome.

So far as the case of the trypanosome is concerned, it is

already quite evident, from figures published during the last year or two, since the use of the iron-hæmatoxylin stain became more general, that the trophonucleus is in the main constituted on the same plan, having most of its chromatin associated with a definite karyosomatic body. Besides Miss Robertson (17), Minchin has shown this to be the case both in various fish-trypanosomes (13) and also in T. lewisi (14). We may mention that during the progress of our work at Rovigno we have obtained a similar result in the case of the trypanosomes in the little owl; and we see that recently Rosenbusch has published figures (20) of cultural forms of these trypanosomes (which he calls "Hæmoproteus noctuæ" and "Leucocytozoon ziemanni") showing this same nuclear structure.

Further, we are inclined to think that in many cases the minute details of the karyosome will be found to be similar, that is to say, as regards the tendency of the chromatin to be located at the periphery of the plastinoid basis, and the presence in the central, clearer zone of a definite granule. Of course, nuclei with a large karyosome may be expected to show this more distinctly than those with a very small karyosome (such as, for example, T. lewisi.) One of us (H. M. W.) has several times observed, in Giemsa preparations of a trypanosome of the chaffinch,[1] an appearance of the nucleus quite similar to that in fig. 37, namely, a conspicuous granule occurring in the centre of a clear zone in the middle of the nucleus; and the interpretation of the whole appearance is doubtless also the same. It is interesting to note that some years ago Laveran and Mesnil, in their account of certain trypanosomes of fishes (7), published a figure of T. remaki, of the pike, which showed the same nuclear appearance. We remember thinking this unusual at the time, as it was quite different from the uniform granular character which the nucleus was generally described as possessing.

These finer details of the karyosome are best revealed,

[1] It is hoped to publish an account of this parasite, and of others in the chaffinch, very shortly.

perhaps, by Twort; in the case of films stained with iron-hæmatoxylin, the stain must be very well extracted, or else the whole karyosomatic mass is too heavily stained for them to be made out. This is evident by comparing our various figures. From many of the figures in the above-mentioned papers we should say that extraction in those cases had not been carried far enough for this purpose. For instance, in most of the resting nuclei of the various trypanosomes drawn by Rosenbusch (l.c.), the karyosome is too dark to show the central granule.

There can be no doubt that this granule or centriole is the intra-nuclear centrosome first described by Schaudinn in the trophonucleus of his trypanosome in the little owl. It is also evident that it acts as a division-centre, and forms an intra-nuclear spindle at the commencement of nuclear division. This phase is well shown by several of Rosenbusch's figures. Again, to compare a dividing stage described from a Giemsa-stained preparation, Minchin, in his account of T. grayi (**11**), figures an intra-nuclear granule at each end of the spindle still connecting two daughter-nuclei, immediately after division has taken place. Hence this intra-nuclear centrosome[1] is doubtless a regular constituent of the trophonucleus of a Trypanosome.

It will be noticed from our figures that, in the Giemsa-stained preparations of T. raiæ, the red-stained part of the nucleus is fairly uniform or homogeneous in appearance. It is more usual, however, for the nucleus of trypanosomes stained by the Romanowsky method to appear granular in structure, apparently consisting of small or medium-sized granules in close contact, and forming a compact mass (cf. the selected figures, either in the article on "Trypanosomes" in Lankester's 'Treatise on Zoology,' or in Lühe's article in Mense's 'Handbuch der Tropenkrankheiten'). This appear-

[1] Moore and Breinl (**14A**) use the term "intra-nuclear centrosome" in a different sense from ourselves, namely, for the entire central body which we regard as the karyosome. They do not seem to have distinguished at all the centriole contained in the karyosome.

ance is quite easily capable of explanation when the known tendency of the Romanowsky stain to be deposited in excess around anything of a chromatic nature is borne in mind. We may suppose that in such cases there is a certain amount of chromatin distributed in the nuclear sap or karyolymph (in addition to that associated with the karyosome); this is most probably in the form of very fine granules, which are of course magnified by the stain to many times their real size. Hence the effect is produced of a granular mass, such as has been so often described. By this means the clear central area, indicating the position of the karyosomatic body, is generally occluded completely and indistinguishable.

We have now to consider, briefly, the hæmogregarine-nucleus. Here, too, there can be no question but that the true nuclear structure is better revealed by stains like iron-hæmatoxylin and Twort than by the Romanowsky method of staining. Nearly all the illustrations of hæmogregarines which we have seen are from parasites obviously stained by the latter method. Prowazek, it may be mentioned, in his paper on H. platydactyli (**16**), has three figures which were drawn from preparations stained by Grenacher's hæmatoxylin, and these also give indications of the same type of structure—an irregular reticulum carrying chromatin-grains and masses of various sizes—which we have found in H. rovignensis. The nuclei in these figures of Prowazek's differ very greatly from those he has drawn from Romanowsky preparations; many of the latter, we are convinced, do not give at all an accurate idea of the nuclear constitution.

Of all the other figures of hæmogregarine-nuclei at which we have looked, those of Börner, in his account some years ago (**4**) of reptilian hæmogregarines, seem to convey most approximately the true idea of the nucleus. From his "Tafelerklärung" we gather they were drawn from Romanowsky preparations; but for this intimation we should have regarded them as from preparations stained by some hæmatoxylin method, both from the appearance of the nuclei and from the manner in which the figures are coloured. We

are inclined to think the nuclei in some of his figures may be possibly a trifle schematic both as regards the uniform size of the granules and the rather suspicious regularity of the reticulum; but in many of the other figures there is, in our opinion, an indication of the nuclear structure which is probably as correct as it is possible to obtain it by the Romanowsky method. In none of Börner's figures is there anything remotely resembling a karyosome, and, in fact, the author expressly mentions that he never observed such a structure in the nucleus.[1]

Numerous figures accompanying the description of new hæmogregarines have been published during recent years, all of them naturally from Romanowsky preparations. It would take too long to cite them; nor is it necessary. It is sufficient to say that in no case can the structural details of the nucleus be deciphered. In all cases it is obvious that the nucleus drawn was still hopelessly overloaded with stain. At the best the nucleus is figured as a dense granular mass, bearing often a strong resemblance to those in our figures from Giemsa-stained preparations, from which it may be

[1] The only instance of which we are aware, where anything resembling a karyosomatic nucleus appears to be present, is in certain figures of Wenyon's (22) on Pl. 12, purporting to represent H. gracilis in the liver of Mabuia. The figures are from preparations stained by hæmatoxylin. We think it most likely that Wenyon has figured besides phases of a hæmogregarine, also phases of a coccidian, the latter being the ones in which the nuclei show a karyosome. His fig. 29 shows undoubtedly the development of typical merozoites of a hæmogregarine (cf. H. simondi); and it is only these merozoites which he figures also in the red corpuscles. His figs. 27, 22, and 31, on the other hand, we consider, represent a Coccidian; the two latter especially appear very like young coccidian schizonts. Since our MS. was sent to the printers the memoir by Hahn (4B) has appeared. We can only point out here that Hahn uses the term "karyosome" in a sense quite different from that in which we understand the word, namely, to mean the entire nucleus when in a condition "devoid of visible chromatin bodies" (p. 331). He terms such bodies "achromatic nuclei" (which seems to us rather a contradiction in terms), and calls them "karyosomes, in the sense that they are the bodies from which the chromatin bodies subsequently arise."

inferred that its structure conforms to that of H. rovignensis and to what we consider is the general plan. At other times nothing but a "splotch" of colour, from which it is impossible to ascertain anything, is depicted.

In conclusion we have only to point out that it seems clear that the nucleus of a hæmogregarine is of a very different type from that of a trypanosome. The former is characterised by its chromatic reticulum, with chromatin grains or masses more or less generally distributed upon it. In the latter the greater part or nearly all of the chromatin is, as it were, condensed around a plastinoid basis, the whole forming the conspicuous karyosome; and in the centre of this plastinoid area is a definite granule, the intra-nuclear centrosome.

So far, therefore, as the hæmogregarines at least are concerned, we are totally unable to agree with Hartmann (4c), who proposes to remove the Hæmosporidia entirely from the Sporozoa, and place them with the trypanosomes and their allies among the Flagellata as a group named Binucleata.

Postscript.

We had not intended to refer in this paper to the nucleus of Halteridium. Quite by chance, however, we have noticed a couple of sentences at the end of Berliner's account of the cytology of certain Flagellates (1), which relate to the structure of Halteridium noctuæ and Leucocytozoon ziemanni, as shown by iron-hæmatoxylin. There is no reference to this point in the title or list of contents, and we have only had our attention drawn to Berliner's figures since our present paper was completed. We refer to Berliner's note because we have ourselves obtained similar indications of the nuclear structure of these parasites during our work at Rovigno. We will only point out here that from Berliner's published figures, and equally from our own preparations, there can be no doubt that the nuclear structure of Halteridium is quite different from that of a hæmogregarine, and, on the other hand, remarkably like that of the

trophonucleus of a trypanosome, in being of the karyosomatic type. Further, Berliner mentions and figures the occurrence at times of a distinct extra-nuclear granule, connected by a fibril with the main nucleus, which he regards as representing the kinetonuclear element of a trypanosome perhaps in a somewhat reduced ("rückgebildet") condition, consequent on the intra-cellular, "resting" condition of Halteridium. We are very pleased to have this independent confirmation, and from iron-hæmatoxylin preparations, of the occurrence of nuclear dimorphism in Halteridium, a feature which one of us (H. M. W.) has already described (**23**) in the case of another species parasitic in the chaffinch, though unfortunately in this instance only Giemsa-stained smears were available. There can be little doubt, therefore, that Halteridium, in regard to its nuclear structure at all events, shows very much closer affinity to the trypanosomes than do the hæmogregarines.

Lister Institute;
November 27th, 1909.

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EXPLANATION OF PLATES 8–10,

Illustrating Professor E. A. Minchin's and Dr. H. M. Woodcock's paper on "Observations on certain Blood-parasites of Fishes occurring at Rovigno."

[The drawings on Pl. 8 are all from Giemsa-stained preparations, those on Pl. 9 are from films stained with iron-hæmatoxylin, and those on Pl. 10 from films stained by Twort's method. All the figures relating to Hæmogregarina rovignensis are magnified 3000 times linear; those relating to Trypanosoma triglæ and T. raiæ 2000 times.]

PLATE 8.

Figs. 1–28.—Hæmogregarina rovignensis. Figs. 1–11 are from the first infected gurnard, figs. 12–27 from the second, and fig. 28 from the third infected fish. Figs. 5–11 are from a very thin smear, which was faintly stained; both corpuscles and parasites are uniformly flattened out, but the nuclei of the parasites come out better than in any other Giemsa-stained smears.

Fig. 1, 2, 5–9, 16, 17.—Ordinary small forms, schizonts.

Fig. 3.—A small form, free from the blood-corpuscle, occurring in a smear from the kidney.

Figs. 4, 10, 18–21.—Large forms of the slender or male type.

Figs. 11, 22–26.—Large forms of the broad or female type.

Figs. 12, 13.—Young individuals of the slender, male type.

Figs. 14, 15.—Young and intermediate-sized individuals respectively of the broad, female type.

Fig. 27.—A double infection of the corpuscle, with two small forms of the parasite.

Fig. 28.—Small form showing a constriction of the nucleus in the middle.

Figs. 29–31.—Trypanosoma triglæ.

Figs. 32–37.—T. raiæ. Fig. 33 is of a small parasite, fig. 35 of an intermediate-sized one; the rest are of large individuals.

In fig. 34 the small vacuolar spaces were originally occupied by black grains similar to those seen in the parasite of fig. 37.

Fig. 38, *a–d*.—T. raiæ, trophonuclei of different parasites (× 3000).

PLATE 9.

Figs. 39–50.—H. rovignensis. Figs. 39–43 are from the first infected fish, figs. 44–50 from the second one.

Figs. 39–43, 46, 47.—Ordinary small forms (schizonts).

Figs. 44, 45.—Young and intermediate-sized individuals respectively of the slender or male type.

Figs. 48, 49.—Large forms of the broad, female type.

Fig. 50.—Large individual of the slender, male type.

Fig. 51.—Trypanosoma triglæ.

Figs. 52–56.—T. raiæ.

Fig. 55.—The trypanosome of this figure is on a different film from the others, one from which the stain has been considerable more extracted.

Fig. 57. *a–i*.—T. raiæ, trophonuclei from various trypanosomes; *e* and *h* are from large trypanosomes, the rest from smaller or intermediate-sized parasites (× 3000).

PLATE 10.

Figs. 58–64.—H. rovignensis. Fig. 58 is on a film from which the red part of the stain (neutral red) has been much less extracted than in other cases.

Figs. 58–61.—Ordinary small forms.

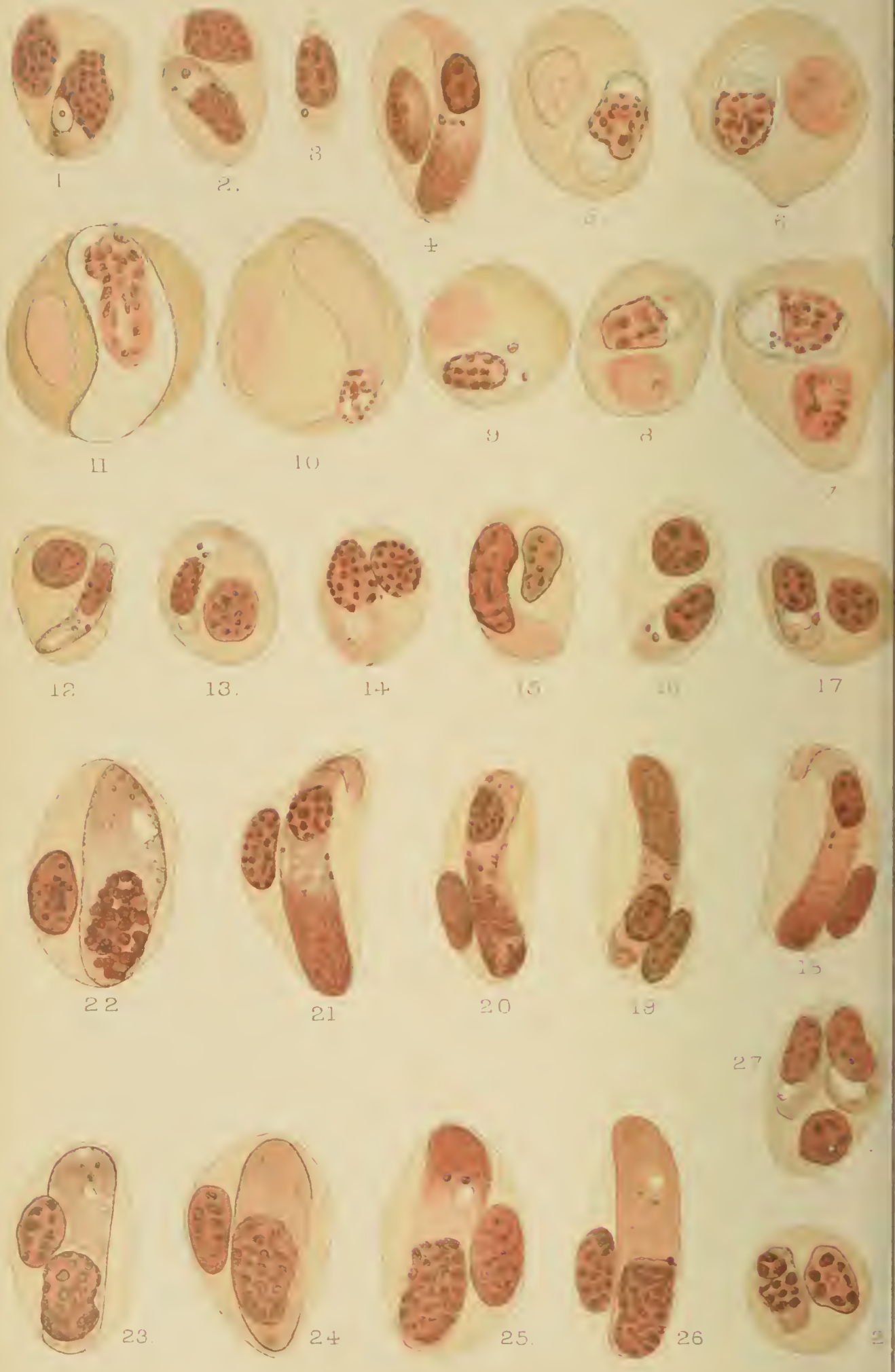
Figs. 62, 64.—Large broad forms.

Fig. 63.—Large slender form.

Fig. 65.—T. triglæ.

Figs. 66, 67.—T. raiæ.

Fig. 68, *a–f*.—T. raiæ, trophonuclei from various individuals; *d–f* from large parasites (× 3000).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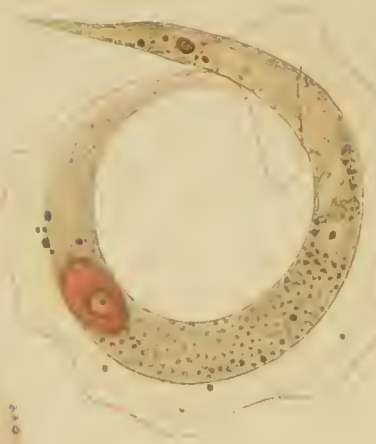

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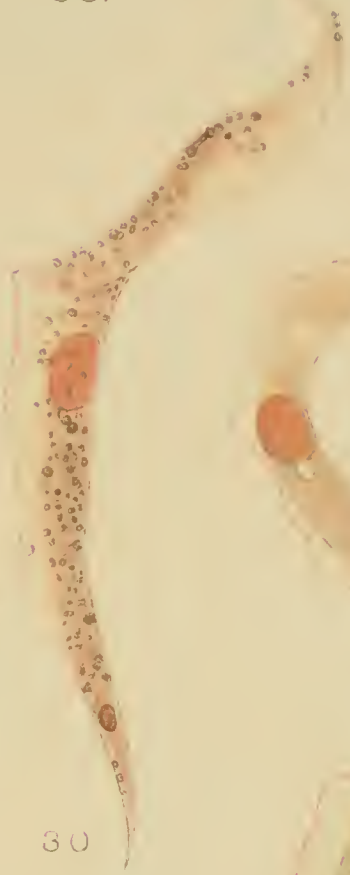

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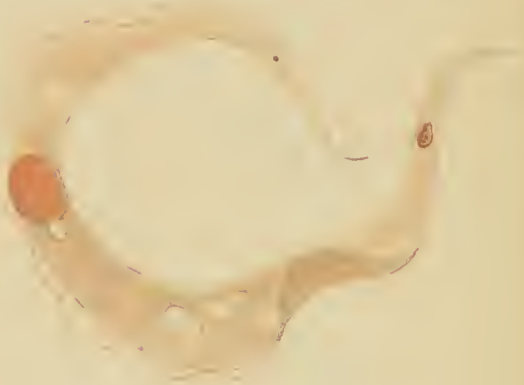

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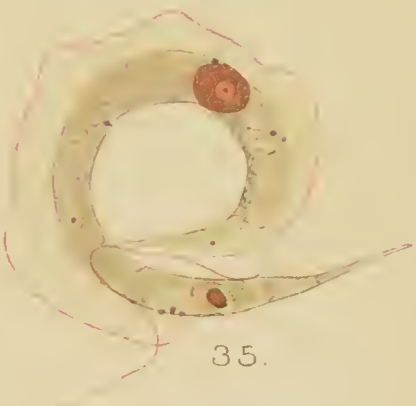

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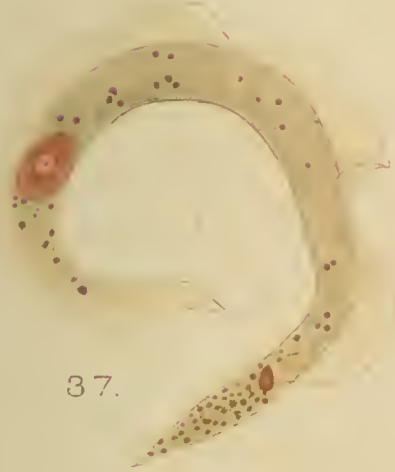

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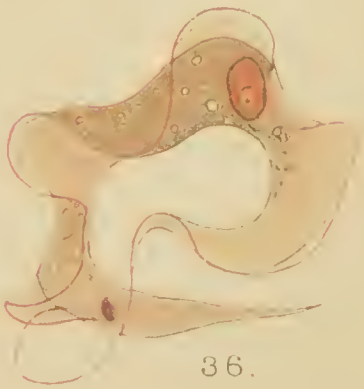

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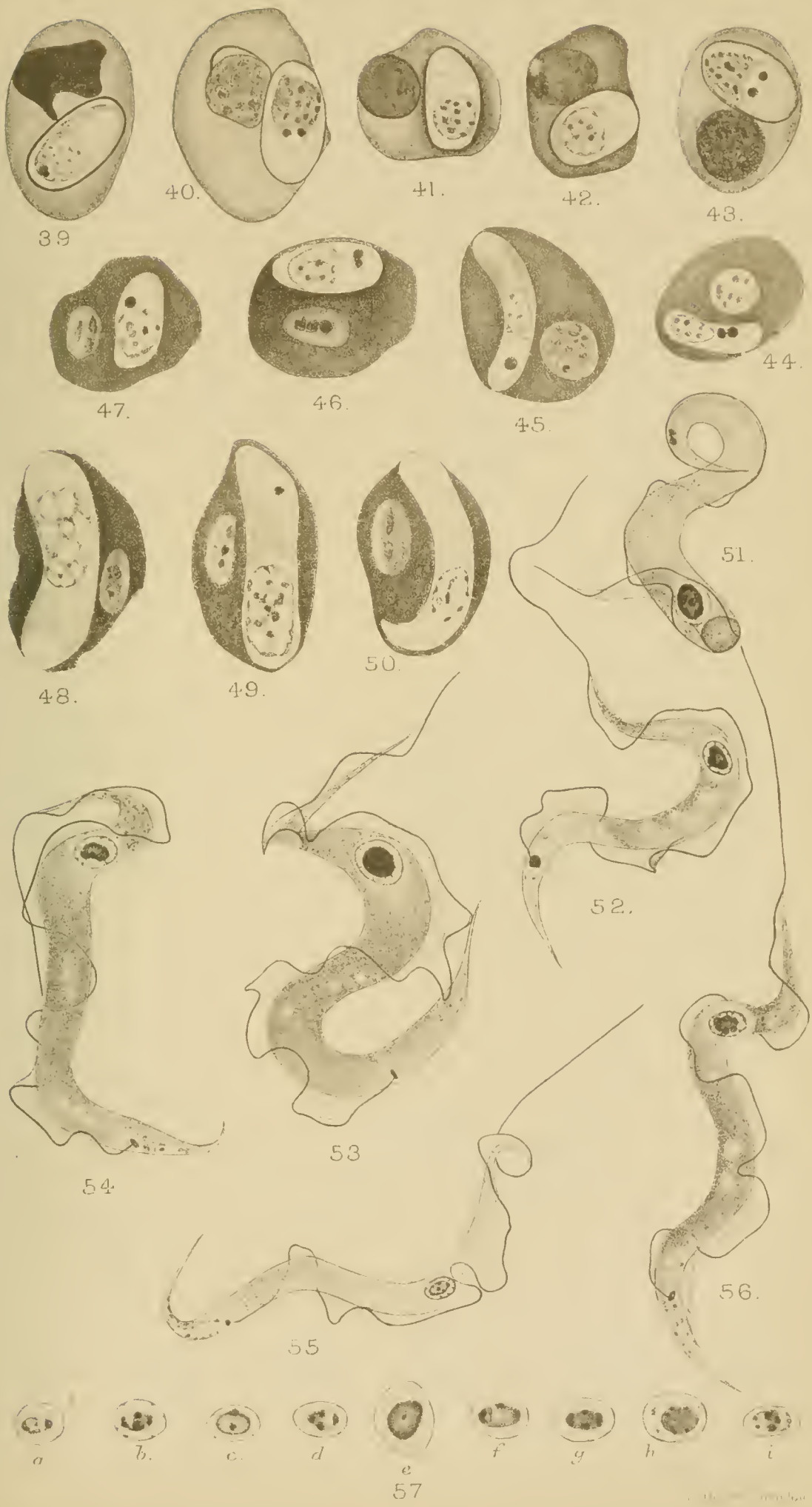
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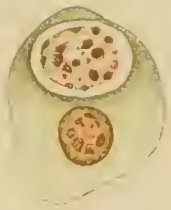

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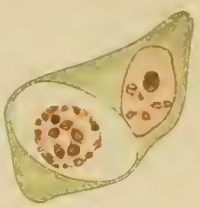

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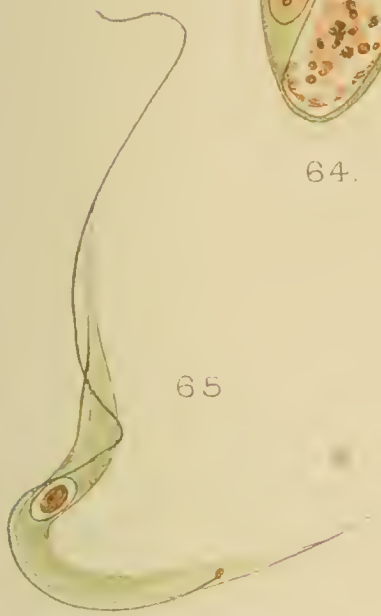

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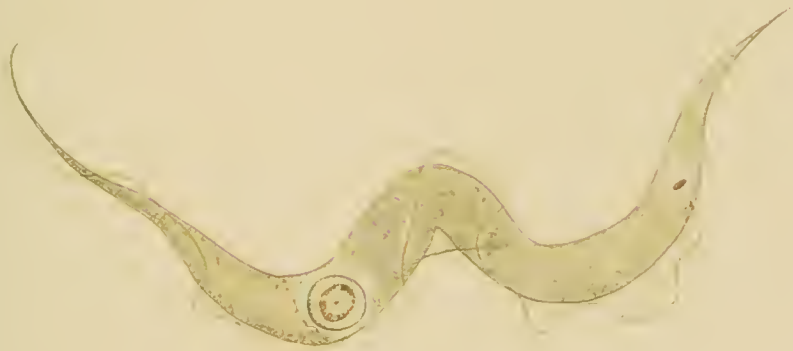


67.

a. b. c. d. e. f

68.

On Ganymedes anaspidis (nov. gen., nov. sp.), a Gregarine from the digestive tract of Anaspides tasmaniæ (Thompson).

By

Julian S. Huxley.

With Plate 11, and 5 text-figures.

Introduction.

In 1907 Mr. Geoffrey Smith was in Tasmania on a zoological errand, his object being especially to investigate the structure and development of Anaspides, the Mountain Shrimp of that country.

After his return to England, he noticed, while examining his sections in detail, some curious structures in the liver, which on investigation proved to be large binucleate cells, obviously of parasitic origin. Turning his attention to the gut, he found that it was in some cases inhabited by large numbers of Gregarines of an unusual type, and surmised that there was a connection between these and the non-motile parasites in the liver.

This was enough to show that Anaspides, so interesting in every detail of its structure, is no less so in regard to its parasites; and, as he had much work of his own on hand, he kindly offered me the congenial task of describing this new Sporozoan, at the same time providing me with all his surplus specimens of Anaspides. For this, and for much help and advice, I must here tender my best thanks; nor must I

forget to express my gratitude to Prof. Bourne for much kind assistance.

Methods, etc.

Preservation.—Some of the Anaspides had been pickled in formalin, some in corrosive sublimate; these latter were much better preserved, and were exclusively used in the work.

Preparation of the Gregarines.—Mr. Smith's specimens of Anaspides had been kept in captivity for some time before they were preserved; and, either they had had very little to eat, or else all the fare provided for them was digestible—at all events their guts were almost empty, save of parasites. Thus it was easy to make preparations of large numbers of the Gregarines by staining the gut and liver-tubes whole in paracarmine for a couple of hours, and then, after taking up to xylol, teasing in Canada balsam on the slide, and removing as much of the débris of the gut as possible, leaving the parasites behind.

This was quite good for general features, but, as I found to my cost later, did not bring out certain important cytoplasmic structures.

Subsequently some more Anaspides were sent over from Tasmania; these had been preserved at the moment of capture, and their guts were filled with a mass of sand, swallowed for the sake of the contained organic fragments. This made matters more difficult. The Ganymedes had to be picked one by one out of the débris by means of a capillary pipette under the binocular microscope. They were then mounted from 90 per cent. alcohol on to a film of egg-albumen smeared over a slide, so that they could be stained with Heidenhain's iron hæmatoxylin, which proved much the best reagent for picking out the details of the complicated structures in the cytoplasm.

Besides making these whole preparations, I had sections

cut of individual parasites, and of the gut and liver of the host. Most of these were stained with iron hæmatoxylin, some with Ehrlich's hæmatoxylin and eosin, and some with methyl-blue eosin by Mann's method. Iron hæmatoxylin was the best for most purposes, but Mann's method was very interesting in revealing some of the complexity of the purely vegetative processes that take place in the nucleus and nucleolus.

It was of course impossible to get any Anaspides over to England alive, and thus several questions of structure and life-history which could probably have been easily elucidated by observations and cultures of the living Gregarine, have had to be left to await the verdict of some investigator who has not got the Tropics between himself and the source of his material.

General Account (Life-history, Habitat, Etc.).

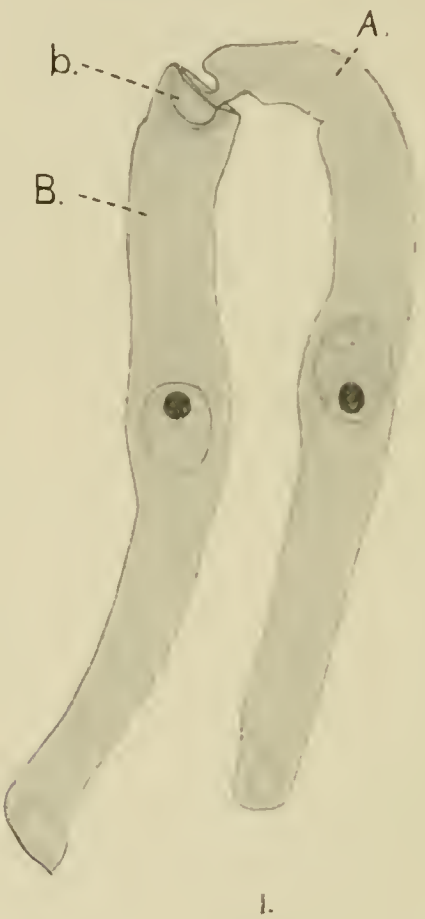
As above mentioned, Ganymedes is a parasite of the Syncaridan Crustacean Anaspides tasmaniæ, inhabiting various portions of its digestive tract. Before proceeding to a detailed account of its structure, it will be best here to give a brief general survey of its life-history, as far as such a continuous record can be pieced together from the mere snap-shots which are all that preserved material can give.

I came across no sporozoite stage. The smallest Gregarines found were only about one-eighth the length of the full-grown motile trophozoite, but otherwise similar in every way. These elongated motile forms, obviously belonging to the class Gregarinida, are in what I shall call the first trophic period, which is spent within the very long mid-gut of the host. Here some are attached to the epithelium (fig. 9), but the majority are found free in the lumen. If the host has recently been feeding, the gut is crammed with sand-grains and organic particles; and when this is the case, the parasites collect between this food-mass and the gut-wall,

where there is plenty of food that they can absorb, and least chance of their being carried away to the exterior.

Sometimes the parasites, instead of having their typical straight or gently-curved form (fig. 1) lie coiled and con-

Text-fig. 1.



1.

An associated couple of Ganymedes, showing the cup-individual (B) grasping in its cup the ball (*b*) of the other associate (A) The ball end of B is abnormal. The cup end of A has a large vacuole within it. The bodies are slightly dilated round the nucleus.

torted against the intestinal wall; and when this is so, many are usually congregated in patches, and are stuck together, presumably by the coagulated secretion of the endodermic cells. What are the reasons for this condition I could not discover.

Finally, a certain number of the Gregarines are found associated in pairs, the attachment being by dissimilar ends (text-fig. 1). Not very many are in this state, but I suspect that the shock of killing, and the subsequent manipulation, manage to sever the connection between a large number of couples, and therefore cannot say if association always supervenes when the parasites reach a certain size, nor what are the proportion of couples to free Gregarines.

Association marks the close of the first trophic period. In the second trophic period the Gregarines are non-motile, have lost all the complex structure they had before, and are characterised by their (probably rapid) growth to a very large size. In this state they are found in the liver-tubes, of which there are twenty or thirty, lying free in the hæmococœle, and not intertwined. It follows that the associated couples must migrate forwards to the junction of mid- and fore-gut, where the liver tubes open, and thence back into one of these. On penetrating a safe distance along the tube, a transformation must take place, the two Gregarines undergoing complete cytoplasmic fusion, a state of affairs known hitherto only in those neogamous forms from Holothurians, Cystobia and allied genera (Woodcock, 6).

These fused couples, looking just like one cell with two nuclei, are found wedged in between the cells of the wall, with a considerable free surface for absorption towards the lumen of the tube. There is often another free surface on the exterior, due, I should say, simply to the growth of the creature, and the consequent forcing apart of the liver-cells (text-fig. 3). For this growth, Ganymedes is here in a very favourable place, since the so-called liver, in addition to producing digestive ferments, is the organ where a great part of the food is absorbed; and so, while the parasites may enter on this period when measuring no more than $70 \times 60\ \mu$, they often attain to the considerable size of $200 \times 130\ \mu$, and I have seen one that measured $300\ \mu$ in its greatest length, though its breadth was only $120\ \mu$. The shape is variable, from a nearly perfect sphere to a long ellipsoid or ovoid.

The two nuclei meanwhile become round and very large, and possess on one side a large lenticular nucleolus.

The next step in the cycle is for the associated couple, while still in the liver-tube, to form a thick resistant coat round itself: in so doing it becomes perfectly spherical, and a process of concentration of cytoplasmic materials must take place, as I have found none of these cysts with a diameter of more than 115 μ, and one only 85 μ across, the average being about 100 μ.

The formation of the cyst wall of necessity closes the trophic periods, and sporogony now presumably begins. I say presumably, for I have seen no spores, nor even any of the preparatory nuclear divisions. Two cysts in the liver of a particular host showed nuclei with central nucleoli emitting chromatin (fig. 17)—a phenomenon very common in Protozoa at the close of vegetative life: and I have found a number of the usual type of cysts free in the gut.

From these facts, and from analogy with other intestinal Gregarines, we must suppose that after the formation of the smooth cyst wall, the couples can be expelled from the liver tubes (while those in the second trophic period remain in place by virtue of their soft surface adhering to the similar surfaces of the liver-cells), that they are then passed out by the anus, and that it is only here, under the stimulus of the changed conditions, that the processes leading to the production of spores can take place.

This being so, it is probable that infection is casual, the spores or sporocysts being taken in with the food—as, indeed, might have been deduced from the feeding habits of Anaspides. The infection is usually heavy (text-fig. 3), and frequently seems to be multiple, cysts, motile Gregarines, and associated immobile forms being often found all in one host. The proportion of infected hosts was over 50 per cent. in the case of those that were captured by Mr. Smith in a small pool on one of the mountain becks of Mt. Wellington; but in those he obtained from a larger piece of water, the infection was nil—or at least no parasites were forthcoming

in the dozen or so of hosts that I examined. The time of year seems to have no effect on any of the processes of the parasites' life.

As regards the effects produced by Ganymedes, no inconvenience seems to be suffered by the organism of the host as a whole, and only trifling damage is done to individual tissues. Those few cells of the gut epithelium to which the Gregarines attach themselves look generally unhealthy, and their nucleus becomes hyperchromatic (fig. 10); and the walls of the liver tubes get more or less distorted by the growth of the large couples in the second trophic phase: but in neither of these ways can any serious harm be done.

After these preliminary remarks, we may now proceed to consider in detail the structure of Ganymedes in its various stages.

Detailed Account.

(i) The First Trophic Period.

Although the size of the smallest free Gregarines seen was only 80—100 μ, yet I could find no points of difference between them and the adults, save that in the young forms the body has not attained to its full size relative to the structures (soon to be described) situated at the extremities. From these small forms all stages may be seen to Gregarines 400—425 μ long, and 23—30 μ broad, though the average size is 250—300 μ × 17—20 μ.

The shape of the body is cylindrical, tapering slightly towards one end, and considerably towards the other. The thinner end is almost certainly anterior in progression, and when attachment takes place, it is by means of a structure at this extremity. This structure in favourable specimens is seen to consist of a sphere connected by a thinner neck to the main body: I propose to call it the ball, and the thin extremity on which it is placed, the ball end. The other end may be called the cup end, for here many individuals possess a perfectly regular hemispherical depression, whose

outside walls continue the lines of the body: the whole is marked off by a circular groove, thus rather resembling the sucker of an Octopus.

Leaving the details of these organellæ for the present, I will now describe the main body of Ganymedes. This is of the usual type seen in motile Gregarines. It is covered with a firm cuticle, the longitudinal striations on which can be easily seen (figs. 6, 10, 11). Just beneath this appears in many cases a pale ectoplasmic layer, lacking the granules of the central endoplasm: and though I have never been able to demonstrate actual myonemes, yet from what we know of other Gregarines it is probable that this layer is the seat of the contractile structures which this free-swimming creature must possess. The endoplasm proper is denser, and contains granules. The whole cytoplasm is of reticular or alveolar structure.

The nucleus lies more or less in the centre of the body: it is ellipsoidal: the folds and processes sometimes seen at one end of it (fig. 15) being probably artefacts. Its breadth is often very nearly that of the Gregarine, and it would sometimes touch the cuticle except that when it is large the body bulges out slightly round it. It possesses a thin but distinct nuclear membrane, within which is a reticulum with granules on the threads—sometimes loose with largish grains (fig. 14), sometimes finer (fig. 15). In addition there is present a deeply staining spherical nucleolus, usually towards the cup end of the nucleus. In it, a thin outer rind usually stains deeper than the central medulla, which is filled with clear vacuoles of various sizes (figs. 14, 15). With Mann's methyl-blue-eosin it stains usually bright crimson to claret-colour, often with a violet crescentic area on one side.

Returning now to the anterior extremity, we find that in some cases there is, as above stated, a distinct stalked sphere (figs. 7—10). This is covered with a cuticle thinner and less firm than that of the body, the two passing into each other round the narrowest part of the neck (fig. 7). The sphere is filled with a quite homogeneous fluid, except at the extreme

front end, where there is usually a sort of pad of fine-grained cytoplasm projecting back into the cavity (fig. 8). In the main body, behind the neck, is another spherical cavity, apparently separated from that of the ball proper, and containing a fluid that is not quite clear, but of a loose reticulate structure (figs. 6, 7). Enclosing the hinder part of these may sometimes be seen a dark crescent of nearly homogeneous material (fig. 9).

So far, so good. In other cases, however, we find quite a different appearance, there being only one cavity present, and all traces of a neck having vanished (figs. 3, 5). Closer inspection shows that the cavity corresponds with that of the true ball, as its contents are perfectly clear, and it has a pad of cytoplasm anteriorly. The dark crescent may come directly behind it (fig. 5), while the thick body cuticle extends completely over it. The question then is, what is the relation between these two conditions?

It seems obvious that the ball can be extruded at will —but in what way? Is it evaginated (pleurecbolic) or is it acrecbolic, and, if the latter, is it pulled out by muscular or elastic action or pushed out by some other means; and how is it retracted? An examination of many Gregarines (a task necessary owing to the absence of living material, but laborious from the small size of the ball— 8—10 μ across), has made it seem probable that it is acrecbolic, and pushed out by the accumulation of a watery fluid behind it. As far as I can make out, the structures and processes concerned are as follows:—The dark crescent (*s.t.* in figs.) is a tissue which has the power of secreting a fluid (*w.*) into a space anterior to it, thus driving the ball out through an opening in the body cuticle. When the ball is retracted, the elastic cuticle would be closed over the anterior end; and when extrusion has taken place, it would press in and form the thin neck. One animal (fig. 2) shows what I suppose to be an early stage of extrusion: the hole is just being enlarged, so that the cuticle at its edge stands out as a well-marked rim (*cut. rim*). In later stages (figs. 4 and 6)

this rim will press against the convexity of the ball and thus be difficult to see; it is only in the early stages of extrusion that its inner surface will form an angle with the surface of the ball, and thus stand out. The pad of cytoplasm (*p.*) is always seen at the anterior end of the ball vesicle, showing that there can be no question of invagination.

Retraction would then take place by the resorption of the secretion; while the ball seems to be kept in place by strands from the ectoplasm (probable muscular layer), for this, and this only, usually extends up the sides of the secreted fluid to the ball vesicle (figs. 3, 5, 6).

When fixation takes place, the condition of things looks somewhat different (fig. 10), and there is an open communication from the ball to the space behind it. Very possibly the cytoplasm at the neck is temporarily dissolved so as to leave this passage-way for the food absorbed by the ball to pass further into the substance of the animal.

Finally, in association, the ball of one is extruded into the cup of the other, and the cup then seemingly contracts so as to hold the ball firm (fig. 9; text-fig. 1). It may be here remarked that the free ball end in the couple in text-fig. 1 is quite abnormal: it was pointed, and contained a pointed cavity within it, but otherwise had none of the typical structure.

The cup-end also presents various difficulties. When well formed its structure is simple enough, and has already been described. But at other times the hollow cup may be quite wanting, the body ending simply in a rounded end with rather thick ectoplasm (fig. 12); or, more often, there are numerous vacuoles beneath the cuticle (fig. 13), with sometimes an irregular aperture in addition (text-fig. 2). What the meaning of these variations is, and whether the cup-end can pass from one state to another, I fear I cannot say.

It was from the presence of the cup that I ventured to call this new genus Ganymedes, though the pedant will perhaps maintain that this name should have been reserved for some

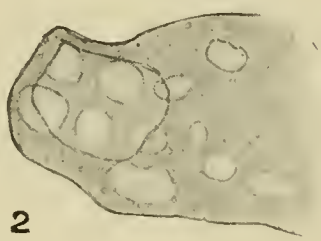
parasite of Aquila. With the specific title anaspidis, however, I think no one will quarrel.

(ii) Second Trophic Phase.

Between the two phases of trophic life no intermediate stages were found, all the couples in the liver having lost every trace of the cytoplasmic structures of the Gregarinoid form. All they possess is a thin cuticle (fig. 18), investing a delicately-meshed cytoplasm.

The nucleus, on the other hand, has increased in complexity (fig. 18). It is large and more or less spherical,

Text-fig. 2.



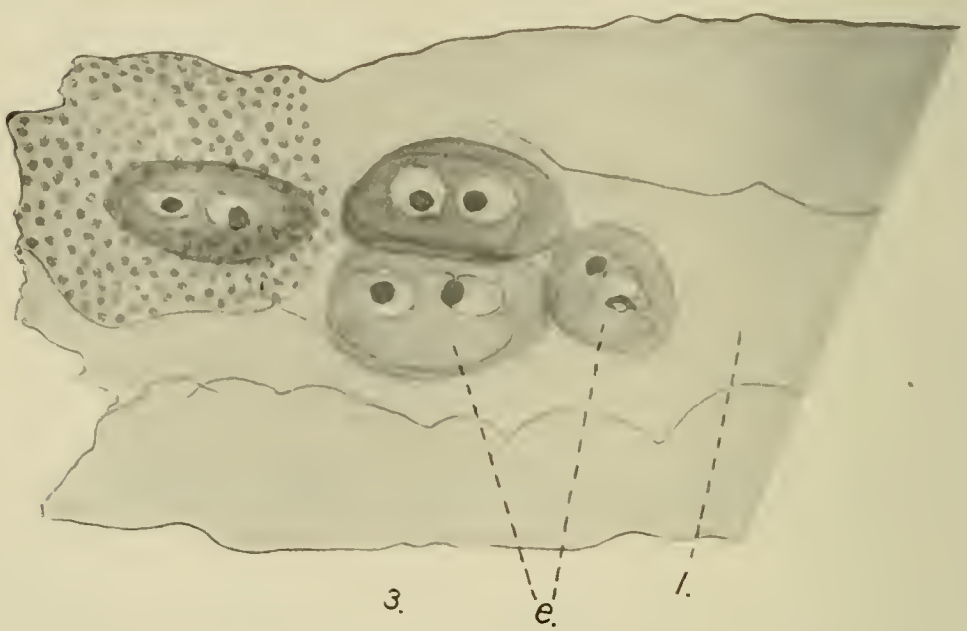
Diagrammatic view of the cup end of a Gregarine, to show the opening on one side, and the numerous vacuolar spaces in the cytoplasm.

with a thin nuclear membrane, and an achromatic network in which there is very little chromatin present. The chief interest lies in the nucleolus, which is peculiar in two ways. First, it occupies an unusual position, right on one side of the nucleus, somewhat like the lens of an eye, with a considerable surface in contact with the cytoplasm—a state of things not, I believe, known in any other Gregarine, though Awerinzew (1) has described something similar for a Myxosporidian; and secondly, it possesses itself another lens-like structure, projecting more or less into the cell-body, and composed of a very pale-staining meshwork, with its outer border not a smooth curve, but formed of the slightly projecting parts of the component alveoli (fig. 18).

This is perhaps the absorptive part of the nucleolus, taking up from the cytoplasm the soluble food which this in its turn has abstracted from the liver-tubes.

In the centre of the nucleolus, abutting on the absorptive part, is often an area, with a reticular structure, staining blue-violet with Mann's method. The remainder is composed of a dense material staining deep red, in which are embedded definite clear pink vacuoles. Towards the cyto-

Text-fig. 3.



Portion of a liver-tube of Anaspides with four couples of Ganymedes in it. The nuclei of the liver cells are represented only in one corner. *l* = lumen of liver tube. The lighter parts of the parasites (*e*) are exposed on the exterior of the liver-tube.

plasm these vacuoles project slightly; when one sticks right out, as at *x*, fig. 18 *b*, it is colourless, showing that the others look pink only because there is red substance above and below them. Towards the nucleus, on the other hand, the vacuoles rarely project, the edge of the nucleolus being usually clean cut. Text-fig. 4 represents diagrammatically another nucleolus in which the absorptive area was extremely large.

The nucleolus thus seems obviously to be the chief agent concerned in the manufacture of food-stuffs (for theories regarding the action of Mann's methyl blue eosin see Léger and Duboscq (2)).

What is the function of the rest of the nucleus in this period remains uncertain, though its large size shows that it must play some important part in metabolism. The chief interest here lies in the behaviour of the nucleolus, which migrates out to enter into direct relations with the cytoplasm at the beginning of the second trophic period, when assimilation begins to be greatest, and at its close, when all

Text-fig. 4.

Section of one of the nuclei of a couple in the second trophic phase. The nucleolus does not project very far, and the surface of the absorptive area is flush with that of the nucleolus, although the area itself is very large.

assimilation ceases, returns, as will be seen later, to the interior of the nucleus.

(iii) Encysted Phase.

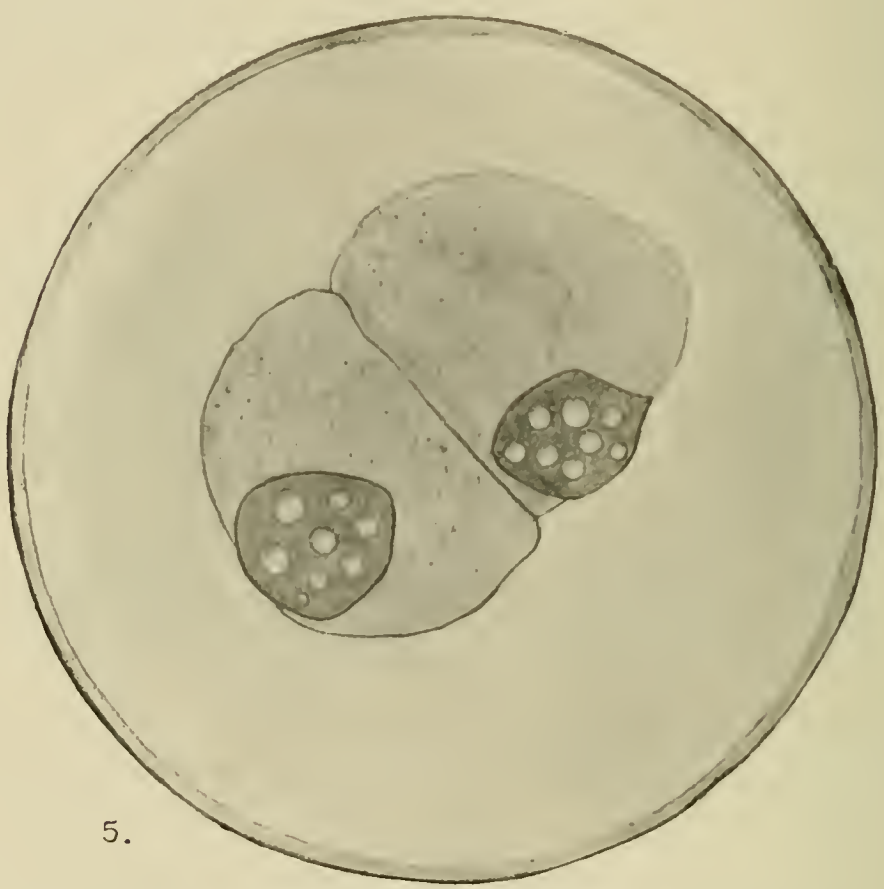
The cyst-wall, though always fairly strong, varies a good deal in thickness. It stains bright blue by Mann's method, bright red with carmine, but not strongly with hæmatoxylin. From it often project radially inwards curious irregular, branching filaments, never reaching much more than a third of the way to the centre, as to whose nature and function I am quite in the dark (fig. 16).

The cytoplasm is reticular, with minute granules on the

threads, and larger, chromatic granules here and there. It always looks denser than in the unencysted forms.

The nuclei in what I take to be the earlier cysts are much like those described for the second trophic phase, except that they stain a little deeper, and that the nucleoli do not project so far out from the surface (text-fig. 5). In the next

Text-fig. 5.



A cyst found in the gut. The nuclei are not actually touching, but very near to each other. The cyst-wall is very thick in this specimen.

stage (fig. 16) the nuclei, bounded only by a very thin membrane, stain quite deeply, as they are almost filled with chromatic granules of various sizes. The nucleolus is still in contact with the cytoplasm, but its outer surface is now flush with that of the nucleus. This outer border of the nucleolus is made up of rows of minute vacuoles, while the

rest is dense, with a clean-drawn boundary towards the interior, and homogeneous except for a few large vacuoles.

To this stage probably belongs the cyst in fig. 19, stained by Mann's method. The nucleolus is blue, having given up most of its chromatin to the nucleus, which is violet with dark purple grains.

In fig. 17 we have another state of affairs: The nucleolus, now retreated from the surface, seems to be giving off chromatin to the nucleus in the shape of hollow spherules. It is itself formed of a single central vacuole, surrounded by a layer of small ones embedded in a dense chromatic cortex (the lower nucleolus is cut tangentially, and so does not show this condition). The nucleus, apart from the chromatic spherules, appears perfectly homogeneous, with no achromatic network, and differs also from the nuclei of other stages in being amœbiform, with "pseudopodia" that can be very clearly seen on focussing up and down.

From what we know of other Gregarines, it is clear that these stages are preliminary to the breakdown of the large trophic nuclear apparatus, and the reconstitution of the idiochromatin to form the gametocyte nucleus. But, as above mentioned, the cysts soon after this pass into the gut and out by the anus, so that their further development must remain for the present unknown.

Conclusions: Systematic Position.

Though here more than ever must we lament the absence of spores, it is still possible to draw some fairly definite conclusions. To start with, Ganymedes is not a Polycystid, nor does it belong to any existing family among the Monocystids. Thus a new family, the Ganymedidæ, must be created, whose characters will provisionally be those of the genus: these may be here conveniently summarised as follows:

(1) The possession by the motile form of a special extensible organ at the front end, which may serve for fixation to the cells of the host.

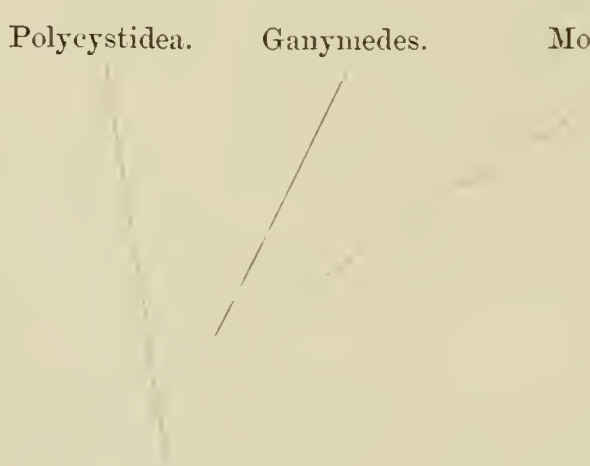
(2) The presence of a special cup-like structure at the posterior end, which co-operates with the epimeritic organ at the anterior end to effect a close union of two individuals during association. Association is thus by dissimilar ends, and lasts for some time.

(3) The eventual complete cytoplasmic fusion of the associated couples, and the existence of a second trophic phase, when the animals grow very fast, but are morphologically quite degenerate.

(4) The position of the nucleolus in this phase, on one side of the nucleus, partly in contact with the cytoplasm.

(5) The habitat, in the gut and liver of Syncaridan Crustacea.

Considering these characters in relation with other members of the class, we find that no known Gregarines inhabit the liver of any Crustacean; none have the nucleolus in the same position; none go through two trophic phases; none have any special structure for association at the posterior end; and none have a protrusible organ of the same sort at the front end. It is thus at least obvious that Ganymedes is the representative of a very divergent line. The suggestion I would make is that, while nearer to the Monocystid type, Ganymedes is partly intermediate between the two great groups of Eugregarines, as represented diagrammatically in the following tree:



In the first place, the ball and the cavity containing the secreted fluid represent with great probability an epimerite and protomerite. True, there is no cuticular septum; but the secreting tissue forms a fairly definite barrier between these on one side, and on the other the deutomeritic posterior part. Here alone, it is to be remarked, do we find the true granular endoplasm. Occasionally, too, this latter can be seen ending off with a definite contour within the secreting tissue (fig. 3). The ball itself, when extruded, would pass for a typical epimerite save for the absence of a septum behind it; but in so far as it is protrusible, it is only paralleled by the anterior extremity of Lankesteria ascidiæ (Siedlecki, 4). This, however, seems to be merely a pseudopodium, or a drop of the hyaline inter-reticular substance of the cytoplasm pressed out through a hole by contraction of the animal, and its extrusibility has obviously been independently evolved.

The fact of its being a parasite of the digestive tract is the second link with the Polycystidea. The only Monocystid gut-parasite whose life-history has been thoroughly worked out is Lankesteria, and this possesses an "epimeritic" organ. The three or four other genera of this sub-class that live in the gut, such as Callyntrochlamys and Ancora, are very insufficiently known; it is even possible that they may be Polycystid in early stages.

Regarding the matter phylogenetically, we find that the early Eugregarine stock must have been motile, Polycystid gut-parasites; their association was by dissimilar ends, and took place only at the very end of the trophic period; and they showed well-marked anisogamy.

One of the first steps towards the typical Monocystid condition was the change of habitat, due very likely in the first instance to the evagination of the full-grown trophozoites from the gut into the cœlom—as takes place to-day in certain insect-parasites at the time of the host's metamorphosis. For a full discussion of the further stages, leading eventually to complete isogamy, coupled with entirely

cœlomic habitat, precocious association, and degenerate structure, the reader is referred to Woodcock (**6**). Suffice it here to say that the course of affairs in Ganymedes must have been somewhat different. It is probable that Ganymedes at first associated only at the close of the trophozoite stage. Some of the couples having migrated into the liver, found it (like the cœlom for other Monocystidea) a safe retreat and abounding in soluble food. Here too the Gregarine could afford to dispense with all the structures necessary for a life in the open gut, and devote all its energies to growing. One might have thought then that Ganymedes would have associated in the sporozoite stage, like Cystobia, and migrated at once into the liver; but, whether non-motile couples below a certain size could be expelled from the tubes or be engulfed and digested by the activity of the liver-cells (see Smith, **5**, p. 536), or from some other cause, Ganymedes has found it necessary to remain in the gut till it has attained a definite bulk, thus presenting to us the phenomenon of two sharply-distinct trophic phases after the sporozoite stage. As the parasites are non-motile when they are about to sporulate, conjugation must needs be precocious, so that no Gregarine shall migrate alone into the liver, and thus be, from the point of view of the species, wasted. For this fairly lasting association some special mechanism was imperative, hence the cup and ball; while the necessity of remaining some time in the gut has led to Ganymedes retaining more of the original Polycystid structures than is usual in the morphologically degenerate Monocystidea. Finally, although the sporogony remains unknown, it may be confidently prophesied that this Gregarine will be found to be completely isogamous.

Thus it will be seen that the Ganymedidæ diverged very early from the Monocystid stock, and possess now many new and peculiar characters intermixed with those they have inherited from the common ancestor. For the complete disentangling of these from each other, further work must be done on Ganymedes, and in addition all

Syncaridan Crustacea should be searched for allied parasites, whose structure would at once give us new standpoints from whence to view the problem.

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4. Siedlecki.—"Ueber die geschlechtliche Vermehrung der Monocystis ascidiae, R. Lank.," 'Bull. Internat. Acad. Sci. Cracovie,' Dec., 1899.

5. Smith.—"On the Anaspidacea, Living and Fossil," 'Quart. Journ. Micr. Sci.,' vol. 53, 1909, p. 489.

6. Woodcock.—"The Life-cycle of 'Cystobia' irregularis, Minch., together with observations on other 'Neogamous' Gregarines," 'Quart. Journ. Micr. Sci.,' vol. 50, 1906, p. 1.

EXPLANATION OF PLATE 11,

Illustrating Mr. Julian Huxley's paper "On Ganymedes anaspidis (nov. gen., nov. sp.)."

Reference Letters for the Figures.

b. Ball-cavity. *c. s.* Cuticular striæ. *ect.* Ectoplasm (probable myocyte layer). *p.* Cytoplasmic pad at anterior end of ball. *s. t.* Secreting tissue. *v.* Vacuoles. *w.* Secreted fluid that accumulates to drive the ball out.

Bor.-car. Borax carmine. *Paracarm.* Paracarmine. *Hæm.* Hæmatoxylin. *M. B. E.* Methyl-blue eosin (Mann's method).

PLATE 11.

Fig. 1.—Large individual at the close of the first trophic stage, with well-formed cup. (Paracarm. × 640.)

Fig. 2.—Ball end of the same, to show the ball being pushed out through the hole in the cuticle; the edges of this hole stand out markedly as a rim (cut. rim). (× 1300.)

Figs. 3–8.—Ball ends of various Gregarines in different conditions.

Fig. 3.—(Semi-diagrammatic). Very slightly extended. Secreting tissue very large, with the granular endoplasm (e_1) ending off within it. Outside is a non-granular layer (e_2), and just beneath the cuticle the still paler ectoplasm, extending on the left to touch the ball-vesicle. (Bor. Carm. × 1875.)

Fig. 4.—Semi-extended. The secretion of the secreting tissue is fairly dense. The double contour of the hinder part of the ball is well seen. There seems to be no ectoplasm. (Iron Hæm. × 1875.)

Fig. 5.—(Semi-diagrammatic.) Completely retracted. Very large cytoplasmic pad (p) with dark grains in it. A large dark granule in the secreting tissue. The ectoplasm extends to touch the ball. (Iron Hæm. × 1875.)

Fig. 6.—Almost extended. The secreted fluid has here a wide-meshed structure. The thick body-cuticle ends abruptly where it touches the ball, which possesses only a thin cuticle. Cuticular striæ are seen on the under surface. No well-differentiated ectoplasm. (Iron Hæm. × 1875.)

Figs. 7 and 8.—(Semi-diagrammatic.) Completely extruded.

In fig. 7 the neck of the ball is well seen, also the more delicate nature of the ball's cuticle. No cytoplasmic pad is visible.

In fig. 8 the ball is directed slightly upwards. The cuticle is distended round the secreted fluid, showing that this is under pressure. (Paracarm., fig. 7 × 1300; fig. 8 × 1875.)

Fig. 9.—Section (5 μ) through the point of junction of an associated couple in the first trophic phase. The cytoplasm of the ball individual (A) is denser than that of the other (B). (M. B. E. × 1300.)

Fig. 10.—Section (5 μ) through the point of attachment of a mobile Ganymedes to a cell of the host's gut. The cuticular striæ are well seen. The ball is thrust into the host-cell, and contains a fluid that is not clear, the reticular structure being probably due to the coagulation of absorbed food. There is an open passage through the neck into a

cavity in the body of the parasite. The cytoplasm contains numerous deeply-staining granules. The nucleus of the host-cell (*n*) is large, darkly-stained, and homogeneous, except for some dark grains. (Iron hæm. × 1340).

Figs, 11–13.—(Semi-diagrammatic). Cup-ends.

Fig. 11.—Cup-end of the Gregarine whose ball-end is shown in fig. 5; (*a*) is focussed near the upper surface, and shows how the cup is separated from the body by a circular groove; (*b*) shows the greatest diameter of the cup. (Iron Hæm. × 1875.)

Fig. 12.—Cup-end of another Gregarine, to show absence of all differentiation. The ectoplasm is thicker at the end than elsewhere. (Paracarm. × 1300.)

Fig. 13.—Section of the cup-end of Gregarine A in fig. 9, to show the numerous vacuolar spaces beneath the cuticle. (M. B. E. × 1300.)

Figs. 14 and 15.—Sections (5 μ) to show the structure of the nucleus in the first trophic phase. (M. B. E. × 1300.)

Figs. 16 and 17.—Sections of cysts.

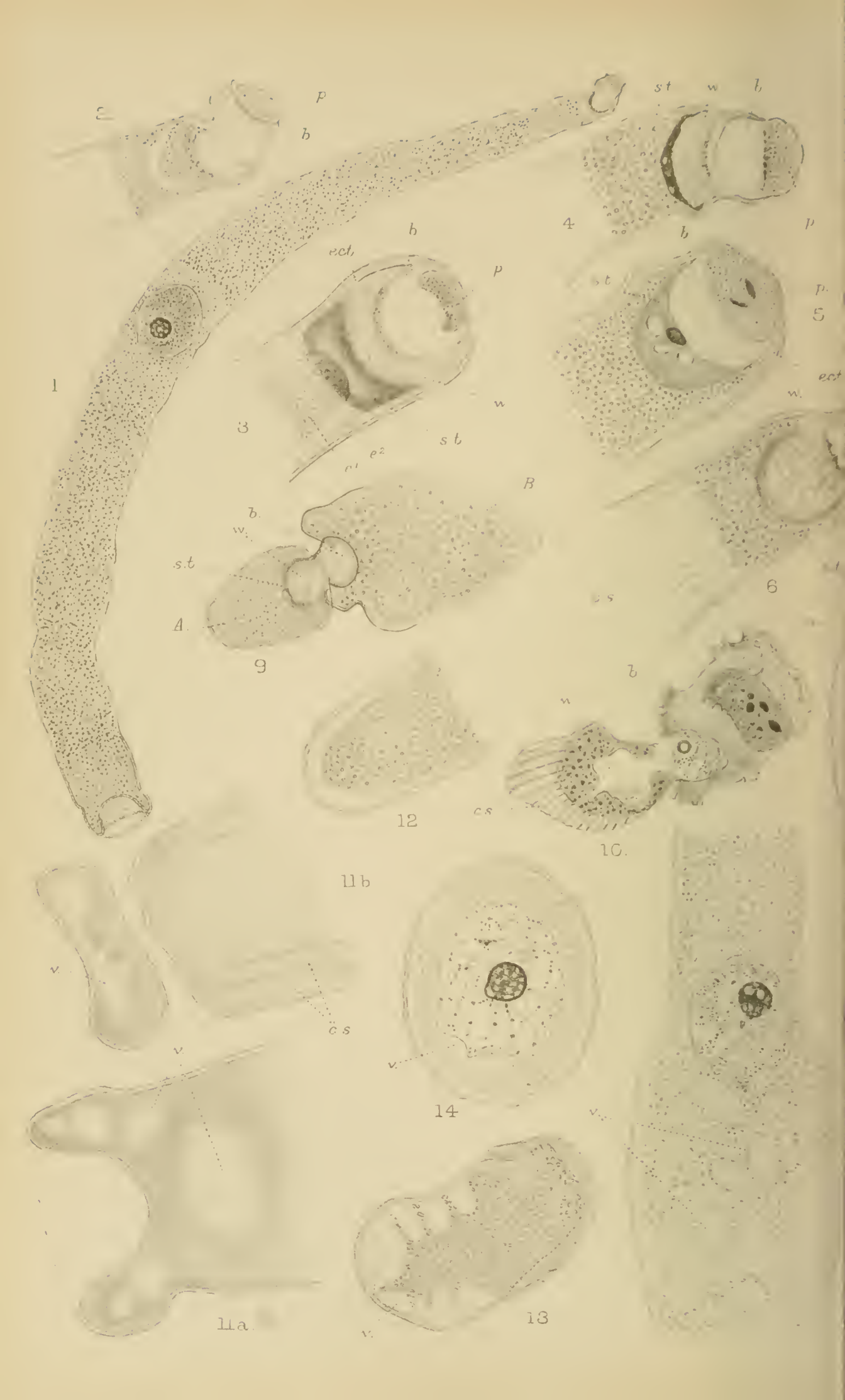
In fig. 16 the filamentous inward projections from the cyst-wall can be seen. Small chromatic granules fill up the nucleus; there is no sign of an achromatic network. The nucleoli are retreating to the interior of the nucleus. (Iron Hæm. 5 μ × 970.)

In fig. 17 the nuclei are amœboid, filled with a homogeneous sap in which are hollow chromatic spherules, apparently emanating from the nucleoli. The cyst-wall is crumpled, and in one place a flap of it has got detached so that its surface-structure is seen. (Ehrlich's hæm. + eosin 10 μ × 800.)

Fig. 18*a*.—Section (5 μ) through an associated couple in the second trophic phase. The reticular nature of the cytoplasm is not indicated. (M. B. E. × 610.)

Fig. 18*b*.—The next section in the series. The nucleolus and the outline of the nucleus are given, more highly magnified. The three areas of the nucleolus and their structures are shown (see text). At *x* a vacuole projects beyond the general surface, and is seen to be colourless. (M. B. E. × 870.)

Fig. 19.—Section of a cyst, to show the alteration in staining reactions of nucleus and nucleolus in this stage (see text). (M. B. E. × 400.)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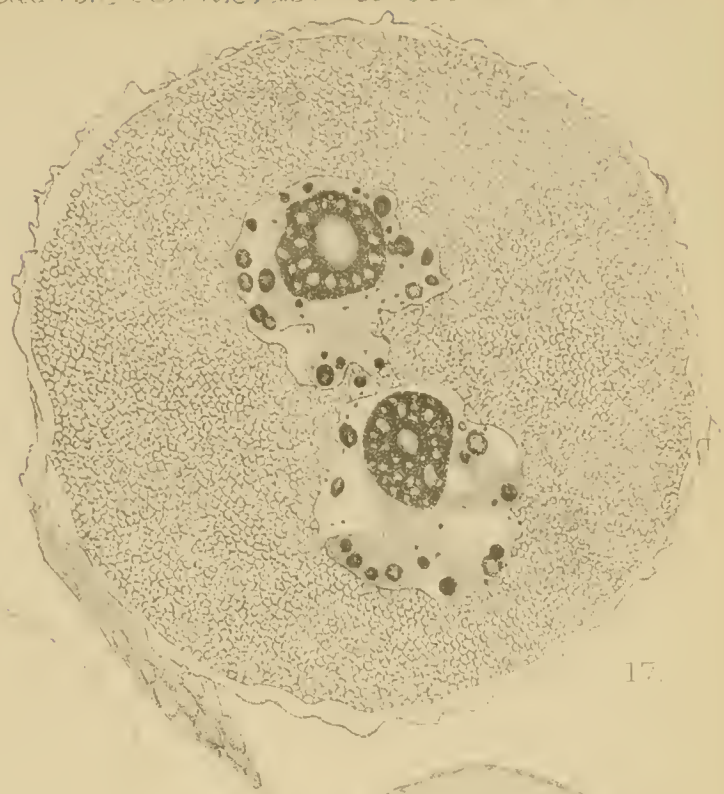

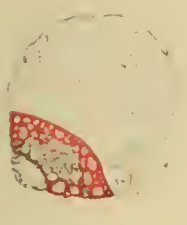

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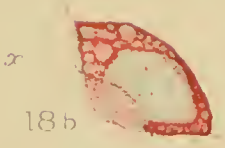
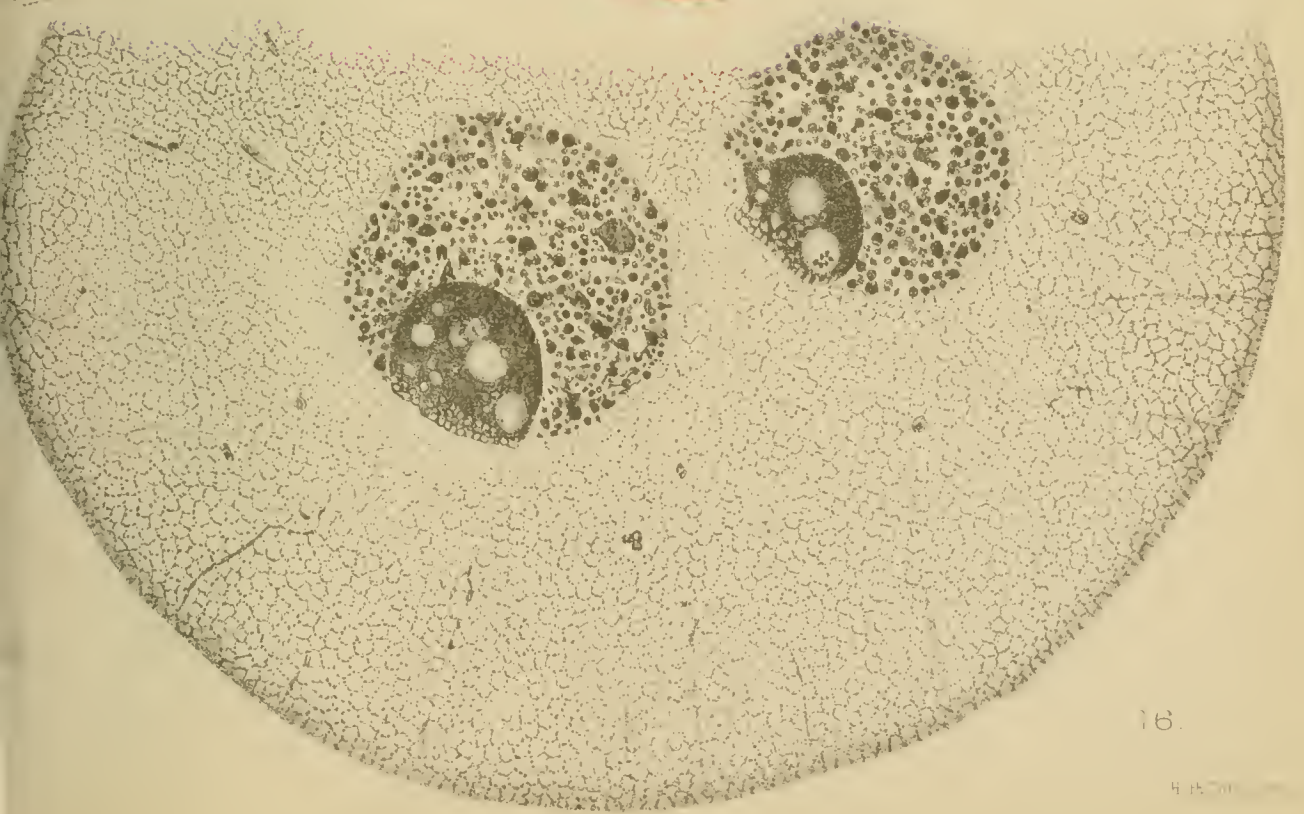


18b.



The Fœtal Membranes of the Vertebrates.

An Address reprinted from the "Proceedings of the Seventh International Zoological Congress" held at Boston, 1907.[1]

By
A. A. W. Hubrecht.

I was honoured by the request of the Executive Committee to give an address at the first meeting of the Embryological Section of the Seventh International Zoological Congress.

I hope that in choosing for my subject the present state of our knowledge concerning the fœtal membranes of vertebrates I can avoid the disadvantages of too much special detail, and can at the same time call your attention to the fact that these fœtal membranes offer a very wide field for theoretical speculation, that may in its turn influence our views concerning certain important phylogenetic problems.

The fœtal membranes of vertebrates are known to occur in reptiles, birds, and mammals. The embryological hand-books tell us that they are absent in amphibians and fishes.

In consequence, a primary subdivision of the vertebrates has been instituted, those with fœtal membranes being classed as Amniota allantoidea, those without them as Anamnia anallantoidea. From this nomenclature any close observer, even when he is not a zoologist, may safely conclude that one of the fœtal membranes carries the name of amnion, the other

[1] At Professor Hubrecht's request this address is here reprinted. It will assist readers in apprehending the conclusions which Professor Hubrecht holds to be rendered probable by the large memoir published in this Journal in November, 1908.

the name of allantois. An older, now more obsolete, subdivision into Achoria and Choriata reveals the presence of a third membrane, the chorion, about which we will have more to say hereafter, and which will explain how this third membrane came to fall—so to say—between two stools, when the division into Amniota and Anamnia was established.

If we now take into account that neither chorion nor amnion nor allantois was ever detected in fishes or in amphibians, then we must recognise that the problem, how these fœtal membranes of the vertebrates did arise, is one well worthy of full consideration.

Up to now attempts to explain their gradual evolution have utterly failed. So, for example, the suggestion of van Beneden and others that the amnion, as a protective membrane, arose in consequence of the early embryo sinking into the yolk-sac, which closed up above it, has long since been abandoned. Also Haeckel's idea that the allantois arose by a precocious segregation of the urinary bladder of an early amphibian which took the habit of carrying blood-vessels, at a very early stage, to the outer wall of the blastocyst, must be dropped by all who object to predestination in evolutionary processes. Whenever an explanation offers itself which does afford a clue to a more logical sequence of events, it should be preferred.

And turning finally to the outer layer, the chorion, who can be satisfied with the lame explanation that the appearance of this membrane is a necessary sequel to the formation of the amnion, which we find inside of it, and which later, in so many orders of mammals, never even arises by folds, which, however, in their turn are necessary to explain the chorion's appearance?

The subsidiary explanation of all the three embryonic envelopes, which I am going to offer you on this occasion, seems to me to have the great advantage of simplifying matters; especially in this sense, that henceforth we can link them all three to one simpler and earlier stage (which must have preceded in the Carboniferous and in earlier geological

epochs) without having to look for incipient stages of any of them among our present ichthyopsids. Nay, we may even say that of this earlier, archaic starting-point evident traces have been preserved in the teleostomes, the dipnöi, and the amphibians, so that we have to reconsider most seriously whether it will be wise to go on subdividing the vertebrates into the two subdivisions of those that *have* and those that *have not* the fœtal envelopes above mentioned.

Now let us consider the facts as they present themselves to us, when we want to test the question whether one single original fœtal envelope could not after all be at the bottom of the three complicated involucra we have just mentioned. As far as I can see, we are only in need of this one assumption, that an invertebrate ancestor was possessed of what we call an exterior larval layer (such as are not uncommon among different worms, and as we find them, with certain further complications, in some arthropods), to be able to explain how, in their vertebrate descendants, chorion, amnion, and allantois gradually came into being.

Part of this hypothetical assumption we see actually realised under our eyes wherever one of the mammals goes through its normal stages of development.

We find that the cell-material out of which the embryo is going to be built up is surrounded by an expanded cell-layer, which takes no part whatever in the composition of the future embryo. Here we actually have our single larval layer that will be stripped off later, and that surrounds what are going to be the formative cells.

In all mammals it is this very larval layer which will become the outer wall of the blastocyst, what we have above called the chorion.

But before following it in its further transformations, we have to ask ourselves, what can be the reason that this outer larval layer, this trophoblast, is so far away from the formative cells of the embryo which adhere to it only at one point?

We have only to recall the fact of the pilidium larva, in which, similarly, the distance between the outer layer and the

cell-material, which is going to be the new worm, is also very considerable, to remove the objection that in this respect mammals would stand isolated. And we may go one step further and say that it is easy to understand why this considerable extension of the outer larval layer has come into existence. When we look back along the line of phylogenetic descent we can imagine that at the period when, for the first time, aquatic animals became inhabitants of the land, four-footed instead of four-finned, and adapted for aërial breathing in addition to their respiration by the aid of gills, it may have been a great advantage to them to become viviparous at the same time, i.e. to keep their developing eggs inside of them, where they are better protected and can be better nourished than outside of the mother. The atmosphere and the dry land offer less favourable conditions for the development of that small amount of protoplasm that forms the primordium of each new being than does the water, and so viviparity is likely to have been a parallel phenomenon to the exchange of the aquatic for the terrestrial existence.

We can see clearly that once an embryonic envelope, one cell-layer thick, being present (on our original assumption, as far back as the invertebrate ancestor), that this one-layered larval envelope could obtain high efficiency for the incipient viviparity if only it bulged out as much as possible, thereby—

(1) Preventing the egg from passing through the genital ducts rapidly and being deposited, so to say, accidentally.

(2) Enabling the egg to adhere in various ways to the maternal tissues, either as a simple mechanical improvement of what was attained (1), or at the same time inducing phagocytotic attacks on that maternal tissue.

(3) Creating the occasion for individual trophoblast cells of this outer layer to absorb fluids either from the uterine cavity or accessory to the phagocytic processes alluded to under (2), and thus accumulating nutritive material inside the blastocyst.

Furthermore, it is equally clear that, once the viviparity having been establised, and the surface extension of the

trophoblast going parallel with it, a yet more efficient mode of nutrition than the one alluded to above under (3) might be obtained if the embryonic vascular system, which was slowly coming into existence on the hereditary plan of development, succeeded in spreading out, in one way or another, on this outer trophoblastic layer, and would enter into osmotic interchange with maternal blood.

Finally, the protection of the embryonic shield during its further development by some sort of appliance resembling a water cushion would, in these incipient viviparous animals, undoubtedly have been a most efficient variation, for the earliest origin of which we have simply to go back to the early stage in which we noticed the formative cells of the embryo adhering to the larval layer, the trophoblast, in one spot only. Suppose that in further development this sessile attachment to have become converted into a circular adhesion —by fluid accumulating between the trophoblast cells and the formative cells, as we see it happen under our eyes in Erinaceus and Gymnura—we then find that the water-cushion, in casu the amnion, took its origin in a most simple fashion, whereas the chorion is in no way dependent on it, but has preceded it as as earlier formation.

The rapid summary here given shows us that the assumption of a single monodermic larval layer is quite far-reaching enough to allow us to understand how, out of it, chorion, amnion, and allantois (the latter as representing one form of early vascularisation of the trophoblast) have gradually come about.

The only change we have to make, in what I might designate the present "fashion" in comparative embryology, is that we look upon the earliest ancestors of mammals not as oviparous, yolk-laden vertebrates, but that we acknowledge them to have been viviparous animals with blastocysts that obtained vesicular shape from quite other motives than an eventual "loss of yolk," such as Rabl has attempted to prove. Here, then, is the place for an appeal to palæontologists. They have no shadow of direct interest in fœtal envelopes which are

never met with in the fossil condition! But they may, nevertheless, be all the more impartial judges when we have to choose between two different assumptions: the one given in the hand-books, according to which mammals must, through the Ornithodelphia, be derived from some oviparous sauropsidian ancestor, or the one here advocated, according to which a viviparous Prototrapod, provided with an adhesive and distending larval layer diverged into various directions, some of the descendants utilising the conditions of growth and development (such as they find them) with the highest degree of intensity and becoming primates, others applying their trophoblast to nutritive purposes in more diverse and less direct ways, becoming the ancestors of most of our other Monodelphia and Didelphia. Others, again, going a certain distance with the preceding, but then acquiring yolk-laden eggs (Ornithodelphia), whilst yet other very effective branchings off in various directions gave rise to the primitive sauropsidian ancestors.

The difference between the sauropsidian and the amphibian descendants of the protetrapods need no longer be so incisive —as those zoologists that divide the Vertebrates into Amniota and Anamnia would make it. The hypothesis here brought forward proposes to look upon what we know as the Deckschicht of the early larval Amphibia and Dipnoi, and even of the teleostomes, as a last remnant of the very larval layer from which we started in trying to explain the fœtal membranes of vertebrates according to what seems to me a simple plan.

We have now to look a little closer into certain details, by which we may be enabled to judge of the greater or smaller degree of tenability of some of the views here brought forward.

We notice that all the Mammalia-monodelphia, that have up to now been observed in very early stages, fully confirm the strong antithesis which in those early stages prevails between the trophoblast and the embryonic cells strictiori sensu. We also notice this in the Didelphia, as far at least as Selenka's figures for the opossum go, although he himself has not interpreted the facts he brought to light in the same

way as I do. Similarly, Wilson and Hill, in their latest paper on the development of the duck-bill, give us figures of sections which make it probable that the distinction between trophoblast and formative cells holds good here, even though the development of yolk has obliterated the sharp outlines of the process.

Again, in reptiles and birds traces of the larval layer have, in later years, been unmistakably noticed. Schauinsland, Mitsukuri, and Mehnert were among the foremost to contribute facts in this direction, although at the same time they failed to see the essential points of comparison with the mammals. This failing on their part is all the more explicable as the bird's egg, which has always served as the prototype even of mammalian development, does not clearly bring out the fundamental distinction that exists between trophoblast and formative matter of the embryo.

The gradual obliteration of this distinction may, perhaps, be ascribed to the fact that in these sauropsids, as in the ornithodelphia, a shell has developed, which naturally tends to relegate any outer larval layer to the pension list.

Concerning the yolk accumulation in the sauropsidian egg, there is no trouble at all to suppose that the vesicular blastocyst of an early viviparous ancestor has gradually become yolk-laden. The contrary assumption, found in the handbooks, that the mammalian egg, while totally losing its yolk, has yet preserved the identical developmental features as the sauropsid, is, in reality, much more difficult to reconcile with sound evolutionary principles.

We have seen that a simple clue to our understanding of the complicated fœtal envelopes of the sauropsids and the mammalia is the assumption of a simple larval layer, one cell thick, among the invertebrate ancestors.

We must be ready to admit that this one factor has undoubtedly given rise to an endless number of variations and modifications in those innumerable families, genera, and species which have come and have gone, ever since the time when viviparity and terrestrial life became an established fact

in the vertebrate kingdom. What is preserved to us in the recent fauna inhabiting this planet is only the faintest echo of the multitudinous and protean changes that have, during the course of time, succeeded one another. And it has been our mistake to attempt to co-ordinate the present stages of development with each other in such a sense that they were expected to represent, in lineary arrangement, the successive evolutionary stages of those fœtal envelopes.

How false the conclusions may be to which this method may lead us is best exemplified by what is at present often taught concerning, e.g., placentation, a phenomenon in which the outer larval layer, the trophoblast, plays such a prominent part. You will find in the text-books that this was started by what is called the diffuse placentation as it is at present met with in many ungulates, in the lemurs, and in certain Edentates. It is my conviction that this doctrine is utterly false. The diffuse placentation is no placentation at all! The horse and the lemur are, by birthright, aplacental animals, much more so than marsupials, such as Perameles and Dasyurus, which have hitherto ranked among the Mammalia aplacentalia. And still, by careful comparison of various data, we can soon discover that the diffuse placentation, and that variety of it which is styled the polycotyledonary, far from being archaic or primitive, is, on the contrary, very largely a secondary modification. Among the living Carnivora we find several intermediate stages, not in the sense that these have been phylogenetic transitions, but in that wider sense that these Carnivora demonstrate the possibility how more intricate placentary structures may finally have led up to a diffuse placentation, as that of the horse and the pig, consequent upon an increase in the area of surface contact between mother and fœtus. What was originally a small surface of intense interchange (Procavia) has then gradually become an extended surface, along which two epithelial layers, one maternal and one fœtal, between the blood of the mother and the blood of the embryo, offered no impediment for a sufficient interchange of nutritive matter and of oxygen.

If we do not accept the starting-point in the placentation-process to be represented in the ungulate arrangement, a proposal which the systematic position of the Ungulata would in itself render doubtful, we must then look for another phylogenetic sequence which will help us to rightly interpret that momentous process of placentation. And here the important results of Hill's investigation of very intense placental phenomena in some marsupials, such as Perameles, have great weight.

We may fairly conclude that kangaroos, phalangers, opossums and other marsupials have only gradually become aplacentary, parallel to those other formidable changes which must have accompanied the elaboration of that peculiar type which we call our recent Didelphia, in which the dentition, the lactation, and those adaptations of the new-born animals for nutrition during their life inside the marsupium form such distinctive characters.

And so if the Didelphia are in reality erratic Monodelphia secondarily modified and with an allantois that has been thrown out of the line of its normal development, with the exception of Perameles, Dasyurus, and in part Phascolarctos, then we have again to look, not amongst them, but amongst the Monodelphia, for such forms that can give us an indication as to what may have been the primitive stage of placentation.

And I may here state that my own researches on the placentation of both primates and of insectivores have led me to the conclusion that we should look in quite another direction than the one alluded to above, which starts from diffuse placentation. In the earlier part of this address I have considered those early phylogenetic stages when, in viviparous, air-breathing tetrapods, the larval layer, the trophoblast, found the most diverse possibilities open to it.

I believe that those forms of which the embryonic trophoblast actually attacked the maternal uterine mucosa phagocytically were the pioneers towards the formation of what has later become the discoid placenta. In some forms, even among our recent mammals, that phagocytic attack is com-

bined with a penetration of the whole blastocyst inside the maternal tissue, e.g. man, anthropomorphæ, hedgehog, Gymnura, and many rodents. This was naturally a far higher position of vantage than any peculiar fixation inside the lumen of the uterus, for now, when once the blastocyst was encapsuled inside its mother's tissues, it could be most thoroughly bathed in maternal blood without any extravasation into the uterine lumen. To take three examples of this we may allude to the guinea-pig, the hedgehog, and man. Still, all these utilise the favourable conditions offered to them, thanks to their situation inside a capsula or decidua capsularis, in a very different manner.

There is a most remarkable amount of similarity between the hedgehog and man, as far as the conditions are concerned, which the mother offers to the young. But then the embryo itself of man has seen its way to much more intense utilisation of these favourable conditions than the hedgehog embryo has. Principally because the vascular system of the hedgehog develops in a sequence of stages, which serve to bring its area vasculosa on the umbilical vesicle in primary contact with the profusion of maternal blood by which the blastocyst is surrounded.

On the contrary, in man this area vasculosa on the umbilical vesicle is not in contact at all with the maternal circulation. In man it is more devoted to hæmatopoietic functions, i.e. to the formation of new blood-corpuscles for the embryonic circulation. But in another respect the human blastocyst has got far ahead of that of the hedgehog, in so far as the developing embryo has succeeded in vascularising its outer larval layer, its trophoblast, at a quite exceptionally early moment, without the aid of any allantoic outgrowth, and simply in consequence of a very early segregation of certain portions of the mesoblast, into which the entoderm sends both blood-vessels and blood-corpuscles. This very early vascularisation of the trophoblast leads to a most intense osmotic interchange between the blood of mother and child—far more intense that what obtains in the hedgehog, where an ompha-

loidean placentation precedes an allantoidean one, the allantois being a vesicular outgrowth, as it is in so many mammals and in all sauropsids.

I cannot refrain from looking upon the vascularisation of the outer larval layer or trophoblast, such as it occurs in man, in the monkeys, and in Tarsius, as the more primitive arrangement of the two. And in that case the presence of a connecting stalk (Haftstiel) and the absence of a free allantois in man, monkeys, and Tarsius is not a secondary simplication, but a primary fact of high importance. What is known as the allantois tube inside the so-called Haftstiel or Banchstiel of man, monkeys, and Tarsius, is not the remnant of what was once a vesicular allantois, but a remnant of that part of the entoderm which has served towards the vascularisation of the trohpoblast. It is this portion of the entodermal surface which will become the free allantois in those other descendants of the primitive tetrapods, which have not adhered to the very direct line of utilising most fully and as early as possible all favourable circumstances. This most direct line leads up straight to the primates. Less direct lines, in which conditions of different or of slower vascularisation have come to the foreground, are, however, represented in various orders of monodelphian mammals, and further in the Didelphia, the Ornithodelphia, and in the different subclasses of sauropsids. In the latter the allantois has grown to the dignity of a separate fœtal membrane, which co-operates to the further ensheathing of the developing embryo, and which carries the blood-vessels for respiratory purposes to the inner surface of the egg-shell, whereas, in the ancestral viviparous forms, the same vessels were more directly distributed over the inner surface of the outer embryonic larval layer, in order to improve the nutritory conditions which had been inaugurated by phagocytic action of the trophoblast cells on the maternal tissues.

This, then, is a short sketch and a rapid review of how the fœtal membranes of the vertebrates may be looked upon if we make certain changes in the interpretations that have

been hitherto adhered to, but by which latter nobody has as yet succeeded in clearing up the actual phylogenesis of these fœtal membranes.

Full and extensive investigations of all those numerous genera of mammals that have not yet been examined will, I hope, in due time give us occasion to complete or to modify the views here advocated.

It was a great pleasure to me to offer them, tentatively, in an address which I was invited to give in the section of embryology of this Seventh International Congress—a section which, with good right, has been called into life for the first time at this meeting in Boston. Embryological problems have been attacked by American investigators with wonderful results, and the lucidity of exposition that is characteristic of so many of your embryological workers is only equalled by the beautiful transparency of the eggs of those marine animals on which so many important researches on cell-lineage have been conducted.

That I have been less clear is not only a congenital defect, but is parallel with the utter hopelessness of our expecting that we shall ever be able to follow the cell-lineage in the deeply hidden and exceedingly small mammalian eggs. Still, a full knowledge of that very cell-lineage would be eminently decisive for many of the questions that have occupied us in the course of this address, to which you have listened with so much patience.

The Structure and Life-History of Crithidia melophagia (Flu), an Endo-parasite of the Sheep-Ked, Melophagus ovinus.

By
Annie Porter, B.Sc.Lond.,
Zoological Research Laboratory, University College, London.

With Plates 12 and 13 and 15 Text-figures.

Contents.

Introduction.

The part played by insects as agents in the transmission of the pathogenic organisms of sleeping sickness and other protozoal diseases gives great importance to the investigation of the parasites found within them. It is necessary for anyone seeking developmental stages of pathogenic flagellate Protozoa to have also a first-hand working knowledge of the possible flagellates that may be purely parasites of the insect involved, for certain stages of insect flagellates may resemble possible developmental phases of such organisms as Trypanosomes. Much useful information regarding stages of flagellates can be gained from the study of such a parasite as Crithidia melophagia (Flu), occurring in the alimentary tract, ovaries, and ova of the sheep-"ked," Melophagus ovinus. This insect, which is blood-sucking, is also known as the sheep-"tick" or sheep-"louse." It belongs really to the Diptera (Hippoboscidæ), possessing extremely reduced wings.

Crithidia melophagia (Flu) was recorded by E. Pfeiffer in 1905, but not named by him. The parasite is of peculiar interest, for I am able to bring forward evidence of a double mode of infection, both hereditary and casual. Swingle (1909) studied the flagellate stages and briefly described infection in the egg of Melophagus. Flu (1908) found parasites in the gut, ovaries, and larva, but was not clear as to the mode of infection (see p. 211).

Owing to conditions of environment it was impossible to conduct the whole of this investigation in a large city. Consequently the work has entailed travelling, and I have to thank many friends in agricultural centres for their kindly help.

Material and Methods.

Many specimens of Melophagus ovinus were examined during a long period of investigation, but owing to the effective operation of the dip laws in England there was

much difficulty in obtaining the "keds."[1] Indeed, it seems probable that the sheep-ked may soon become almost extinct in England. Those obtained came in very small numbers from many localities in the south of England, namely, Sussex, Hampshire, Kent, Middlesex, and Gloucestershire. I also received a number of keds from different parts of Scotland, but these never contained the Crithidia.

Many of the Melophagus, however, were infected by a fungus (see Appendix II). Where fungus was present Crithidia very rarely occurred. I shall show later, from experimental evidence, that this fungus was fatal to the Crithidia (p. 210).

Unlike Swingle (1909), who found that practically every Melophagus he examined in Nebraska was infected with Crithidia, I found that this was very far from being the case. Much depended on the locality from which the Melophagus was obtained. The more heavily infected individuals came from the southern districts of England. Often entire stocks of keds from one locality proved to be uninfected. Again, it was impossible to keep keds alive more than three days after their removal from the sheep.

Both young and adult Melophagus and many puparia in all stages of development were carefully examined. Raising puparia naturally upon a sheep was tried, but was not an easy matter, and as one could not be sure of having infected keds, there was always a percentage of uninfected puparia.

For observations of the living organism two methods of procedure were followed. The alimentary canal was isolated and divided into œsophageal, crop, stomach, intestinal and rectal portions, which were separated one from another. These were either teased with needles, mounted in 0·75 per cent. salt solution, and covered, the cover-slip being carefully vaselined, or the contents of the isolated portions of the gut were expelled by gentle pressure, and these only were examined, being mounted as before. Alkaline methylene

[1] In this paper I shall frequently use the term "ked" to denote Melophagus ovinus.

blue and neutral red were occasionally used as intra-vitam stains and were sometimes useful.

For fresh preparations used in work on hereditary infection, the ovaries and gut were dissected out very carefully, kept as far as possible relatively in situ, and mounted in 0·75 per cent. NaCl solution. The behaviour of the Crithidia visible through the walls of the gut and their action when they passed out from it were then most carefully watched.

I have attached very great importance to the study of the living organism in all its phases.

For making permanent preparations the alimentary tract of the Dipteran host was carefully removed and divided into portions as before. These isolated portions were usually teased very finely and fixed wet. Formalin vapour and osmic acid vapour were chiefly used for instantaneous fixation of the hanging-drop preparations, which were then spread. The preparations were subsequently treated with methyl or ethyl alcohol. Corrosive-acetic-alcohol (Schaudinn's fluid) and Bouin's fluid (slightly modified and containing a little alcohol) were also used for fixation.

Various stains were employed. Giemsa's stain gave some pretty results; thionin acted rapidly and well; iron-hæmatoxylin, carefully differentiated with iron-alum, was very serviceable; while gentian violet and Delafield's hæmatoxylin were of great use, particularly in obtaining details of the membrane and flagellum.

In the investigation of Crithidia melophagia, as in all other flagellates on which I have worked, I found that preparations mounted in neutral Canada balsam were superior to dry films or to films mounted in any other media.

Preparations of ovaries, eggs, and puparia were treated similarly. Special methods adopted are detailed in the section dealing with hereditary infection (p. 204).

Distribution of the Parasite in the Host.

The Crithidia parasitic in the alimentary canal of Melophagus are often mixed with the blood obtained by the ked

from the sheep. This blood from the sheep in the œsophagus, crop, and anterior part of the stomach of Melophagus is always fluid, and of an extremely bright red colour. That in the remaining part of the stomach is duller red but fluid, and in the intestine the blood, now semi-digested, is always darker in hue, sometimes brownish or greenish, while in the extreme rectum it is black. The enhanced red colour in the anterior portions of the alimentary canal has been shown experimentally to be associated apparently with the presence of an anti-coagulin in the digestive tract of the sheep-ked (see Appendix III).

Crithidia can be found throughout the length of the alimentary canal of Melophagus ovinus. In the anterior parts of the canal they are small, rounded, non-flagellated forms, which, when they come in contact with the blood, rapidly develop and divide, the products of division becoming the typical flagellates found throughout the rest of the canal. The parasites, after this rapid development, pass backwards towards the partly digested blood, which would appear to be a medium more suited to their requirements. In the posterior third of the stomach there are large numbers of young flagellates which form great aggregation rosettes (Pl. 12, fig. 43) and clumps, while true division rosettes are also present (Pl. 12, fig. 56).

In the intestine the same holds good. When many Crithidia are present in a ked, they usually swarm in the fore-part of the intestine. Repeated division occurs in the intestine, so that small flagellates are found in the rectum. Most of these attach themselves to the gut-wall or to débris and encyst, the resting (post-flagellate) stage of the parasite then being found on the walls of the rectum and in the fæces.

The ovaries and ova serve as places in which a kind of post-flagellate development occurs, the ova being penetrated by flagellate forms of Crithidia, which rapidly lose their flagella and ultimately round themselves off, and pass through a resting stage (Pl. 13, figs. 57–94).

The Malpighian tubules of Melophagus ovinus are sometimes invaded by Crithidia melophagia, but this is not common.

Parasites were more numerous in female than in male keds.

Repeated investigation of sheep's blood failed to show the presence of any flagellate therein. Flu and Swingle obtained similar results. C. melophagia is, then, purely a parasite of Melophagus ovinus.

Movements.

The movements of C. melophagia are very vigorous. The parasites are even more active than C. gerridis (see Porter [1909], p. 352). As in C. gerridis, the membrane takes an important share in locomotion, but the movements of the body of C. melophagia are not so noticeable as in the parasite of the water-bug.

When C. melophagia was examined under the water immersion (2·5 mm.) objective, the movements of the less active organisms could be analysed. In progression the organism moves with its flagellum foremost, and the latter executes vigorous, slightly spiral, boring movements. The body also aids in progression, for waves pass from the posterior end towards the flagellum, causing a series of peristaltic-like swellings. The body of the parasite seems to become shorter during this period, and then by relaxing to move forwards. The bead-like swellings due to undulatory movements are more noticeable in certain areas, and in the living organism myonemes could be sometimes seen both on the body and in the membrane in these regions. Flu has also figured myonemes on some of the parasites he drew, and observation of them in life confirms his work, but it was with the greatest difficulty that I could find myonemes in stained specimens (Pl. 12, figs. 17, 18, 40, 42, 45).

The body of C. melophagia, compared with that of C. gerridis, is relatively rigid, but slight twisting movements do occur. The previous workers on C. melophagia are agreed

as to this rigidity. The anterior end, to which the flagellum and undulating membrane is attached, is naturally more flexible than the posterior end, and its movements are more marked.

Movements of contraction of the posterior end of the body of C. melophagia result in a temporary concentration of the protoplasm around the nucleus of the organism. The body then resembles a short, thick pear, drawn out at its anterior end into a long, narrow stalk. Sometimes the body remains in this condition, which is fairly common in forms about to encyst, and in such forms withdrawal or degeneration of the flagellum, followed by the secretion of a thin gelatinous wall, completes the encystment. In other parasites from the stomach, where no encystment occurs, this concentration of the protoplasm in the nuclear region is not so marked, and when relaxation occurs the organism is propelled forward with a very slight jerk, and repetition of the contraction follows, as has been before described. The jerking is never so marked as in Herpetomonas, for the membrane has the effect of producing smoothness of motion.

Reversal of the direction of motion occurs and is very rapid. The flagellum swings out, describing a semi-circle, of which the body acts as the diameter for an instant, but the force of the movement of the flagellum is so great that the body also swings outwards in a line with the flagellum, and the organism moves away, not exactly in the same course as before, but in one at a very small angle to it. The path of the organism is frequently parabolic in nature.

Many peculiar movements can be observed when C. melophagia is endeavouring to free itself from débris in the lumen of the gut. Much writhing, both of the flagellum and body of such a parasite, is then seen, and the organism often swings round and round, the point of attachment serving as the centre of rotation. If the posterior end should be attached, the flagellum executes violent lashings and spiral movements, these latter not being, as a rule, very noticeable in the normal organism.

Occasionally I have seen the flagellum and membrane of specimens of C. melophagia torn away from the body, and for a few seconds after, the flagellum executed intermittent flickers or lashing movements before it finally became still.

Aggregation-rosettes (Pl. 12, figs. 41, 43; Pl. 13, figs. 95, 96) are common in C. melophagia. Rosettes seem to move fairly as a whole, and I have watched them rotate rather quickly. Each individual of such a rosette is attached by its flagellum to débris, usually epithelial in nature, and moves up and down in a slightly inclined plane.

In division the movements of the daughter organisms are very noticeable. I will defer the description of their motion until division is discussed.

During encystment in the rectum of the host, which occurs with some of the parasites, movement of the nucleus towards the flagellar end of the organism occurred. I have also seen the migration of the nucleus from the mid-region of the body to near the flagellum during periods of violent movement of the latter organella. I have never seen migration of the blepharoplast in living organisms under similar conditions, though it may occur at times, since blepharoplasts can occasionally be found in the post-nuclear region (Pl. 12, figs. 40, 42), as well as by the side of the nucleus (Pl. 12, fig. 33) in different stained specimens. By far the commonest position for the blepharoplast is the pre-nuclear one. The other movements occurring during encystment will be described in the section of the paper dealing with that subject (see p. 200 and text-figures 1–10).

Morphology.

The life-cycle of Crithidia melophagia may be conveniently divided into three stages, which gradually merge into one another. They are—the pre-flagellate, flagellate, and post-flagellate stages. The morphology of these forms may now be described.

The Pre-flagellate Stage.

The early pre-flagellate stages of C. melophagia are more or less oval or rounded bodies (Pl. 12, figs. 1–6), varying from 4·5 μ to 6 μ long, and from 1 μ to 4·5 μ broad. They are most abundant in the fore-gut of young Melophagus, but the pre-flagellate stage is passed through with great rapidity and is easily missed. This probably accounts for the very brief references to these small forms by Flu and Swingle.

The protoplasm of the pre-flagellate forms is very finely granular (Pl. 12, figs. 1–5). The nucleus is usually round and not quite central in position (Pl. 12, figs. 1, 9–12). The bar-like blepharoplast (kinetonucleus) is very deeply staining, and lies either below (Pl. 12, figs. 2, 10) or to one side of the nucleus (Pl. 12, figs. 1, 6). A chromatophile area with its chromatin in a very diffuse condition is sometimes fairly prominent, and from this a fine thread arises, which grows outwards, forming the flagellum (Pl. 12, figs. 9, 10), and appearing to draw out the end of the body with it (Pl. 12, figs. 11–13), while the periplast of the body forms the membrane (Pl. 12, figs. 14–20). The posterior end elongates at the same time (figs. 16–18) and the flagellate form (Pl. 12, figs. 19, 20) is assumed. This development is in accord with that of C. gerridis and C. tabani, and I have watched these processes in living specimens of both C. gerridis and C. melophagia.

Division of pre-flagellate forms can occur before the development of the flagella (Pl. 12, figs. 3, 4). This will be described in the section dealing with division.

The Flagellate Stage.

The mature flagellates vary very much in size, the variation being due to division and growth. Very large forms (Pl. 12, figs. 44, 45) may be as much as 50 μ to 75 μ long, this measurement including the flagellum,[1] while short forms just flagel-

[1] It is almost impossible to differentiate between the limiting areas of the body, the membrane and the free flagellum of C. melophagia, as so much variation occurs in different specimens.

lated (Pl. 12, figs. 18, 19) in the crop, or the small forms produced by division prior to encystment (Pl. 12, figs. 20, 21; 99) are very much smaller (12 μ to 20 μ long). The breadth of the flagellates varies from 1·5 μ to 2·8 μ.

The protoplasm of C. melophagia is very slightly alveolar or almost hyaline, differing therein from the more alveolar protoplasm of C. gerridis. There is no suggestion of large permanent vacuoles or of a cyto-pharynx. Occasionally the protoplasm is more granular at the posterior end (Pl. 12, figs. 30, 34) and slight alveolation occurs there. At the anterior end, near the origin of the flagellum, the remains of the chromatic area, from which the flagellum arose, sometimes persist.

The nucleus (trophonucleus) of C. melophagia is oval (Pl. 12, figs. 21–24) or rounded (figs. 26, 30, 32) and somewhat vesicular. There is a fair amount of chromatin present, which may consist of a number of very fine granules, evenly distributed (fig. 32), or the chromatin may be concentrated into about eight masses (fig. 44), or, as is often the case, the chromatin is present in the form of bars (figs. 25–29), which sometimes extend across the whole breadth of the nucleus (figs. 34–37), less frequently across part of its breadth (figs. 24, 42), or in an even more rare condition dots and bars occur in the nucleus of the same organism (figs. 30, 39). In certain cases the chromatin of the nucleus may be concentrated into a central mass (fig. 23).

The nuclear membrane is fairly distinct in most of the specimens I have examined. I think that such a membrane must be present to keep together the nuclear material during the migrations of the nucleus seen during life.

The blepharoplast (kinetonucleus) of C. melophagia is very evident in a stained preparation, for it colours deeply whatever stain be employed. Like the nucleus, it can also be seen in life as a small bright refractile bar. In some cases it is slightly bowed or curved (Pl. 12, fig. 32), or oval (Pl. 12, fig. 34). It is dumb-bell-shaped in forms about to divide (Pl. 12, fig. 44). The blepharoplast, which is typically

rod-like, usually lies transversely across the organism (Pl. 12, figs. 21–28). It is exceptional to find it in any position other than anterior to the nucleus, though on a few occasions the blepharoplast was at the posterior end of the body (Pl. 12, figs. 40, 42), but in these cases the flagellum originated in a pre-nuclear position.

As a rule the blepharoplast shows no differentiation of structure (Pl. 12, figs. 21–39), but sometimes in dividing forms, in which the blepharoplast is dumb-bell shaped, there seems to be a concentration of chromatin in the ends of the dumb-bell (Pl. 12, figs. 40, 44, 45). A clear area (Pl. 12, fig. 31) is often present around the blepharoplast.

Chromidia are present, scattered in the general protoplasm (Pl. 12, figs. 25, 37, 39). They stain in the same way as the nucleus, and less densely than the blepharoplast. The occurrence of such chromatoid granules at division (Pl. 12, fig. 45) suggests that they have been given off from the nucleus into the general protoplasm, and exercise some controlling influence over the same.

The undulating membrane and the flagellum.—The flagellum originates from a chromatic area in the pre-flagellate form, and is attached to the body by a narrow membrane (Pl. 12, figs. 21–46), which is a periplastic outgrowth of the anterior end of the body. There is but one flagellum in any single, undividing individual (Pl. 12, figs. 21–39). The flagellum is thick, but gets thinner towards its free end (Pl. 12, figs. 40, 45). At times it appears to show very fine transverse striations.

In stained specimens the membrane sometimes shows myonemes (Pl. 12, figs. 39, 42, 45), though, curiously enough, the myonemes were much more obvious in some of the living specimens that I examined. Flu described myonemes in C. melophagia, but figured the myonemes as accompanying a central spindle. This latter feature I have never seen.

A basal granule (blepharoplast of Minchin) is often present (Pl. 12, figs. 17, 27, 33, 42, 45) between the point of origin of the flagellum and the blepharoplast (kinetonucleus).

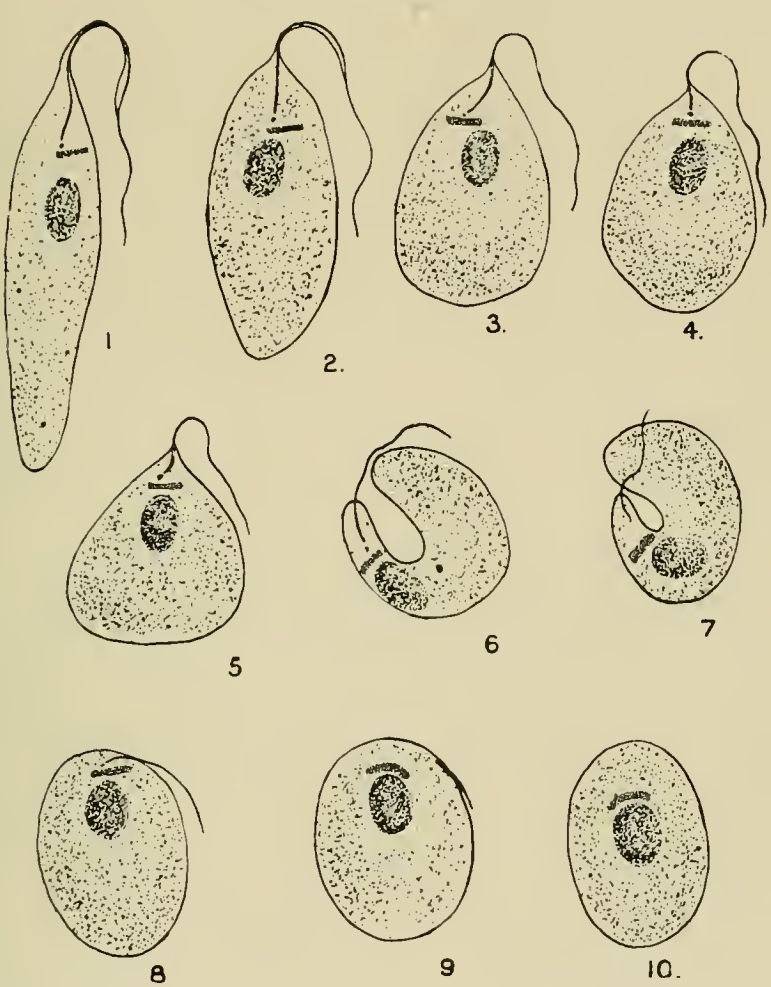
The Post-flagellate Stage of C. melophagia in th Rectum of Melophagus ovinus.

The preparation of Crithidia melophagia for life outside the body of the host occurs in the rectum of the sheep-ked. Large numbers of small flagellates (Pl. 12, figs. 27–29) are present in the hind gut, also some forms in process of division (Pl. 12, figs. 97, 98). The small forms attach themselves to the wall of the rectum and encyst there, but encystment can be watched when the rectal contents are expressed on to a slide and examined under the microscope. The flagellate (text-fig. 1) at first executes violent lashing movements with its flagellum, and during this motion migration of the nucleus nearer the flagellar end of the organism frequently occurs (text-fig. 2). At the same time the body of the Crithidia shortens and thickens (text-figs. 3, 4; Pl. 13, fig. 100), waves of contraction passing rhythmically down the body, which gradually may become somewhat triangular (text-fig. 5; Pl. 13, fig. 101). The flagellum meanwhile shortens (text-figs. 5, 6), and the organism may bend on itself (text-figs. 6, 7) during this period. Concentration of the protoplasm occurs, the flagellum becomes less wavy (text-fig. 7), and, little by little, it contracts nearer the body (text-figs. 8, 9; Pl. 13, figs. 102–106) and is withdrawn, the parasite becoming oval (text-fig. 10; Pl. 13, figs. 109–112). The organism at this time becomes surrounded by a thin layer of refractile, gelatinous substance, which rapidly hardens to form a closely adherent resistant cyst-wall. The oval bodies (Pl. 13, figs. 109–114) so produced are post-flagellate forms, which become detached from the walls of the rectum, and pass out with the fæces of the ked, from which fæces they can be recovered. These cysts, which measure from 2·5 μ to 5·5 μ by 1·5 μ to 3 μ, serve for the infection of other Melophagus ovinus.

All Crithidia melophagia do not go through a post-flagellate stage in the gut of their host. Some, after passing a portion of their existence as flagellates in the gut of the ked,

pierce the walls of the alimentary tract and make their way to the ovaries of the ked, where their development is continued.

TEXT-FIGURES 1–10.



Encystment of Crithidia melophagia in the rectum.

Text-figs. 1–5.—Parasite rounding off and flagellum disappearing.
Text-figs. 6–7.—Show bending of parasite on itself.
Text-figs. 8–10.—Final stages in loss of flagellum and assumption of typical cyst form.

Swingle (1909, p. 104) has described thick-walled cysts. I have but rarely seen the thick-walled forms (Pl. 13, fig. 114), most of the cysts found being thin-walled.

Longitudinal Division.

The longitudinal division of the living organism has been frequently watched. While the movements of the dividing flagellates are noticeable, those of the smaller dividing pre-flagellates are far less marked.

When a flagellate is about to divide, the protoplasm of the posterior end concentrates somewhat in the nuclear region, and the organism appears to shorten. The protoplasm migrates from the centre of the parasite towards the sides, so that a comparatively clear area is left at the centre (Pl. 12, fig. 46). The greatest change at this stage is seen in the blepharoplast and flagellum. The blepharoplast becomes slightly dumb-bell-shaped (Pl. 12, figs. 44, 45) and gradually constricts into two (Pl. 12, fig. 46). The flagellum splits rapidly at the body end (Pl. 12, fig. 46), and then, more slowly, the halves become free. The nucleus meanwhile becomes slightly indented in the median line (Pl. 12, fig. 46) and then gradually constricts into two, the halves migrating to the periphery (Pl. 12, fig. 47). During this nuclear division the daughter-flagella execute very vigorous lashing movements, and a constriction appears at the flagellar end of the parent organism. A split appears at this end (Pl. 12, figs. 47–49), and, at the same time, vacuoles in the clear median area fuse, and thus the extension of the split is facilitated. The daughter-organisms rapidly separate from one another, their appearance at times being suggestive of diverging curved calipers (Pl. 12, figs. 51, 52). At length the two are practically in a straight line (Pl. 12, figs. 53–55), in which condition they remain for a short time and then finally separate.

The division of the pre-flagellate forms is initiated by the division of the blepharoplast, and is followed by the division of the nucleus and the appearance of vacuoles. A slight split appears at one end (Pl. 12, fig. 3), and the organism remains in this condition until the flagellum of each half has partly grown, when final separation is effected by their movements.

Sometimes repeated division of a pre-flagellate form occurs

and a rosette (Pl. 12, fig. 4) is produced, but the rapidity of the process of formation of flagella causes short duration of the rosette stage. On the other hand, repeated longitudinal division of flagellated individuals occurs, and as the individuals so produced do not separate immediately, rosettes (Pl. 12, fig. 56) are formed. In division, the posterior ends of the daughter-organisms are the last parts to separate. As the daughter-forms remain in proximity and themselves proceed to divide with rapidity, true division-rosettes are formed, in which the posterior ends of the organisms are central, while the flagella radiate out from the common centre. Such division-rosettes (Pl. 12, fig. 56) differ from the aggregation-rosettes (Pl. 12, figs. 41, 43; Pl. 13, figs. 95, 96) where the organisms become attached by their flagella. The distinction between the two forms of rosettes has not been shown by previous workers on C. melophagia.

Longitudinal division results in the formation of both equal and sub-equal daughter forms.

While the occurrence of equal longitudinal fission is the commoner (Pl. 12, figs. 50, 54, 55), I have seen cases of marked inequality in the size of the daughter-parasites, the one being very thin and narrow, the other considerably broader and thicker (Pl. 12, figs. 51, 53). As the entire process of sub-equal division has been watched in living organisms, there is no possibility of it being mistaken for anything else. The polymorphism resultant on division is strongly against the idea that there are sexual forms of Crithidia, and I have never seen the slightest indication that there is sexual dimorphism, in C. melophagia, C. gerridis, Herpetomonas jaculum, H. muscæ domesticæ, H. culicis, and a new Herpetomonas from Vespa crabro, all of which I have examined in the living condition (see Porter [1909] on C. gerridis and H. jaculum).

Division, usually twice repeated, is found to occur in parasites destined to encyst, and the resultant forms are very small. The first division is of the usual flagellate type (Pl. 13, fig. 97). The process of the second division rather

resembles that of the pre-flagellate stages, for before it is accomplished the flagella have almost disappeared. Sometimes no flagellum is visible at all, and the parasites look like dividing cysts.

On rare occasions the posterior end of a flagellate has divided before the anterior end (Pl. 13, fig. 98).

The Hereditary Infection of Melophagus ovinus by Crithidia melophagia.

Casual infection of Melophagus ovinus by the ingestion of post-flagellate cysts of Crithidia melophagia is fairly easily observed. The development of the parasite in the egg can only be studied with difficulty. I now wish to give a fuller account than exists up to the present of the processes leading up to the birth of Melophagus infected with Crithidia melophagia.

The first point to be determined was the way in which the Crithidia reached the egg. Infected Melophagus were carefully dissected so that no rupture of the gut was made. The ovaries also were dissected out and kept as far as possible in the position beside the gut that they occupied in life. Crithidia could be seen through the gut-wall moving actively about. Suddenly they concentrated in one place and soon began to pass through the wall, their posterior (blunt) end first. They rapidly swam direct to the ovaries and penetrated them in the same way, that is, with the non-flagellar end first. The flagellum was very rarely used as a boring organ to allow of the passage of the organism.

Penetration of the ovaries of their host by the parasites occurs in other cases, e. g. C. gerridis, H. jaculum, but the ova are apparently unattacked and the flagellates simply degenerate. But in the case of C. melophagia the organisms (Pl. 13, figs. 57, 59) make their way rapidly to the ova, to which they cling, whether the ova are mature or immature. In some cases one Crithidia only enters the egg (Pl. 13, fig. 58); at other times several penetrate it at once. In

penetration the blunt end of the flagellate enters the egg first. Occasionally the flagella are cast off as the Crithidia pass into the egg and remain on the outside.

In the case of older ova, the parasites seem to penetrate the egg at a definite spot (Pl. 13, fig. 58), which probably becomes the mouth of the embryo. Parasites invading older embryos enter by the embryonic mouth. Like Swingle I did not find parasites in the milk-glands or milk of Melophagus.

In investigations of the stages of C. melophagia in the egg and puparia I found that smear preparations were preferable to sections. Greater rapidity of manipulation and thinner preparations could be obtained by this means.

The method adopted was to prick the egg or open the young puparium and express the contents on to a slide. The contents were at once fixed and then were allowed to flow over the slide, so that no artificial spreading was required, and therefore no mechanical distortion or tearing of the parasites could occur. The preparations so made contained much fatty matter. The slides were treated with ether to remove the fat, and then after washing with absolute alcohol were stained and mounted in the usual manner.

Once within the egg the parasite gradually loses its flagellum (Pl. 13, figs. 61–63). This may be cast off entire, for flagella are found floating freely in the vitellus of eggs that had been treated with the utmost care in the manner previously detailed. In many cases the flagellum appears to be gradually absorbed (Pl. 13, figs. 64, 66). Longitudinal division of the flagellates in the egg may occur, though rarely.

The protoplasm of the Crithidia then concentrates round the nucleus and blepharoplast (Pl. 13, figs. 64–69) and the parasite gradually becomes more or less rounded (Pl. 13, figs. 70–73). Multiple division of both nucleus and blepharoplast then occurs (Pl. 13, figs. 74–77), and the daughter-blepharoplasts appear to pass outwards towards the periphery (Pl. 13,

figs. 76, 77). A "plasmodial"[1] form (Pl. 13, figs. 75, 77) is thus assumed. The protoplasm collects around the nuclei, and gradually fragmentation of the "plasmodium" occurs, the result being the formation of a number of small bodies, which rapidly round off, forming definite resting bodies (Pl. 13, figs. 78–81). Sometimes these resting bodies remain in proximity to one another, so forming groups (Pl. 13, figs. 80, 81). The parasites now measure only 1·5 μ to 4 μ long and 1 μ to 2·5 μ broad. Sometimes one chromatic mass (Pl. 13, fig. 82) only can be distinguished. Often both nucleus and blepharoplast (Pl. 13, figs. 80, 81, 83, 84) are present.

As the embryo grows the rounded forms of the parasite in the stomach (which is the chief cavity within the young Melophagus) also grow (Pl. 13, figs. 82–84). The Crithidia then undergo multiple division, small rosettes (Pl. 13, figs. 85–88), analogous to pre-flagellate rosettes, being produced. The division clusters may separate, giving rise to small, pear-shaped or ovoid individuals (Pl. 13, figs. 89–94), or they may remain as a rosette (Pl. 13, fig. 88) for some time. Whether the Crithidia remain as groups or become isolated as oval non-flagellated bodies, they undergo no further development for a considerable period. In fact, when the young Melophagus is hatched, a month after extrusion of the puparium, there is still no further differentiation in the parasite.

Freshly hatched Melophagus do not contain the fully developed flagellates, but the rounded or pear-shaped pre-flagellate forms (Pl. 13, figs. 92–94) and rosettes (Pl. 13, fig. 88) may be present. The parasites appear to lie dormant for a day or two, during which time the young insect does not appear to suck blood. Soon after the first meal of blood is taken, rapid development of the pre-flagellate forms occurs, and the adult flagellate form of the Crithidia is quickly assumed.

[1] A plasmodium is really a multinucleate mass of protoplasm formed by fusion of small amœbæ. However, the term is sometimes used, as in describing certain Haplosporidia, for a multinucleate mass of protoplasm formed by division.

Casual Infection.

The method of cross-infection in many species of Crithidia has not been demonstrated, but in the cases known the casual or contaminative method seems to prevail. The post-flagellate stages of Crithidia gerridis and C. tabani are known, and the cysts of these parasites are shed in the fæces of the insectan hosts. The crithidian cysts are swallowed by new hosts when they feed on material accidentally contaminated by the fæces of their neighbours. The cysts then develop in the alimentary tracts of the new hosts. Melophagus ovinus also becomes infected with its Crithidia by the casual method.

When studying C. melophagia I have noticed that the fæces of Melophagus ovinus are voided near spots on the sheep from which blood has recently been sucked (particularly is this the case at times of extrusion of puparia); that the fæces contain crithidian, post-flagellate cysts, and sometimes active flagellates; and that other Melophagus, feeding at the same spot, have thrust their proboscides into the semi-fluid fæces to reach the blood of the sheep. Ingestion of cysts under such circumstances is easy. The ingestion of fæces has been seen particularly well when a number of keds have been kept confined to a small area of the sheep's body.

At shearing a slight injury was caused to one sheep, and the keds seemed to collect round the small bleeding patch. Their habits were carefully observed then, and were similar to those described above. I do not agree with Swingle that casual infection is only a remote possibility; to my mind it is a certainty.

A modified contaminative cross-infection is rendered possible by the cannibalistic habit of Melophagus ovinus. The keds have been seen to attack one another, the point of seizure invariably being at the end of the abdomen near the anus. When a ked so attacked has been freed from its aggressor and then dissected, I have found that the abdominal cavity was almost empty, the viscera having been devoured

by the attacking ked. By this cannibalistic habit it is possible for Melophagus ovinus to acquire practically every stage of Crithidia melophagia direct, and this is probably a subsidiary method of spreading the parasite.

Environmental Effects.

Crithidia melophagia shows less response to slight changes of environment than does C. gerridis or Herpetomonas jaculum, both of which I have studied. Nevertheless, under certain conditions remarkable effects have been produced by relatively simple means, and these may now be recorded.

(1) Response to light.—Increased intensity of white light produces increased velocity of movement of Crithidia melophagia.

Green light somewhat retards the movements of the organism. This is also the case with Herpetomonas jaculum.

Intense light causes aggregation-rosettes of C. melophagia to separate.

C. melophagia lives very much longer in diffuse light than in bright light.

(2) Response to changes of temperature.—C. melophagia can live at a temperature just below that of the blood of the sheep, but the flagellates are killed at a temperature above 40° C.

At room temperature (15° C.) the parasites will live for several hours.

(3) Response to change of medium.—Though the flagellates normally live surrounded by fluid blood (a discussion of which will be given in Appendix III), yet they can live in other media and can resist the effects of such media to varying degrees.

(*a*) Tap-water when added to the parasites in the gut-liquid seemed to have little effect. Though the movements of the flagellate became slightly more active, this was possibly

due to the greater space in which the parasites could move, the débris being distributed over a greater area than before.

(*b*) 0·75 per cent. NaCl solution increased the activity of the parasites.

Five parts of tap-water added to one part of 0·75 per cent. NaCl solution containing Crithidia caused the flagellates to move more rapidly, the spiral boring movements of the flagellum becoming more exaggerated.

(*c*) Caustic potash.—Two per cent. solution killed all the Crithidia within a minute; 1 per cent. potash solution killed them in from seven to twelve minutes, but their bodies were not dissolved, this pointing to the chitinoid nature of the thin periplast or ectoplasm.

(*d*) Acetic acid.—One third per cent. aqueous solution had the effect of swelling the parasites, which then died.

(*e*) Grape-sugar.—A most remarkable effect was that produced on C. melophagia by a solution containing a very small amount of grape-sugar. When this was added to the parasites they commenced to divide very rapidly, and many divisions occurred. To ascertain if there were a connection between this division and the occurrence of sugar in the natural medium of the parasites, some experiments were made. The results were as follows:

(i) Sheep-serum contains a very small amount of grape-sugar.

(ii) The liquid obtained when wool cut from the sheep was boiled with water and then concentrated also showed traces of sugar. There were, then, these two sources from which the ked probably could obtain minute quantities of sugar. It is possible that the traces of sugar may take a small share in stimulating division of C. melophagia, which goes on more rapidly in the stomach of the ked than elsewhere.

(*f*) Fresh blood (human or sheep's) added to a preparation of living Crithidia caused the parasites to move away to areas where the blood was somewhat less concentrated, where they proceeded to divide rapidly.

(*g*) Dilute glycerine killed C. melophagia almost at once. Vaseline had the same effect after a very short time.

(4) Effect of a parasitic fungus of Melophagus ovinus on C. melophagia.—The presence of a fungus in Melophagus ovinus has already been mentioned. As I very rarely found the fungus and Crithidia co-existing in a ked, it was deemed advisable to find out any possible interrelation of the two parasites. The Malpighian tubules of the ked—often blocked with fungus—were the most heavily infected organs. Fungus taken from the Malpighian tubes was crushed with a little water. The emulsion, which probably contained an enzyme, was added to a preparation of actively moving C. melophagia. The movements of the flagellates slowed at once, their protoplasm became much more vacuolated, and the parasites appeared to burst. After seven to nine minutes no living Crithidia were to be seen.

The fungus-infected Melophagus ovinus seems widely distributed. Specimens from Scotland were practically always heavily infected with it, and some keds from each locality tried in England also were infected. These keds very rarely contained Crithidia. The fungus seems to have a pathogenic action upon the flagellate, and I believe that the co-existence of the fungus and Crithidia for long together is almost impossible.

General Remarks.

Regarding the previous work done on the genus Crithidia, I have already noted most of the memoirs dealing with the subject in my paper on Crithidia gerridis (1909). Consequently the remarks now appended relate especially to the flagellate of Melophagus ovinus.

E. Pfeiffer (1905) first briefly described a flagellate as occurring in the gut of Melophagus ovinus. He mentions that L. Pfeiffer had seen and recorded the parasite in 1895. The flagellate stage only was described, and no definite name was given to the organism, which was stated to be "like a trypanosome."

P. C. Flu (1908) published an account of the flagellate under the name of Crithidia melophagia. Flu stated that he saw parasites in the ovary of Melophagus, and small forms in the larva, but was unable to determine the mode of infection of the host.

L. D. Swingle (1909), working in Nebraska, wrote a description of the parasite, calling it C. melophagi. From a private communication I learn that Swingle's work was completed, but not published, before Flu's paper appeared, thus accounting for the specific name melophagi (described as new), which cannot stand. The chief value of Swingle's work lies in the fact that he described rounded and "plasmodial" stages of the parasite as occurring in the egg of the host.

While Swingle was working in Nebraska, I was investigating the parasite independently in England. It gives me great pleasure to be able to confirm Swingle's work, and to add many more details concerning the modes of infection of the parasite and its general life-history.

Summary.

(1) Crithidia melophagia is a flagellate occurring in the alimentary tract, ovaries, ova, and puparia of Melophagus ovinus.

(2) The parasite has three stages in its existence, a pre-flagellate stage (Pl. 12, figs. 1–20), passed chiefly in the crop and fore-gut of the insect host, a flagellate stage (Pl. 12, figs. 21–44), occurring chiefly in the posterior two thirds of the gut, and a post-flagellate stage, occurring either in the rectum and fæces (Pl. 13, figs. 97–114) or in the ova and pupæ (Pl. 13, figs. 57–94).

(3) The pre-flagellate stage is passed through very rapidly. These parasites are small, usually oval bodies, 1μ to 4·5 μ by 4·5 μ to 6 μ, with round nuclei and bar-like blepharoplasts. The flagellum arises near the blepharoplast from a chromatophile area. Division of pre-flagellates may occur (Pl. 12, fig. 4).

(4) The flagellate forms are from 12 μ to 75 μ long, and 1·5 μ to 2·8 μ broad (including the flagellum). The general protoplasm is slightly alveolar. The nucleus is vesicular. The blepharoplast is well marked, rod-like, usually anterior to the nucleus, and generally homogeneous.

Chromidia may occur as isolated granules.

(5) The undulating membrane and flagellum are well marked. There are indications of myonemes (Pl. 12, figs. 40, 45) in some stained specimens, but the myonemes are more evident in some living specimens. The membrane is of great use in securing smoothness of motion. The flagellum is long and forms a chromatic edge to the membrane. A basal granule may occur near the root of the flagellum.

(6) The post-flagellate stage in the host's rectum (Pl. 13, figs. 97–114) gives rise to resistant (resting) bodies that are passed out as cysts with the fæces and serve to infect new hosts. The cysts measure, on the average, 4 μ by 2·5 μ. The flagellates divide, usually twice, and the four small forms thus produced lose their flagella, become round, and then invested with a thin gelatinous wall.

(7) The post-flagellate stages in the ova and puparia of Melophagus (Pl. 13, figs. 57–94) serve for the hereditary transmission of C. melophagia. The flagellates pass through the wall of the gut near the anterior ends of the ovaries, swarm towards and enter the ovaries and penetrate the ova—the posterior (aflagellar) end of the parasite being used in penetration. Within the ova each parasite loses its flagellum and becomes ovoid or rounded (Pl. 13, figs. 64–73). Nuclear multiplication follows and "plasmodial" forms are produced (Pl. 13, figs. 74–77). These give rise to small, rounded bodies (Pl. 13, figs. 83, 84) about 3 μ by 2 μ which undergo multiple fission to form rosettes (Pl. 13, fig. 88), which give rise to the typical pre-flagellates.

(8) The young Melophagus do not show flagellates until after their first feed of blood, the blood stimulating the pre-flagellates to form flagella.

(9) Multiplication of C. melophagia by longitudinal

division occurs in both the pre-flagellate and the flagellate stages of the parasite.

(10) Infection of Melophagus ovinus with C. melophagia is either hereditary or casual. In the case of casual infection the insects ingest the post-flagellates voided with the fæces of other Melophagus.

(11) A very dilute solution of grape-sugar causes the parasites to divide. There are only traces of sugar in both sheep-serum and wool extract.

(12) Sheep's blood or human blood added to the Crithidia also increased the rapidity of their growth and division.

(13) A fungus present in the Malpighian tubules and gut of the ked (see Appendix II) has a rapid, fatal effect on the Crithidia.

(14) An anti-coagulin is present in the salivary glands, stomach and intestine of Melophagus ovinus (see Appendix III).

(15) A new spirochæte, S. melophagi, was found in the gut, ovaries and puparia of a few of the Melophagus examined (see Appendix I).

Appendix I.

On the Occurrence of a Spirochæte, S. melophagi, n. sp., in Melophagus ovinus.

I wish to record the occurrence of a rare spirochæte in the gut, ovaries and puparia of Melophagus ovinus. The spirochæte was observed in life in the above-mentioned organs of a very few of the Melophagus examined, and at very different periods of the year (February, September, October). Very few spirochætes occurred, and consequently it is impossible to give full details regarding size and structure. The organisms seen were from 10 μ to 30 μ long and were narrow. They vary in length, some being practically half the length of others, indicating the probability of transverse division. As some parasites were thicker than others there

is the inference that longitudinal division takes place. This would be in accordance with the behaviour of other spirochætes, for Fantham (1907–8–9) has shown that both forms of division occur in S. balbianii and S. anodontæ. I (1909) also have observed the same, while the joint researches of Fantham and myself (1909) have demonstrated that both directions of division occur in S. recurrentis and S. duttoni, and that there is a periodicity in the direction of division.

The movements of S. melophagi are fairly active, and are of the typical spirochæte nature, namely, of forward progression accompanied by spiral or corkscrew rotation on its course.

The occurrence of S. melophagi in the ovaries, ova and puparia of the ked is of much interest, for it indicates that the spirochæte is transmitted hereditarily. Hence Melophagus ovinus can transmit both Crithidia melophagia and Spirochæta melophagi to its offspring.

Appendix II.

Note on a Fungus found in the Malpighian Tubules and Intestine of Melophagus ovinus.

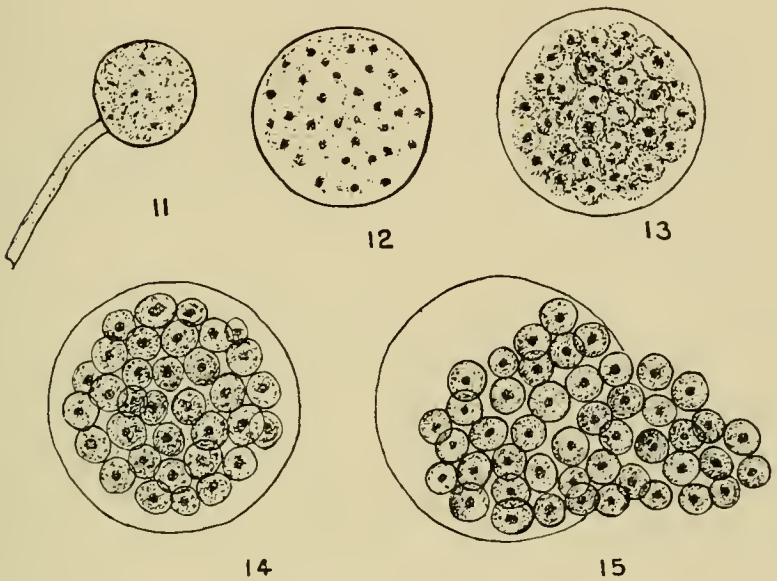
A fungus was present in many specimens of Melophagus ovinus examined, especially those obtained from Scotland. Crithidia were not seen in the "keds" received from Scotland, and I have shown experimentally that the action of the fungus is fatal to the flagellate.

The fungus occurred chiefly in the Malpighian tubules of the insect, and to a lesser extent in the intestine. The Malpighian tubules were frequently blocked by the fungus. A brief description of the fungus may now be given.

The hyphæ were long and filamentous with few septa. Many spores were produced. At the extremity of some hyphæ globular heads were formed, possibly due to sexual processes. The globular bodies contained many nuclei (text-fig. 11) fairly evenly distributed through the protoplasm.

Nuclei and protoplasm then shrank away from the wall of the rounded body—provisionally called a sporangium (text-fig. 12)—so that a space intervened. Segregation of the protoplasm round the nuclei followed (text-fig. 13), and a morula-like body resulted. The morula differentiated into a mass of rounded spores (text-fig. 14), each of which formed a spore coat for itself. The sporangium ultimately ruptured

TEXT-FIGURES 11–15.



Fungus parasitic in Melophagus ovinus.

Text-fig. 11.—Hypha with globular head.
Text-fig. 12.—Differentiation of nuclei within the head (sporangium).
Text-fig. 13.—Spores forming in sporangium.
Text-fig. 14.—Mature sporangium.
Text-fig. 15.—Dehiscing sporangium.

(text-fig. 15), and the numerous small spores were set free. Some spores remained in the Malpighian tubes, others passed out into the intestine and were voided with the fæces.

Parasitic fungi have been previously recorded in insects, for example, in the house-fly, caterpillar, mosquito. The fungus mentioned by Schaudinn in Culex was probably a member of the Entomophthoreæ, or related thereto. The

fungus infesting Melophagus ovinus seems to be more nearly allied to the Peronosporeæ.

I learn from a private communication that a similar fungus was found last year by Dr. H. B. Fantham, of Cambridge, in the alimentary tract and Malpighian tubes of the grouse-fly, Ornithomyia lagopodis. From examination of a preparation of the fungus of Ornithomyia, kindly lent to me, I believe that the fungi of the grouse-fly and the sheep-ked are very closely related.

Appendix III.

On the Occurrence of an Anti-coagulin in the Alimentary Canal of Melophagus ovinus, and its Significance in Relation to Crithidia melophagia.

The pronounced and peculiar brightness of the blood in the crop and fore-part of the stomach of the keds examined was noticed very early in the investigation. The blood of the sheep in the stomach of keds that had not fed for as long as three days was still practically fluid and had not coagulated much, while twelve to twenty-four hours after feeding the blood had not coagulated at all. This led me to suspect that an anti-coagulin, such as had been described in a tick (Argas persicus) by Nuttall and Strickland (1908), was present here also, and a series of tests were performed at different times which verified this inference. Every test that I performed had the same result—coagulation was delayed.

The method of testing was simple. Separate emulsions of the salivary glands, stomach, and intestine of Melophagus ovinus were made with 0·75 per cent. NaCl solution. A known quantity—about 0·5 c.c. of human blood from a pricked finger—was then mixed with the same quantity of organ-emulsion, while for control purposes the same quantity of blood mixed with 0·75 per cent. NaCl solution was used. The test fluid and the control fluid were taken up in small glass capillaries, and the test was applied by blowing out the

liquid at stated times and noting when coagulation occurred in each. Typical results of these experiments are tabulated below:

A. Adult Melophagus.

Experiment.	Coagulation period of blood and organ-emulsion.	Coagulation period of blood and ·75 NaCl solution.
(1) Salivary gland	20 min.	7–8 min.
(2) ,, ,,	22 ,,	8 ,,
(3) Intestine	14 ,,	8 ,,
(4) ,,	14 ,, 30 sec.	8 ,,

Obviously an anti-coagulin was present, for considerable delay of clotting occurred.

B. Young Melophagus.—Here the interval between the clotting of the test and control preparations was noted. A few typical results are given:

(1) Blood mixed with emulsion of the salivary glands clotted nine minutes after the control.

(2) Emulsions of intestine added to blood caused the latter to take three times as long to clot as the control preparations took.

Comparing the behaviour of the emulsions of the salivary glands of young and of older keds, the anti-coagulin seems to be more strongly developed in the salivary glands of the older keds, while a similar comparison between the intestinal emulsions would tend to show that the anti-coagulin was more abundant in the intestines of young keds.

The temperature at which the anti-coagulin was destroyed was also investigated. It was found that below 50° C. the anti-coagulin would act. At about 55° C. its action was checked. When 60° C. was reached it was destroyed.

Human blood mixed with emulsions of any part of the alimentary canal at once assumed the vivid red hue so noticeable in the blood removed from the gut of the keds.

The red blood-corpuscles of the sheep, seen *en masse*, appear far brighter on adding emulsions of the gut of the ked containing the anti-coagulin. When much water was added to normal blood, hæmolysis occurred, and the colour

of the solution so obtained was made much brighter when an emulsion of crushed salivary glands of the ked was added to it. The leucocytes of the sheep's blood occurring in the gut of the ked do not appear to be affected in any way by the anti-coagulin.

Anti-coagulin appears to be found in all parts of the alimentary canal of the ked and to decrease in amount from before backwards. As before mentioned, I determined experimentally that freshly shed, and therefore fluid, blood acted as a stimulant to division of the Crithidia. This artificial condition is the counterpart of the natural condition of the blood within the fore-gut of the ked. There, owing to the action of the anti-coagulin, the freshly ingested sheep's blood does not clot, but remains fluid. It is probable that Crithidia within the gut are stimulated by this fluid blood, and divide rapidly. I obtained similar results in the case of Herpetomonas jaculum, where "division of the flagellate Herpetomonad takes place rapidly under natural conditions after ingestion of blood by the host" (Porter [1909], p. 382). If the Crithidia are in the pre-flagellate condition the rapid multiplication is followed by the outgrowth of flagella, after which the organisms separate and pass further down the alimentary canal. The presence of anti-coagulin, from the salivary glands, in the contents of the fore-gut of the ked may be the cause of the rapidity with which the pre-flagellate stage of Crithidia melophagia is passed through, the blood, kept fluid by the anti-coagulin, acting as a stimulus to further development.

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EXPLANATION OF PLATES 12 AND 13,

Illustrating Miss Annie Porter's paper on "Crithidia melophagia."

[All figures were outlined with an Abbé-Zeiss camera-lucida, using a 2 mm. apochromatic (Zeiss), or $\frac{1}{12}$ inch achromatic (Zeiss) objective, and compensating oculars 8 and 12 of Zeiss. The magnification is in all cases approximately 1500 diameters, except where otherwise stated.]

PLATE 12.

Figs. 1–20.—Pre-flagellate Stages.

Fig. 1.—Pre-flagellate with round nucleus, bar-like blepharoplast. No flagellum. Crop. Giemsa.

Fig. 2.—Oval pre-flagellate. Blepharoplast slightly constricted. Crop. Delafield's hæmatoxylin.

Fig. 3.—Dividing pre-flagellate. Crop. Delafield's hæmatoxylin.

Fig. 4.—Division rosette of pre-flagellates. Two individuals again dividing. Crop. Delafield's hæmatoxylin.

Figs. 5–8.—Elongating pre-flagellates. Crop. Thionin.

Fig. 9.—Large preflagellate, with round nucleus, rod-like blepharoplast, flagellum just differentiating. Crop. Giemsa.

Fig. 10.—Rounded form. Flagellum longer than in fig. 9. Crop. Giemsa.

Fig. 11.—Smaller parasite with large nucleus and long flagellum. Crop. Delafield's hæmatoxylin.

Fig. 12.—Parasite, showing elongation of flagellar (anterior) end of the body. Crop. Giemsa.

Figs. 13 and 14.—Crithidia with elongated posterior ends. Anterior part of stomach. Giemsa.

Fig. 15.—Pre-flagellate with posterior blepharoplast. Crop. Giemsa.

Fig. 16.—Parasite with anterior end more developed. Crop. Giemsa.

Figs. 17 and 18.—Almost mature flagellates, membranes showing myonemes. Crop. Giesma.

Figs. 19 and 20.—Practically adult flagellates. Fore-part of stomach. Thionin.

Figs. 21–43.—Flagellate Stage.

Fig. 21.—Small flagellate. Nucleus with chromatin in granules extending part way across the body. Rod-like blepharoplast. Intestine. Giemsa.

Fig. 22.—Flagellate, with well-marked myonemes on the body. Stomach. Gentian violet. × 2250 approximately.

Fig. 23.—Parasite, with flagellum almost continuous with the blepharoplast. Nucleus with central chromatin. Stomach. Delafield's hæmatoxylin.

Fig. 24.—*Crithidia* showing blepharoplast posterior to the nucleus —an uncommon condition. Stomach. Giemsa.

Figs. 25, 26.—Flagellates showing chromidia in their posterior ends. Chromatin of nucleus in bars. Stomach. Giemsa.

Figs. 27–29.—Parasites with somewhat pointed posterior ends. Chromidia present in fig. 29. Intestine. Thionin.

Figs. 30, 31.—*Crithidia* showing somewhat alveolar protoplasm. Stomach. Thionin. × 2250 approximately.

Fig. 32.—Flagellate with blunt posterior end, round nucleus containing large chromatin granules, and extending across complete breadth of body; blepharoplast curved. Stomach. Thionin. × 2250 approximately.

Fig. 33.—Parasite with scattered chromidia. Blepharoplast slightly posterior to and to the side of the nucleus. End of crop. Giemsa.

Fig. 34.—Crithidia with large oval blepharoplast. Stomach. Giemsa.

Fig. 35.—Narrow parasite. Intestine. Giemsa.

Figs. 36, 37.—Longer parasites with many chromidia. Stomach. Iron-hæmatoxylin.

Fig. 38.—Flagellate showing alveolar protoplasm, nucleus and blepharoplast almost in contact. Intestine. Thionin. × 2250 approximately.

Fig. 39.—Long form. Nucleus with chromatin arranged in bars. Oval blepharoplast. Membrane distinct. Intestine. Giemsa.

Fig. 40.—Long parasite with thick flagellum. Myonemes present on body. Blepharoplast showing constriction, so about to divide. Chromatin of nucleus in large masses. Stomach. Delafield's hæmatoxylin.

Fig. 41.—Small aggregation-rosette, showing entanglement of large and small flagellates. Stomach. Giemsa.

Fig. 42.—Flagellate with rounded nucleus and posterior blepharoplast. Basal granule near root of flagellum. Myonemes in membrane. Intestine. Iron-hæmatoxylin.

Fig. 43.—Large rosette. Many parasites shown aggregated around a piece of débris. The flagella serve as points of attachment, therein differing from a division-rosette. Common in stomach and intestine. Delafield's hæmatoxylin.

Figs. 44–56.—Stages in Division.

Fig. 44.—Parasite showing constricted blepharoplast with clear area around it. Chromatin in nucleus arranged in masses at periphery. Intestine. Thionin. × 2250 approximately.

Fig. 45.—Stage similar to fig. 44. Well-marked myonemes on body and membrane. Giemsa. × 2250 approximately.

Fig. 46.—Parasite with both nucleus and blepharoplast constricted. Flagellum beginning to split at base. Stomach. Delafield's hæmatoxylin.

Fig. 47.—Flagellate with anterior end of body, nucleus and blepharoplast all divided. Stomach. Delafield's hæmatoxylin.

Figs. 48, 49.—Somewhat rounded parasites; bodies of daughter-forms not yet diverging from one another. Stomach. Thionin.

Fig. 50.—Daughter-organisms forming a V. Stomach. Giemsa.

Figs. 51, 52.—Further stages in the divergence of the bodies of the

daughter-forms. The flagella have interlocked. Intestine. Delafield's hæmatoxylin. The parasites represented in fig. 51 divided sub-equally.

Fig. 53.—Sub-equal division. Daughter-organisms are almost separated. Intestine. Delafield's hæmatoxylin.

Figs. 54, 55.—Parasites about to separate. Stomach. Giemsa.

Fig. 56.—True division-rosette. The separation of the daughter-individuals takes place from the flagellar end backwards, so that in a rosette the posterior ends of the organisms are centrally directed. Stomach. Thionin.

PLATE 13.

Figs. 57–94.—Stages of the Parasite in the Ovary, Eggs, and Puparia.

(The eggs in figs. 58, 64, 65 are represented diagrammatically.)

Fig. 57.—The flagellate as it penetrates the ovary. Delafield's hæmatoxylin.

Fig. 58.—Flagellate in the act of penetrating a young egg, the blunt end of the parasite being used. Thionin. The egg of Melophagus ovinus is represented diagrammatically.

Figs. 59, 60.—Flagellates from ovary. Flagella somewhat reduced. Giemsa.

Figs. 61–63.—Flagellates from within the egg. Giemsa.

Figs. 64, 65.—Rounding-up forms of C. melophagia within eggs. Delafield's hæmatoxylin and fresh preparations. Eggs of Melophagus represented diagrammatically.

Figs. 66–72.—Series of parasites showing successive stages in shortening and rounding-up of flagellates when within the eggs. Delafield's hæmatoxylin.

Figs. 73, 74.—Parasites showing nuclear division. Very young puparium. Giemsa.

Figs. 75–77.—"Plasmodial" stages of C. melophagia in developing puparia. Peripheral blepharoplasts seen. Giemsa and fresh preparations.

Figs. 78–81.—Rounded parasites resulting from plasmodial forms. Delafield's hæmatoxylin.

Figs. 82–84.—Parasites produced by growth of forms similar to those shown in fig. 81. Giemsa.

Figs. 85–87.—Rosettes of somewhat oval parasites from young puparium. Delafield's hæmatoxylin.

Fig. 88. — Well-defined division-rosette from mature puparium. Giemsa.

Figs. 89–91.—Dividing forms. Mature puparium. Giemsa.

Figs. 92–94.—Parasites resembling pre-flagellates produced from cyst. Mature puparium. Delafield's hæmatoxylin.

Figs. 95, 96.—Small aggregation-rosettes. Intestine. Thionin.

Figs. 97–114.—Post-flagellate Stages in Rectum.

Fig. 97.—Parasite dividing prior to encystment. Intestine. Thionin.

Fig. 98.—Uncommon form of division, occasionally seen in living specimens. Rectum. Giemsa.

Fig. 99.—Small form. Flagellum in process of absorption. Rectum. Giemsa.

Fig. 100.—Parasite showing concentration of protoplasm in the region of the nucleus. Rectum. Giemsa.

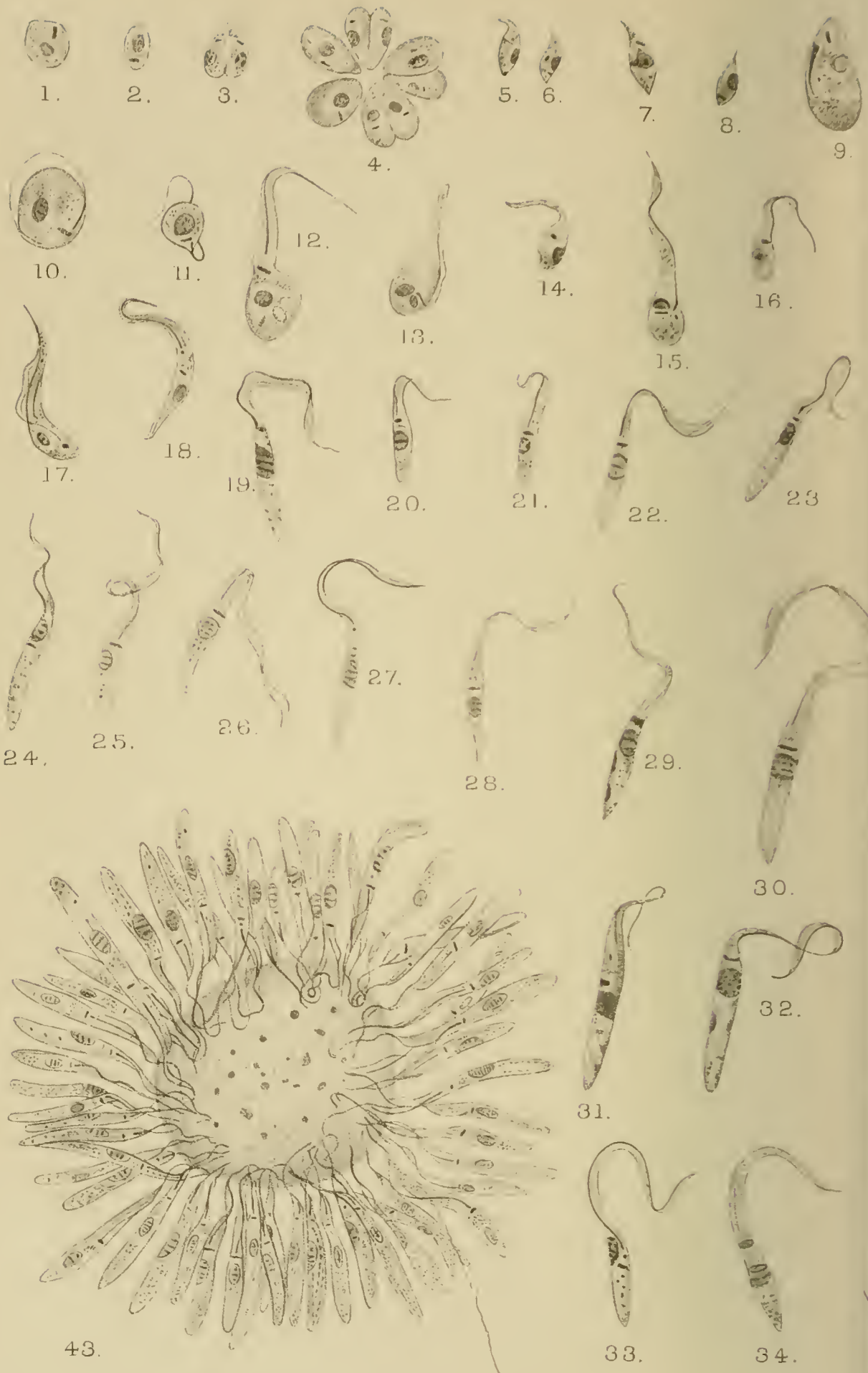
Fig. 101.—Form common in rectum. Body much flattened. Flagellum disappearing. Delafield's hæmatoxylin.

Figs. 102–108.—Parasites showing progressive disappearance of flagellum. Rectum. Thionin.

Figs. 109–112.—Post-flagellate cysts from rectum. Giemsa.

Fig. 113.—Post-flagellate cyst from fæces of *Melophagus ovinus*. Giemsa.

Fig. 114.—Thick-walled cyst. Rectum. Giemsa.



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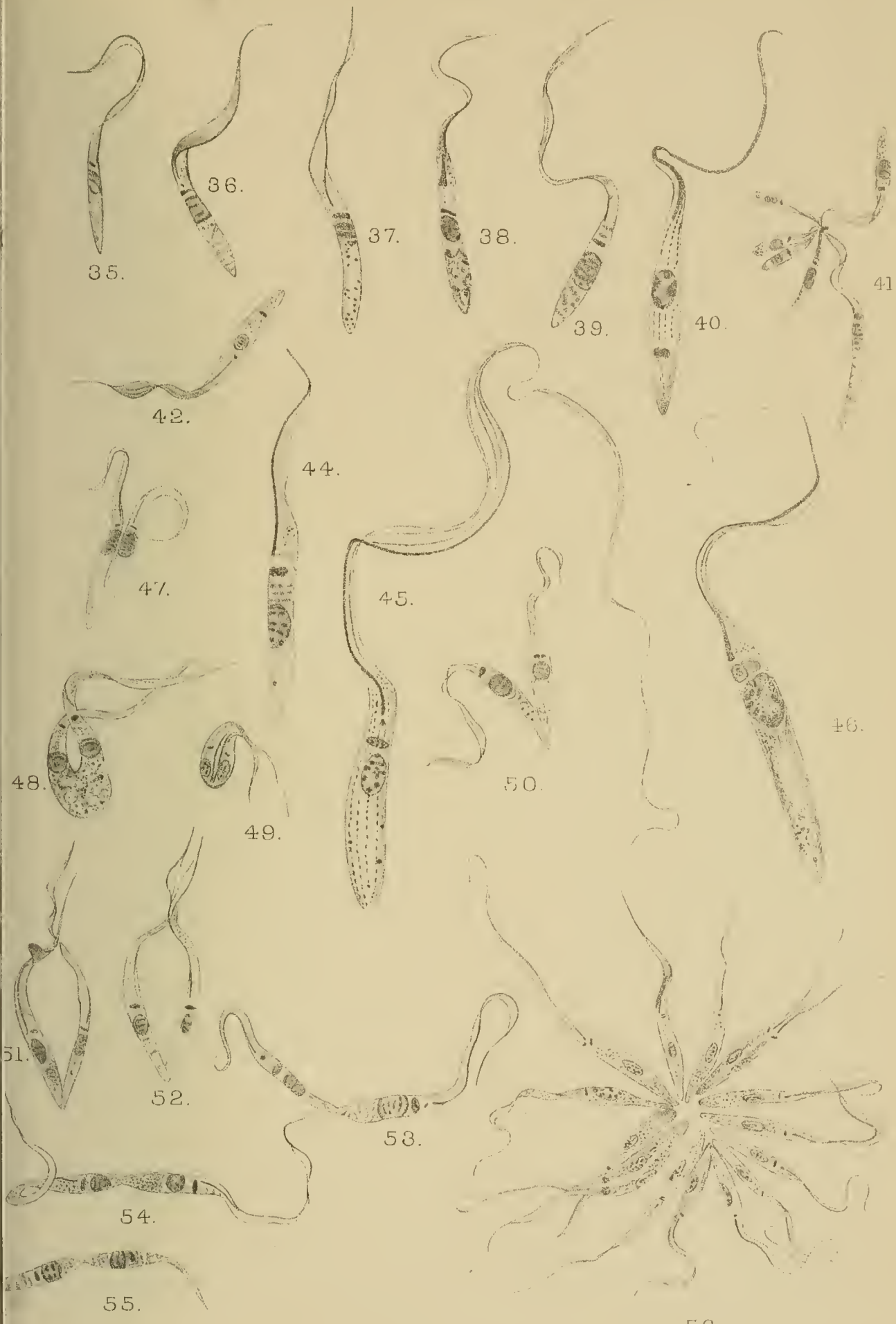

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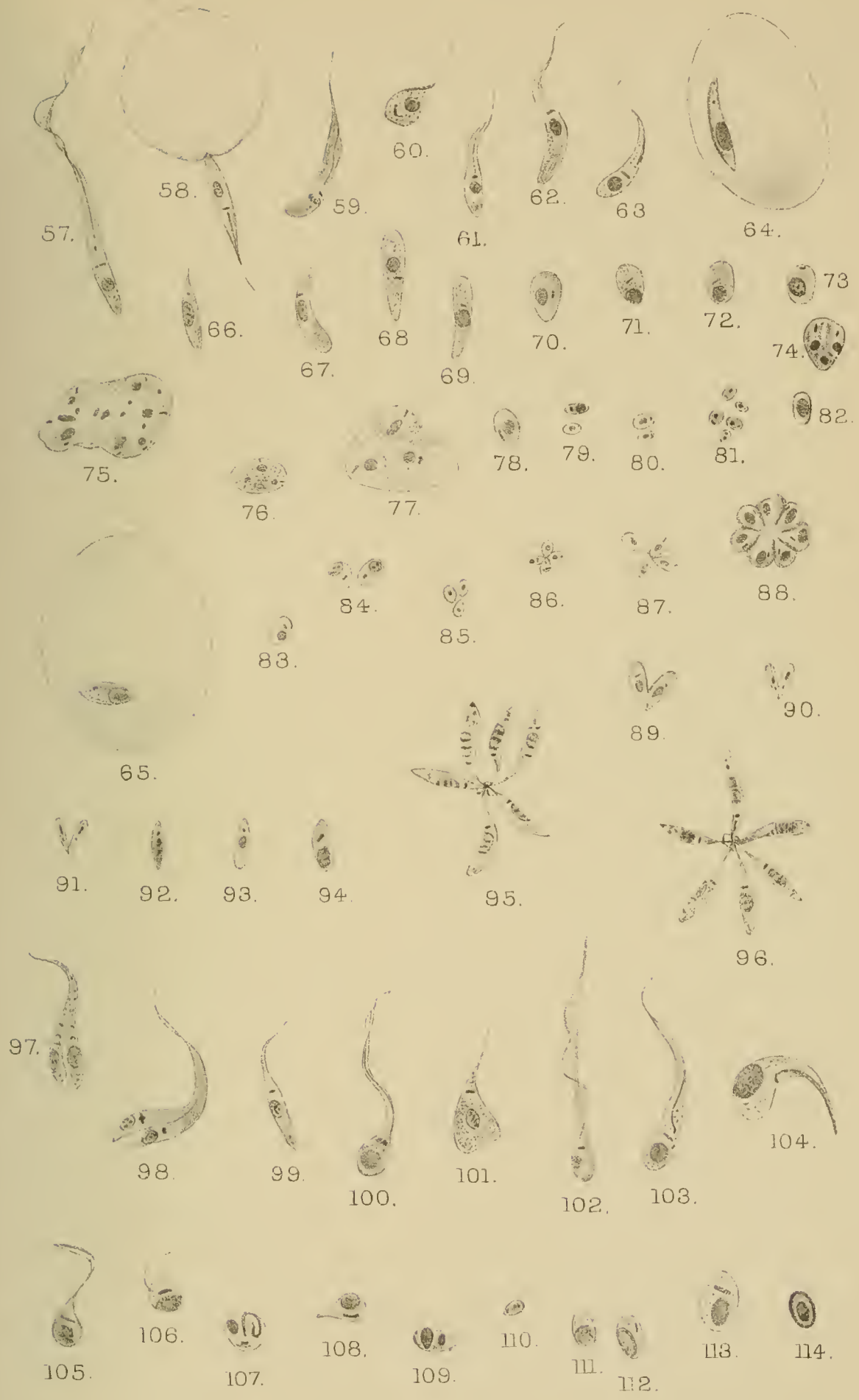

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CRITHIDIA MELOPHAGIA.

Studies in the Experimental Analysis of Sex.

By

Geoffrey Smith,
Fellow of New College, Oxford.

With Plate 14.

3. Further Observations on Parasitic Castration.

During my occupation of the British Association Table at Naples this winter I took the opportunity of re-examining certain points connected with the effect of Sacculina neglecta on the spider-crab Inachus mauretanicus, with the especial purpose of trying to settle the exact way in which the gonad of infected individuals degenerates and is absorbed. The mid-winter months being the most favourable season at Naples for finding numerous individuals of Inachus very profoundly modified by the presence of the parasite, I was able to re-investigate many crucial stages in the modification of the external and internal sexual organs, with the result that, while certain new facts of interest have come to light, I see no reason whatever for departing in any respect from the statement of facts made in my earlier work, or from the deductions drawn from them ('Naples Monograph,' No. 29, Chap. V). In this paper, besides giving the results arrived at in respect to the degeneration of the gonad, I propose to describe certain new instances of infected Inachus which afford incontestable proof that male crabs with differentiated though reduced male internal organs can assume all the adult female secondary sexual characters. It will also be shown both for the male and female sex that the effect of parasitic castration can on no account be

interpreted as a return to a juvenile undifferentiated condition.

In Part 2 of these studies it has already been pointed out that this interpretation is ruled out by the facts, and this was also pointed out in my earlier work, but not in so detailed and categorical a form, with the unfortunate result that Professor T. H. Morgan, in a recent paper on "Sex Determination" ('Journal Exper. Zoology,' vol. vii, 1909, pp. 343, 344), has adopted this very explanation of my observations. Thus he writes: "The broad abdomen of the castrated male might be considered to correspond to the juvenile state. The only external structure cited by Smith that might seem to indicate that the characters of the castrated males are female rather that juvenile ones is the presence of hairs on the abdominal appendages of Inachus, absent in the young crab, but present in the adult female. Such evidence would not in itself be conclusive, since the presence of hairs may be due to increase in size or to a later moult rather than to latent female characters. Smith concludes that the male sex, and probably the male sex alone, can be so radically modified in its sexual nature as to assume a perfect external hermaphroditism. If, on the contrary, we assume that we have here, not hermaphroditism, but an imperfect development of male characters combined with the juvenile condition, we might offer a plausible explanation of the facts."

I am sorry that any want of explicitness on my part should have misled Professor Morgan, but I cannot accept the statement that the only characteristically adult female character, cited by me as being assumed by the infected males, is the presence of hairs on the abdominal appendages. I pointed out in my earlier work ('Naples Monograph,' xxix, pp. 67, 70 and 71) that in the young stages of the female, before the adult breeding form is assumed, the abdomen is a comparatively small flat plate, whereas in the adult it becomes suddenly widened and also takes on a hollowed trough-like shape, so that the two forms of abdomen are absolutely distinct morphological structures, distinguishable from one

another at a glance (see figs. 1, 2, 3 and 4, Pl. VII, 'Naples Monograph' and again 'Quart. Journ. Micr. Sci.,' vol. 54, Pl. 30, figs. 10, 11, 13, 14). Now, when the infected males take on the female external characters they have never been found to assume the juvenile flattened form of abdomen which characterises the young stages of both males and females, but they invariably take on the hollow trough-like form characteristic of the adult breeding female and of her alone (see the numerous figures on the plates referred to above). That is the first objection to the view that the alteration of the male is merely towards a juvenile condition, and anyone who will examine the series of specimens exhibited in the South Kensington Museum or in the Oxford Museum, or those deposited by me at the Zoological Station at Naples, will at once perceive the entire morphological difference of the abdomen in the young and adult female, and the identity of the modified male abdomen with that of the adult female.

Secondly, with regard to the abdominal appendages. It is not a question of the mere presence or absence of a few hairs, as Professor Morgan has unfortunately been led to suppose. The abdominal appendages of the juvenile and adult individuals differ as radically, if not more radically from one another, than the form of the abdomen. In the young form of the female these appendages are short, stout and rod-like, and provided with a very few short bristles, as shown in Pl. 14, fig. 7 of this paper. In very young males similar appendages are present, but they are lost at a very early stage indeed, only the two anterior appendages being kept as the copulatory styles. The form of these two appendages in the young male is shown in Pl. 14, figs. 1 and 2.

The adult female, at the same moult at which it acquires the characteristic adult form of abdomen, assumes a totally different kind of appendage of the form shown in Pl. 14, fig. 4. Here it is seen that instead of being stout and rod-like with a few stiff hairs, as in the young females, the appendage has become transformed into two wisp-like branches, the exopodite being densely clothed with long plumose hairs, the

endopodite, now a slender-jointed structure, being furnished with exceedingly long pointed hairs for the attachment of the eggs. The structure of these abdominal appendages in the adult female, adapted as they evidently are for reproductive purposes, is as morphologically distinct from that of the young individuals of either sex as anything very well could be.

Now let us inquire in what form the infected modified males assume the abdominal appendages. The answer is plainly given by reference to Pl. 14, fig. 4. This figure is an actual camera drawing of the second abdominal appendage of an infected individual, which was proved to be a male by the presence of a copulatory style of a somewhat modified form (Pl. 14, fig. 3), and internally by the presence of testes and vesiculæ seminales of a typical character on either side. The testis and vesicula seminalis of one side of this individual are shown in Pl. 14, fig. 10. The form of the abdominal appendages (Pl. 14, fig. 4), of which there were four on each side in addition to the copulatory styles, is identical with that of a normal adult female; in fact, since this figure serves equally well to depict the abdominal appendage of a normal adult female, I have not considered it necessary to give another figure, which would simply mean repeating the same structures.

The infected male individual to which figs. 3, 4, and 10 on Pl. 14 refer is a particularly favourable type for showing conclusively that the abdominal appendages, when assumed by the infected males, are of the characteristically adult female type. As a matter of fact a commoner condition is that shown in Pl. 14, fig. 5. In this infected male the copulatory style was greatly reduced (fig. 6) and the abdominal appendages were also developed in an imperfect condition, with almost complete suppression of the endopodites. Nevertheless, the characteristic plumose hairs are present on the exopodite, which is of a slender shape, thus conforming to the adult type of female appendage and not really approaching to the juvenile condition. This figure might equally well refer

to the abdominal appendage of an infected female, in which the endopodites are very frequently thus reduced.

We have now examined in some detail two of the most important characters in which the infected male Inachus is modified by the presence of the parasite Sacculina, viz. the shape of the abdomen and the form of the abdominal appendages, and we have seen that Professor Morgan's attempt to explain the modification of the male as a return to a juvenile condition is quite at variance with the facts. But we have still two more points to consider, which render that explanation still more impossible.

The most important of these two points is the fact that in a certain small percentage of cases the infected males, on recovery from the parasitic disease, have been observed to have regenerated the gonad, and to have developed large ova measuring about 1 mm. in diameter and full of the reddish-coloured yolk characteristic of the mature ova of the female Inachus. Professor Morgan himself admits the cogency of this fact, so that I need not labour it here, its significance, indeed, being obvious.

The second point is one which I have only been able to settle finally during my recent visit to Naples. In my earlier work (loc. cit., p. 68) I inclined to the view that the presence of Sacculina caused the young females under 13 mm. in carapace length to assume prematurely the adult type of abdomen and abdominal appendage, and I emphasised this point as being of importance in precluding the view that the effect of the parasite was merely to arrest development or cause a return to a juvenile state. By a careful examination of the large amount of material put at my disposal by Dr. Lo Bianco this winter, I have found that this premature assumption of adult characters by infected females undoubtedly occurs. During December and January all the uninfected females of carapace length up to 14 mm. had the immature juvenile form of abdomen and appendage, but all the infected females measuring from 6–14 mm. had the fully adult type of both those structures. The real theoretical significance of

this fact, which has an important bearing on the whole meaning of parasitic castration, will be discussed later, but it has been introduced here as a final nail in the coffin of the theory which attempts to explain the effects of parasitic castration as due to arrested development or the assumption of juvenile characters. Possibly the use of the term "parasitic castration" has had something to do with perpetuating this unfortunate error, the analogy between ordinary operative castration or mechanical removal of the gonads and their degeneration owing to the presence of a parasite being, as Professor Sedgwick has pointed out, extremely small. In parasitic "castration" the degeneration of the gonad is not brought about by the parasite mechanically removing or attacking the gonad, but by its setting up a deep-seated alteration of the metabolism of the host which secondarily reacts on the gonad. We may now enter into the question of the method of degeneration of the gonad. In the above paragraphs I trust that the following conclusion has been thoroughly vindicated. The modification of the male Inachus by the parasite Sacculina consists in the assumption by the male of adult female sexual characters to a greater or less degree of perfection; in neither sex can the modification be ascribed to arrest of development or the assumption of a juvenile immature condition.

As I have shown in my earlier work (loc. cit., pp. 72–74) the degenerate condition of the ovaries and testes with their ducts in infected Inachus is due to two causes: firstly, an arrest of growth, so that the gonad tends to remain in the same condition as it was when infection took hold, and secondly, to an actual absorption of the tissues of the gonad and their final disappearance, a process which was often accompanied by an actual irruption of the roots of the parasite into the germinal tissues. The arrest of growth of the gonad and the first stages of degeneration, at any rate in the male, were shown to be independent of the irruption of the Sacculina roots.

The method of absorption and disappearance of the gonad

was not clearly made out, and it was my chief object this year to obtain some idea of how this process takes place, to observe, for example, whether phagocytosis takes any active part in it.

The condition of arrested growth without any signs of active degeneration is well exemplified by the testis and vesicula seminalis figured on Pl. 14, fig. 10, which was dissected out of the perfectly modified male whose appendages are represented in figs. 3 and 4. In Pl. 14, figs. 8 and 9, are drawn, on the same scale, the vesicula seminalis and a small portion of the coiled testis of an uninfected male of the same size, showing that the gonad of the infected individual has remained very small and undeveloped. Spermatozoa were entirely absent from the infected individual, whereas the vesicula of the normal individual was crowded with them. There is, however, another point to be observed. Investing the gonad of the normal individual is a thin sheath of connective tissue with flattened, darkly staining nuclei (*c.s.*, Pl. 14, fig. 9). In the infected individual this sheath is seen to be of proportionately greater thickness.

In Pl. 14, fig. 11, is shown a portion of the testis of an infected male in which the process of absorption of the gonad has proceeded to a considerable extent. In three places small disconnected masses of testicular cells (*t.*) are seen lying ensheathed by connective tissue; between the disconnected pieces of germinal tissue nothing remains but the connective-tissue sheath. By staining such preparations with a triacid stain, e. g. Ehrlich-Biondi, small globules are seen lying between the germinal nuclei and the sheath, which take up the orange stain. These globules may be looked upon as degeneration products of the germinal tissue in process of absorption. In none of the preparations which I have made of degenerating gonads is there any sign of phagocytosis, the degeneration appearing to take place by some process of auto-digestion.

Turning to the degeneration of the ovary, Pl. 14, figs. 12 and 13, we find exactly the same process. Fig. 12 represents

a portion of degenerate ovary of an infected female, in which islets of ovarian tissue containing disintegrating ova are seen encapsuled in the connective-tissue sheath. Fig. 13 is a high power drawing of a small portion of the ovary showing the clear distinction between the germinal nuclei (*N*), the nuclei of the connective-tissue sheath (*cs*) and the degenerating ova.

In a very great number of infected crabs dissected no trace could be found of the remains of a gonad; and in these, allowing for a certain number in which I overlooked the degenerating remains, one must suppose that the process of encapsulation by connective tissue and auto-digestion had led to complete disappearance. I am unable to state for certain whether the connective-tissue sheath plays an active part in the absorption of the germinal tissue; the chief part is clearly due to a simple disintegration of the same nature as is now known to occur in the destruction and absorption of the larval organs of insects during metamorphosis. In this latter process it was formerly held that phagocytosis played the principal part, but it is now known that a process of auto-digestion by fluids is at least as active an agent.

To conclude this part, I will attempt to outline, in a more satisfactory manner than was possible before, an explanation of why it is that the presence of a parasite should bring about such profound physiological and morphological changes in its host.

We must clearly define, in the first place, what these changes essentially consist in. It has been shown in my earlier papers, and I trust still more fully brought out in this paper, that the effect of Sacculina on Inachus is to cause the infected individuals of both sexes to assume adult female characteristics. This results not only in transforming the males into hermaphrodites with preponderating female characters, but also in hastening on the assumption of adult female characters by immature females. The problem, therefore, resolves itself into this, Why should the presence of Sacculina cause the host of either sex to become adult female in nature?

Let us examine what the process of becoming adult involves in an ordinary female crab. Plainly the most important change is the rapid elaboration of yolk material which accumulates in the ovary, causing the latter to grow to a very great size. This elaboration of food material in the ovary is the fundamental point in which the adolescence of the female gonad differs from that of the male. In the male gonad at maturity we have an immense multiplication of nuclei and of chromatin but a small development of cytoplasmic material and no deposit of yolk; in the female we have the exact opposite of this process. The most important part, then, in the process of becoming adult female, is the active elaboration of yolk material.

We have arrived, therefore, at this point of the argument: that the presence of Sacculina causes the crab of either sex to become adult female in nature, and the most important activity of this state is the elaboration of yolk material. Can we prove that the presence of Sacculina actually causes its host of either sex to produce yolk material? I believe we can. If the roots of Sacculina which fill the body of an infected Inachus be examined, they will be found to be packed with small globules of an oily material, and if the roots are stained with such a mixture as Ehrlich-Biondi's tri-acid stain it may be observed that the Sacculina roots take up the same constituent in the stain, namely the acid fuchsin, as the yolk of an adult female crab's ovaries. From the observed contents of the Sacculina roots and from their reaction to stains it is clear that they are elaborating from the blood of the Inachus of both sexes a closely similar yolk material to that which is normally accumulated in the ovary of a healthy adult female Inachus.

The effect of Sacculina on Inachus is therefore to force the latter to elaborate yolk material of a similar kind to that which is normally developed in the ovary of the female at maturity. As the Inachus elaborates it the Sacculina abstracts it, so that it does not come to be deposited in the gonad until after recovery from the disease, when, as we have

seen, the yolk-containing ova may be formed in the gonad of either sex.

Meantime the continued production and circulation in the blood of the infected Inachus, whether male or female, of this yolk material, or rather of the substances from which the yolk is built up, is accompanied by the production of the secondary sexual characters proper to the adult female. These yolk-forming substances, or substance, are therefore identical with the "sexual formative substance," whose existence we deduced in Part 2 of these studies. We may summarise the above argument as follows: The Sacculina roots require for their nourishment a substance in the blood of the crab which they can work up into yolk material. This substance is provided for them in the female sexual formative substance, which is circulating in small quantities in normal male crabs as well as, in greater quantities, in female crabs. But the Sacculina roots must have the power, not only of abstracting this material from the crab's blood, but also of forcing the crab to go on forming this substance in excess. This may seem to be a great assumption; but it is exactly here that a very close parallel can be drawn between the phenomenon we are dealing with and the general processes of immunity to parasites and organic poisons. Immunity has been interpreted, especially by Ehrlich, to mean that when a poison acts upon an organism it combines with and anchors certain organic molecules, which are then regenerated in excess and poured out into the blood-stream as antibody. If we suppose, therefore, that the Sacculina roots anchor the molecules of the female sexual formative substance, and this, from the fact of their forming yolk material, they appear to do, it is in accordance with the facts of immunity to suppose that the molecules of the sexual formative substance, wherever they are formed, will be regenerated in excess.

The continued operation of this process, namely, the production of female sexual formative substance in the blood-stream, and its abstraction by the Sacculina roots, would

account for all the observed phenomena, viz. the development of adult female characters, which are dependent on the presence of this substance in quantity in the blood, and the abortion of the gonad owing to the Sacculina roots seizing on its proper nutriment and not permitting it to grow or develop. Nevertheless in the case of the hermit-crab infected by Peltogaster, Potts has shown that small eggs may be formed in the gonad, even while the parasite is still vigorous, showing that the excess of sexual formative substance has to some extent been seized on by the gonad.

In the above manner it appears to me that we not only gain a clear idea of the process involved in "parasitic castration," but the phenomenon, instead of appearing an isolated curiosity of a wholly inexplicable nature, falls into line with the well-known reactions to parasitic infections which are classed under the category of immunity. The clue to the whole theory rests in the truth of the statement that "parasitic castration" consists in the assumption by the infected individuals of adult female characteristics, owing to the development within them of the female sexual formative substance. If this statement of the case is rejected by the reader on the evidence which I have adduced, he will naturally reject the theory proposed to account for it, and if he can succeed in framing a different and more satisfactory theory which will include all the facts I shall be very well pleased.

But any attempt to explain "parasitic castration" by vague analogies with the effects of operative castration, or by referring the whole phenomenon to arrested development or appearance of juvenile characters, is certainly foreordained to failure.

The explanation here offered of parasitic castration differs from that which I proposed in my first work ('Naples Monograph,' xxix, p. 82, et seq.) only in its greater precision, not in its general outline. In my original statement of the theory I ascribed the alteration of the male to an adaptive response of the metabolism in order to make good the drain on the system caused by the presence of a parasite. The metabolism

was represented as changing from the katabolic male condition to the more anabolic female, and this change was supposed to be effected by the development in the body of the female sexual formative substance. It is clear that this theory is fundamentally the same as that now proposed, but being couched in rather vague and general language, it seems to have made very little impression even on those who unreservedly accepted my statement of facts. By showing, firstly, that the assumption of the adult female condition involves an active elaboration of yolk material, and secondly, that the Sacculina roots actually withdraw some substance from the crab's blood from which they manufacture a yolk substance closely similar to that normally deposited in the eggs of the crab, and also by emphasising the fact that in reality both sexes of the host react in exactly the same way to the parasite, it has been possible to express the theory in a far more objective manner.

Summary of Part 3.

(1) The effect of Sacculina on male Inachus consists in the assumption by the male of adult female characteristics, and can in nowise be ascribed to arrest of development or acquisition of juvenile or immature characters, as suggested by Professor T. H. Morgan.

(2) The effect of Sacculina on young immature females of Inachus is to force them to assume prematurely adult female characteristics.

(3) The absorption of the gonad of infected Inachus of both sexes is brought about by a process of ensheathment with connective tissue and auto-digestion, phagocytosis apparently playing no part.

(4) The reason why Sacculina causes the assumption of the adult female state in Inachus is found in the facts (1) that the roots of Sacculina elaborate a yolk-substance from the blood of Inachus of a similar nature to that which is elaborated in the ovaries of an adult female Inachus; (2) that in order to elaborate this yolk substance they take up from the

blood of Inachus the female sexual formative substance, which is the necessary material for forming the yolk; (3) that the female sexual formative substance, being anchored by the Sacculina roots, is regenerated in excess; (4) that the presence of the female sexual formative substance continually circulating in large quantities in the body-fluids of the infected crabs causes the production of adult female secondary sexual characters, and, when the parasite dies, of yolk-containing eggs.

4. On a Case of Parasitic Castration in a Vertebrate.

Although numerous cases are now known of the presence of a parasite causing arrest of development or degeneration of the reproductive organs in various invertebrates, no clear instance of this process has been reported, so far as I am aware, among vertebrate animals as the result of bacterial disease of organs other than the reproductive organs themselves. Of course, where the reproductive organs themselves are the seat of infection, a certain amount of atrophy or degeneration may naturally result, but we have here to deal with a case of parasitic castration, analogous to the case of Sacculina on Inachus, or of Entoniscus on various crabs, where the reproductive organs are not themselves necessarily attacked by the parasite, but are secondarily affected by the general disturbance of the metabolism, set up by the presence of a parasite in other parts of the body.

During December, 1909, I received a pure-bred Gallus bankiva cockerel for breeding purposes. It belonged to the breed known as the Indian Jungle Fowl, a breed which has departed very little from the wild Gallus bankiva. The bird when it arrived appeared in good health; the plumage was in good condition, the comb and wattles well developed and red, the spurs fully developed, the tail carried erect, and the bird crowed in the normal manner. Its age was one year and a half. About two weeks after it arrived it showed signs of sickness and a tendency to mope in the straw at the back of its run. These symptoms became gradually worse, and at the beginning of February the whole

appearance of the bird was changed: the comb and wattles were greatly shrunken, and instead of being bright red were unhealthy pink patched with grey; the skin round the eyes was bloodless; the tail was carried drooping, and the bird never crowed. The bird was isolated and treated with purgatives, but the illness continued, the comb and wattles having withered by the middle of April to about half their original size. The spurs and plumage were unchanged, save for the fact that the tail was always drooped. The bird was killed and dissected on April 8th.

The post-mortem examination showed that it was suffering from very acute avian tuberculosis. The liver was interpenetrated with whitish calcareous nodules swarming with the characteristic tubercle bacillus, while the whole course of the alimentary canal, pancreas and spleen was covered with similar swellings, some of them of the size of a pea, also full of living bacteria. Only the alimentary and lymphatic organs were infected, the lungs, kidneys, and testes being entirely free of infection.

Although the testes were uninfected, it was at once apparent that they were very remarkably reduced in size, measuring only 10 mm. in length by 5 mm. in breadth, whereas in a normal cockerel of the same breed and age, at the same time of year, they measured 40 mm. in length by 25 mm. in breadth. The vasa deferentia were also reduced in size, and this was especially noticeable in the coiled lower part of the tubes where they pass into the vesiculæ seminales; no spermatozoa were present.

Sections of the testes showed the testicular tubes intact, with a regular lining of germinal epithelium cells with nuclei in a resting condition. There was no sign of any mitosis or of any other stages in the process of spermogenesis. The testicular tubes, in fact, presented the appearance characteristic of immature birds of a few weeks old.

In a certain number of the tubes degenerating germinal cells with abnormal nuclei could be seen.

In contrast to this extreme reduction and arrest of develop-

ment in the germinal part of the glands, the interstitial cells, forming islets everywhere between the testicular tubes, were well marked.

There was no trace of infection by the tubercle bacilli in either testis.

It is clear from the course of the disease and from the post-mortem examination that the reduction of the comb and wattles and the atrophy of the testes went hand in hand with the acute development of the tuberculosis. We know from numerous experiments that the effect of the removal of the testes in Gallus is to arrest the development of the comb and wattles; otherwise, except for the loss of the crowing and the drooping of the tail, the other secondary sexual characters are not affected. We have seen that as the bird in question became ill, the principal symptom was the reduction in the comb and wattles, and the post-mortem showed that the testis must have been accompanying these organs in a process of atrophy.

We have, therefore, in this case, an instance of parasitic castration caused by a bacterial infection of a vertebrate host, exactly parallel to the cases of parasitic castration in various Invertebrata caused by such various parasites as Crustacea, Sporozoa, and worms of various kinds. In a great number of these cases the effect of the parasitic castration is to arrest the development or cause the atrophy of the primary and secondary sexual characters without actively calling forth the production of the female sexual characters in the parasitised male. In other cases (as far as we know only in the Crustacea) besides the suppression of the sexual characters both primary and secondary proper to the infected individual, we find the active assumption of female characters by the parasitised male, as described in Parts 2 and 3 of these studies. The particular case just described belongs, as far as the evidence goes, to the former of these two categories, *i. e.* that in which certain of the male sexual characters atrophy without the active assumption of female characters. The principal interest attaching to this case

consists, firstly, in establishing a bacterial disease of a vertebrate as a cause of parasitic castration and thus extending the operation of this principle to two new classes of organisms, and secondly, in bringing out the correlation between the activity of the testes and the development of the comb and wattles of Gallus bankiva. In the next part this correlation will be dealt with more fully on an experimental basis.

LETTERING.

C. S. Connective tissue sheath. *En.* Endopodite. *Ex.* Exopodite. *N.* Germinal nuclei. *O.* Ovary. *T.* Testis. *V. S.* Vesicula seminalis.

EXPLANATION OF PLATE 14,

Illustrating Mr. Geoffrey Smith's paper on "Studies in the Experimental Analysis of Sex."

All the figures refer to Inachus mauretanicus (Lucas).

Fig. 1.—First abdominal appendage (copulatory style) of normal uninfected male. × 5.

Fig. 2.—Second abdominal appendage of normal uninfected male. × 5.

Fig. 3.—First abdominal appendage of infected male "A." × 5.

Fig. 4.—Second abdominal appendage of infected male "A." × 5. (This figure might serve equally well for the abdominal appendage of an adult female.)

Fig. 5.—Second abdominal appendage of infected male "B." × 5.

Fig. 6.—First abdominal appendage of infected male "B." × 5.

Fig. 7.—Second abdominal appendage of normal uninfected female, before adult condition is assumed. × 5. (The adult form of this appendage is practically identical with that given in fig. 4.)

Fig. 8.—Vesicula seminalis of a small normal male, measuring 14 mm. carapace length. × 20.

Fig. 9.—Coils of testis of the same male. × 20.

Fig. 10.—Vesicula seminalis, duct, and coils of testis of infected male "A." × 20.

Fig. 11.—Portion of testis of an infected male, showing absorption of germinal cells in connective-tissue sheath. × 30.

Fig. 12.—Portion of ovary of an infected female, showing absorption of ova and germinal cells in connective-tissue sheath. × 30.

Fig. 13.—Another portion, higher magnification, of ovary of infected female. × 60.

Huth, Lith^r London.

Some Observations on a Flagellate of the Genus Cercomonas.

By

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With 19 Text-figures.

In the present paper I shall describe a flagellate of the genus Cercomonas, a genus first created by Dujardin, in his 'Historie Naturelle des Zoophytes Infusoires,' published in 1841. Since Dujardin's original description numerous flagellates have incorrectly been attributed to this genus, so much so that Klebs, in his 'Flagellatenstudien' (1893), says that this genus has not been defined with sufficient accuracy, that it has been confused with Heteromila and Bodo by the overlooking of the tail flagellum, and that the genus Cercomonas must be rejected. It is undoubtedly true that the genus Cercomonas is very confused, and this confusion has been considerably heightened by the description of Cercomonas from the intestine of man and other animals. Davaine (1854) was the first to record the presence of Cercomonas in the evacuations of a man suffering from cholera. Without going into the question of the correctness or otherwise of Davaine's conclusions, it is undoubtedly a fact that many observers, noting the presence of active flagellates in the intestinal contents, have attributed them at once to the genus Cercomonas, and as a result of this various species of Trichomonas, Lamblia, and possibly other flagellates have been included in this genus. In the present instance the flagellate to be

described was found in the fæces of a patient in the Albert Dock Hospital at the London School of Tropical Medicine. This patient was infected with Entamœba coli, and in order to observe changes in the encysted forms of this amœba some of the fæces were placed in a clean glass-stoppered bottle. In the course of a few days it was noticed that large numbers of flagellates were present. It is probable they had developed from cysts which must have been present in the fæces. On first examination it was seen that these flagellates corresponded very closely with the original description of Dujardin for the genus Cercomonas, and for this I took them to be. On more careful examination I found that the tapering posterior end was in reality a second flagellum, and that this could be traced along the surface of the body to which it was attached as far as the insertion of the long anterior flagellum. The presence of this posterior flagellum and its attachment to the body required very careful observation to make out, for it can only be clearly seen in certain portions of the animal, and it is quite conceivable, as Klebs maintains, that Dujardin overlooked this posterior flagellum. Dujardin's original description of the genus is as follows:

"Genre Cercomonas.

"An. arrondi ou discoïde, tuberculeux, avec un prolongement postérieur variable, en forme de queue, plus ou moins long, plus ou moins filiforme.

Les Cercomonas ne different absolument des Monads que par un prolongement postérieur, formé par la substance même du corps qui s'agglutine au porte-objet, et s'étire plus ou moins, de manière à n'être tantôt qu'un tubercule aminci, tantôt une queue allongée transparente, tantôt enfin un filament presque aussi fin que le filament antérieur, et susceptible d'un mouvement ondulatoire; mais bien souvent j'ai cru voir les Monades passer par degrés l'état de Cercomonas."

A comparison of this description with that now to be given will show how closely the two agree.

The occurrence of this flagellate has been described above. By transplanting into other media I have been able to keep

cultures of this flagellate free from other Protozoa for about a year, and it is only that circumstances preventing me from continuing these observations I now describe what results I have already obtained.

Method of Observation.

I have found the best liquid culture medium to be hay infusion to which a small quantity of fæces has been added. The flagellates will live and multiply in hay infusion alone, but, as in other thin media, the numbers of flagellates are always very small, so that any observation is difficult to make. In the thicker medium the numbers are not only larger but the movements of the flagellates are slower and accordingly more easily followed. For keeping stock cultures small test-tubes were used as in bacteriological methods, but for making observations hanging-drops in the moist chambers of Max Schultze were most useful. In these hanging-drop preparations the flagellates would live for weeks, till finally, all nutriment being used up, encystment followed. By the addition of fresh nutriment to the hanging-drop the culture would commence again.

In addition to the liquid medium I have found the solid agar medium used for the culture of amœbæ most useful. It was first employed for the culture of flagellates by Berliner. This observer, working with Copromonas major, found that on the solid medium the flagellates multiplied rapidly till enormous numbers were present. I can fully confirm this, and for the study of the details of nuclear division the presence of such large numbers of dividing forms is very useful. The medium I employed differed slightly from that used by Berliner. For the culture of amœbæ I have used with success the medium first invented by Musgrave and Clegg, and I have found it equally good for the flagellates at present under discussion. I have employed it in the ordinary Petri dishes. By unveiling the dishes the progress of the culture may be watched under the low powers of the microscope. A very useful method for the

use of this medium, and one which will allow observations to be made with high powers, is the following: A long cover-glass (1½ inches) is taken and carefully cleaned. On a clean slide ridges of Czokor's wax, first recommended to me by Professor Minchin, are so arranged, about an eighth of an inch high, that the cover-glass will form the lid of a box. Some of the medium is melted by placing the test-tube in boiling water, and a small drop of this is allowed to fall on to the cover glass, which is lying on the top of the hot-water oven. By careful tilting of the cover-glass the melted medium will form a very thin layer over the cover-glass, which is then removed so that the medium may solidify. The surface of the medium is then inoculated with a small quantity of material from a previous culture and the cover-glass inverted on the wax ridges. By means of a hot wire and more wax the whole may be completely sealed up. It is most essential that not the smallest opening be left, or it will be found that the medium will quickly dry and the culture end.

In this way it is easy to follow the multiplication of the flagellates with the $\frac{1}{6}$ in. objective, and if the film of medium has been made sufficiently thin the oil-immersion may be employed.

In every case where the flagellates grow in the solid medium their chief nourishment seems to be the numerous bacteria that grow at the same time.

For studying the flagellates in the fixed and stained condition the cover-glass method has been mostly used. Some of the liquid medium or some of the culture scraped from the surface of the agar is spread on a clean cover-glass, and without allowing it to dry it is dropped, film side down, on to the surface of some fixing fluid. Another method of obtaining a film from the agar cultures is this: A cover-glass is dropped on to the surface of the agar culture in a Petri dish. It is gently pressed down till its surface is seen to have touched the culture. On raising it with a needle it will be found that a layer of the culture is adherent to the cover-glass, and it may be fixed as before.

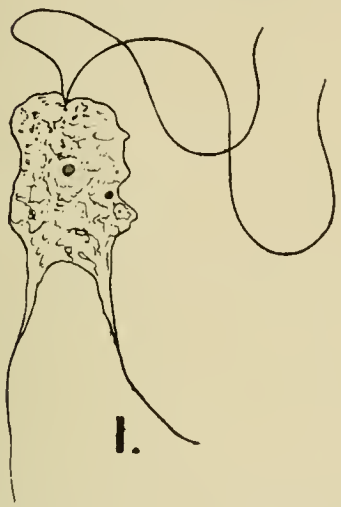
For fixing the flagellates the most useful fixative has been Schaudinn's mixture of two thirds saturated aqueous solution of sublimate and one third alcohol, slightly acidified with acetic acid. This has been used in the manner just described by Schaudinn or in a slightly modified form. The films are best stained with iron-hæmatoxylin.

Description of the Living Flagellates.

When examined in a drop of liquid medium on a slide the

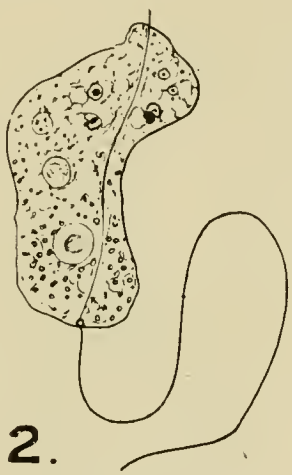
Text-figs. 1–8.—Drawings from life.

Text-fig. 1.



Amœboid form in early division stage.

Text-fig. 2.

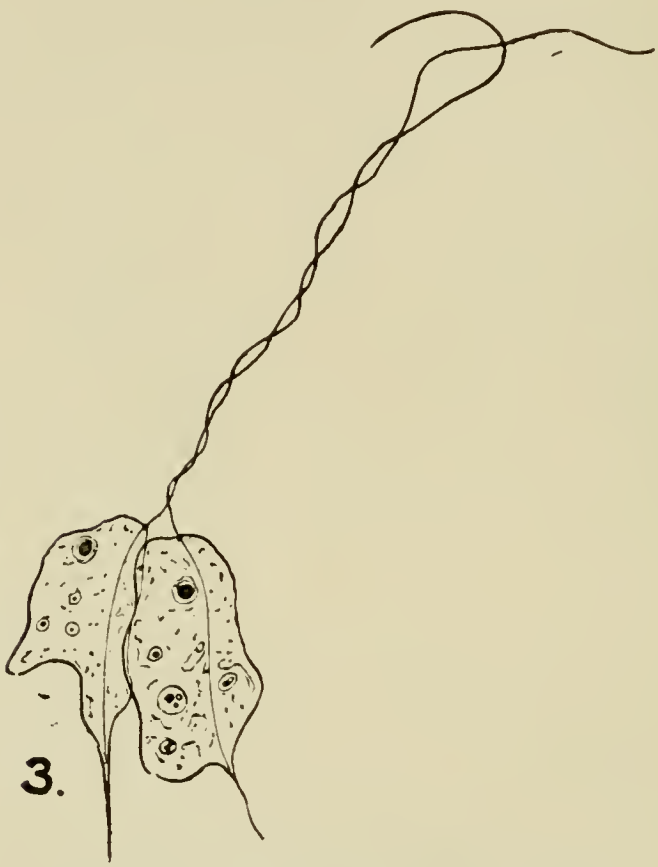


Amœboid form.

flagellates appear as pear-shaped organisms, with a long flagellum, about twice the length of the body, arising from the blunt end. The posterior end of the body is, as a rule, drawn out to a fine and tapering point. By the constant lashing of this long anterior flagellum the animal is drawn along. Sometimes the flagellum is, as it were, hooked around some distant object, and by its flexion pulls the body towards this point. The posterior end of the body, which, as stated above, is also a flagellum, moves much less vigorously than the anterior. Its movements may be quite passive, being only the accidental changes in position produced by the changes

in shape of the body. At other times there is a distinct to-and-fro or lashing movement, but at its maximum it is much less violent than that of the long anterior flagellum. The protoplasm of the body may be continued along this posterior flagellum for a considerable distance. On very careful focussing it can be seen that the posterior flagellum

TEXT-FIG. 3.



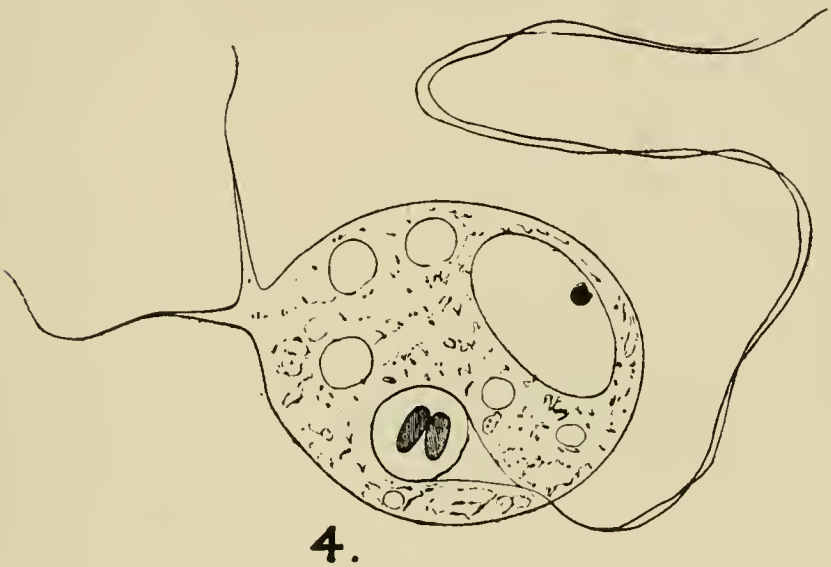
Two amœboid forms with entangled flagella.

is attached to one side of the body, and really arises from the insertion of the anterior flagellum. This is very well shown in some of the figures, e.g. 3, 5, 9. When the body is viewed in certain positions it is seen that it is distinctly flattened along the line of attachment of the posterior flagellum (fig. 10), and when the posterior flagellum is moving at its maximum rate this flattened edge of the body shows slight but distinct undulatory movement, reminding one most strikingly of the

movements of the blood inhabiting Trypanoplasma. Indeed, this flagellate in many respects occupies a position intermediate between the genus Bodo and Trypanoplasma.

The nucleus is clearly visible in the living animal. There is a distinct membrane, and at the centre of the nucleus is a large karyosome. The nuclear membrane is drawn out at one pole towards the insertion of the two flagella, and occasionally a clear line may be detected connecting the apex of

TEXT-FIG. 4.



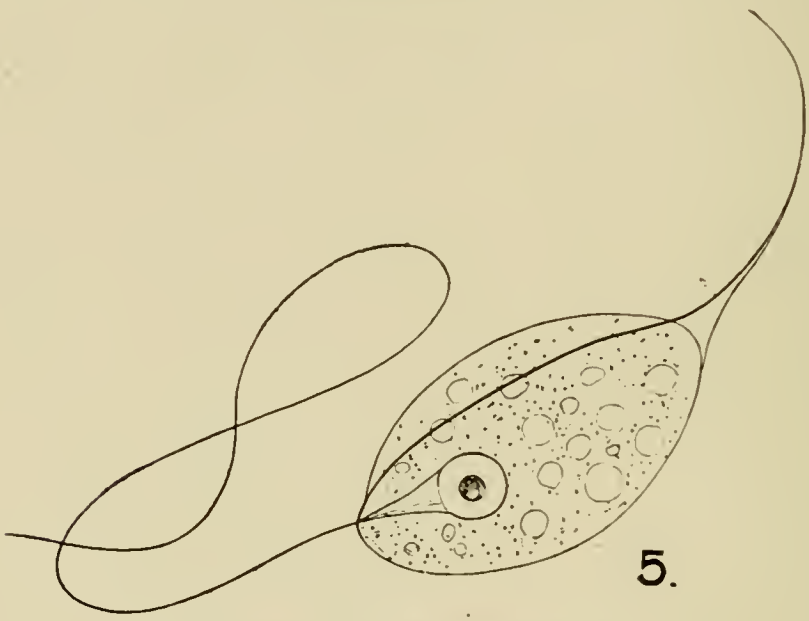
Division-stage of free-swimming form.

the nucleus with the base of the two flagella. The details of these structures are much more evident in the fixed and stained films. The protoplasm of the body contains food and other vacuoles, but contractile vacuole is not present. Sometimes the nucleus is surrounded with refractile granules, having the same greenish line and refraction as the karyosome within the nucleus. These may be present in sufficient numbers as to completely obscure the nucleus. Similar granules occur in the protoplasm of encysted forms (fig. 6). These granules stain deeply, and are possibly of a chromatin nature.

In the hanging-drop preparations especially this organism

exhibited a peculiar polymorphism. In the central part of the hanging drop, where the fluid was deep, the flagellates had the typical pear-shaped appearance, with the long, tapering, posterior extremity. At the sides of the hanging drop, where there was only a thin layer of moisture on the cover-glass, the typical pear shape was lost and the flagellates had the appearance of amœbæ. When first I observed this I thought my culture had become contaminated with an amœba, but the

Text-fig. 5.



Ordinary free-swimming type.

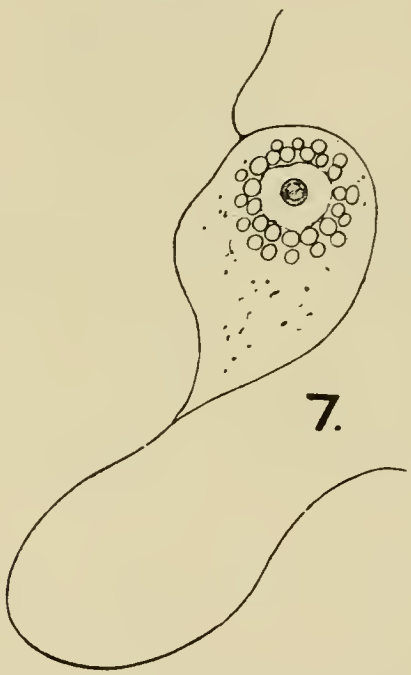
presence of the long anterior flagellum and the short posterior one disproved this idea. It was possible to watch a single individual swimming in the deep part towards the edge. On reaching the shallow part the character of the organism changes at once to the amœboid form. Pseudopodia are protruded and withdrawn, and the animal creeps about in a typical amœboid manner. All this while the long anterior flagellum is lashing to and fro, but appears powerless to draw the animal across the surface of the cover-glass. It is only in the deeper part of the hanging drop that the flagellum is useful. The posterior flagellum is often not visible, and its prolongation across the surface of the body is more difficult to detect.

When seen it is inert and only moves in a passive manner. It seems to take little share in movements of progression.

On the surface of the agar medium the organism is generally of the amœboid form.

At the edge of the hanging-drop preparations or on the surface of the agar it is easy to watch these amœboid forms ingesting food by surrounding objects with pseudopodia. As a rule the amœboid forms contain many more food-vacuoles than those swimming in the deeper layers.

TEXT-FIG. 7.



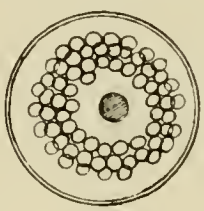
Less regular encysted form.

Reproduction is by longitudinal division. There is first multiplication of the flagella, whether by new formation or division of those already existing has not been determined. The nucleus next divides. The karyosome is divided into two parts, and finally the elongated nuclear membrane becomes constricted and two nuclei are formed. After a short time the protoplasm becomes drawn out and finally a constriction appears, which ultimately ends in complete division. The process of this division is very readily watched on the cover-

glass cultures described above. Both the amœboid and the free-living forms divide in this manner, but on account of the more sluggish movements of the former they are more readily kept under observation.

In the cultures encysted forms commence to appear after a few days. In the liquid cultures they are to be found in the scum on the surface or in the deposit at the bottom. On the agar cultures the cysts appear in the older parts of the culture. On this medium the margin of bacterial growths spreads over the surface, and in this margin the actively reproducing flagellates are to be sought. In the oldest part of the culture no free flagellates can be found, but only the cyst.

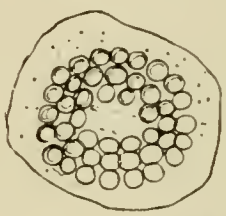
TEXT-FIG. 6.



6.

Encysted forms showing refractile granules surrounding nucleus.

TEXT-FIG. 8.



8.

Free form with refractile granules. Probable preparation for encystment.

In the fresh condition these cysts appear as slightly brownish spherical bodies, with a wall of double contour.

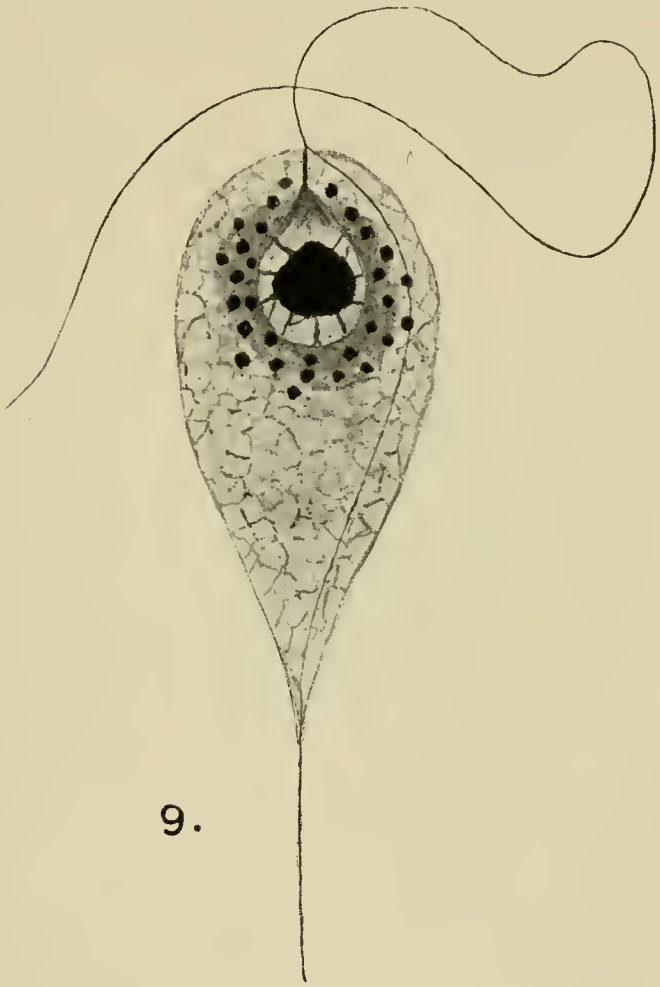
At the centre of the cyst is the spherical nucleus, which has similar characters to that of the free form, except for the prolongation towards the flagella. The nucleus is surrounded by the bright refractile granules, which were described as occurring in some of the free forms. It is probable these granules are of a chromatin nature, and that they arise from chromatin passed out from the nucleus, though this process has not been followed.

Though these organisms have been kept under observation for a year or more conjugation has not been seen, nor has any sexual process been detected. It is possible that some sexual process is bound up with the encystment, but as the

entrance into and emergence from the cyst has not been directly observed and no multiplication within the cyst could be seen nothing definite on this point can be stated.

Text-figs. 9–19.—Drawings from stained preparations.

TEXT-FIG. 9.



Free-swimming form with granules round nucleus.

FIXED AND STAINED SPECIMENS.

In the fixed and stained specimens, in addition to the details which were so clearly visible in the living organism, others could be made out.

The protoplasm of the body has a marked alveolar structure. The anteriorly placed nucleus shows a large, deeply staining

karyosome, while connecting this latter body to the nuclear membrane is a coarse linin network. All the chromatin of the nucleus appears to be concentrated in the karyosome. The prolongation of the nuclear membrane towards the

TEXT-FIG. 10.

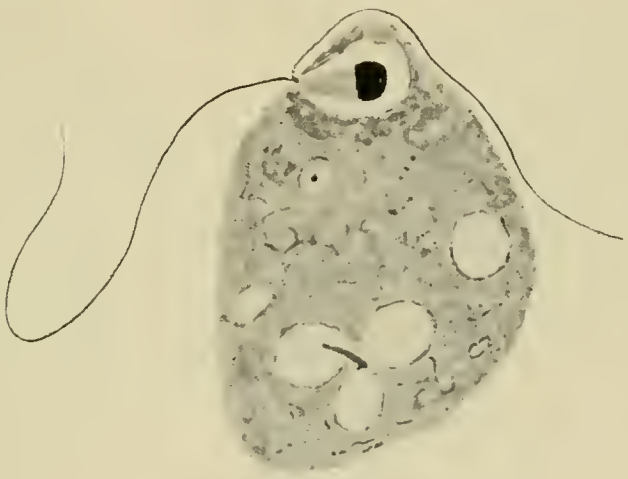
Side view of free form showing the flattened side along which the flagellum runs.

flagella is clearly shown, while the base of these organs is connected to the apex of the nucleus by a rod-like rhizoplast. In some cases the drawn-out apex of the nuclear membrane shows longitudinal markings, which converged toward the rhizoplast, while in others there is a connection in the form of a more deeply staining pyramin between this body and the

karyosome (fig. 11). Prowazek describes for Cercomonas longicaudia a "ein Art undeutlichen Zwischenfibrille," which connects the karyosome to the insertion of the flagella. Prowazek figures this connection as a dark line running from the karyosome to the apex of the nucleus, but I have not been able to detect any structure as definite as the one he figures.

This flagellate is a very excellent illustration of the fallacy of relying for detail on the old dry Romanowsky methods of

TEXT-FIG. 11.



11.

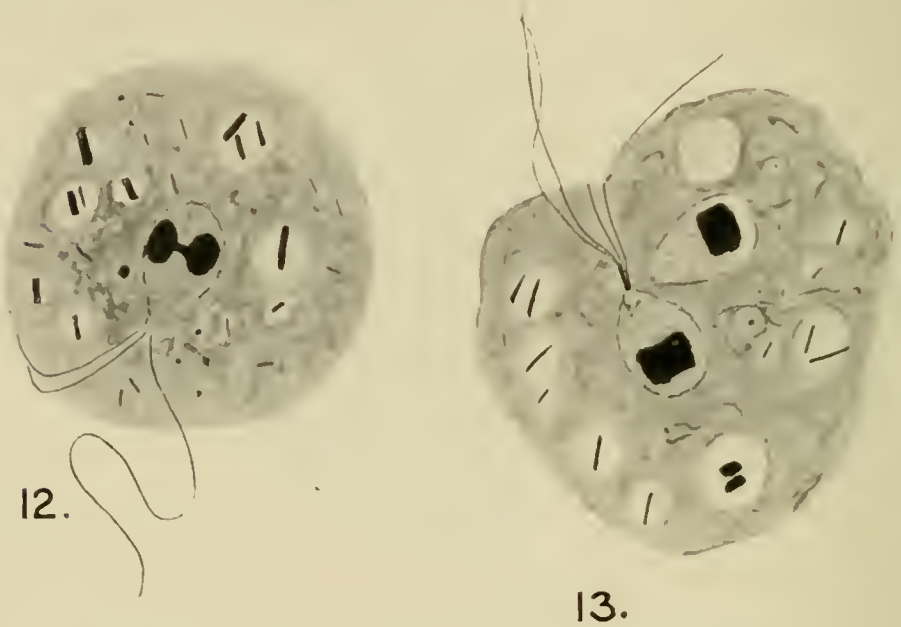
Shows connection of karyosome and rhizoplast.

staining. The nucleus of this organism is clearly visible in the living condition. There is a definite nuclear membrane. At the centre of the nucleus is a large refractile karyosome, while the space between this body and the nuclear membrane is free from granules. The nuclear membrane is drawn out at one point towards the insertion of the flagella. Now if a film of the material containing this flagellate is allowed to dry as in the usual method for the preparation of blood for staining trypanosomes, and stained by one of the modifications of the Romanowsky method, the result may be very beautiful from the colour point of view, but totally misleading in the structure of the nucleus. This latter organ appears in these

dried films as an irregular clump of red staining granules. In other words, its appearances are like those of the nuclei of trypanosomes in similarly prepared films. In films fixed and stained by the wet method described above the structure of the nucleus is comparable with the appearances to be made out in the living organisms.

The details of longitudinal division can be followed in the

TEXT-FIGS. 12, 13.



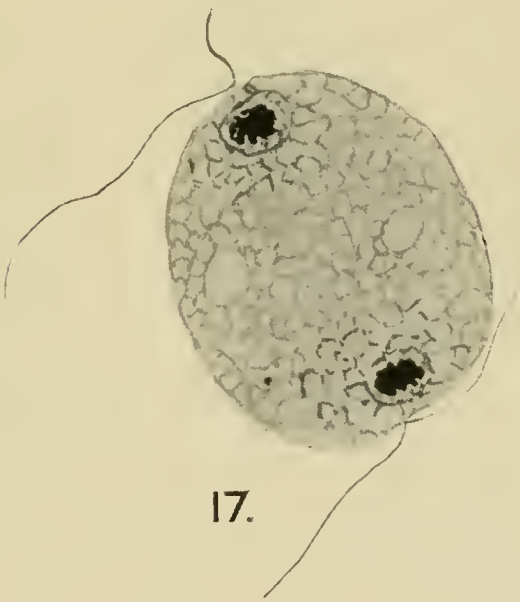
Dividing forms.

stained preparations. The large karyosome becomes elongated and constricted, and finally divided into two parts (fig. 15). I was never able to detect within the karyosome a centriole, spindle, and æquitorial plate, as described by Berliner in the division of Copromonas major, but the division takes place in an amitotic manner, resembling that of Copromonas subtilis (Dobell). Most usually the karyosome becomes distinctly dumb-bell shaped as in fig. 12, but at other times the division is along the longitudinal axis of the elongated karyosome, the resulting daughter-karyosomes each being elongated (figs. 15, 16). Following the division of the karyosome the nuclear membrane elongates while the daughter-

karyosomes separate. The flagella are duplicated at this stage, but they still have a common rhizoplast, which is inserted into one point of the elongated nuclear membrane, which is drawn out slightly at this point towards the anterior end of

TEXT-FIGS. 14-16.

TEXT-FIG. 17.

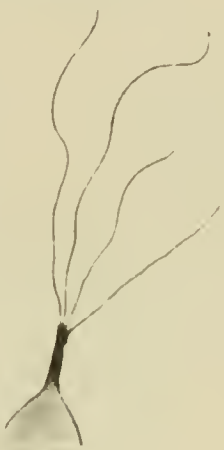


Dividing forms.

the body of the flagellate. Division of the nuclear membrane commences by a constriction at the point opposite the insertion of the rhizoplast. The division is completed, and the two nuclei, each with an apex, are connected to the base of the rhizoplast. The rhizoplast finally divides longitudinally, so that there result two nuclei, each with a rhizoplast and two flagella. The exact method of origin of the flagella I was

unable to trace, though some of the appearances seem to indicate the formation of two new ones by outgrowth from the rhizoplast. In fig. 18 is the nuclear apparatus of a flagellate partially broken up on the film. It shows very clearly the single rhizoplast with the duplicated flagella. The last stage in the division process is thus the splitting of the rhizoplast, while the first stage is the multiplication of the flagella and the commencing division of the karyosome. After complete division the nuclei pass to opposite poles of the body (fig. 17),

Text-fig. 18.



18.

Part of nucleus, rhizoplast, and flagella of partly broken-down individual, to show the multiplication of the flagella before division of the nucleus and rhizoplast.

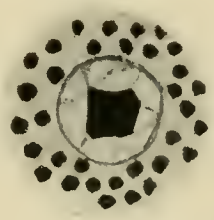
and after a varying interval of time the body is divided into two equal parts.

The bright refractile granules which were described above as occurring in the protoplasm around the nucleus in the encysted forms and in some of the free forms appear in the stained specimens as dark-staining granules. Whether these are chromatin granules of the nature of a chromidium or whether they are capable of some other interpretation cannot be definitely stated, since their fate has not been followed. They certainly stain as chromatin, and their presence within the cyst (fig. 19) would seem to suggest the possibility of

their being nuclei of spores destined to escape from the cyst and ultimately to develop, with or without conjugation, into the adult flagellate form. Though cysts have been constantly kept under observation and every inducement possible to encourage the emergence from the cyst has been tried, I have never been fortunate enough to witness this process. That it does occur is borne out by the experiment of adding dried cysts to fresh medium, resulting in a culture of flagellates.

In the stained preparation certain appearances are capable of interpretation as a conjugation of the flagellates, and some of the nuclear appearances as processes of maturation, but as no undoubted conjugation was observed in the living flagel-

Text-fig. 19.



19.

Cyst showing dark-staining granules surrounding the large central nucleus.

lates I refrain from describing these. Without the control of observation on the living forms descriptions of conjugation and the accompanying nuclear changes are of little value, since the possibility of error in interpretation is very great. For Copromonas major Berliner has described from stained preparations such a process of conjugation, but without the necessary controls it is always possible that abnormal or involution forms have been mistaken for such stages.

In rich cultures of the flagellates there is a very great variation in size. Some individuals are comparatively large, reaching a length of 15 μ or more, excluding the flagella. Others are very minute, being not more than 2–3 μ in longest diameter. All intermediate sizes are to be met with in the cultures. The encysted forms have a diameter of about 6 μ or

7 μ. These cysts will withstand drying at ordinary laboratory temperatures, and are capable of giving rise to fresh cultures when brought into suitable media.

Nomenclature.

It is certain that Dujardin's original description of the genus Cercomonas is incomplete, but it seems to me quite clear from his account that he was dealing with flagellates similar to the one described in this paper. Though he did not definitely state that the fine drawn-out posterior extremity of the body was a flagellum, still, he says that it was at times so fine as to resemble the anterior flagellum, and that it was capable of independent movements. Further, in his table of classification he divides the Monads into two groups. In the first he includes forms with "un seul filament flagelliform," while in the second those with "plusieurs filaments ou appendices." The genus Cercomonas appears in the second of these groups as a form with "un second filament ou appendice postérieur." It is therefore quite evident that Dujardin regarded this posterior termination of the body as of the nature of a flagellum. Stein and Blochmann describe the genus Cercomonas as having a drawn-out posterior end, though they do not describe a definite flagellum. The genus Cercomonas was not accurately defined by Kent or Bütschli, and to Klebs the confusion seemed so great that he proposed the rejection of this generic name and the substitution of Gruber's name Dimorpha, which was created for a bi-flagellate showing at certain stages definite heliozooid characters. In this genus Dimorpha Klebs included forms which he identified with those described originally by Dujardin as Cercomonas, and he suggests that this observer has overlooked the second flagellum. We have seen how near Dujardin was to definitely describing this second flagellum, so that the action of Klebs in rejecting this genus is hardly sound. It seems to me clear that the forms described by Dujardin really possessed two flagella, though

he failed to see this clearly. On this account I think it safer to retain the genus Cercomonas for flagellates of the character described in this paper, viz. flagellates with an anterior blunt end from which arises a single long flagellum and a posterior tapering end also with a flagellum, traceable over the surface of the body towards the insertion of the anterior flagellum. This conclusion is come to by Prowazek also, who figures Cercomonas longicauda with two flagella arising from the nucleus.

The specific name of this flagellate is difficult to determine. Dujardin named several species of Cercomonas, though he was careful to state that he was far from regarding these as true species, but as a convenient means of distinguishing the forms met with in different infusions. From the figures of Dujardin and Stein it is possible that the flagellate belongs to the species longicauda, so that the flagellate described here may be assumed to be Cercomonas longicauda Dujardin.

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Some Observations on a New Gregarine (Metamera schubergi nov. gen., nov. spec.).

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With Plates 15 and 16.

Contents.

Introduction.

While working at Heidelberg in 1906, under Professors Bütschli and Schuberg, the latter kindly called my attention to a new species of gregarine in the gut of Glossosiphonia complanata L. (Clepsine sexoculata), and suggested its further investigation. The preceding summer, while busied with a recently discovered coccidium occurring in the leech Herpobdella atomaria Car. (= Nephelis vulgaris), Professor Schuberg turned his attention to Glossosiphonia complanata, which occurs in company with Herpobdella in the Neckar and occasional ponds in the Heidelberg district. Deeming it probable that two forms so alike in habit and environment might harbour the same parasites, he dissected

several specimens of this leech, and, though the results were in the main negative, he found several animals infected with a species of gregarine. Reference to the literature proved the parasite to be identical with a species briefly mentioned by Bolsius in 1895 (2), and the subject of a more detailed but still fragmentary paper in 1896 (3). Beyond a superficial study carried on incidentally during his work on the Glossosiphonia Bolsius seems to have paid no further attention to the parasite, which remained unnoticed until 1900, when Castle (5), in an exhaustive treatise on the N. American Rhynchobdellidæ and their parasites, mentions having observed the gregarine seen by Bolsius in about half the specimens of Clepsine elongata which he examined. He adds, however, that he only finds the animals in the stomach diverticula, and never in the intestine or crop, as indicated by Bolsius in his diagrams. Castle also mentions encysted protozoa which he found in C. fusca, and suggests the possibility of their relationship to the form in G. complanata. The cysts he found in the muscle-layers of the body-wall, so that they probably have nothing to do with the gregarine in question.

Lühe (14) quotes the parasite as having been mentioned by Bolsius, and suggests that it probably belongs to the tricystid gregarines.

The gregarine is thus a new and previously undescribed form, for which I propose the name Metamera schubergi.[1]

In the preparation of the sections and the study of the living animal, during the last few weeks of my stay in Heidelberg, Professor Schuberg assisted me most kindly in every way in his power; and it is due solely to him that I was able to obtain Bolsius' principal pamphlet. My thanks are also due to Geheimrat Prof. Bütschli, whose practical suggestions I found of the greatest value.

[1] The form which appears most closely allied as regards structure of the trophozoite is Echinomera. A study of the life-history, however, has revealed points of difference which seem to warrant the creating of a new genus for the form under consideration.

By the kindness of Professor Sedgwick, who allowed me a free hand in the laboratory of the Imperial College of Science, S. Kensington, I was able eventually to complete my study of the sections. And in this connection I must express my indebtedness to Mr. C. C. Dobell, who is at present lecturing at the College. His unrivalled knowledge of protozoan life-history and technique has always been most generously placed at my disposal, and has proved of the greatest value in the preparation of this paper.

Material and Methods.

The leech which serves as host to Metamera schubergi is Glossosiphonia complanata Linn. A few specimens of Hemiclepsis marginata were also found infected. The leeches live under stones in shallow water—running by preference—though I have found them in smaller numbers in still pools. The material was collected at Heidelberg from the shallows left by the summer fall of the Neckar in the neighbourhood of the electric power station, below the new bridge, and also from the opposite bank, along the wall separating the skating rink from the river itself. The leeches are fairly common, and may be found clinging firmly to the under-side of stones at the water's edge, especially in the numerous lumps of red sandstone which litter the shore everywhere.

Recently I examined some specimens of Glossosiphonia complanata sent me from the neighbourhood of Cambridge, and found them well infected.[1] These latter were obtained in January, when the leeches are hard to find owing to the scanty vegetation in the ponds in winter. In all the specimens I examined from this source I only obtained one cyst, and that a very small and early one.

The leeches can be kept for an indefinite period in a good-sized glass jar, provided the water be aërated by passing bubbles of air through it. Food is not necessary, though a

[1] For this I have to thank Mr. Harding, and also for his kindness in assisting me to determine the species.

few small water-snails are much appreciated. Owing to the transparent nature of the integument in Glossosiphonia, the parasites are visible in the living leech ; and if the latter be forcibly pressed between two slides provided with wax corners, and examined under a low magnification, the gregarines may sometimes be detected in the stomach diverticula and intestine. Unfortunately, however, this method of diagnosis is by no means infallible, as the numerous pigment-cells with their clear nuclei look very like gregarines, and render accurate observation impossible. The gregarines occur in the hindermost stomach diverticula and the intestine, just as indicated by Bolsius in his diagram. The cysts are found in the same regions of the alimentary canal, but are especially numerous in the intestine.

Examination of sections shows that cysts can develop as far as the sporoblast stage in the intestinal canal of the host, though they are often expelled with the fæces at a much earlier stage in development.

In sections just above the anus no cysts were to be seen. This part of the gut was almost occluded by a mass of cephalonts and some sporonts of a peculiarly blunt outline. The leech from which these sections were cut had previously evacuated fæces containing a few very early cysts among a greater number in which sporoblasts could be distinguished. As many as ten cysts have been counted in one section.

To obtain the gregarine, the infected leeches were partially dried on blotting-paper and the under-surface opened by three incisions—two parallel and close to the margins, and one at right angles to the long axis of the animal, at about the junction of the middle and anterior thirds. The flap of tissue was then carefully turned backwards towards the anal sucker, the animal being placed in a watch-glass containing normal saline solution. The gut-contents were thus emptied into the saline, together with connective tissue, which is of no account. By the aid of a hand-lens the gregarines could now be seen sticking to the bottom of the glass, or still fixed to fragments of the host-tissue. These latter are useful in

studying the structure of the epimerite, as this organ, in the course of the teasing out, is very easily torn away, leaving decapitated individuals which may be confused with true sporonts. By gentle coaxing with a pipette the gregarines can be freed from the bottom of the watch-glass and transferred to a slide for further handling.

Preparations in toto were made originally under a cover-slip provided with wax feet, and the various reagents drawn through with blotting-paper. In this way, by fixing the gregarines with alcohol and glacial acetic acid (9 : 1), a large number of animals may be treated under one cover-slip, which is an obvious advantage. More recently I made some preparations by fixing the selected gregarines in a watch-glass with picro-acetic acid (3 : 1) and adding the various fluids by means of a pipette and eventually picking out and mounting the stained gregarines under a low magnification. I consider the former method of treatment the more satisfactory and certainly less laborious. As stains for these preparations I used Grenacher's alcoholic carmine solution and Schuberg's modification of Mayer's acid carmine. This latter solution, being acid in reaction and not neutral, has the power of penetrating the cuticle, and in employing it the preparations must be very rapidly washed through with $\frac{1}{2}$ per cent. solution of HCl to prevent precipitation of the carmine during the further treatment with the alcohols. Leeches destined for sections were fixed either in Gilson's fluid or in the above-mentioned alcohol and acetic mixture. Gilson's fluid should act for two or three hours, and the sublimate constituent be most carefully washed out with iodine-alcohol or a solution of KI in 75 per cent. alcohol. As staining reagents hæmatoxylin (Delafield's) and eosin, safranin, and Heidenhain's iron-hæmatoxylin were employed. Owing to the paucity of material, the laborious expedient of applying both methods in succession on the same preparation had to be employed. It was found that hæmatoxylin and eosin were satisfactory for the cephalonts and sporonts, but gave very incomplete and misleading results with the nuclear changes of the encysted forms, which

were defined much more distinctly with the iron-hæmatoxylin method. All tissues were embedded in paraffin, with chloroform as the intermediary fluid.

Culture of the cysts.—To obtain the ripe spores the cysts were simply placed in the moist chamber, where, in the course of seven or eight days, the spores were developed. The cysts were either placed simply on a slide in a drop of Neckar water or under a cover-slip provided with wax feet. The cysts dehisced by simple rupture after about seven or eight days. Cysts placed in normal NaCl solution in the moist chamber did not develop successfully.

Structure of the Trophozoite.

The body is divided by septa into epi-, proto-, and dentomerite, and is elongated in form (figs. 1–6). Some individuals have a more thick-set appearance than others, especially in the extreme hinder end of the gut, where the gregarines are often crowded together. The animal measures about 150μ by 45μ. At the posterior end of the dentomerite there are often present indications of further subdivision of the body, and occasionally as many as three complete segments are seen (fig. 4). This segmentation is not confined to gregarines of any peculiar build, being present in both long and short forms, and it varies in the degree of development of the segments. It was present in about a third of the gregarines examined alive in Heidelberg, and is also very distinct in the preparations of these animals made at the time. The Cambridge gregarines also showed segmentation, though it was distinctly less in evidence, both in the living animal and in carmine preparations of it. It appears to vary greatly —from the very faintest indication to quite definite septa. It must be stated in this connection that no segmented gregarines were seen in the sections of the infected leeches, though constantly found in preparations made by teasing out the host-tissues. This compels one to consider the possibility of injury during extraction being the cause of this segmenta-

tion, although the stained preparations do not in the least degree support this suggestion.

The epimerite is a dome-shaped structure. It is provided with short club-like processes, recalling those of Echinomera, but often branched, arranged in a dense ring around the line of junction with the protomerite, and also on the roof of the dome (figs. 4 and 5). These latter processes are markedly shorter than those of the ring, and decrease in size as the apex of the epimerite is approached. The processes are perforated at their somewhat clubbed ends by small pores, clearly to be seen in the freshly mounted living gregarine by the aid of a $\frac{1}{12}$ in. oil-immersion lens. Judging from analogy with such forms as Echinomera and Pterocephalus (Nina), and also from the appearance seen in sections across the point of fixation to the host, there is no doubt that fine pseudopodia are protruded through these pores, which fix the gregarine to the intestinal mucous membrane of the host. The fixing apparatus is by no means easy to identify, as, owing to the unavoidable roughness of the dissection, the gregarines are rudely torn from their moorings, and almost invariably carry away with them a crown-like fringe—derived from the host-cells—which surrounds the epimerite in the zone of the processes, and obscures all details of its structure (fig. 3).

When kept under observation for some time—say an hour or so—in NaCl solution, a curious phenomenon ensues. Just at the line of junction between the protomerite and epimerite a bubble-like vacuole appears, which gradually increases in size, and carries with it the fringe of host tissue with the embedded processes till they sit like a crown on its upper pole, sometimes symmetrically, sometimes displaced to one side. Having reached a diameter about equal to that of the protomerite the vacuole bursts, and the gregarine is suddenly deprived of its epimerite (fig. 2). This vacuole formation has been seen by Léger and Duboscq to occur in Pyxinia (**14**), and in my opinion has a probable bearing on the mooted question regarding the fate of the gregarine epimerite, in the

transition from cephalont to sporont. Frenzel (14) believed the epimerite to be absorbed in a manner similar to the assimilation of a tadpole's tail. He found among numerous cephalonts with large epimerites individuals with but a minute projection from the protomerite, and he regarded this as a scene in the gradual absorption of the epimerite. The sudden disappearance he regarded as pathological, and due to changes in the surrounding medium. My own observations point to the same conclusion. The vacuole formation quoted above is plainly due to plasmoptysis, which can be followed under the microscope from its earliest onset to the bursting of the bubble. Further, when the gregarines were examined in a special solution of egg-albumen, NaCl and camphor, as prepared by Professor Bütschli, the vacuole formation was considerably delayed; a fact explicable on the ground that the solution more nearly resembles the natural environment of the gregarine.

The behaviour of the finger-shaped processes also points to the epimerite being absorbed rather than directly thrown off when the cephalont becomes free. In gregarines which are normally lying free in the gut the processes are never to be seen (figs. 1 and 6). The epimerite is still present, but the processes have been withdrawn during the process of separation from the mucous membrane; just as they are absorbed in Echinomera when the cephalont becomes free in the gut (17). This applies to all the free-lying specimens seen in sections, and to a solitary living form which, together with several cysts and some fæces, was pressed out through the anus during examination of a leech between two slides (fig. 1).

In the living sporont (fig. 1) the extreme anterior end of the animal is quite transparent and devoid of granules, a few of which, separate from the main endoplasmic mass of the epimerite, may be seen showing Brownian movement along its anterior border. After some time the whole granular body of the gregarine appears to shrink back somewhat into the cuticular sheath which envelopes it, and this clear area

enlarges proportionally until almost the whole of the conical knob which forms the epimerite is clear of granules. During this process all three divisions of the endoplasm are still quite distinct. By the time this stage has been reached osmosis asserts itself, and the vacuole formation mentioned above commences (fig. 2). In sections, however, the free-lying sporonts all show a curious thickening of the extreme anterior end of the epimerite, which behaves towards stains in the same way as the rest of the cuticle, being, in fact, a thickening of the latter anteriorly (fig. 6). It seems a feasible explanation of this structure to say that it represents the cuticular constituents of the numerous processes of the epimerite, which have been retracted on the animal becoming free. It may here be mentioned that Lühe (**14**), in his review of the gregarines generally, pronounces in favour of the casting off of the epimerite as the typical way in which the cephalonts become free.

The nucleus lies in the deutomerite. It consists of a nuclear membrane enclosing a clear ground substance, in which lie a large vacuolated karyosome and a number of masses of chromatic substance (fig. 7). The specimens from which figs. 3 and 4 were drawn were very faintly stained owing to excessive washing out, but some other preparations stained with Grenacher's carmine confirm the appearances seen in sections, especially as regards the vacuolated nature of the karyosome. The nuclear area is about 18 μ in diameter; the karyosome measures about 8 μ, and as a rule contains one very large vacuole and several small ones. The large chromatin masses are scattered irregularly throughout the nucleus, and are of varying shape. The nuclear membrane is well marked, and in common with the karyosome and the chromatin masses stains deeply with both Delafield's hæmatoxylin and Heidenhain's iron-hæmatoxylin. The ground substance takes on a very faint blue tinge with iron-hæmatoxylin. In some of the sections the karyosome has yielded almost completely to the differentiating iron alum, and appears grey by contrast with the black chromatin masses. In

these cases its vacuolated structure is very plain (fig. 7). As a rule, however, the karyosome shows very deeply stained in the adult nucleus. Besides the nucleus there are usually to be seen scattered throughout the body patches of a substance which stains deeply with chromatin stains. These patches have been described by Berndt (1) and others, and are especially numerous in the protomerite. Comes (7) has recently shown that these appearances in Stenophora are probably due to metabolic products, and are not nuclear. There are also deeply stained granules in connection with the epimerite processes in sections stained with iron-hæmatoxylin, as described by Schellack in Echinomera hispida (17).

Cyst-Formation and Development of the Spores.

The act of association of two animals to form a cyst has not been observed in the living animals. As indicated above, in the sporont the epimerite tends to become less prominent, while a pad of cuticle forms anteriorly. Simultaneously with this shortening of the long axis of the body the protomerite increases in breadth and bulges, particularly around the edges of the apical cuticular pad. From sections it would seem that the two animals come together with their epimerites in contact. A ring of cuticle now arises around the base of the terminal pad in one animal. Into the cup formed by this ring the cuticular pad of the other gregarine is inserted, while external to, and dovetailing with the ring of the cup, a similar ring of cuticle arises in the second animal (fig. 37). In very young cysts in which the nuclei of the two animals are still unaltered the above arrangement of the parts is very clear; but as development proceeds the septum of cuticle dividing the encysted sporonts becomes increasingly irregular. In this region in the earlier cysts there are patches of deeply stained material suggestive of membrane, which are probably the remains of the cuticle of the contiguous epimerites (fig. 13).

Behaviour of the nucleus preparatory to the

formation of the first two daughter-nuclei.—Although the material which I was able to collect was very limited, I was fortunate in obtaining one leech very heavily infected. In the intestine of this animal I found numerous cysts, and also an enormous number of adult gregarines mostly fixed to the gut-wall. A study of these sections has revealed several phases of the first division of the nucleus, though to elaborate all the stages is impossible without further examples, which I hope shortly to procure. In order, therefore, to make the most of this limited material, I employed first hæmatoxylin (Delafield's) and eosin, and then after decolorisation with acid alcohol, re-stained by Heidenhain's method. This latter method revealed numerous important facts quite indiscernible with the original staining. My thanks are due to Dr. Pembrey, of Guy's Hospital, who very kindly provided me with all the necessary apparatus for staining.

For some time at any rate after a definite cyst-wall has formed, the nuclei of the encysted gregarines remain apparently unaltered. Then the chromatin masses begin to fragment, with the result that chromidia are formed within the limits of the nuclear membrane. Simultaneously, this membrane becomes increasingly thin, and the karyosome throws out masses of substance from its interior, becoming in consequence markedly reduced in size. These masses are more or less spherical and of distinct outline; they stain very deeply, showing black with iron-hæmatoxylin. Their number and size vary greatly (figs. 9–14). At times one large mass is present, almost equal in size to the original karyosome; at others, numbers of small masses are seen. The actual process of extrusion of one of these masses is shown in fig. 36. After their extrusion, the main karyosome-relic shows a blue colour with hæmatoxylin and eosin, as contrasted with the more purple hue shown by the intact karyosome and the chromatin masses of the trophozoite nucleus. The extruded masses on the other hand behave throughout, as regards stains, like the chromatin masses. After the fragmentation of the

chromatin masses and the breaking up of the karyosome have proceeded for some time, a new structure appears in the nucleus. In close proximity to the main karyosome residue, which is seen lying near the periphery of the nucleus, an ill-defined mass appears which takes up nuclear stains very definitely. The earliest appearance of this mass is shown in fig. 9 before the chromidia formation has progressed very far. A slightly later stage is shown in figs. 10 and 11, where the nuclear area presents a homogeneous appearance, without any signs of the chromidial elements being discernible, while the neighbourhood of the main karyosome residue is occupied by a somewhat elongated mass, showing faint longitudinal striation (fig. 11). The relative size of this mass, which I will call the "achromatic mass,"[1] is shown in figs. 9, 10, 11. It will be noticed that the various products of the karyosome are in close connection with it.

At this stage, the absence in my preparations of any structures distinguishable as definite chromosomes or centrosomes is to be emphasised. The achromatic mass stains deeply with iron-hæmatoxylin, but yields to the differentiating iron-alum before the karyosome and its products become decolorised.

The next stage in the division represented is shown in figs. 12 and 13. The achromatic mass has increased in bulk and definition, and has become more drawn out. The striation is very marked, and for the first time in the course of the division the true chromosome element appears. At each pole of the achromatic mass, which is now distinguishable as a true spindle, there is a small black mass of chromatin; while converging towards this mass, like the ribs of a basket, are seen deeply stained streaks of granules of chromatin, arranged upon the spindle-fibres and obviously *en route* for the respective poles of the figure. It may here, again, be seen that the spindle stains very deeply with chromatin stains, and

[1] I call this structure the "achromatic mass" because of its function —as seen in its later development—and not on account of its staining properties.

it is only on very thorough differentiation that the chromosomes are rendered visible. The spindle fibres appear to merge with the terminal chromatin mass. Distal to this there is no true astral arrangement visible.

Each terminal chromatic aggregation now gives place to a definite vesicular structure, situated at the poles of the spindle and forming the centre of a definite astral radiation (figs. 14 and 15). Simultaneously with the appearance of the vesicle, the chromatin streaks and granules disappear from the spindle, so that the more definite the terminal vesicle, the fewer the chromosomes on the spindle. Fig. 12 shows a ring-like arrangement of the terminal chromatin aggregation at one pole of the spindle (*a*), while fig. 15 shows a true polar vesicle containing definite granules of chromatin, in one instance arranged indiscriminately around the circumference, in the other accumulated at one point upon it. These vesicles are the points upon which the very definite spindle-fibres converge, and measure from $1\frac{1}{2}$–$2\frac{1}{4}$ μ across. In figs. 14 and 15 it will be noticed, firstly, that—apart from the granules within the vesicles and the karyosome products—there are practically no other discrete chromatin elements to be seen; secondly, that some of the spindle-fibres plainly run down into the midst of the nuclear area and the karyosome remnants, where these latter are not already lying on the spindle. In fig. 15 will be seen, lying close to the large irregular karyosome residue, a collection of deeply stained granules, which are connected with the karyosome and with each other by deeply stained strands. They have probably been recently thrown out from the karyosome, which is much distorted from its original spherical shape.

The latest stage of the first division represented among my slides was unfortunately injured before anything more than a rough drawing had been made of its structure (fig. 16). It represented the spindle very much drawn out, just before the final separation of the two daughter-nuclei. There was at each pole a well-marked vesicle, containing numerous granules of chromatin, and distal to this vesicle was a mass of achro-

matic substance, showing within it a granule of deeply stained substance. The figure was very suggestive of the state of affairs seen in fig. 18 *a* and *b*, with, however, a single polar granule. The sparsity of material unfortunately renders a complete account of the first division-phenomena out of the question. From a careful study of the slides at my disposal I suggest the following as the more striking points, the significance of which I shall revert to later on (see p. 278). Firstly, the depth to which the spindle proper stains with both Delafield's and Heidenhain's hæmatoxylin: secondly, the proximity of the karyosome to the origin of the achromatic mass, and, later on, the very definite spindle-fibres running down in among the karyosome remnants and the site of the old nucleus: thirdly, the absence of regular chromosomes such as can at any stage be outlined or counted with anything approaching certainty: fourthly, the vesicles at the poles of the later spindles, which form the centres of definite astral figures. The nature of these vesicles it is difficult to decide. Are they centrosomes or incipient daughter-nuclei? As will be seen later, the daughter-nuclei are strikingly vesicular; and the fact that, if these vesicles are considered as centrosomes pure and simple, there are no other defined chromatic elements in the spindle figure, seems to indicate their being early stages of the daughter-nuclei. This being the case, the centrosome must be sought either in one of the granules on the circumference of the vesicle, or distal to the latter. On this point, though tempted to an explanation, I dare not base a theory upon a drawing so diagrammatic as fig. 16.

Proceeding to the further division of the daughter-nuclei, all uncertainty about the centrosome vanishes. In the earliest stages, where eight or nine nuclei are present in each cyst (fig. 17 *a*, *b*, and *c*), the astral radiations are very marked, and the centrosome consists of a deeply stained mass at the periphery of the nuclear vesicle, from which emanate the striæ. These, where they spring from the centrosome, are extremely obvious. In fig. 19 *c* and *d*, stained with hæma-

toxylin and eosin, the centrosome is differentiated into a faintly stained peripheral portion—the centrosphere—in the centre of which is a black centriole; this also shows in fig. 20 stained in the same manner.

In studying the various generations of daughter-nuclei several interesting points demand attention. They present an infinite variety as regards the arrangement of their chromatin. Except when actually drawn out into a spindle they are invariably vesicular in structure; and, in the great majority of cases, in the earlier stages at any rate, they contain a distinct karyosome. This is of interest in that in Echinomera hispida, described by Schellack (17), where the karyosome invariably appears in the daughter-nuclei, its origin is referred to the unpaired chromosome of this form, which chromosome thus has a function allotted to it. In Stylorhynchus, which also shows this phenomenon, there is, however, no such unpaired chromosome (11). The fate of these daughter-karyosomes in Metamera schubergi is not certain. The corresponding spindle figures do not show any traces of karyosome fragments in their neighbourhood. On the other hand, in such stages as shown in figs. 19 *c* and *d*, where the nucleus is on the point of elongating into a spindle, the karyosome seems to be extruding part of its substance. If this is so, the process is one of immediate and complete solution, and not exactly parallel with the behaviour of the adult karyosome. It must be clearly understood that, as the figures show, a karyosome cannot be always with certainty identified in these daughter-nuclei. There are always present masses of chromatic substance of varying sizes, and their arrangement is at times such as to make the distinction impossible. In the daughter spindle-figures, as with the first division, there is again no definite chromosome formation. The chromatic elements are sometimes discernible as streaks and granules near the poles of the spindle; sometimes the deep black appearance of the spindle-fibres, alone present, suggests that these latter may be conveying chromatin in very minute particles. A constant feature of these young

spindles is a black mass of deeply staining matter at the extreme poles. In some early spindles shown in fig. 18 *a*, the earliest actual daughter spindle-stage to hand, this polar mass is seen as two adjacent granules or centrioles lying in a definite centrosphere showing radiations. In fig. 18 *b* these two granules are connected by a deeply staining link. This I interpret as the early division of the centrosome, occurring almost before the daughter-nuclei, which in the figs. 18 *a* and *b* are distinguishable as faint vesicles, are free from their parent spindle. In this connection it is of interest to note that the daughter-nuclei always appear provided with two centrosomes. I have not been able to discover any with a solitary centrosome. This is in keeping with the above suggestion as to the early division of the centrosome in the history of each daughter-nucleus. As the daughter-nuclei become smaller their division-figures become less complicated, while the chromatin becomes arranged as a single mass rather than as separate particles. Some of the smallest spindles still show occasionally distinct chromatin elements near their poles, but the majority do not. There appear to be no definite astral rays distal to the terminal mass of chromatic substance (figs. 21 *d*, 23, and 24 *b*). Finally all traces of spindle-formation disappear, and the nuclei are reduced to mere masses of chromatin about 1 to 1·5 μ in size. These are arranged on the periphery of masses of protoplasm, after the fashion of a typical so-called Perlenstadium, and the protoplasm soon becomes mammillated round each nucleus with the formation of gametes (fig. 25).

That part of the protoplasm which does not take part in the formation of the gametes—the Restkörper—contains a few nuclei which have not kept pace with the general division (fig. 25 *b*). These laggard nuclei are present here and there in all sections of the later daughter-divisions, and are noticeable in that they are larger than their more numerous companions. Similar nuclei have been noticed by Léger and Duboscq in Hoplorhynchus (13). Scattered throughout the later cysts are also seen a number of round clear bodies

(fig. 25 *a*) stained very faintly with iron-hæmatoxylin. They are most obvious in cysts containing gametes or sporoblasts, and have not been seen in the earlier cysts, at any rate in the same form. Their size varies considerably, and they appear to be products of the original karyosome which have lost most of their staining properties, and which have become more obvious owing to the splitting up of the protoplasm entailed in gamete formation. The majority are rather too large to be referred to the daughter-karyosomes. The main residue of the original karyosome is often to be found, deeply stained, in these later cysts.

The gametes are very like those described for Lankesteria ascidiæ by Siedlecki (18), and show no signs of sexual differentiation (fig. 26). Considering the fact that there is at no time in the history of the encysted animals any difference in structure, and that the nuclear changes are practically coincident, this isogamous type of gamete is what one would expect. Conjugation has not been observed in the living animal, owing to my studies being interrupted by my departure from Heidelberg. Fig. 27 shows, however, what is practically certain to be a zygote. The gametes measure about 3 μ, and are roughly circular in outline. Their nuclei consist of small masses of chromatin with no definite vesicular structure. The zygote measured over 4·5 μ, and contained two distinct nuclei. Several cysts were found containing sporoblasts, (figs. 28 to 33). These are ovoid bodies measuring 6 μ by 4 μ, and containing large vesicular nuclei. These sporoblasts gradually acquire a spore coat, and grow in size somewhat during the process (fig. 33), so that in a cyst of sporoblasts one or two may be detected with the outline of a formed spore (fig. 34). The fully formed spore is shown in fig. 35. The nuclear changes resulting in the formation of the sporozoites have not been made out, nor did I obtain a view of a free sporozoite. It was easily seen, however, in optical sections of the living spores that eight sporozoites were arranged peripherally around a granular mass of residual protoplasm. The spores measure 9 μ by 7 μ, and are navicelliform, provided at each end with a little peg-like projection (fig. 35).

Discussion of Some Special Points in the Life-Cycle.

In the description of the trophozoite mention has been made of the traces of further segmentation shown occasionally at the posterior end of the deutometrite in Metamera schubergi. The presence of segmentation in some gregarines, apart from the three fundamental divisions of the body, is a well-established fact, Léger (12) having described a form, Tæniocystis, where this phenomenon is so well marked as to make the animal resemble a small cestode. Porospora (13) also shows a segmentation, which, however, appears to be somewhat different in nature, as the animal is said to be capable of obliterating its segments merely by stretching itself out during movement.

In Metamera schubergi the segmentation is always confined to the posterior end of the deutomerite, and is not constantly present. In their full development these posterior septa appear in every way as definite as those of the anterior part of the gregarine; but in some animals, on the contrary, it requires the most careful focussing to demonstrate their existence. I am unable to explain the significance of these septa; whether they mark a certain period in the life-cycle or whether they are due to some form of plasmolysis I cannot say. They are, however, sufficiently often present to form a striking feature of this gregarine.

As regards the explanation of the phenomena shown in the division of the nucleus, it is difficult to discover anything of the nature of a precedent in the current description of this stage. The vacuoles described by Cuénot (6), Prowazek (16), and others, in close proximity to the sporont nucleus, or by Siedlecki (18) within the latter, have not been seen in Metamera schubergi. From the proximity of the commencing achromatic mass to the actively disintegrating karyosome, I suggest that this latter body supplies material —more or less, it is impossible to say—which will assist in the formation of the two daughter-nuclei. Another point, to

which attention has been frequently called, is the intense staining capacity shown by the achromatic mass, both at its first appearance and later in the fully formed spindles. This applies equally to Delafield's hæmatoxylin and to Heidenhain's method, which latter is known to stain plastin-substance darkly. Now the chromosome material, when first detected, is seen as streaks lying on the spindle-fibres near the poles; or, when the fibres are seen in optical section, as a line of contiguous granules (figs. 12 and 13). No preparation showing an equatorial arrangement of the chromosomes was obtained, although, of course, this does not prove the non-existence of such a stage. Fig. 12 shows some of the chromatin streaks directly continuous with the well-marked terminal mass; and it is thus possible that this mass represents a collection of chromatin which has been delivered by the spindle-fibres. I suggest, therefore, that throughout the division the spindle-fibres are carrying chromatin in a form unrecognisable as discrete particles, until it undergoes condensation towards the poles of the figure. With the appearance of the vesicles the chromatin elements disappear from the spindle, leaving only the few scattered granules of figs. 12 and 15. These vesicles would thus appear to have been formed from the chromosomes of the earlier stages, and supposing them to be indeed daughter-nuclei, it is conceivable that they go on growing at the expense of chromatic substance still uncondensed in the spindle-fibres, until finally they become free as the first pair of daughter-nuclei. This theory would account for the staining properties of the spindle; and the absence, at the earliest stage of the division, of definite chromosomes.

As regards the origin of the chromatin of the daughter-nuclei, there is nothing upon which to dogmatise. We have the fragmentation of the original chromatin masses, which proceeds until the resultant particles are indistinguishable, and we have the breaking-up of the karyosome, both of which might supply a source for the chromatin. That this chromatin is being in some way drawn up on to the spindle

from the débris of the old nucleus is obvious from figs. 14 and 15.

Siedlecki, in his work on the karyosome of Caryotropha (19), reviewing the rôle played by this body in Coccidia, points out that while in some types the karyosome plays a purely vegetative part, in others it has definite responsibilities regarding the reproductive functions. The latter appears to be the case in Metamera schubergi. If, as I believe to be the case, the daughter-nuclei reform their karyosomes, may not these daughter-nuclei—which are, after the upheaval of the trophozoite nucleus during its first division, presumably sexual in nature—throw some light on the functions of the karyosome? If the latter be purely vegetative in function, why should it recur in the daughter-nuclei, which, with their two centrosomes, are plainly not in a vegetative condition?

In the face of the facts it is certainly a reasonable suggestion that the original karyosome consists of two elements at least. The one of these is thrown out at the first division of the nucleus, and is of no further use in the formation of the daughter-nuclei; the other is of vital importance in the propagation of the species, as realised in the sexual gametes. In the daughter-karyosomes only one of these components persists—i. e. that part essential to nuclear division; the other part—for which, in the active reproductive processes now proceeding no need remains—is not represented. Thus, in the daughter-spindles no karyosome remnants are seen. This is hardly the place for a discussion on the binuclearity hypotheses, so ably dealt with by Dobell (8), but the above-mentioned differentiation of the karyosome constituents is sufficiently suggestive. On the one hand, the vegetative and reproductive elements of Goldschmidt's theory may be seen in the original karyosome residue and the so-to-speak more intense daughter-karyosome respectively. On the other hand, one is equally justified in assuming that the karyosome residue merely represents elements whose life is over and whose functions are exhausted, while the perpetuated remainder persists in the daughter-karyosomes, which are

thus thoroughly equipped for their part in the ceremony of division.

It will be noticed that, except in fig. 15, where the vesicles attain their maximum development, there is no true striation shown distal to the polar aggregation; in other words, although the spindle-fibres are throughout very distinct, the centrosome element is not. This, again, suggests a bearing on the origin of the centrosome. On the one hand, as Dobell (8) points out, we have a binucleate condition held as the starting-point in the development of the centrosome; on the other there are observers, such as R. Hertwig, who believe the centrosome to be a specialisation of the central spindle, so that the spindle in the Protozoa is equivalent to centrosome + spindle of the Metazoa. Without wishing to claim originality for the suggestion, I may say that the first division figures of Metamera schubergi have all along pointed forcibly to a most interesting lack of differentiation and specialisation between the various constituents. The chromatin is not marked off in the form of distinct chromosomes, nor are the centrosomes—assuming my interpretation of the figures to be correct—distinguishable as such. The three elements, chromatin, spindle, and centrosome, act in concert in the formation of the first two daughter-nuclei, and it is difficult to say where one begins and the other ends. I suggest, therefore, that the evidence afforded by Metamera schubergi tends to support Siedlecki's view, expressed in connection with his work on Caryotropha (8), that "we have in a protozoan cell . . . but a single and simple nuclear apparatus before us," and not a binuclear arrangement.

In conclusion, with reference to the apparent isogamy shown by this gregarine, it will be noticed that we have another apparent exception to what Léger (13) deems the general rule in gregarines, i. e. anisogamy. In this connection the recent work of Brasil (4) and Hoffmann (10) on Monocystis, which had previously been considered isogamous, is interesting. The work of the latter emphasises the futility of drawing conclusions from stained preparations.

He showed that a very definite anisogamy was visible in the living cysts, which, however, became much less marked in the process of fixing and staining. This may be so in Metamera schubergi, but, considering isogamy as the more primitive condition, it is possible that this gregarine, whose first spindle suggests a phase in the evolution of karyokinesis, may also exhibit true isogamy.

I hope in the spring to renew my acquaintance with this species, and to be able to complete its life-history.

Diagnosis of Metamera schubergi n.g., n.sp.

A cephaline gregarine belonging to the family Dactylophoridæ (Léger).[1] Trophozoite ca. 150 μ by 45 μ. Epimerite subconical, with apex excentrically placed, and surrounded by numerous branched, digitiform appendages. The deutomerite sometimes (not always) shows a secondary septation into one to three segments in the region posterior to the nucleus. Conjugation isogamous, no sexual differentiation being observable at any stage in the life-cycle. Cyst dehiscing by simple rupture. Spores navicelliform, containing eight sporozoites, and measuring 9 μ by 7 μ.

Hosts: Glossosiphonia complanata (Heidelberg and Cambridge) and Hemiclepsis marginata (Heidelberg).

Guy's Hospital,
London, S.E.,
February, 1910.

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EXPLANATION OF PLATES 15 AND 16,

Illustrating Mr. H. Lyndhurst Duke's paper on "Some Observations on a New Gregarine (Metamera schubergi nov. gen., nov. spec.)."

PLATE 15.

[Figs. 1 and 2 were drawn from living animal, figs. 3 and 4 from preparations fixed with alcohol and acetic acid and stained with Schuberg's modification of Mayer's acid carmine. Figs. 5, 6, and 16 are diagrammatic. Figs. 7–18 were fixed with Gilson's fluid and stained with Heidenhain's iron-hæmatoxylin. All these figures were drawn with Zeiss oc. 6, obj. 2 mm. apochromatic. Figs. 9, 10, 11, and 17, 18 are to scale at magnification of 2000. Figs. 12, 13, 14 are drawn on a slightly smaller scale.]

Fig. 1.—Living sporont expressed through anus of leech.

Fig. 2.—Same sporont as Fig. 1, showing bubble-formation.

Fig. 3.—Cephalont with epimerite embedded in fragment of host-tissue.

Fig. 4.—Showing optical section of epimerite.

Fig. 5.—Diagram of structure of epimerite, etc.

Fig. 6.—Diagram of sporont with cuticular pad on epimerite.

Fig. 7.—Nucleus of trophozoite.

Fig. 8.—Nucleus showing fragmentation of chromatin masses and extrusion process of karyosome.

Fig. 9.—Sporont nucleus showing earliest appearance of the "achromatic mass," with fragmentation of the karyosome.

Figs. 10 and 11.—Successive sections of another nucleus showing slightly later stage than fig. 9.

These three figures (9, 10 and 11) are drawn from same cyst.

Fig. 12.—First division of sporont nucleus showing at (*a*) the ring arrangement beginning at the pole; also the streaks of chromatin and the spindle-fibres in optical section. The two poles are respectively at the extreme upper and lower focus. One of the chromatin streaks is seen running into the polar aggregation.

Fig. 13.—An early cyst, containing two associated individuals, with remains of epimerites seen at the centre. Nuclei at stage of first division. In upper animal the polar aggregation and the chromatin streaks are very marked. (Combined from two successive sections.)

Fig. 14.—First division of the sporont nucleus at a somewhat later stage than figs. 12 and 13. Shows polar vesicles more distinct. Also the distinct fibres running down into neighbourhood of original nucleus and karyosome.

Fig. 15.—First division of sporont nucleus at a later stage than fig. 14. Vesicles fully formed and fibres running down towards karyosome. The vesicles here shown were 6μ apart, lying respectively at top and bottom focus.

Fig. 16.—Diagram of first spindle just before final separation of first two daughter-nuclei.

Fig. 17, *a*, *b* and *c*.—Earliest stage of daughter-nuclei, eight or nine in cyst.

a. Shows centrosomes connected by a thick band.

b. Shows chromatin bunched as an early spindle figure.

c. Shows karyosome.

All from same cyst.

Fig. 18.—Somewhat later daughter-nuclei at end of division.

a. Shows two centrioles at each pole; also one daughter-vesicle. (The section has not passed through the left vesicle.)

b. Shows division of the centriole with poorly developed daughter-vesicle. (The vesicle at the right end of the figure lies outside the plane of this section, and is therefore not seen.)

c. Shows a separated daughter-vesicle.

All from same cyst.

PLATE 16.

[Figs. 21–34 and 36 were fixed with Gilson's fluid and stained with Heidenhain's iron-hæmatoxylin. Figs. 19 and 20 were stained with Delafield's hæmatoxylin and eosin. Figs. 19–34 were drawn at magnification of 2000. Fig. 35 is not to scale, being relatively too large.]

Fig. 19 (*a–e*).—From same cyst. Somewhat later daughter-nuclei. All show karyosomes. *c* and *d* show early stage of spindles, and the karyosomes in a state of activity.

Fig. 20.—Showing differentiation of centrosome into centriole, and centrosphere in a daughter-nucleus of same stage as fig. 19.

Fig. 21.—Similar daughter-nuclei showing karyosomes: also corresponding spindle.

Figs. 22 and 23.—Later stages of daughter-nuclei, mostly showing karyosomes; also corresponding spindles.

Fig. 24.—Smaller daughter-nuclei and spindles.

Fig. 25.—Shows the Perlenstadium, with a single free gamete. Notice the clear karyosome remnants (*a*), and the residual nuclei (*b*).

Fig. 26.—Gametes.

Fig. 27.—A zygote with two unfused nuclei.

Figs. 28–32.—Sporoblasts. Figs. 29 and 31 show these in transverse section.

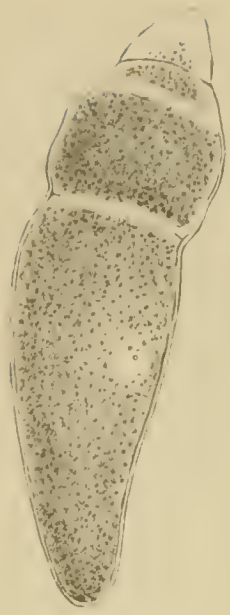
Fig. 33.—Shows a sporoblast assuming shape of spore.

Fig. 34.—Shows a spore coat in process of developing.

Fig. 35.—Fully formed spore, with sporozoites in optical section.

Fig. 36.—Shows the karyosome in the act of extruding some of its substance.

Fig. 37.—Diagram to show method of apposition of associating sporonts in a cyst.



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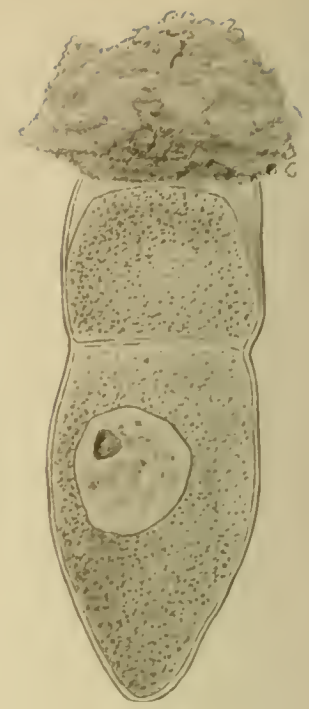

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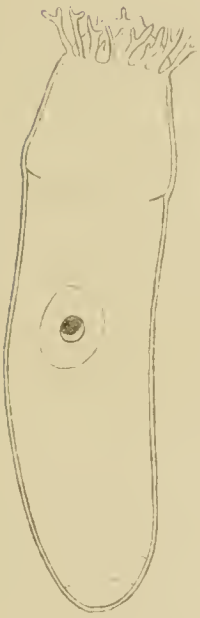

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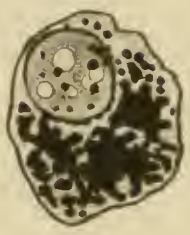

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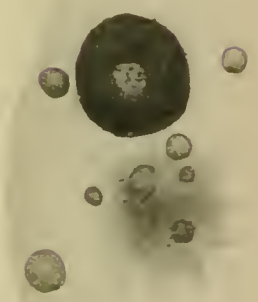

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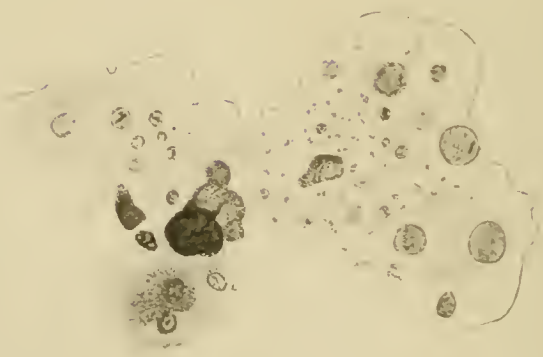

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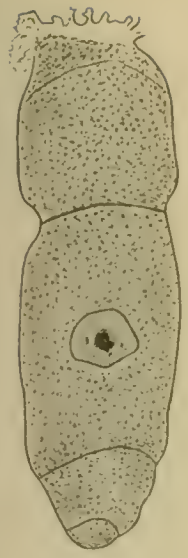

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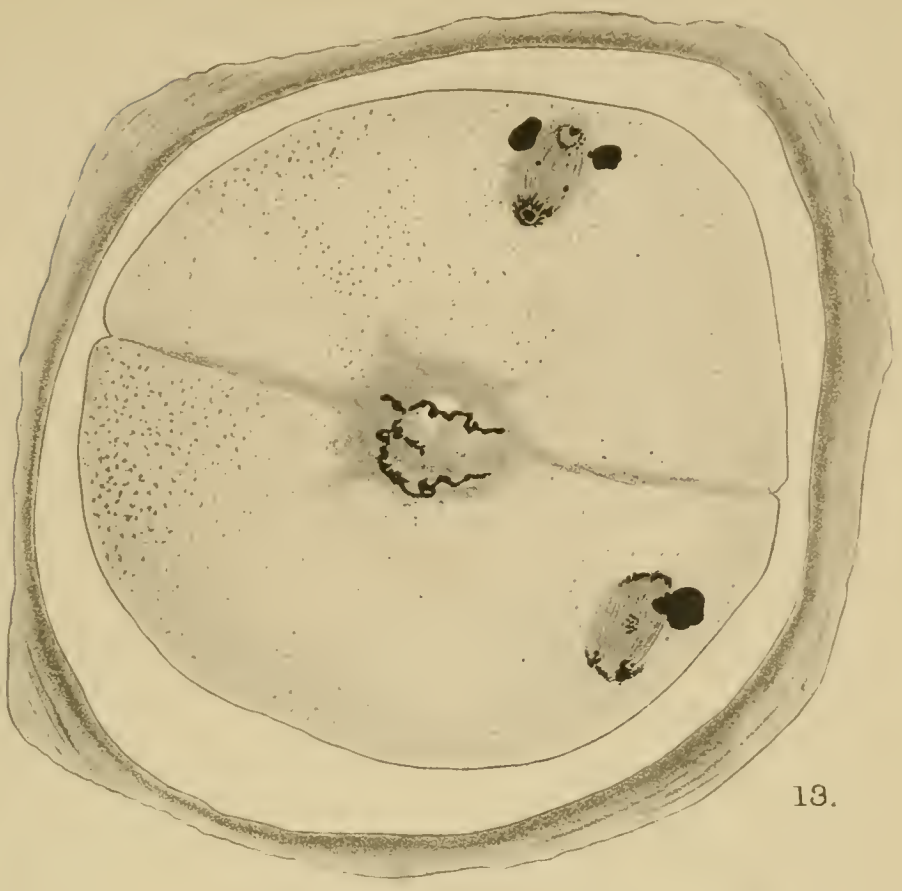

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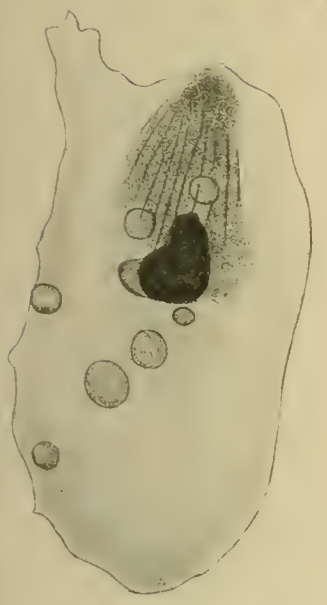

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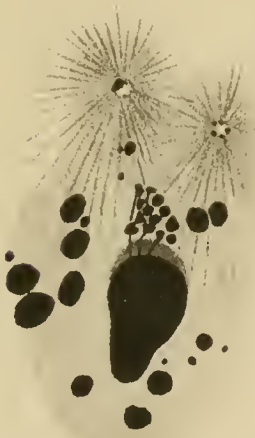

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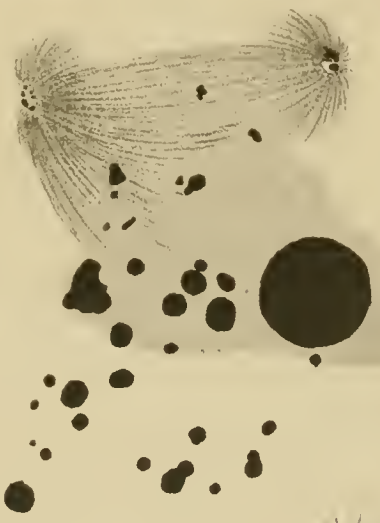

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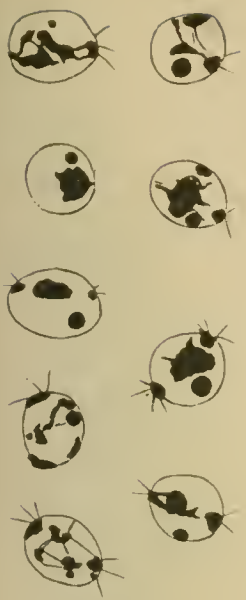

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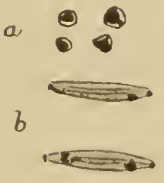

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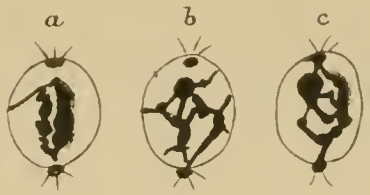

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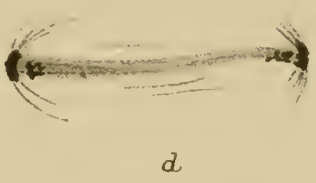

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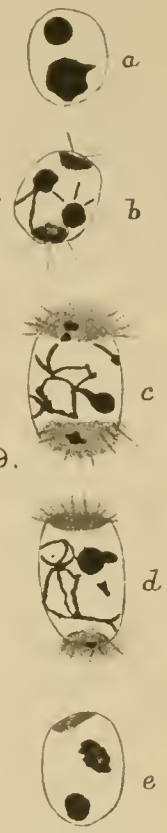

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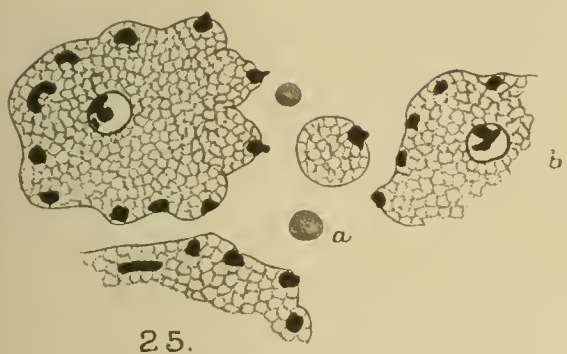

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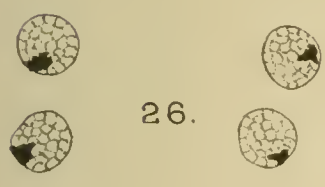

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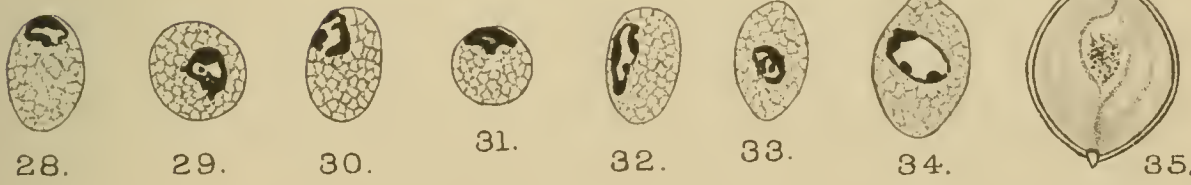

28. 29. 30. 31. 32. 33. 34. 35.

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On the Anatomy of Histriobdella Homari.[1]

By

Cresswell Shearer, M.A.,
Trinity College, Cambridge.

With Plates 17–20 and 5 Text-figures.

Contents.

[1] I have to thank the Director and members of the staff of the Marine Biological Association of Plymouth for their kind attention and interest in my work while at Plymouth.

1. Introduction, Material, and Methods.

Our knowledge of the anatomy of Histriobdella is based on the papers of Van Beneden (1858), Foettinger (1884), and Haswell (1900). Of these, Foettinger's account is the most extensive, while Haswell's paper is perhaps the most valuable. Both accounts contain a more or less detailed description of the internal structure and organisation of the adult. Several years ago I described the presence of solenocytes in connection with the nephridia of Dinophilus. This discovery rendered it probable that these peculiar structures would also be found in Histriobdella, with which Dinophilus shows many relationships. Moreover, the different description of the nephridial system given by Haswell in Stratiodrilus from that of Foettinger for Histriobdella called for a re-investigation of these organs. For these reasons the present work was begun. I was soon led to undertake a detailed examination of the animal. It is some twenty-five years since the publication of Foettinger's paper, and during this interval the European species of Histriobdella has received no further attention. In the following account I have endeavoured to clear up Foettinger's description of several of the organs. I have had the advantage of having made use of the methyl-blue method of intra vitam impregnation, which has proved most valuable. With its use I have experienced no difficulty in determining the number of the nephridia and their relationship to the segments, and to make out new details in their structure quite impossible from ordinary sections of fixed material.

Good methyl-blue[1] preparations of the nephridia can be obtained by placing the lobsters bearing the parasites in small tanks of sea-water, to which sufficient blue has been added to colour the water a light shade. It is necessary for the animals to remain in the blue two or three days before it appears in the nephridia. As the blue is rapidly absorbed by the living tissues of the lobster, an additional quantity has

[1] This is "soluble blue," and not methylene blue.

to be added to the water from time to time. With good air-circulation and a little attention, a medium-sized lobster can be kept alive for several weeks in a tank of four or five litres capacity without change of water.

At the end of the second day the blue will have collected in dark granules on the walls of the nephridial canals, so as to outline these clearly. By this time it has been discharged from the nervous system and the sensory cells of the epidermis. About the bases of the legs of the head it shows a tendency to remain some time after it has disappeared from the brain. It is retained alone by the nephridia on the third day. Here it collects in dense masses on the courses and openings of the canals.

In the study of these methyl-blue preparations I have made use of long, thin cover-slips, such as are used in preparing large serial sections instead of ordinary slides, on which to mount my preparations. The use of a thin cover-slip used as a slide allows of the preparation being examined from each surface, as desired, under an oil-immersion lens. It is thus possible to trace a nephridium first on one side of the preparation, and then turn the slide over and trace it further on the other surface.

Histriobdella is a somewhat difficult animal to fix. The only reagent that has given uniform results is a saturated sublimate solution, with 5 per cent. acetic, used boiling hot. Hermann's solution and Flemming and the osmic acid mixtures give very irregular results, and are not to be depended on for their action. One lot of material will be excellent, while the next, fixed with the same solutions and under the same conditions, are useless. Picro-acetic and Bouin's solutions, used hot, give good results, but not as good as material fixed with sublimate-acetic. Picro-sulphuric was used for preparations to be studied whole, on account of the excellent preservation it gives of the external form. As stains, the following have given satisfaction: Hæmacalcium and Benda's iron-hæmatoxylin, paracarmine, lithium-carmine followed by Lyon's blue for eggs.

The nephridial canals are remarkably difficult to recognise in sections on account of the retraction they invariably undergo during fixing. It is impossible to trace them with any degree of certainty through consecutive sections. For this reason I have relied mainly in my investigation of the nephridia on methyl-blue impregnation preparations of living material. The figures accompanying the present paper therefore represent the appearance of the nephridia in living material. It is necessary to use the highest powers of the microscope to determine the structure of the nephridia, and even then the eye requires considerable practice and training to distinguish the motion of their cilia. It is difficult to convey any idea of the extreme delicacy and minuteness of these structures. The necessity of being compelled to use immersion-lenses for their study excludes the use of any of the ordinary dark ground systems of illumination. Doubtless these would offer an excellent means of investigating structures of this nature in an animal so transparent as Histriobdella, if they could be used successfully with the immersion-lens.

Of great service in the study of the methyl-blue preparations is, I have found, the use of a number of sodium glass screens of different shades, such as are used in orthochromatic photography to vary the exposure from five to fifteen times.

To obtain a uniformly constant light I have used an ordinary Welsbach gas lamp, with standard screens. This gives a light much superior to that of ordinary daylight in bringing out the finer structure of the nephridial canals.

For sections I have used the ordinary paraffin and the paraffin celloidin method. The sections were cut of the uniform thickness of 7 μ. In the reconstructions of the nervous system shown in figures I have used a method which is in part a modification[1] of that described by Woodworth ('Zeit. f. wiss. Mik.,' xiv, 1897, p. 15). Each section, of which there

[1] This I owe to my friend, Mr. E. W. Nelson, of the Marine Biological Association, Plymouth.

were about eighty, was first outlined on paper with the aid of a camera lucida, and the nervous system carefully marked in. Each of these drawings was then measured transversely from side to side, and the measurements plotted out on millimetre paper, allowance being made for the magnification between sections. The nervous system was also measured, and likewise put in, all the distances being doubled to give an axial line. The ends of the plotted points were then joined up, and an outline of the external form and the nervous system obtained. The figures were then reduced to their present size, and at the same time transferred to ordinary drawing-paper by means of an eidograph. In the sagittal section shown in fig. 15 the dorso-ventral diameter was taken instead of the transverse. By this means the relationship of the ganglia to the segments can be accurately determined in a way that would be impossible with the ordinary reconstruction methods (figs. 15, 21, 28).

2. Historical Review and General Remarks on Habits, etc.

Histriobdella was discovered and briefly described by J. P. van Beneden (1) in 1853. He found it as a parasite on the eggs of some lobsters obtained from Ostend. He considered it a larval Serpulid, and placed it among the Polychætæ. Subsequently, in 1858, he (2) pointed out that it was an adult form. From its peculiar structure he remarked that it could not be easily classed with any known group of animals, although some of its features he thought were such as to place it among the leeches. He gave a more or less detailed description of both sexes, and figured the eggs and immature young.

To Foettinger (8) we owe the most extensive account of this animal. He describes the nervous system, nephridia, reproductive organs, and, in fact, was the first to give a detailed account of its anatomy based on sections. He supported the conclusions of Edouard van Beneden that it was an Archiannelid, placing it near Polygordius, but separate from it,

in the family Histriodrilides. In his opinion many of its characters show its inferiority in organisation to Protodrilus. Among these the absence of any trace of the circulatory system, the feeble internal segmentation, marked by the complete absence of dissepiments and the small number of segments. On the other hand, the presence of well-developed ganglia points towards a higher organisation than that possessed by any known Archiannelid. Again, the complicated sexual apparatus of the male is different from anything at present found in this class. The presence also of chitinous jaws with striated muscles and the anterior and posterior feet he considered as distinguishing it as a type superior to Polygordius.

More recently Haswell (**13**) has obtained, as already mentioned, a freshwater species from the branchial chamber of a Tasmanian crawfish. In the possession of cirri it differs externally slightly from Histriobdella. Haswell pointed out, among other new features, that the lateral organs which Foettinger considered penes are in reality organs that function as claspers, while the penis, as in Dinophilus, is a median unpaired structure. While Foettinger described the seminal vesicles he was unable to trace their ducts to a common receptaculum as Haswell has done in Stratiodrilus. The nervous system of Stratiodrilus seems to be different however from that of Histriobdella, in being more highly differentiated. In Histriobdella the ventral nerve-cord is still in complete continuity with the epidermal layer, while in Stratiodrilus it is situated much deeper. This difference may be in great part due to the close union of the epidermal and sub-epidermal tissues in contrast to those of Stratiodrilus. A more important difference is the separation shown by the two component halves of the ventral nerve-cord in Histriobdella, and the somewhat different position and number of the ganglia.

The greatest difference, however, is shown in the excretory system. It is impossible to reduce this to a common type. In Stratiodrilus the crossing and branching of the canals

in the anterior region, and their course in some instances through more than one segment, seems to preclude any comparison with Histriobdella.[1] Again, in Stratiodrilus the interior feet are retractile, and can be completely drawn into the head. This is not the case in Histriobdella, where the distal joint alone is retractile. The main mass of the foot is incapable of retraction, even under the action of strong reagents.

Histriobdella was found by van Beneden and Foettinger on the eggs of the European lobster, and was considered by them a parasite on these alone. It is, however, like Stratiodrilus, normally an inhabitant of the branchial chamber and gills. It passes to the eggs of the female from the gill-chamber when these happen to be present,[2] returning to the same situation when the eggs are hatched and the egg-membranes shed. In the branchial chamber it is quite difficult to detect at first, on account of its almost colourless condition and the fact that in this situation it does not show the excitable movements exhibited while on the eggs, but crawls slowly, keeping close to the mucous membrane. Examination of the branchial surface of the carapace, however, once the eye has become accustomed to distinguishing them, seldom fails to show their presence in this situation in either of the sexes. They prefer the carapace to the gill surface, as it affords a better footing, and the long hairs under which they move prevent their being readily brushed off. To the bases of these they attach their eggs in great numbers, especially towards the margin of the carapace, where the hairs are long and numerous. Comparison of the parasites from the "berry" with those from the chamber shows no difference between them, except that the jaws of the parasites from the chamber

[1] Professor Haswell informs me that since the publication of his account of Stratiodrilus he has re-examined the nephridia and has re-confirmed his statements regarding them.

[2] According to Herrick this takes place once in two years. "The Reproductive Period in the Lobster," 'Bull. of U.S. Fish Commission,' vol. xxi, 1901, p. 161.

seem a little better developed than those of the "berry." The parasites are evidently able to migrate rapidly from one situation to the other. On female lobsters whose eggs are about to hatch, many of them have already migrated to the gill-chamber. A certain number, however, are always to be found on the old egg-membranes, although the eggs have been hatched and the membranes are much discoloured with age, showing that the breeding period had passed some time. I have taken females in this condition, and placed them in tanks with air circulation and kept them under observation. In the course of several weeks the membranes drop off, but no parasites are found about the tank, showing that they have all taken refuge in the gill-chamber. In the gill-chamber and on the eggs both sexes are present in equal numbers. When the lobster ova are well advanced and about to hatch, the male Histriobdellid would seem to preponderate over the female. On the ova the immature young are found in greater numbers than in the gill-chamber.

Frequently a large female can be seen carrying a male attached to its back by means of its claspers. These would seem to throw out some sticky secretion, for once the male has taken hold of the female it is unable readily to let go, and gets carried about by the female although it makes violent efforts to free itself.

In the gill-chamber, as on the eggs, the parasites show the same tendency to collect in small groups, huddling close together and crawling over and over one another. When disturbed they separate, to re-collect shortly in another group. Why they do this is not obvious, as the individuals are sometimes all males or immature young, in which the sexual organs have not yet developed. This habit of collecting in groups therefore can hardly be for the purpose of the impregnation of the females.

I have examined a considerable number of "berried" crabs and rock lobsters, both at Plymouth and Naples, without finding Histriobdella. They would seem to be exclusively confined to the lobster.

Nothing is known regarding geographical distribution beyond the fact that Histriobdella is common on the lobster of the Channel region. J. P. van Beneden (2), as already mentioned, obtained it at Ostend. He also states in his second paper that he had observed it on the lobster of the Norwegian coast. I have been unable to find it on the lobster at Naples. My observations were, however, limited by the rarity of this animal in the Bay of Naples. I only had the opportunity of examining a few adults. So far it has not been reported as occurring on the American lobster.[1] It is remarkable that an animal of such peculiar structure should be represented in Europe by a single species, while its nearest ally should be found in fresh-water streams of Tasmania.

Little is also known of its life-history and habits. If a small mass of lobster ova with the parasites is placed in a watch-glass of sea-water, it will be noticed that they never crawl on any foreign body brought in contact with them. When left to themselves they collect in groups, twisting their bodies together, and remaining quiet for long periods. On being disturbed they show singular excitement, twisting themselves violently and throwing their heads rapidly from side to side, all the time remaining firmly attached by their powerful hind legs. From time to time they can be seen to bite one another with their strong jaws.

While the parasite can be obtained from the branchial chamber or "berry" of almost any lobster on the coast of England, the manner in which it gains access and passes from one host to another has not been determined. Like most parasites, it has limited powers of locomotion, being unable to swim, and crawling very slowly. It has no larval stage that might assist in its distribution. The eggs are attached in capsules to the lobster ova, and the young undergo their entire development within this capsule, emerging in almost the adult condition. There can be no larval

[1] Professor Herrick informs me that he has never found it on the American lobster.

stage during which it can live, either internally or externally, on some other host.

The parasites are able, however, to pass from one host to another without apparent difficulty. This can be readily demonstrated by placing a lobster in a solution of neutral rose in sea-water until the parasites it bears are stained, and then placing it in company with a number of normal unstained lobsters. In the course of a day many of the stained parasites will be found to have gained access to the normal lobsters, while many unstained parasites will be found on the stained lobster. This takes place readily in large tanks where the animals have room to keep well apart. How this passage is accomplished under these conditions I have been unable to observe, as the female lobster is very shy when "in berry," and unsociable, strictly avoiding its mates and companions.

Both Foettinger and Haswell have drawn attention to the remarkable chitinous jaws with which Histriobdella is furnished. Haswell has made a careful study of these in Stratiodrilus, and has shown how the movements of the component parts of the mechanism are brought about. In Histriobdella the jaws are almost identical, as far as I can determine, with those of Stratiodrilus. Foettinger represents them as furnished with many more teeth than I can find to be the case. Their use is not known, as neither Foettinger nor Haswell have made any observations on this head. Unfortunately the intestinal contents are reduced to such a fine amorphous condition as to afford no evidence as to the animal's food. It is probable that the parasites feed on small algæ to a certain extent, as the intestinal contents are usually of a greenish tint. Diatoms occasionally are present, and in some instances would seem to compose the greater portion of the food. This is so in the case of the parasites living on the "berry." In the parasites of the gill-chamber they seem absent, and the intestinal contents consist of a fine brownish mass, among which reddish granules are seen. It is certain that the jaws are not used for tearing the membranes of the lobster's ova as has been supposed.

When the animals are excited they have a rapid way of opening and closing the jaw teeth, but they are never seen to use them to tear open the lobster ova. When suddenly disturbed they sometimes secure themselves by means of their jaws to the egg membranes. They possess the power of protruding the jaw apparatus considerably beyond the mouth orifice, and in crawling they are sometimes seen to seize some object in front of them by protruding their jaws in this manner, after the fashion of many Polychæts.

Histriobdella is remarkably sensitive to any changes in the sea-water. The circulation of water through the branchial chamber of the lobster insures their receiving a continual change of water under normal conditions. Likewise, on the "berry" the water is kept in constant circulation round them by the ceaseless motion of the lobster's swimmerets. With any slight impurity of the water they fall off their hosts, and are found on the bottom of the tank in a half paralysed condition. They are quickly killed by the addition of small quantities of fresh water, and die very readily when exposed to bright light. This is of interest when it is recalled that Stratiodrilus is found in fresh water.

Fertilisation takes place internally. The male drives its penis through any portion of the body-wall of the female. In one instance I saw a male drive its penis into the head and discharge a considerable quantity of spermatozoa. These could be seen under the microscope working their way down into the generative segment. In many cases the males fertilise young females without eggs, and the spermatozoa apparently remain in the body till the ova develop. Many females can be observed carrying spermatozoa but no eggs.

The female exercises apparently little choice in the selection of a site in which to deposit her eggs. On the "berry" these are usually attached to the membranes of the lobster ova, while in the branchial chamber the carapace side is the one selected. They are usually deposited in groups of four or five, and this would indicate that these are all deposited at one time by the female. The eggs are all of one size, and it is

impossible to distinguish the sex of the immature young. They develop at once on being laid, showing that they have already been fertilised within the body. Even when the female is isolated in pasteurised sea-water the eggs develop immediately on being deposited; no sperm can therefore fertilise them in the sea-water.

The egg-laying is done at night, as every morning fresh capsules are to be seen adherent to the coatings of the lobster "berry." The eggs within these are always in segmentation or gastrulation stages. They are laid in great numbers, so that it is easily possible to obtain all the stages of development up to the time the young worm leaves the capsule. Development is direct and would seem to be rapid, for by the end of the fortieth hour the young are fully formed and appear ready to quit the capsule. On leaving this they move about the gill-chamber or pass immediately to the lobster ova, where they soon attain maturity. They are readily distinguishable at this time by their small size and the undeveloped condition of the generative segment. The young of both sexes resemble the female in shape. Van Beneden (2) has figured a number of the young stages, and Haswell (13) mentions that he has obtained a number of the stages in the development of Stratiodrilus.

Regarding the nephridia, Foettinger (8) stated that in the male there were five pairs of these organs, while in the female there were four. Each nephridium consisted of an intracellular tube running backwards on the border of the longitudinal muscle-strands. They turn in sharply towards the median line, to terminate ventrally, on the surface of the succeeding segment to that in which they arise, in a small pore. He could observe no internal openings or funnels. Their heads at their point of origin are on the dorsal surface; since they terminate on the ventral surface they run backwards in an oblique plane between the dorsal and ventral muscle-bands. The first pair arise in the neck segment close to the head, and run backwards to terminate on the ventral surface of the second segment. The second pair arise in the

posterior portion of this segment, and terminate in a similar manner in the third segment. The third pair arise in the third segment to terminate in the fourth. In the female the third and fourth pairs overlap, while in the male the fourth pair arises much farther back between the posterior portion of the fourth and the anterior border of the fifth segment.

In Stratiodrilus, on the contrary, according to Haswell, the nephridial system would seem to extend into the head region. Each nephridium at its anterior end divides into an external and an internal branch. The external branch runs forward into the head, while the internal crosses over to join the internal branch of the opposite side. From the fact that the motion of the cilia of this pair of organs is always from behind forward, their openings are probably in the head. The other nephridia are not branched. "In the female an apparently continuous line of cilia is traceable backwards on each side from the head canals to a point some little distance behind the second cirrus, where a canal is clearly traceable, which, after bending round in a loop, opens on the exterior on the ventral side. But as the direction of the movement of the cilia is from before backwards in the posterior part of this line, it would appear probable that there are two pairs of canals in this anterior region in the female. In the male, on the other hand, there is no such evidence of division, the pair of nephridia which branch in the head being traceable backwards, without change in the direction of the cilia, nearly as far as the bases of the second cirri, at which point they bend in and terminate in the cœlom in the middle line." In the fourth segment, according to Haswell, it is probable that the oviducts represent the nephridia, while in the male they are represented by the vasa deferentia. In both sexes, in the fifth segment there is a pair of organs (beginning in a loop in the male) which run back in the caudal region to terminate near the anus. The direction of the movement of the cilia in these organs is from behind forwards. Thus, in the male there are three pairs of organs, while in the female there are four; so that the nephridia do not partake of the metamerism

of the body, Stratiodrilus having the same number of segments as Histriobdella. In no part of the canals were ciliary flames observed.

3. General Description of the Nephridia.

From the inspection of figs. 1, 7, and 9, it will be seen that the nephridia have much the same positions as those assigned them by Foettinger (8). Apparently in the male the fourth pair, figured by him in the genital region, have no existence. Like the female, the male has only four pairs of organs. It will be seen that they are the narrow, delicate, S-shaped structures he has described (figs. 4, 5, 6, 10, 14), running in the mesodermic tissue of the body-wall. Their position in sections can be seen in figs. 37 and 43. Each organ takes its origin in a small space—a prolongation or part of the general blastocœlic cavity that surrounds the gut—in the anterior portion of the segment to which it properly belongs, and runs back to terminate on the ventral surface of the following segment near the median line. It arises in a knob-like process that projects slightly into the space. This process is thick-walled, and sometimes contains refractive granules. It is shown in fig. 14. Its structure is difficult to determine, and especially the relationship it bears to the space. What I take to be the real head of the organ is shown in section in fig. 42. Here the space into which it projects is surrounded by darkly staining nuclei. These are not seen in the living condition. It bears no cells that have any resemblance to solenocytes, and these structures would seem to be entirely absent in Histriobdella. In a number of preparations it was obvious that the internal ends of the canals were closed, and that they did not open into the space into which they project.

The main portion of the nephridial canal is a thin-walled intra-cellular tube, the anterior end of which contains a few refractive granules and nuclei. It runs directly backwards in an oblique plane, and is much longer than the terminal

portion. It reaches its greatest length in the case of the second nephridium (fig. 14). Frequently the lumen can be seen to be enlarged into small spaces or lacunæ. These would seem similar to the spaces I have described on the nephridial canals of Dinophilus. A number of these are usually seen on the course of the second organ (fig. 6). One large one is often found on the posterior part of the third. From the terminal portion of the canal they seem to be absent. The nephridial flagella pass through their centre while their walls themselves are unciliated. It is possible they are due to the somewhat abnormal conditions under which the parasites are kept in the process of their impregnation with methyl-blue, as I have never been able to observe their presence in the unstained living Histriobdella, although something like their appearance can be detected in sections.

The terminal portion of the nephridial canal turns towards the median line, close to which it ends in a darkly staining pore (fig. 14). About this the blue usually collects in thick granules, which can sometimes be seen vibrating to and fro in the fluid escaping from the canal. The lumen of this terminal portion is greatly restricted in size.

Throughout the length of the nephridial canal the ciliary action of the flagella in their interior can be plainly observed during life. The movement of this is always in the one direction—from before backwards—and I have never observed any reversal of this motion as Haswell has described in Stratiodrilus. Despite numerous observations, I have been unable to determine whether the flagella are derived from the walls of the canal or from the knob-like head of the organ. In some preparations they seemed derived from the wall, in others they seemed derived from the nephridial heads. In sections they would seem to be derived from the walls.

In no case can the canals be seen dividing, as Haswell has described in the case of the first pair in the male of Stratiodrilus. I am quite positive in saying no such division takes place in Histriobdella. They run through

only one segment, in every case terminating in the next segment to that in which they arise in the manner similar to the nephridia in Annelids.

In both sexes the first three pairs of nephridia hold the same position, but the fourth varies according to sex. In the male it is situated much farther back—at the junction of the generative with that of the following segment, while in the female it is much more forward—in the anterior portion of this segment. In the female the third and fourth pairs overlap and cross one another in different planes.

The most posterior nephridium in Stratiodrilus is in that segment that would correspond to the fifth in Histriobdella. In neither of the sexes are organs found in this region in Histriobdella.

Foettinger, in figuring a pair of nephridia in the region of the penis, evidently mistook the motion of the cilia in the vas deferens, or the slit in the vesiculæ, for the ciliary motion of excretory organs. The slit in the vesiculæ was first described by Haswell in Stratiodrilus, and as such was evidently overlooked by Foettinger. They are even better developed in Histriobdella than in Stratiodrilus. They are edged with very stout cilia, that could readily be mistaken for nephridial flagella. With methyl-blue it is easy to determine, however, that no excretory organs exist at this point in Histriobdella.

In Histriobdella, unlike Stratiodrilus, the nephridia partake to some extent of the metamerism of the body. In the third and fourth segments this is masked in the male by the great development of the reproductive organs. In the case of the nephridia there has resulted a pushing forward in the female of the fourth organ, while in the male this has been reduced in size and moved backwards.

Unlike Dinophilus, we do not find the sharp specialisation of the different parts of the nephridial canal into a thick-walled anterior excretory portion and a thin-walled posterior part. It is more uniform throughout in structure.

A. The First Nephridium (figs. 1, 7, 9, 37, and 42).

The first nephridium arises in the segment immediately behind the head, and opens on the exterior in the second segment. The head of the organ I have never been able to see plainly in the living condition, as it is hidden by the muscle-bands. These are always undergoing contraction during life; the lumen of the canal in its anterior portion is continually compressed, and thus the action of its cilia rendered very intermittent. The head of the organ at its point of origin is very close under the epidermis; in one case seeming to be almost under the limiting membrane of the epidermis. In section the head of the organ appears as shown in fig. 42, which is taken in a horizontal plane in the dorsal region of the first segment. The space into which the nephridial head projects is shown surrounded by a number of darkly staining nuclei. The canal with its flagella is shown cut in section in the body-cavity. The actual projection of the head into the space is not seen in this section.

The neck segment is very clear, and were the canal prolonged into the head, as in Stratiodrilus, it could easily be seen at this point passing into the head. As this can never be done, it is apparent that the organ takes its origin in the neck segment and is not prolonged into the head. It is also certain that it does not divide and send a branch to join one from the opposite side, as in Stratiodrilus. Throughout its course it is a simple, unbranched, intra-cellular tube, being in the same position in both sexes.

B. The Second Nephridium (figs. 1, 6, 7, 9, 14, and 37).

The second nephridium arises in the anterior portion of the second segment, and runs back to terminate in the anterior part of the third. It is much the longest, being twice the length of the first. Its course is straight backwards along the border of the muscle-bands. The action of its cilia is much more constant than that of the others, and for this reason it

is the one most readily observed. The main portion of its canal is slender and thin-walled. The general course of the organ is shown in figs. 1, 6, 7, 9, 14, and 37. It will be seen from these figures that Foettinger observed the organ only at the point where it passes from the second to the third segment, and that he was unaware of its considerable extension into the anterior region of the second segment. In the female the segments through which it stretches are somewhat more compressed, and for this reason it appears in the female shorter than in the male.

c. The Third Nephridium (figs. 1, 3, 4, 10, and 13).

The third nephridium has much the same position in both sexes. It arises in the anterior part of the third segment and runs back to bend outwards in the male and slightly inwards in the female, and terminates in the anterior part of the generative region. When the body is retracted it overlaps the posterior third of the second. The head of the organ, as already mentioned, at its point of origin is in the normal condition on a level with the opening of the second. It is situated close under the epidermis, as in the case of the first nephridium, and on the dorsal surface. It runs backwards, and about the middle of its course makes a sharp turn ventralwards (fig. 5). In the female it overlaps considerably the fourth, its opening on the exterior being internal to the course of this organ. While in the male it makes only one turn outwards, in the female it is S-shaped, the terminal portion running inwards (fig. 3).

d. The Fourth Nephridium (figs. 1, 3, 10, and 13).

In the male the fourth nephridium arises in the posterior part of the generative segment in the region immediately behind the clasper. It runs backwards and terminates in the anterior part of the caudal segment. Its course is short and somewhat difficult to observe. It is much the smallest of all

the nephridia, and its action more feeble than that of the others, as the motion of its flagella is the first to stop when the animal is compressed under a cover-slip. It, however, assumes a much darker colour on impregnation with methyl-blue than do the other nephridia, and for this reason seems to play a considerable part in the excretion of waste products. Its position in the male has been correctly indicated by Foettinger, who remarks that no organ is to be found in this position in the female.

Unlike Stratiodrilus, the organ does not begin in a loop or run back so far in the tail region, but opens on the exterior just over the line of separation between the generative and the caudal segments (fig. 12).

4. Body-Cavity and Nephridia.

As in Dinophilus, there is an extensive blastocœlic cavity surrounding the gut, which sends prolongations into the head region, and also into the anterior and posterior feet. It has been described by Foettinger as lined by a more or less definite cœlomic epithelium. I cannot find that this is strictly the case. The gut surface of the cavity is covered by a delicate cuticle, in which at rare intervals are seen small flat nuclei. It is difficult to say if this membrane is a definite structure or a mere secretion from the blastocœlic ends of the cells of the gut-wall. The somatopleuric side of the cavity is not lined by any such membrane. The longitudinal muscles, as in Stratiodrilus, are surrounded by a similar delicate cuticle, but no nuclei are to be seen in it as in the gut membrane. I believe in neither of these cases can this membrane be considered a true peritoneal or cœlomic epithelium. No mesenteries are present, nor can I observe the fusion of the gut to the dorsal ectoderm as mentioned by Foettinger. The gut is more or less closely applied to the dorsal wall, but I cannot find that any true fusion takes place.

In the head the blastocœlic space sends prolongations into

the feet, and forward on the under side of the brain. It is more or less separated from the cavity of the trunk by the neck muscles and the narrow constricted condition of this region. Its extension into the posterior feet is in free communication with the trunk, so that in living preparations the eggs in the female can sometimes be forced into the leg portion of the cavity by slight compression of the cover-glass. They slip back, however, to the main blastocœlic space surrounding the gut when this pressure is removed. The whole of the blastocœlic cavity is very irregular in outline, and is divided, as has been described, in the trunk region by the oblique muscle strands into two lateral chambers.

In every respect it corresponds with the same cavity surrounding the gut in Dinophilus. There is this difference, however, that the numerous brown granules seen in it in Dinophilus are wanting in Histriobdella, although Histriobdella, like Dinophilus, has no specialised vascular system. It is sharply divided from the sac of the ovary, there being no communication between the two. When the ova are forced into the blastocœlic space of the hind limbs the sac of the ovary is either pushed with them, or is definitely ruptured, and the ova pass directly into the blastocœle. Both at the anterior and posterior regions the wall of the ovary is considerably thickened where it crosses the blastocœlic space between the body-wall and the gut. In the male the sac of the testis is likewise sharply cut off from the blastocœlic space in the anterior and posterior part of the generative region. Histriobdella, like Dinophilus, shows the primary and secondary body-cavity existing together, but sharply divided from one another. The nephridia, as in Dinophilus, are in relation with the blastocœlic cavity alone.

From the fact that we get two nephridia in the generative region in the female, there is considerable reason for concluding that the oviduct and its funnel can hardly represent a transformed nephridium as Haswell has suggested. The arrangement of the ganglia and the external appearance of the

segmentation bear out the conclusion that in the male and female this region is composed of two segments. Haswell, in Stratiodrilus, states that "in the fourth segment the nephridia are probably represented in the female by the oviducts, in the male by the vasa deferentia." I have shown in the male and female that two nephridia are present in the generative region, although holding slightly different positions in the two sexes. It is therefore impossible that the oviduct and vasa deferentia represent transformed nephridia, unless we consider the generative region to be composed of three segments, for which there is no evidence.

In my paper on the nephridia of Dinophilus tæniatus I have given some reasons for opposing the view brought forward by Schimkewitsch (**28**) and Harmer (**12**), that the oviducts and vasa deferentia in the male of this animal represent modified nephridia. Here there are four pairs of close solenocyte-bearing nephridia in the male and five in the female. They show the same primitive relationship with the blastocœlic cavity as do those of Histriobdella. Harmer's suggestion is that in the male the fifth nephridium has been modified into the vesiculæ seminales and vasa deferentia, while it remains unmodified in the female as the fifth nephridium. In the male he holds that one of the pairs of nephridia has lost its primitive relationship with the blastocœlic cavity, and here becomes highly modified into the large ciliated apparatus of the vesicula seminalis and the vasa deferentia. The principal evidence relied on by Harmer in making this comparison is the resemblance of the funnel-like opening of the vasa deferentia into the cavity of the testis, to the funnels with which he thought the nephridia were furnished. I have shown that these do not exist, and that the nephridia of D. tæniatus are definitely closed. Therefore the funnels of the vasa deferentia cannot be derived through modification from those of the nephridia.

In Histriobdella and Dinophilus, I believe the oviducts, funnels, and vasa deferentia represent structures

belonging to an entirely different set of organs from those of the nephridia, viz. the cœlomoducts of Lankester's nomenclature.

5. Muscular System.

The muscular system has been described by Foettinger, whose account is correct in its main particulars. The muscles of the trunk region, as described by him, consist of two groups, the dorsal and ventral longitudinal, and the irregular oblique or transverse muscles. It is to these last that I wish to call particular attention in the present account, as they are only mentioned briefly by Foettinger.

In addition to this I have been able to add new details in the division and arrangement of the fibres of the longitudinal muscles that escaped Foettinger's observation.

A. Longitudinal Muscles.

The chief muscles of the body are these powerful longitudinal bands. They have already been described by Foettinger in considerable detail. They consist of two dorsal and two ventral sets. Each band is composed of from twenty to thirty fibres, flattened dorso-ventrally. They are attached by their outer margins to the cuticle, while their free edges project into the body-cavity. In the generative segment their number seems reduced, but this is due to their confinement within a limited space—against the gut dorsally and the nerve-cord ventrally. In the caudal region they spread out, forming a more or less complete wall round the segment, only interrupted dorsally by the gut and ventrally by the nerve-cord. They split up in the head and tail regions, sending fibres to the jaws and the anterior and posterior feet. In the head dorsally they converge on one another, uniting in the median plane, and are inserted in the anterior surface of the jaw apparatus. The ventral bands, on the other hand, divide into two sets of fibres, the outer of which split again to supply fibres to the anterior and posterior surfaces of the

anterior feet, while the other set run forward and are inserted ventrally into the anterior part of the jaw mechanism. In the posterior region each band splits likewise, the ventral sending fibres to the foot of the same side, other fibres crossing to be inserted in the small appendage of the posterior limb. The dorsal send part of their fibres into the leg on the same side, while the internal ones cross over to be inserted in the leg of the opposite side, these fibres thus forming a cross dorsal to the anal part of the gut. The dorsal longitudinal bands give off a few fibres to the two segments of the caudal region, which run towards the median line and are inserted into the cuticle. It is due to the action of these fibres that the contraction of the caudal segment is brought about.

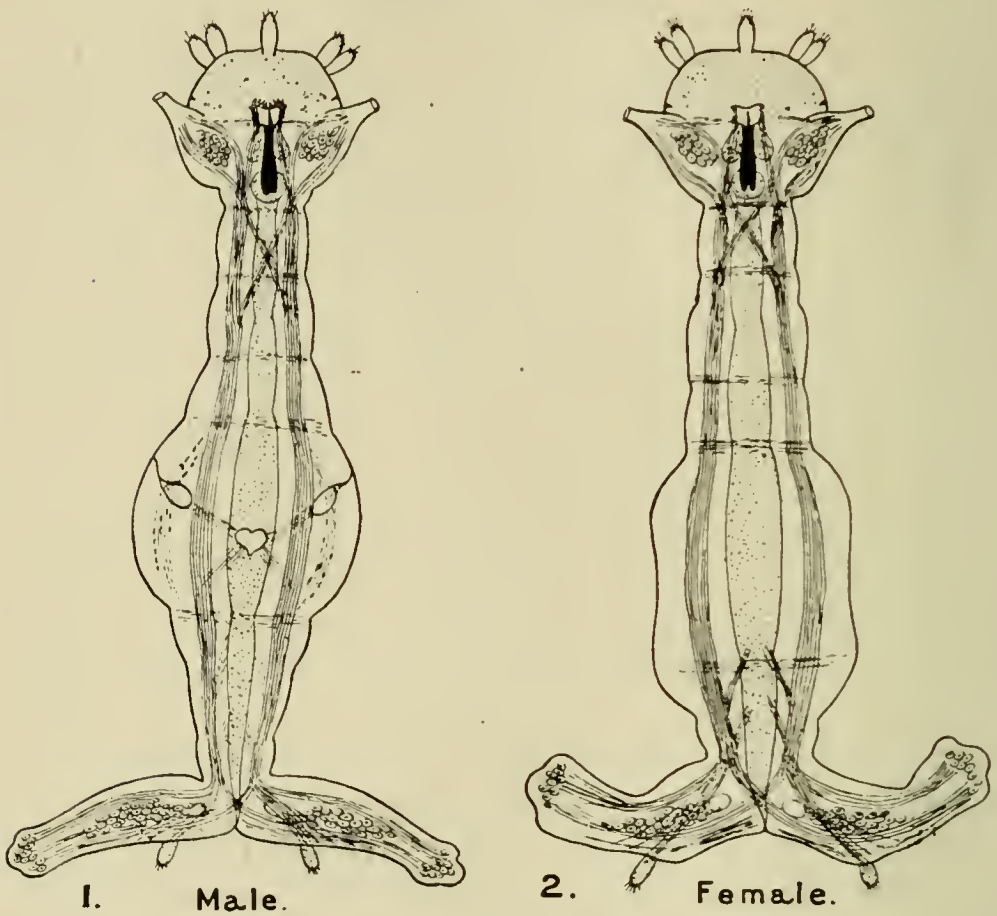
As already mentioned, the ventral bands split in the head region into two sets of fibres. One of these runs forward to be inserted in the anterior part of the jaw apparatus, while the other supplies the extensor and flexor surfaces of the anterior feet. The manner of insertion of these last is somewhat peculiar. The fibres of the external side of the longitudinal band are not inserted immediately into that side of the foot nearest them, but run to the anterior surface of the organ, while those of the inner side of the band cross these to run to the posterior surface. In addition to these there are also other fibres, derived from the bands of the opposite side of the body, that also run to the anterior face of the foot. These fibres form a cross ventral to the anterior end of the stomach. Besides these there are some strands that run from the same side of the foot directly towards the median line, and appear to be inserted into the anterior end of the jaws. All these are inserted into the distal joint of the foot. The course of these different fibres can be readily understood on reference to text-figs. 1 and 2.

B. Special Muscles of the Generative Segment.

In the anterior and posterior part of the generative segment, in the intersegmental region, a few transverse fibres are present, running beneath the epidermis.

In the male special muscles are developed in relation with the claspers and the penis. These are similar in their arrangement to the same muscles of Stratiodrilus. The penis possesses a pair of protractors and retractors. The retractor muscles also function as the retractors of the claspers. They

TEXT-FIGS. 1 AND 2.



The muscles seen from the dorsal side in the male and female. The division of the longitudinal bands in the head and caudal regions is also represented.

run from the base of the penis to the base of the claspers, and by their contraction at the same time retract the penis and claspers. The claspers have also, as in Stratiodrilus, a set of protractor muscles, which run obliquely forwards and inwards in the generative segment, and also a few fibres that run from the bottom of the clasper sheath to the anterior lip of the same.

It will be seen that there is some difference between the arrangement of the main muscles in Histriobdella as compared with Stratiodrilus. In the neck region I cannot find the complicated crossing of fibres shown by Haswell in his fig. 1. Nor in the posterior legs can I distinguish some of the fibres he represents. The muscular system of Stratiodrilus is much better developed, and the presence of cirri and the retractile condition of the anterior feet give it a more elaborate muscular system than that of Histriobdella.

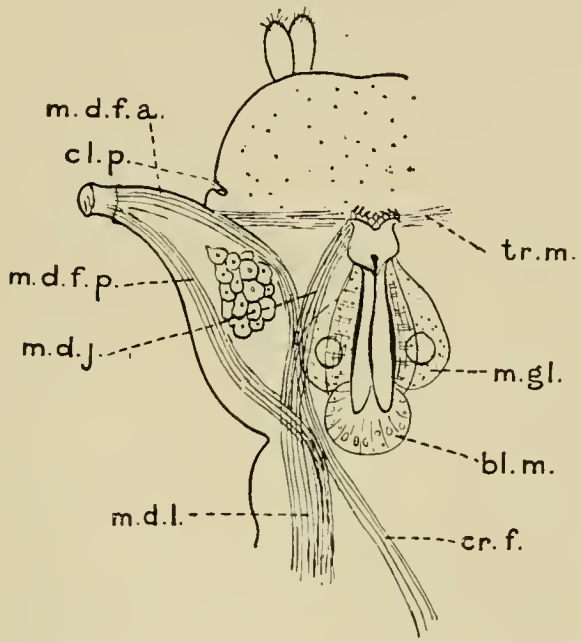
c. Oblique Muscles.

If we examine a number of transverse sections we will see the body-cavity traversed occasionally by oblique strands (figs. 39, 40, 41, 43, and 44). Foettinger mentions their resemblance to the oblique muscles of Protodrilus, but he was somewhat uncertain as to their nature. He says, "Je n'ai pu m'assurer si elles étaient de nature musculaire" (p. 457). They divide the body-cavity, as in the Archiannelids, into a circular portion surrounding the gut and a right and left lateral chamber. In some of my sections they form almost a continual sheet of fibres, and they are much better developed than one might suppose from Foettinger's remark. They are found as irregular bundles crossing the body-cavity from the head to the tail region. They are well marked in the posterior part of the head; commencing at a point on a line with the chitinous jaws, they are continued back into the neck region in an unbroken succession. In the middle of the segment they almost disappear, while they are more prominent in the intersegmental regions. In the anterior and posterior parts of the generative segment they are also present, but are entirely missing from the middle in the male, being interrupted by the muscles and accessory glands of the penis. Anteriorly they divide the testis in two portions, forming a right and left chamber (fig. 39). In the anterior part of the first segment they are shown in fig. 43. Here, during part of their course, they touch the wall of the gut.

In Stratiodrilus their presence has been observed by Haswell (**13**), who states: "Throughout the body slender oblique bundles occur at fairly regular intervals, running from the cuticle of the lateral surface to that of the ventral near the nerve-cord" (p. 306). Here, however, they would seem to be much less developed. I think there is no doubt that they correspond to the oblique muscles of Polygordius. It is interesting to note that the nephridial canals, as in Polygordius, are always within the limits of the lateral cavities formed by them. Another point of similarity consists in the manner of their insertion into the dorsal body-wall. They spread out in a fan-like manner, as Hempelmann (**15**) has shown takes place in Polygordius (see his text-fig. 14). This same arrangement of the fibres, it will be seen, is found in Histriobdella (fig. 41). The anterior and posterior feet, in addition to the fibres they receive from the longitudinal bands, also possess a special musculature of their own. In the anterior foot this consists of a series of parallel fibres that run from its base to the distal, flat, saucer-like pad of the foot. The foot itself is composed of two parts, a distal retractile portion and a larger non-retractile, cone-shaped basal portion. Some of the fibres are applied closely to the cuticle of the outer part of the basal portion, while those of the bands, as already mentioned, are inserted into the retractile distal portion. They surround and run into the basal gland of the foot. There is a collection of granular mucus cells at the base of the foot, abutting internally on the jaw musculature. They stain deeply with carmine, each cell having a darkly granular periphery, with a clear centre hollowed out in a small cavity. The gland gives off a number of straight tubes, that open on the pad surface of the foot. They run up amongst the muscle-fibres, and can be readily distinguished from these by the manner in which they take the stain. The gland pours out on the surface of the pad some sticky secretion, by means of which the animal is enabled to obtain a firm hold. In the case of the posterior limbs a similar, but larger, gland is present. It extends from the wall of the gut out of

the centre of each leg to the commencement of the outer third, where it gives off a mass of fine, darkly staining tubules, which open on the pyramidal pad of the foot. This gland is able to pour out a copious viscid secretion. Frequently, when the animals are irritated, this secretion can be

TEXT-FIG. 3.



3.

Head showing the muscles in connection with the feet and the jaws. *bl.m.*, Bulb-like muscular organ of the jaws; *cl.p.*, ciliated pit of the head; *cr.f.*, crossed strands of the dorsal longitudinal muscles; *m.d.f.a.*, dorsal longitudinal muscles running into anterior surface of the foot; *m.d.f.p.*, dorsal longitudinal muscles running into posterior surface of the foot; *m.gl.*, salivary gland of the mouth; *m.d.j.*, dorsal longitudinal muscle running to jaw apparatus; *tr.m.*, transverse muscle-strands running into the feet.

seen pouring out from the ends of the tubules, forming minute round drops on the end of the foot. Like the anterior limb, the posterior has some muscular fibres apart from those it receives from the longitudinal muscle-bands. These are a delicate set of fibres just under the cuticle on the posterior surface, that run from the extremity to be inserted on either side of the anus. In addition to these there are some oblique

fibres, as in Stratiodrilus, but they are but feebly developed. A considerable prolongation of the blastocœlic cavity takes place into the posterior limbs, running out along each leg between the muscle-fibres and the glands. Into this space the ova in the female are sometimes forced when the animal is compressed under a cover-slip, showing that it is in free communication with the cavity surrounding the gut.

The movement of the limbs takes place alternately, the head being swung from side to side with the movement of the feet. It is a most remarkable sight to see the animals rear up, as they sometimes do, on their hind feet, and stand executing movements with their head while they remain firmly attached with their powerful hind feet. They also crawl quite readily, by means of the feet, on the underside of the surface-film of the water. In the ordinary movements of crawling the glands do not appear to throw out any secretion on the pads of the feet; only when they are disturbed do they pour out a thick secretion, which firmly attaches the feet to the surface on which they happen to be. While the animal violently twists its head and body, it never moves its feet. This hold is remarkably firm. On the lobster ova the parasites can be seized by the middle of the body by means of a pair of fine forceps, under a dissecting microscope, and the body pulled off, leaving the feet still attached, the limbs having been torn from the body without loosening their hold.

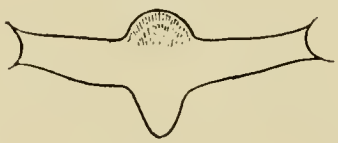
As already mentioned, the front limbs in Histriobdella differ from those of Stratiodrilus in that they are non-retractile. I have never been able to observe any retraction of the feet in the living condition, or in preserved specimens treated with different reagents.

6. Digestive System.

The digestive system is sharply specialised into a number of divisions. These are readily seen in the figure of an immature parasite (fig. 30). Here they are more marked

than in the adult. A more or less slender œsophagus leads dorsalwards and backwards from a quadrilateral-shaped mouth (text-fig. 4). This, although small, is capable of considerable expansion. It is completely everted in allowing the jaws and teeth to be protruded in the act of biting. It commences in a slight ciliated depression, which rapidly deepens into a groove in the anterior part of the head. The œsophagus terminates, on a line with the posterior boundary of the jaw musculature, in a narrow constriction leading into the stomach. It is difficult to say where the mouth ends and the œsophagus commences. The mouth and œsophagus are lined throughout with fine cilia, those of the œsophagus being much stouter than those of the mouth.

Text-fig. 4.



4.

Showing the outline of the mouth when partially closed.

The stomach may be defined as that portion of the intestinal tract lying between the first and the third segment. Its wall is composed of a single layer of cubical cells. It is for the most part uniform in thickness. The rounded internal ends of cells project irregularly into the lumen and are ciliated. At the anterior end, near the œsophagus, the cells are very columnar and contain many granules. They have probably to do with the elaboration of the digestive secretions, as they are seen to be very opaque after the animals have taken food. Those of the ventral wall in this part are somewhat larger than the dorsal. The nuclei are always placed at the bottom of the cell, that is, farthest from the internal ciliated surface. In the anterior region they are long and oval in shape, while in the middle and posterior regions they are spherical, and the cells themselves cubical in outline. In the posterior

region of the stomach the wall is relatively thin in comparison with that of the anterior part, and its cells on the ventral side are furnished with very long, dense cilia.

About the middle of the end of the third segment the stomach contracts into a narrow mid-gut, which runs through the generative region to widen somewhat in the caudal region into a more or less straight hind-gut. The lumen of the intestinal tract, from the stomach backwards, is greatly reduced in size, and, in the contracted condition of the animal, somewhat folded on itself. The character of its ciliation is also different from that of the stomach. At the point where the stomach passes into the mid-gut there is a sort of valve formed by the thickening of the stomach-wall. A similar valve is found at the point of union with the hind-gut. The wall of the mid-gut is relatively the thinnest part of the tract, and its cells are not of the marked yellow colour of those of the stomach. The course of the mid-gut is irregular, from its being slightly folded on itself. That of the hind-gut is comparatively straight, but its lumen is irregular and wavy in outline, due to the irregular thickening of the wall at different points on its course. Throughout the generative segment the gut is very closely confined against the dorsal body-wall. The anus is dorsal. The cells of the hind-gut are of a character quite different from those of the other parts of the tract. They are quite irregular in size, and extend into the lumen so as to make its outline very broken, as if thrown into a number of convolutions. In no part of the wall of the stomach or gut are any contractile muscular fibres to be seen. In the body-cavity, ventral to the anus, and close to the point where the gut joins the body-wall to form the anus, there is usually present a conspicuous cell on either side. The anus itself is an oblong, vertically placed, T-shaped slit placed more towards the dorsal than the ventral side of the animal. It is apparently kept closed by some contractile fibres of the cuticle which function as a sort of sphincter muscle.

The digestive tract of Stratiodrilus agrees in all essen-

tial details with that of Histriobdella as far as can be judged from Haswell's somewhat brief description. There is the same reduction of the tract in the generative region, this being much greater in the female than in the male, and its expansion into a more or less large hind-gut in the caudal region.

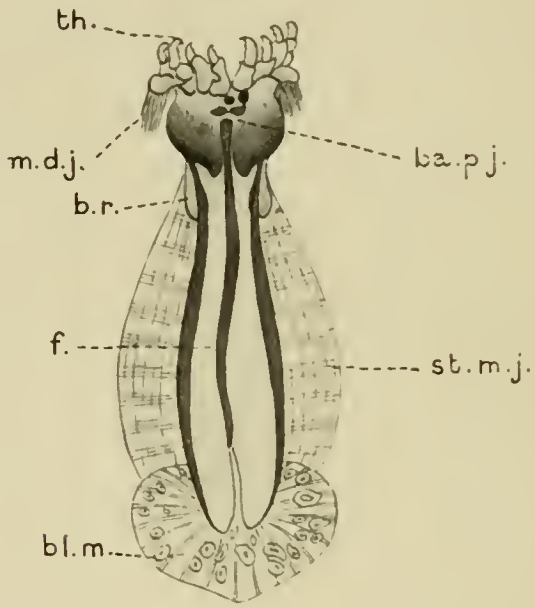
As compared with Dinophilus there is a greater difference. Yet with the exception of the peculiar mid-gut portion of the tract, which is a development due to the peculiar condition produced by the presence of a special generative segment, there is considerable resemblance between Histriobdella and Dinophilus, and in many of the finer histological details there is a very close resemblance. In the first place, the appearance of the cells of the stomach, each composed of a single layer of ciliated cells, the yellow vacuolated appearance of their protoplasm, and the basal arrangement of the nuclei, are the same in the two. The terminal dorsal position of the anus and the configuration of the œsophagus and pharynx are remarkably the same in both.

According to Nelson (**25**) there is a feeble strand of muscle-fibres that act in Dinophilus as sphincter ani, as in Histriobdella. Throughout the stomach region there is a lack of muscular strands, and the stomach is not supported by mesenteries, but is closely applied to the dorsal wall, as in Histriobdella. The blastocœlic surface of the stomach, as in Histriobdella, is covered with a fine cuticle.

The jaw apparatus of Histriobdella is very similar to that of Stratiodrilus. Haswell has given an extensive description of this, so that I need only briefly consider it. As in Stratiodrilus, it consists of two portions—the upper and the lower. The upper consists of a median rod (fig. 36), which Haswell has called the fulcrum. This is slender, round, and slightly curved; it articulates by means of a number of basal pieces with a series of jointed arms, each terminating in a curved tooth (text-fig. 5). It lies in the median plane dorsal to the two blades of the lower jaws, being set at a different angle to these. Its length is somewhat less

than these last. The cubical basal pieces with which it articulates support four arms on each side, each being composed of three or four pieces, the last of which is fashioned into a sharply-curved claw-like tooth. This is strongly serrated on its inner edge. One difference between the jaw parts of Histriobdella and Stratiodrilus consists in the length of the middle joint of these arms. They are much

Text-fig. 5.



5.

Jaw apparatus. Enlarged figure showing the structure of the teeth and the arrangement of the jaw-muscles. *ba.p.j.*, basal piece of jaws; *bl.m.*, bulb-like muscular organ of the jaws; *f.*, fulcrum; *m.d.j.*, strands of the dorsal longitudinal muscle-bands running to the jaws; *st.m.j.*, striated muscles of the jaws; *th.*, teeth.

longer in Histriobdella, and allow of the teeth being folded back in the mouth or œsophagus to a greater extent than in Stratiodrilus. When at rest in the ordinary position the teeth are not folded back to their full extent. The middle piece of the arm projects at right angles to the jaws, and in this position the most anterior part of the arm is the distal joint, the tooth being strongly flexed. When the

arms are folded to their full extent the fulcrum is drawn back on a level with the extremity of the blades of the lower jaws. These are paired throughout. They consist of two long wide blades, thickened at their outer margins, and articulating at their basal ends with two curved wedge-like pieces, the pointed end of the wedge being directed forward in the ventral lip of the mouth. Ventrally they articulate with one another in the median line, and turn up dorsally to form a support for the upper jaws. The upper anterior angle of each plate is turned outwards and backwards, some of the fibres of the dorsal longitudinal muscle-bands being inserted into it. The internal interior edges of these plates are finely serrated, and evidently assist the teeth in their action. As far as can be judged from Haswell's figures, the shape of these plates differs slightly in Histriobdella from that of Stratiodrilus. They fold up dorsally to a greater degree. The main portion of the lower jaws are the wide blade-like portions which project backwards parallel with one another. They are widest behind, and taper slightly in front, where they articulate with the wedge-like portions. Connecting the upper with the lower jaws are the pieces that Haswell distinguishes as "bridles." Into the posterior extremities of these are inserted the powerful striated muscles. Apart from their action in binding together the jaw-sets I have not satisfactorily determined their function. They would seem to be composed of a single curved piece in Histriobdella, and its chitinous substance is broken up into a number of dark hairs where the muscle is inserted, giving it a furred appearance. The powerful nature of these fibres shows that their action in pulling on the bridles has to do with some essential movement of the jaws. It is likely that the actual process of biting is brought about by their contraction, as Haswell has suggested, while the fulcrum has merely to do with their protrusion and opening. In addition to these there is the peculiar bulbular muscular organ, not unlike the sub-œsophageal muscle pad of Dinophilus. This is attached to the posterior ends of the ventral surfaces of the lower

jaws. Its fibres form an oval mass attached directly to the jaw blades. Into this mass some of the striated muscle-fibres are inserted. Its action is hard to understand. It is well shown in Foettinger's figures. In the movements of the jaws the lower blades are sometimes seen to separate considerably from one another posteriorly, and it is possible this motion is brought about by them. What this movement has to do with the teeth I have been unable to observe. This muscular organ appears to be wanting in Stratiodrilus, as it is not shown in Haswell's figures.

On either side of the jaws about their middle there protrudes laterally a small pear-shaped gland composed of from three to four large granular cells with conspicuous nuclei (text-figs. 1 and 2). This gland opens into the mouth or the anterior part of the œsophagus, and is evidently of a mucous nature, as it absorbs the methyl-blue colour very strongly when the parasites are placed in it for a short time. The protoplasm of the gland-cells is finely granular, each having a very large, darkly staining nucleus with a prominent nucleolus. The duct of the gland converges and opens on the ventral side of the mouth. The posterior portion of the organ lies against the muscular pad of the ends of the lower jaws, while its dorsal surface touches the cuticle of the dorsal surface of the head.

In position and structure it is in all respects similar to the glands occupying the same position in Dinophilus, and undoubtedly answers the same purpose. In Protodrilus, also, similar glands are present. It appears to have been overlooked by Foettinger. In fig. 1 of his paper he shows a mass of tissue on either side of the jaws, which in great part belongs to these salivary glands, and not to the jaw muscles, as he evidently thought. Haswell makes no mention of its presence in Stratiodrilus, although it is probably present here also, for he shows a number of round cells in the position that it occupies in Histriobdella.

7. The Nervous System (figs. 15, 21, and 28).

The nervous system extends throughout the body, and is composed of a brain, œsophageal commissures, and ventral nerve-cord, with ganglia at intervals corresponding to the external segmentation. The brain is situated well forward in the head, its main mass being anterior to the oral opening, and close to the dorsal surface. It is composed externally of a mass of nerve-cells surrounding a clear fibrous core. The nerve-cells are distributed over its dorsal surface. Behind, the brain is deeply cleft ventrally, descending in lateral lobes on either side of the anterior part of the mouth. This cleft runs forward, forming a small closed sinus in the anterior end of the organ.

The brain terminates rather abruptly at a point about on a line with the anterior third of the jaws; here it gives off two fine commissures that run directly ventralwards and backwards, connecting it with the first ganglion of the ventral nerve-cord. At the point where these come off some fibres go to the anterior legs, and others run directly backwards in the dorsal region. They probably correspond with the "nerfs sympathiques" of Foettinger. In addition to these, the brain supplies nerves to the anterior tentacles.

The commissures are closely applied to the œsophagus, and are difficult to follow in sections on account of their small size.

At about on a line with the posterior boundary of the brain, and slightly in front of the anterior feet, there is a small ciliated pit on either side of the head. The anterior lip of this protrudes slightly, forming a sort of papilla. This pit is undoubtedly sensory in nature, and appears to have some fine nerve-fibres running to it from the brain. The nerve-cells of the dorsal surface of the brain are distinctly differentiated from the cells of the ectoderm. They are recognisable by the elliptical outline of their nuclei, and the marked way in which they take the stain when treated with the hæmatoxylin mixtures. As compared with the ectoderm

cells, their nuclei are rich in chromatin. This peculiarity renders them distinguishable from the supporting cells of the surrounding tissues. Some of the ganglion cells are clearly multipolar, but axons and dendrites are not recognisable. At the base of the tentacles the cells are bipolar, one process going into the tentacle while the other enters the neuropile. They form a dense mass of cells on the anterior dorsal surface of the brain-core. They are, however, quite distinct from it, only sending a few fine threads into its substance. In the median plane a small space, a prolongation of the general blastocœlic space, extends up under the brain, and separates them from the core, dividing them into two lateral masses. The central core of the brain is composed of a dense mass of interwoven nerve-fibres. It is distinguishable by its yellow colour and its non-nucleated character. It is remarkable that both in relation with the brain and the ventral cord the nerve-cells seem quite apart, and outside the fibrillar part of the nervous system. Their relationship seems closer with the ectodermic tissues of the head and the mesodermic and ectodermic tissues in the trunk than with the fibrillar material of the nervous system in these regions.

The fibres of the ventral portion of the neuropile seem to run from side to side, while those of the superficial layers run more longitudinally. In sagittal sections it is lenticular in outline, and in the median plane is divided by a transverse fissure into an anterior and posterior part. Haswell also shows these divisions in the brain of Stratiodrilus (fig. 8). This division is only limited to the median plane; laterally the neuropile swells out into two large lobes on either side. Thus it consists, as in Dinophilus, in a median and two lateral lobes, the median being in turn divided into an anterior and posterior portion. In the figures of the brain accompanying this paper these divisions do not show, as the brain surface is taken from the ganglion cells and not from the central core. Behind the brain, and dorsal to the muscular apparatus of the jaws, there is a second accumulation of nerve-cells. These may possibly have to do with the

innervation of the jaw muscles; they are dorsal and median to the œsophageal commissures. I have been unable to make out their connection with the muscles. They take up methyl-blue much more readily than do the other cells of the brain, and retain it considerably longer.

The ventral nerve-cord, like the brain, consists of a similar central fibrous core, surrounded with nerve-cells. The two halves of the cord are separated in the intersegmental regions, joining up in the middle of the segments to form a ganglion. From what can be judged from Haswell's drawings, in Stratiodrilus this separation is much less than in Histriobdella. Unfortunately, most of the sections drawn by Foettinger are taken through the middle of the segments, and do not properly illustrate the extent to which the two portions of the cord separate in the intersegmental regions. The two halves of the cord are crescentic in transverse section, the nerve-cells being imbedded on the ventral surface. Where the cords unite these cells are drawn out laterally to form considerable masses on either side.

The main ganglia, as already mentioned, correspond closely with the five main segments into which the trunk is divided. The first is situated in the anterior region of the first segment, and is of considerable size. The second is somewhat smaller, and is situated about the middle of the second segment. It has fewer nerve-cells, and, like Stratiodrilus, it is placed nearer the first than the third ganglion. The third is the largest, taking up the greater part of the length of the cord in the third segment, and having a great number of nerve-cells. Between all the ganglia in the intersegmental regions of the anterior segments the component parts of the cord separate as already mentioned; between the third and fourth ganglia this is hardly perceptible, and from this point backwards to the tail region the two portions of the cord are in close union, with the exception of a small area near the end.

The fourth ganglion is the largest of all, and occupies the middle of the generative segment. The fifth is in the middle of the caudal segment. The position of these ganglia can be

seen from the reconstructions shown in figs. 15, 21, and 28. In these figures the nervous system is seen from the ventral side. The outlines of the cord and ganglia have been measured from the nerve-cells, as far as these could be roughly differentiated from the surrounding tissues.[1] From fig. 15 it will be seen that the main mass of the fourth ganglion lies just in front of the penis, but many of its cells extend backwards in the region dorsal to the penis. Here they would almost seem to form a second division of the ganglion. I have not attempted to determine its structure, which differs considerably from that of the other ganglia, on account of the great size of its lateral parts. A few of its cells are distributed on the penis sheath. Past the fourth ganglion the cord diminishes rapidly, but enlarges again rather suddenly in the interior part of the caudal region. It is the second in this segment that is the largest. In the posterior region the cord divides to run into the posterior feet. At this point a number of nerve-cells are arranged, forming quite a mass. It is difficult to decide whether each of these ganglia is to be considered as representing a segment. If so, then there are three main ganglia in the segment itself, and counting the mass of cells at the termination of the cord, it would be composed of four segments. Foettinger came to the conclusion that it was one segment, formed by the partial fusion of three metameres.

In the female there are a number of differences in the configuration of the nervous system, due to the somewhat different size of the segments as compared with the male. This is most pronounced in the generative region. The absence in the female of the penis and accessory glands results in the almost complete disappearance of the cord and ganglia in the posterior part of the generative region, and throughout this portion of the body the cord and its ganglia are much less prominent than in the male. In the absence of the penis the cord retains its ventral position. In the caudal region, on

[1] In the reconstruction of the male nervous system shown in fig. 15 no allowance has been made for the dorsal curvature of the cord in the generative region.

the contrary, the cord and ganglia are much the same as in the male (fig. 15).

In Stratiodrilus the cord and ganglia are much the same as in Histriobdella. In the male the fourth ganglion is opposite the claspers. After this the cord is very much reduced where it passes dorsalwards over the penis. In Histriobdella this reduction is not so marked. In the caudal region also the ganglia are smaller. Haswell remarks, regarding the nervous system of the caudal region of Stratiodrilus, that "the ventral chain may be described either as represented by a single elongated ganglion imperfectly divided into five or six portions, or as consisting of five or six imperfectly separated ganglia" (p. 315). The nerve-cells are arranged on the ventral surface of the cord, and the lateral ganglia are much better developed in Histriobdella, especially those of the generative region. They send fibres into the cirri. The second ganglion would seem to be double.

Haswell has drawn attention to the fact that the nervous system in Stratiodrilus does not show the complete union with the body-wall tissues as does that of Histriobdella. I think, however, no great importance can be attached to this point. The separation shown by the nervous system in Stratiodrilus is due in great part to the thinness of the body-wall as compared with Histriobdella, and not to a more highly differentiated condition of the system itself.

It is of considerable interest to compare the nervous system of Histriobdella with that of the Archiannelid it resembles most, that is, Dinophilus. From the study of a species closely allied to D. gyrociliatus, Nelson (**25**) has determined the main structure of the central nervous system in considerable detail. In the first place there is a marked separation of the two parts of the ventral nerve-cord in the intersegmental regions, much more so than in Histriobdella. Unlike Histriobdella they do not unite to form the ganglia, but are joined by commissures, the two portions of cord remaining separated throughout their course. There

are four well-marked ganglia corresponding to the four main segments of the trunk. In addition to this, there are a few cells that probably form a fifth, corresponding with the somewhat reduced caudal segment. If we compare the reconstruction figure he gives of the nervous system with that of either the male or female Histriobdella given in the present paper, it will be seen that, with the exception of this greater separation of the cords, there is a remarkable resemblance in the general configuration of the nervous system of the two forms. The brain and the œsophageal commissures are much the same. In transverse sections the cords hold similar positions in the ectoderm. The ventral sinus found in the brain of Histriobdella, it would seem, is also present in Dinophilus as a small closed cavity in the brain substance itself.

In minor histological details they bear a striking resemblance to one another. The brain is clothed dorsally and laterally with a mass of nerve-cells, having the peculiar granular nuclei so characteristic of these cells in Histriobdella. They are similarly differentiated from the supporting tissue cells. The circum-œsophageal commissures are better developed, however, in Dinophilus, and pass backwards round the œsophagus just below the dorsal longitudinal muscle-strands. The centre of the brain is composed of a mass of clear fibrillar material that stains with difficulty. As in Histriobdella, fibres are given off by the œsophageal commissures at the point where these leave the brain. They are much bigger in the case of Dinophilus, and are more easily traced through consecutive sections. In Histriobdella there are no pre-oral commissures, and the ganglia are more circumscribed and definite than in Dinophilus.

As compared with the nervous system of Protodrilus there is a greater difference than in the case of Dinophilus. This is due to the lack of ganglia on the ventral cord. In Protodrilus the ventral cord shows no ganglionic divisions corresponding to the external segmentation. This is very

slight, being shown only by the ciliated rings. Internally it is better marked by the dissepiments and the nephridia. The two halves of the cord remain separate throughout their course, uniting at their ends in a small ganglion. Such a nervous system can hardly be compared with that of Histriobdella.

According to Pierantoni (26), the nerve-cells in Protodrilus are equally as difficult to distinguish from the surrounding tissues as in Histriobdella. While retaining their primitive position in the ectoderm, they send fibrils to the tentacles and the digestive system. In the ventral cord there would seem to be no localisation whatever of the nerve-cells corresponding to the segmentation.

8. Sense-Organs.

Among the sense-organs of Histriobdella are to be classed the five tentacles of the head and the palps of the posterior legs. All these receive nerve-fibres from the central nervous system, and are armed with short, stiff, sensory hairs. The most essential of the tentacles appears to be the median one of the head. In the larva this is the first to appear, and its nerve supply in the adult would seem to be greater than that of the others. In addition to the tentacles, scattered over the cuticle of the body are a number of cells of a sensory nature that stain readily with methylene blue.

On the dorsal lateral parts of the head are the sensory pits described by Foettinger. These, as already mentioned, are very small, and placed a short distance in front of the anterior feet. Foettinger has sought to compare them with the ciliated grooves of Archiannelids. They measure about 14 μ in their longest diameter, and are oval in outline. They are therefore much smaller than the long grooves of Protodrilus and Polygordius. In the bottom of the pit are placed a few fine sensory hairs. As described by Foettinger, the anterior edge of the pit is developed into a slight lip or ridge that is capable of being folded completely over the pit and of

obliterating it. From the way in which this lip is protruded and the pit opened when the animal is feeling its way or examining any small object it may come across in crawling on the bottom of a watch-glass, it is evident that the pit functions in some way as an organ of taste or smell. It appears to receive a set of nerve-fibres from the brain. There is no doubt that these pits correspond to the ciliated pits of the Archiannelids, despite their small size. They are present in both sexes. According to Haswell they are not present in Stratiodrilus.

9. The Reproductive System.

The reproductive organs in the male consist of a testis, paired in its anterior part, two vesiculæ seminales, two vasa deferentia, and a median penis. Dorsal to each vesicle is the so-called granule gland (fig. 11). In relation with the penis there is a gland of unknown function, as in Stratiodrilus.

In the female the organs consist of a large sac or ovary filling the whole of the generative region. On its ventral surface this is furnished with a paired oviduct, armed with a large funnel, the dorsal lip of which only is ciliated. On the course of the oviducts and close to their external openings are the ampullæ or shell-glands.

a. In the Male.

The testis in the male when fully developed fills the anterior and middle third of the generative region. Its extreme anterior end is separated into a right and left portion, its middle portion is fused in the median line. Behind it ends somewhat abruptly in front of the penis. The remaining posterior third of the generative region is taken up with the penis and its accessory glands. This portion is sharply divided from the anterior two thirds by the limiting membrane of the testis. This fact has not been clearly shown by Foettinger. He seems to have overlooked the well-defined

nature of the limiting membrane, and fails to show the sharp manner in which the testis is shut off from the general blastocœlic cavity surrounding the gut. He states that the testis takes up the whole of the generative region, which is not the case, for the penis and its glands take up the posterior third as I have mentioned. The anterior paired portion of the testis is shown in section in fig. 39, while the main unpaired portion is shown in section in fig. 35. Internally the testis is filled with a number of oval bodies, the spermatidia (figs. 27 and 35). These consist of a number of nuclei with granular chromatin, arranged round the circumference of a small mass of cytoplasm. In the region close to the anterior end of the testis they form a solid mass, while in the middle they crowd its cavity as a number of oval bodies. The mature spermatozoa are found in the spaces of the testis cavity between them.

If we regard the generative region as due to the fusion of two segments, then this conclusion is supported by the arrangement of the nephridia and the ganglia. The testis itself takes up the first and largest of these, while the penis and accessory glands take up the second. The division between the testis and penis portion comes at just that point we should naturally conclude that it should from the position of the ganglia.

In the female the double nature of the generative region is not so clear as in the male, and the metamerism is masked by the extensive prolongation backwards of the ovarian sac. In the young female, however, the ovary is confined to the anterior two-thirds. The double nature of the generative region then is almost as distinct in the female as in the male.

The vesiculæ seminales are found in the posterior part of the testis, and are pear-shaped bodies with their pointed ends directed forwards. They are readily recognised on account of the large quantities of sperm with which they are always crowded. Leading into the lateral surface of each vesicle is a fine duct from the granule gland.

These are a mass of large mucus-like cells that lie against the inner surface of the cuticle of the body-wall of the generative region. They secrete a granular mucous substance which

they discharge into the vesiculæ. Each gland is composed of about twenty cells, arranged in a single layer, laterally, against the wall of the segment. They fill up the greater part of the middle third of the region. They commence anteriorly, just behind the orifice of the retracted claspers, and stretch back to a point, on a line behind the vesiculæ on either side. Dorso-ventrally they extend from the border of the dorsal longitudinal muscles round the sides of the segment to the border of the ventral bands. Their cells have a waxy appearance, and their cytoplasm, which is relatively large in amount, is very finely granular. Each cell possesses a round nucleus and a dark karyosome. On a line with the vesiculæ the dorsal cell of each group gives off a fine duct, that crosses the space of the testis cavity and runs into the ventral external surface of the vesicle of the same side. The wall of this tube is also, like the protoplasm of the cells of the gland, finely granular. About its middle there are usually two large nuclei embedded in the wall. Where the tube runs round the outer surface of the vesiculæ it is much thickened, and this appears to be due to the accumulation of drops of the gland secretion in its lumen (fig. 31).

The vesiculæ are roundish bodies with thin walls. The lateral and ventral third of their cavities is taken up with the mucous secretion derived from the granule glands. This, in sections of fixed material, projects upwards into the cavity in a mass of finger-like digitations. On the outer ventral surface of each vesicula there is a small slit. Its edges are armed with short stout cilia. It was the motion of these that Foettinger evidently mistook for the presence of a pair of excretory organs in this region. Through this slit the spermatozoa gain an entrance into the vesiculæ.

The vas deferens leads out from the posterior ventral portion of each vesicle and turns in towards the median line, and is continued as a small tube to the base of the penis. It is of considerable diameter, and forms a sac-like canal on either side. At the base of the penis the vasa deferentia of both sides meet, forming a small receptaculum seminis,

which lies between the two lateral halves of the organ. During life this is always full of very actively moving spermatozoa.

The penis is a firm, semi-solid, pear-shaped body, the pointed end being directed backwards. It is always carried retracted within the sheath. Unlike Stratiodrilus, it is not composed of black chitinous material similar to that of the jaws, but of some transparent substance, sufficiently rigid, however, to enable its being driven through the firm cuticle of the female in the act of copulation. It is protruded through the quadrilateral-shaped mouth of the penis-sheath by the action of the strong protractor muscles. The organ itself is composed of two lateral blades, the spermatozoa being ejected through the median canal between them during copulation.

In relation with the dorsal surface of the penis on either side, and taking up the lateral posterior corners of the generative region, are the so-called accessory glands of the penis (figs. 11 and 40). These are large vacuolated groups of cells forming oval masses running up to the dorsal surface on either side of the gut. From each gland a small duct leads down to the penis, and is inserted laterally about its middle. This opens into the canal on the penis on its ventral side. The gland-cells are divided into an anterior and posterior group. In horizontal sections the gland appears as a four-lobed structure, posterior and dorsal to the base of the penis. The anterior and smaller of these groups is composed of numerous cells, while the posterior, although larger, consists of fewer cells. The cytoplasm is granular and very vacuolar. This is shown in fig. 40, where their anterior ends come in the section on either side of the gut. In transverse section the gland will be seen to be composed of two groups of cells, one of which is much smaller and more dorsal than the other. This is wedged in against the gut on either side. Towards the posterior region of the gland the cells are somewhat larger. The largest of these contains a vacuole of considerable size. This probably acts as a receptacle for

the gland secretion. It is connected with the penis by a strand of cytoplasm that runs to its ventral side, and is continuous across the median line with a strand from a similar cell from the opposite side. Posterior and ventral to this are a number of small, darkly staining cells. They are lenticular in shape, with prominent nuclei. They fill up the corners between the large cells. The largest cell of the gland is placed about the middle or slightly towards its posterior end. The section shown in fig. 24 passes just behind its posterior border. The nuclei of the smaller cells are rod-shaped, and frequently bent in a semi-circular form. On the inner wall of the gland, close to where it abuts against the penis-sheath, are a number of darkly staining masses of nuclear material. The ends of all the gland-cells converge on the penis. When the cells are charged with secretion their nuclei are seen to be large and round, with a well-marked karyotheca. The karyoplasm is collected into a darkly staining karyosome. In the cells that have discharged their secretion, on the other hand, the nuclei are invariably long and rod-shaped, with a uniformly staining karyoplasm, and no karyosome.

In fig. 23 are represented some of the cells of the posterior group under high magnification. The cytoplasm forms a superficial layer which throws threads across the vacuolar interior of the cell. The nucleus is always situated about the middle of the cell and is of considerable size, and contains a darkly staining karyosome.

The compartment of the generative region holding the glands is sharply separated from the anterior part of the segment, which contains the testis, as already explained. This is clearly separated from the granule cells and the vesiculæ, which are within the limits of the testis proper, and enclosed by its membrane.

The region of the accessory gland is often seen distended with the accumulation of secretion within the gland. With dark ground illumination this appears opaque and whitish in colour. In the surface view of a living preparation the two

portions of the gland appear somewhat as shown in fig. 11. The anterior lobe seems distinctly separated from the posterior. The function of these glands is problematical. They doubtless pour some secretion into the canal of the penis during copulation, which assists in this act in some way.

They were first described by Haswell in Stratiodrilus, where they are much larger and somewhat different in appearance from those in Histriobdella. They seem to have been overlooked by Foettinger, although he plainly figures them in his sections. He evidently mistook them for a portion of the testis. That they are separate structures from this can be easily seen in horizontal sections. They correspond to the similar glands found in connection with the male organs in so many Turbellaria, as in Proxenetes, Provortex, and Plagiostoma.

Under the heading of the male reproductive organs come the claspers. These are usually carried retracted, only being protruded when the males are impregnating the females. Under the action of strong reagents during fixation they are sometimes extended, in which case they are always seen projecting ventralwards and never laterally. Each clasper is furnished with a protractor and a retractor muscle that runs to the base of the penis, as already explained. At the base of each organ there is a large mucous cell with a large nucleus. This, in the retracted condition, occupies the anterior wall of the clasper-sheath, and is a conspicuous feature in a transverse section through the anterior region of the generative segment. In a full-grown male the cell is very large. A fine duct leads from it to the tip of the organ and pours some adhesive secretion on the surface of the clasper, similar to that poured on the surface of the feet. This cell is shown in fig. 2. The anterior lip of the orifice formed by the retraction of the organ forms a marked projection which overlaps the orifice (fig. 13). When the organ is extended this lip is obliterated, as shown in fig. 9. The gland cell then occupies the middle of the clasper. At the top of the organ there are a few short, stiff hairs. I have already mentioned that once

the male has seized the female by means of the claspers its grip is immediately rendered secure by the gland secretion, and then the male is only able to free itself from the female with difficulty. Sometimes the male can be seen being carried about by the female, making violent efforts to free itself. The claspers never seem to be used for any other purpose than that of seizing the female, and are never extended to enable the animal to hold more securely when an attempt is made to brush them off the lobster ova.

B. In the Female.

The ovary in the female holds the same position in the generative region as the testis in the male. It has a more sac-like appearance, however, and its lining membrane is thicker than in the case of the testis. In the anterior and posterior regions of the segment there is not the great thickening of the wall seen in the male. It is more uniform in thickness, and the contour of the limiting membrane throughout more distinct. In sagittal sections in the median line it appears as a long chamber lying ventral to the gut (fig. 22).

Foettinger's account of the oviduct and funnel is correct, the funnel being large and collapsible, ciliated on its dorsal side only. It projects downwards into the ventral region of the middle third of the generative segment. Its ventral lip is a short distance from the nerve-cord on either side; its dorsal lip is the longest, and almost meets that of the opposite side in the median line. The funnel is composed of a large number of flattened cells, a conspicuous one being usually seen in the edge of the dorsal lip. The cilia are remarkably stiff and short. It leads into a small, round ampulla which is usually crowded with spermatozoa. This leads into a still larger one, the walls of which are drawn out in a number of digitations. This functions as a sort of shell-gland. Its lumen is filled with a granular secretion that forms the egg-capsule. A short canal leads from the second

ampulla to the exterior. When the ovary is full of ova it is sometimes difficult to see the funnel and oviduct, as its lumen is obliterated by compression against the body-wall.

The inner surface of the ovary is closely invested with a thin layer of nucleated cells—the true cœlomic epithelium. It is from this in the anterior region that the primitive ova arise. This takes place close to where the sac abuts against the end of the third segment. Here certain of the nuclei are much larger than the rest. They are the oögonial cells. They have relatively little cytoplasm and large, transparent nuclei. A considerable number of them are seen at this point in different stages of development. The fact that the oögonia arise from a small, circumscribed portion of the anterior end of the ovary, and not from its epithelial surface in general, recalls the condition described by Nelson (**25**) in Dinophilus conklini, which differs from the other species of this group, D. vorticoides, D. tæniatus, and D. gigas, in that only a small portion of the ovary likewise gives rise to the oögonial cells. It is evident that the epithelium of the middle and posterior portions of the ovarian cavity play no part in their formation. As they pass backwards and become the primary oöcytes, the epithelium of this part of the cavity throws out processes that attach themselves to the growing oöcytes, folding up round them and forming a supporting matrix crowded with small nuclei. They furnish them with the material for their growth, but beyond this take no part in their formation. As the oöcyte grows these follicle cells diminish rapidly in size, and their nuclei undergo degeneration, becoming long and granular. They appear to have something to do with the formation of the yolk-granules, but how this is accomplished is not plain. These arise in situ, as nothing similar to them can be distinguished in the follicle cells, which are always clear and transparent. At the time of their formation the granules are also clear and transparent, and only acquire their dark appearance after they have been formed some time. For this reason the small oöcytes, although highly granular, are almost as transparent as the

oögonial cells. By the time the oöcytes reach the middle of the generative region they turn dark brown in colour. In a few days they increase greatly in size. Their outline becomes regular, and the superficial layer of their cytoplasm seems to stain much more intensely than the deeper portion. Their nuclei become large, round, and transparent, and are readily distinguishable in the living animal. There appears to be no yolk-nucleus present, but the germinal nucleus goes through a number of changes during the formation of the deutoplasm, that probably has to do with the great elaboration of this material.

The mature eggs are found in the posterior region, where they take up the greater part of the ovarian chamber. They measure from 80–200μ in their longest diameter, according to the size of the female. They are oval in shape and somewhat flattened. They are highly granular, the granules being very uniform in size.

Unlike Stratiodrilus, there may be a number of ripe eggs within the chamber at one time, although one usually predominates in size over the others. In the violent movements of the animal small fragments of the egg are sometimes broken off by compression against the gut, or from friction against the other eggs of the cavity. These are seen to move about the cavity quite freely, and, by some peculiar cohesive process, are capable of joining up with the egg again. This can be seen taking place under the microscope. The fragments have a membrane of their own, and may be seen lying against the egg from which they have separated. The membrane between them breaks down, and they flow together rapidly.

Normally the ripe ovum is almost divided in two portions by its compression against the gut. When a ripe ovum is discharged its place is immediately taken by the next in size. I have never actually observed the female in the act of depositing her eggs; as I have mentioned, this takes place usually at night. From the fact that the funnel in the female is well forward at the generative region and the ripe ova are

sometimes far back at the caudal end of the ovary, they have to move some considerable distance forward before they can find exit through the oviducts. In passing through the second ampulla the egg is surrounded by its capsule, which binds it firmly to the membranes of the lobster's ova, or the hairs of the carapace surface of the branchial chamber.

I have already drawn attention to the fact that the oöcyte commences to prepare for the first maturation division and the extrusion of the polar bodies when it has acquired only a portion of its yolk material. It is noteworthy that only one of the oöcytes undergoes this change at a time. It is the most advanced and the largest. While the amphiaster is seen in this egg, I have never observed it in any of the younger ones, although some of these to all external appearances are as large and as mature as the one in which it has appeared.

As the oöcyte prepares for maturation its staining reaction changes. Up till this time the superficial layer of its cytoplasm stains darkly, while the deeper portions surrounding the nucleus do not take the stain. With the appearance of the maturation spindle the staining reaction of the cytoplasm becomes uniform throughout the cell.

The first sign of approaching maturation is announced by the changes undergone by the nucleus. It is distinguishable in the living egg as a clear spot in the middle of the dark granular cytoplasm. By a number of changes, which I have not followed in detail, the chromosomes form, the germinal vesicle breaks down, and the amphiaster of the first polar body forms. This at the moment it appears is very small, but grows rapidly with the growth of the egg. From the time it appears to the time it reaches its full dimensions it at least trebles its length, while the egg grows considerably in size. From measurements made of the length of the central spindle, from centrosome to centrosome, and the diameter of the egg in its longest axis, it was found that from the time the central spindle was clearly visible to the time it ceased to grow it trebled its length, while the egg a little more than doubled

its longest diameter. The spindle seems to grow with the egg. The size of the amphiaster is always proportional to that of the ovum. In the large female, where the eggs are almost double the size of those of the small ones, the spindle is correspondingly larger. The size of the spindle is apparently determined by that of the cell.

In Limulus, according to Munson (23), the growing centre of the egg is the vitaline body. This, in the early stages, presents all the appearances and features of the centrosome and sphere, and, in fact, is the centrosome of the dividing oögonia. In later stages it remains as the definite centrosome in the cytoplasm. Thus it appears as the primitive basis or centre of growth of the cytoplasm, building this in part from the granules supplied by the follicle-cells. In Histriobdella growth does not seem confined to the region near the amphiasters, but seems to take place generally throughout the cytoplasm of the egg. No yolk-nucleus or vitaline body is present. In sections of fixed eggs the cytoplasmic material in the immediate vicinity of the spindle is markedly less dense than in the peripheral region of the ovum. In some sections the middle of the ovum appears as a space, in the middle of which is the spindle with its chromosomes.

The ovum goes through a portion of maturation during the time it is still adding material to its cytoplasm. While the achromatic threads of the amphiaster can be readily seen in the living egg, the chromosomes cannot be detected without staining. At the end of the prophase eight chromosomes are found in the equatorial plate of the spindle.

The astral rays are much less definite than the strands of the central spindle. While the former seem in the living egg as if due to the arrangement of the yolk-granules in definite lines, the latter appear as actual threads running between the granules themselves. In speaking of the astral rays Wilson (32) says: "A careful study of their relation to the meshwork in the Echinoderm, and in many other forms (especially in Nereis, Thalassema, Lamellidoris, and Asterias), leaves no doubt in my opinion that they are actual

fibrillæ, that thread their way among the crowded alveolar spheres. In my best preparations the astral rays appear like wires bending to and fro among the alveoli" (p. 13). "From a study of Toxopneustes one would be led to the conclusion that they arise in rows of granules or microsomes, held together by the continuous substance" (p. 15). These words exactly describe the appearance of the astral rays in the living egg of Histriobdella.

Towards the centre of the astral figures the rays appear as continuous fibres, while peripherally they break up into rows of granules. I believe in both the asters and the central spindle the granules do not build up the achromatic figure, but are merely incidental to it. This is borne out by the fact that they are less numerous within it than in the surrounding cytoplasm. For this reason the area of the amphiaster in the living egg is always the most transparent. The archoplasm can be distinctly seen as a clear substance running between the microsomes.

The less dense nature of the astral rays, as compared with the fibres of the spindle, has been clearly demonstrated recently by Lillie (19) on centrifugalised eggs, where the egg-granules are readily driven through the substance of the astral rays, while they are stopped and forced to go round that of the spindle.

The chromosomes in Histriobdella are arranged round the periphery of the equatorial plate. Each chromosome lies directly against one of the spindle-fibres. These run from one centrosome to the other without any break in their continuity. It is obvious that the chromosomes have no proper mantle-fibres, and that the number of fibres composing the spindle is in excess of that of the chromosomes. In sections the number of fibres can be counted. There are twenty, while there are only eight chromosomes.

The centrosome itself is not distinguishable as a distinct point or granule in the living egg, but its position is indicated by a small area where the fibres of the astral rays and those of the spindle all converge on one another. No sphere can be distinguished.

In the early stages, during the formation of the central spindle, its fibres in part appear to arise outside the area of the nucleus. In one instance I was able to distinguish the spindle-fibres beyond the still evident remains of the nuclear wall. The centrosome clearly arises beyond the limits of the nucleus, and from the reticulum of the cytoplasm, and its presence can be clearly detected before the dissolution of the nuclear wall.

Much has been written on the origin of the spindle and the centrosomes as to whether they are of nuclear or cytoplasmic origin. It has been established that the spindle-fibres may arise from either. In the case of the mantle-fibres they arise almost invariably from the nucleus, while the spindle substance proper arises from the cytoplasm, as has been shown by Meves (22) in Salamandra, Calkins (3) and Ishikawa (17) in Noctiluca, Flemming and Heidenhain (14) in leucocytes. In cases where no central spindle is present the astral rays seem to arise from the cytoplasm, as in a number of plants, some worms, as Thalassema, according to Griffin (11), and in a number of Annelids as described by Mead (21). In other cases from the nucleus, according to Flemming (7), Rückert (27), Wilson (33), and Korschelt (18).

According to Watase (31) the centre of the aster is merely the point where the greatest number of cytoplasmic filaments meet, the centrosome thus produced giving rise in turn to the spindle filaments. Thus the spindle-fibres originate from the centre of the aster, and not from the nucleus. This is clearly shown in the case he instances of the blastomeres of Loligo, where the nucleus remains a clear area in the middle of the central spindle. There is a short period in the formation of the spindle in Histriobdella when almost the same conditions are shown. Again, the observations on eggs that have been artificially fertilised by salt solutions clearly point to the origin of the spindle quite independent of the nucleus. According to Wilson (34) all degrees exist between the asters that lie remote from the nucleus and of undoubted cytoplasmic origin, and those close beside it.

When the amphiaster attains the prophase, it remains in this stage until the egg is fertilised and deposited in the sea-water. If this does not take place, or if the conditions for egg-laying are unfavourable, it apparently remains in this state indefinitely, not making any further progress.

In one instance I was able to keep a large female under observation for the greater part of a week with the amphiaster of its largest egg in the prophase. At the end of this time the fibres of the central spindle and the astral rays were as distinct as at first, and showed no evidence of dissolution. It is evidently contact with the sea-water that is necessary to cause the completion of maturation and the extrusion of the polar body.

The spindle is of considerable size, measuring from 50–60 μ from centrosome to centrosome. It can be readily seen in the living egg with the aid of a good hand-lens. As the animal moves and the egg outline is changed by compression against the body-wall, it does not change the position of its main axis with regard to that of the egg. According to Hertwig's well-known law, as the result of the interaction of the nucleus and protoplasm the spindle comes to lie in such a position that its longitudinal axis corresponds with the axis that passes through the greatest protoplasmic mass. In figs. 18–20 are shown the position of the amphiasters in the egg as it has undergone change. The axis of the spindle, it will be seen, does not always correspond with that of the main axis of the egg, but on the whole it lies very close to this, and the cytoplasm always shows a tendency to group itself symmetrically about the spindle. I have made a number of observations that seemed to show that the form of the egg does not greatly affect the direction of the spindle-axis.

In fig. 32 is shown the egg when it has undergone considerable pressure in its long axis through contraction of the animal. The spindle shows no appreciable shortening as the result of this pressure. In fig. 18 the egg shows the commencement of two furrows running into the cytoplasm, due to compression against the gut. In fig. 32 a small portion

has been broken off the posterior end. This subsequently joined up with the egg again.

No polar body is given off by the egg while it remains within the cavity of the ovary. I have had a female under observation for several days, and have been able to follow the growth and maturation of a particular egg from the first without seeing the formation of any polar body taking place.

I have mentioned that the male is often seen to fertilise the female while she is without eggs and still immature and in the larval state. In these females the sperm can be seen working their way through the tissues and finally collecting in the oviduct. I believe this invariably takes place. Whether the sperm, once in the oviducts, retain their vitality till the female reaches maturity and bears eggs I have been unable to determine. It would seem that it is immaterial whether this does or does not take place. The female is usually fertilised over and over again before she reaches maturity and bears eggs, so that fertilisation is probably effected by the last supply of sperm she may happen to receive. It is clear that the presence or absence of ova in the female play no part as a factor in fertilisation.

No matter where the sperm are injected into the body of the female—and the male exercises no choice in this respect—they seem to collect ultimately in the ampullæ of the oviducts. It would seem as if some substance in this situation exerted a chemotactic influence over their movements, causing them to collect here from all parts of the body.

The sperm are frequently seen in the blastocœlic cavity in small masses beneath the gut. In this situation they are still shut off from the cavity of the ovary and the eggs.

In the anterior end of the ovary, crowded among the small oögonial cells, are frequently seen small masses of sperm. These appear to have undergone considerable change and to have partially lost their tails. It is probable that these sperm have gained access to the ovary by way of the oviducts. It is remarkable, however, that in the posterior region of the ovarian cavity no sperm are seen free among the ova, but they

would seem to be confined to its anterior region. Fertilisation takes place within the ovarian chamber, as a large oblong sperm-nucleus is always found in the ovarian egg, in which the amphiaster has appeared. This always lies at some distance from the spindle and close to the egg-membrane, while the spindle is centrally placed. I have been unable to determine at just what stage in the growth of the ovarian egg fertilisation takes place. As the egg is seen to increase considerably in size after the amphiaster has appeared, and as the sperm nucleus is always found in the ovum when this is present, it is possible that the egg is fertilised at a stage in which the yolk-granules are first beginning to appear. The fusion of the pro-nuclei takes place only after the polar body is extruded, and this takes place when the egg has been deposited in the sea-water.

In Stratiodrilus Haswell has observed the fertilisation of the egg taking place within the ovarian cavity.

In Dinophilus tæniatus, according to Harmer (**12**), the same conditions hold regarding impregnation and fertilisation as in Histriobdella. The penis is inserted anywhere under the skin, the act of copulation taking place repeatedly with the same female. He says, "the act of copulation has no relation to the maturity of the ova of the female, nor is it prevented by the fact that the female has already received an ample supply of spermatozoa by a preceding operation" (p. 13). The spermatozoa can be seen collected in small masses beneath the gut. Fertilisation is therefore internal. The polar bodies are given off apparently when the eggs reach the exterior, or shortly after they are deposited in the sea-water.

In the ripe egg, after the amphiaster of the first polar body has been formed in this manner, a remarkable occurrence can be brought about, which demonstrates most clearly the semi-solid nature of the spindle itself. In compressing the cover-glass on a preparation of a living parasite I happened in several instances to rupture the body-wall in the vicinity of the ovum. The egg-envelope was also broken

at the same point. The yolk-granules then rapidly poured through the opening into the sea-water, and carried the amphiaster with them. It held together as a semi-solid body, and could be seen turning over and over as it was pushed along by the granules. Once in the water outside the body the granules tended to disperse, while the amphiaster remained with its immediate surrounding granules, apparently a solid body. It remained like this for several minutes until it finally dissolved and disintegrated. I have tried to represent this taking place in fig. 8. The asters go first, while the central spindle still remains intact. This seems to show that the substance of the spindle is of firmer texture than that of the asters and centrosphere; and this is borne out, as I have mentioned, by the actual appearance of the archoplasmic substance of the asters as compared with the sharp, definite structure of the spindle. The yolk-granules adhere and seem almost a part of the archoplasmic substance of both asters and spindle, the amphiaster really appearing as a mass of brown yolk-granules held together by the thread-like archoplasmic substance. As the spindle begins to dissolve the yolk-granules can be seen being liberated from the transparent substance of the archoplasm and moving away in the sea-water. In fact the whole process of the dissolution of the amphiaster, as seen under an oil-immersion lens, is similar to that of some gelatinous substance slowly dissolved by the action of sea-water. That the spindle has some considerable rigidity is borne out by the fact that it keeps its shape, and can be seen rolling over and over as it is drawn along in the sea-water. It shows no tendency at first to flatten under the pressure of the cover-glass. This is always considerable, although its corners are supported as much as possible by wax feet, as the capillary attraction invariably draws the cover-glass down somewhat in the middle. It is not till the spindle has begun to dissolve that this rigidity is lost, when it undergoes flattening. It at the same time becomes more transparent, the archoplasmic threads appearing as if actually undergoing dissolution by the sea-water, leaving the dark

yolk-granules behind them arranged in positions that had previously been held by the archoplasm. There is a short period during which the spindle almost remains alone, the asters having completely disappeared from either end. It is at this time that the spindle can be seen to roll over as it is pushed farther and farther away from the point of rupture in the body-wall by the escape from the egg of fresh cytoplasm.

In a number of experiments I subsequently ascertained that this rupture of the egg and extrusion of the spindle will not occur if the egg is far back in the body-cavity. The body-wall ruptures at its thinnest part, which is well forward in the generative region. If the egg has to move forward some considerable distance under pressure, before it can begin to flow through the rupture the amphiaster is usually broken and destroyed. It takes place most satisfactorily when the egg is only a short distance from the point of rupture of the body-wall. It can only be observed to occur when the amphiaster itself is fully mature. When not fully formed it dissolves immediately any movement of the yolk-granules takes place. Unless, moreover, the rupture in the body-wall is fairly large, the amphiaster is usually broken in the act of being forced through, being destroyed by the granules pushing it through from behind.

In one instance the central spindle had the appearance of being composed of a mass of distinct threads, some of which on one side of the spindle had been injured and broken, the yolk-granules appearing as small grains entangled in these fibres.

I think this observation clearly demonstrates the truth of a suggestion that has been put forward, that the achromatic threads and amphiasters are firm structures, or at least more rigid than the reticulum of the cytoplasm. Gardiner (**10**), in his paper on the egg of Polychœrus caudatus, states (p. 89), "That the amphiaster is much more rigid than the surrounding cytoplasm is shown by two instructive preparations which were the result of accident. Ova containing

amphiasters in the stage now under discussion were ruptured just before the worm containing them was placed in Hermann's fluid. The cytoplasm had flowed or been pressed out of the ovum, carrying with it the amphiaster. In both cases the cytoplasmic network had been completely bent and twisted into a confused snarl. The achromatic rays were somewhat, but not nearly so much distorted, but the centrospheres were almost unchanged. From this I infer that the amphiaster and the rays are, on the whole, much more rigid than the cytoplasmic network or the cytoplasm from which they are formed." Evidently the same thing took place in this instance as I have observed in Histriobdella, where the large size of the spindle and the granular nature of the egg renders the various steps in the process clearly visible under the microscope.

By pricking the egg-membrane of Allolobophora, Foot and Strobell (9) have been able to get the egg contents on the slide, and there photograph it after fixation. "By this method the germinal vesicle, and sometimes even the spindle, flow out of the egg-membrane intact" (p. 201). Some excellent photographs are shown of these in figs. 125–130 of this paper. In Allolobophora, as in Histriobdella, the early stages of the first maturation division are gone through by the egg while it is still within the receptacula ovorum.

10. Conclusion and Summary.

Harmer (12) was the first to point out that Histriobdella was more closely related to Dinophilus than to any other Archiannelid, although Pierantoni (26), in his recent monograph, has placed Histriobdella and Dinophilus as an appendix to the Polygordidæ (including Protodrilus). Schimkewitsch (28) has contended that Dinophilus is closely related to the Rotifers, and Haswell (13) has put forward a similar claim for Histriobdella. In Histriobdella it is certain that the parasitic mode of life has resulted in a peculiar specialisation, which, combined with its direct mode

of development, renders its relationship hard to determine, and hides the primitive characteristics of its organisation. That the Rotifers themselves are likewise a highly specialised class of somewhat uncertain affinities is an opinion that is gaining ground, since so much doubt has been thrown on their supposed relation to the Annelid trochophore. The work of Wesenberg-Lund (**20**) has shown that the most simple and trochophore-like of the Rotifers are probably the most highly specialised and the farthest removed from the Annelids. Yet the clearly segmented plan of both Dinophilus and Histriobdella, it must be admitted, is essentially similar to that of a Chætopod. This, combined with the clearly Polychæt nature of egg-segmentation in Dinophilus, is sufficient to place these forms in direct connection with the Annelids, quite apart from either Protodrilus or Polygordius.

Under the heading of the various organs I have already gone into a more or less detailed comparison of Histriobdella with Dinophilus, so that it is only necessary to review the subject here from a more general standpoint. In both forms the animal consists of a distinct head and trunk, the latter composed of relatively few segments. In both the nervous system consists of a well-defined brain or neuropile, and a double ventral nerve-cord, with metamerically arranged ganglia. In Dinophilus these are formed by transverse commissures, while in Histriobdella the two parts of the cord unite directly to form the ganglia. The external segmentation corresponds with that of the nervous system. Dinophilus does not possess the feet, cirri, or tentacles that so clearly mark segmentation in Histriobdella. But the metamerism is less definitely shown by the ciliated bands, mucus glands, and the ring-like constriction of the body into a series of segments. On the other hand the nephridia show a more matamerically placed arrangement than they do in Histriobdella. In both (with the exception of Stratiodrilus) the nephridia open to the exterior in the segment following that in which they arise, as in Annelids. In Histriobdella the muscular system shows a very high

degree of development, and for this reason can hardly be compared with that of Dinophilus; in both, however, the main musculature consists in a series of longitudinal ventro-lateral and dorso-lateral muscles. The alimentary canal shows the same divisions, although differing considerably in the relative proportion of its parts. The strong chitinous jaws are wanting in Dinophilus. In each the cavity surrounding the gut is a primitive blastocele with no definite epithelial lining. This cavity sends prolongations into the head. The equivalent of the cœlom in both is represented by the cavity of the reproductive glands. In the male these consist of a more or less paired testis, vesiculæ, vas deferens, and median penis, and in the female a large ovarian cavity, paired or unpaired, with oviducts.

With Annelids Dinophilus shows a closer relationship than Histriobdella, mainly due to its less direct development. In fact the development of Dinophilus brings it into line with that large group of animals such as the Polychætæ, Echinoidæ, Gephyrea, Lamellibranchs, and the Gasteropoda, in having the ectoderm arising from the first three quartettes, mesoderm from the left posterior cell of the fourth quartette (4 D.), and the endoderm from the remaining cells. In the derivation of a large part of the ectoderm of the trunk from the posterior cell of the second quartette the resemblance to the Polychæt Annelids is most pronounced. This is further enforced in the origin of the bilateral cleavages in the cross cells and in the products of 2 D. "The transition from the spiral type of cleavage to the more specialised bilateral type occurs in precisely the same directions as in the Polychæts. Moreover the second bilateral divisions of the cells of the posterior arms of the cross continue this resemblance. All these characters, if such they may be called, when viewed as a whole point in no uncertain way to the descent from the Annelid stem, and at a point not far from that at which the Polychæta arose" (Nelson, p. 728).

The weight of our evidence, furnished by recent work on the morphology and embryology of Dinophilus, is strongly

in favour, therefore, of a close relationship with Annelids. The general ciliation, the caudal appendage, ciliated rings, nervous system, general configuration of the head, trunk, and alimentary canal are what are found in a number of Annelids, and most clearly in such a form as Ophyotrocha. Nelson (**25**) has even suggested that the pre-oral nerve commissures can be satisfactorily explained by deriving them from the nerve-ring of the Trochopore. He comes to the conclusion: "On the whole, Dinophilus can best be considered as a very young Polychæt worm, retaining some of its larval features, with setæ and parapodia undeveloped, and whose peritoneum and cœlom have been transformed into a generative organ" (p. 135).

The relationships of Histriobdella to Polygordius and Protodrilus have been gone into fully by Foettinger (**8**), Harmer (**12**), and Haswell (**13**), so I need not repeat their arguments for this relationship here. It seems to me, from the Archiannelid point of view, it is important to determine what features of Histriobdella are primitive, and what have been derived from its peculiar mode of life. Eisig (**5**) has gone so far as to suggest that in Histriobdella we have to do with a highly modified, possibly degenerate animal, and not an Archiannelid at all. If Histriobdella is a degenerate form then it must be a degenerate Chætopod as Haswell (**13**) has pointed out. "If we are to take this view, we must at the same time acknowledge that side by side with the supposed degeneration, there must have gone on a special development in certain directions; that, while the definite characters of the segmentation became lost, a special set of locomotor organs with an elaborate musculature became evolved." "This view appears to me to involve difficulties so great that they render the degeneration theory extremely improbable, and it seems to me more in accordance with the facts of the case to conclude that the Histriobdellidæ are really primitive Annulates, and that the rudiments of their specialised features have been inherited from forms lower in the scale" (p. 327).

Apart from any degeneration I agree with Haswell (13) that the relationship of Histriobdella with Polygordius "is extremely remote, and not such as to justify their inclusion in the same class." The absence in Histriobdella of a blood-vascular system, a distinct prostomium and peristomium, the presence of mouth opening well forward in the head, chitinous jaws, and complicated generative apparatus in the male, paired limbs, and mucous glands, clearly separate it from Polygordius and Protodrilus, placing it quite apart from these forms. With the Rotifers, on the other hand the relationship is undoubtedly more pronounced. Haswell has pointed out that all the main features of Histriobdella can be traced to this class, although in general features the resemblance is greater perhaps with the Gastrorichia than with the Rotifers proper. The chitinous jaws of Histriobdella can be readily homologised with the mastix of Rotifers. In the absence of solenocytes and the general similarity of the nephridia of Histriobdella to the flame-cell type nephridia of Rotifers, we have a further resemblance. In both the cuticle is firm and shows a tendency to contract into ring-like folds. In both, also, the generative organs, especially in the male, can be reduced to the same plan.

In Paraseison we have a Rotifer not unlike Histriobdella in many of its features. The body is elongated and worm-like, with a distinct head bearing the mouth at its anterior extremity. In the middle of a very rudimentary coronal disc which bears no ciliated apparatus are four small bundles of hairs, placed in two pairs. Behind the mouth are found the orifices of two glands, similar to those found on the anterior feet of Histriobdella. On the top of the head is a small tubercle representing the dorsal median tentacle of Histriobdella. There is a narrow œsophagus, which leads into a large cylindrical stomach. There is no gut, and the stomach, which is not ciliated, is definitely closed. But this condition has plainly been evolved within the limits of the genus, as it is not characteristic of other Rotifers. It is

noteworthy that Paraseison, like Histriobdella, is parasitic, being found on the gills of the Crustacean Nebalia. It is undoubtedly with such forms as Paraseison among the Rotifers that Histriobdella must be compared. The greatest objection to the comparison of Histriobdella with the Rotifer is encountered in regard to the nervous system. This in Histriobdella is already so elaborated, and of that type found among the higher Annelids, as to be hardly comparable to the diffuse, and less differentiated, and centralised system of Rotifers.

I cannot agree with Haswell that Zelinka's (36) discovery of a sub-œsophageal ganglion in Callidina and Discopus renders this comparison more easy. A further difficulty is found in the absence of any true metamerism in the Rotifers. This difficulty is possibly not so great when we consider the arrangement of the transverse muscle-cells in such a rotifer as Discopus synaptæ. Leaving aside any comparison, therefore, of the nervous system, it nevertheless remains a fact that Histriobdella undoubtedly resembles the Rotifers more closely than any other group of animals.

If Histriobdella is related to the Rotifers it becomes necessary to determine the relationship of Dinophilus to the same class. Schimkewitsch (28) was the first to point out the similarity of the caudal appendage in Dinophilus to the foot of the Rotifer. In Dinophilus, as in the Rotifer, this is used in attaching the animal. In both forms there is a marked sexual dimorphism. But as Nelson (25) has pointed out, the caudal appendage in Dinophilus resembles more that of some of the polytrochal annelid larvæ than the foot of the Rotatoria, and the sexual dimorphism can have arisen within the genus, as it is found in other groups of the Annelida besides the Rotifers. One striking difference between the Rotifers and Dinophilus is the apparent total absence of a definite mesoblast in the Rotifers, while it is clearly present in Dinophilus, where it has the same cell-origin as in Polychæts. In Rotifers the mesoblast would seem to be represented by the germ-cells alone, and it is

necessary to suppose that the Rotifers separated from the main stem of the Annelida at a stage earlier than that of the formation of a definite mesoderm, while Dinophilus arose only after the cœlo-mesoblast had definitely appeared. On the whole, Dinophilus is not so closely allied to the Rotifers as Histriobdella. Unfortunately our lack of information with regard to the development of the cœlo-mesoblast in Histriobdella prevents our forming any opinion as to how much it resembles the Rotifers in this respect.

It is remarkable with regard to the Rotifers that, despite their wide distribution and their great number of species, so comparatively few marine forms should be known. What has become of these if they have ever existed? Are forms like Belatro and Hemidasys (Claparède, 4), Turbanella (Schultz, 29), or the Echinoderes (Zelinka, 37) to be looked upon as the modified descendants of a marine branch of these animals? Here we have a marked metamerism coupled with the main features that characterise both Histriobdella and the Rotifers. It is possible that it is with some of these somewhat obscure groups that the relationship of Histriobdella really lies.

In conclusion, it may be stated that our present knowledge does not warrant us farther than to conclude that Histriobdella is a highly specialised form, retaining many Rotiferan features, and that it is to be grouped with Dinophilus as a primitive Annulate, but not directly related to Polygordius and Protodrilus.

Summary.

(1) Histriobdella homari is a normal inhabitant of the branchial chamber of the European lobster. It is found in equal numbers throughout the year, on both the male and female.

(2) The anterior feet of the head, unlike those of Stratiodrilus, are non-retractile.

(3) There are four pairs of nephridia in both sexes. They are closed, and are of the primitive flame-cell type similar to

those of Rotifers. Unlike those of Dinophilus, they bear no solenocytes.

(4) There is a pair of salivary glands in connection with the mouth.

(5) There are fewer teeth in the jaw-apparatus than Foettinger has represented.

(6) The ventral nerve-cord is composed of two portions, which separate in the intersegmental to unite in the segmental regions, in prominent ganglia. The metamerism of the nervous system corresponds with that of the external form.

(7) In the male there is a complicated generative apparatus. It is similar in all respects to that of the male generative apparatus in Stratiodrilus.

(8) Fertilisation takes place internally. The largest egg is usually seen in the prophase stage of the first maturation division. The amphiaster and the spindle can be seen to pass out through the body-wall with the cytoplasm, when the egg is ruptured by pressure. It remains for some seconds intact in the sea-water surrounded with yolk-granules.

(9) In the equatorial plate there are eight chromosomes in the first maturation division.

(10) Histriobdella is to be placed close to Dinophilus. It retains many Rotiferan features, and is more closely connected with this group than Dinophilus. Histriobdella and Dinophilus show distant relationship with Polygordius and Protodrilus, but cannot be classed with them as true Archiannelids.

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35. Zelinka, C.—"Die Gastroctrichen," 'Zeit. f. Wiss. Zool.,' Bd. xlix, 1890, p. 209.

36. ——— "Studien über Raderthiere," 'Zeit. f. Wiss. Zool.,' xliv, p. 396, Bd. xlvii, p. 353, Bd. liii, p. 1, 1885–1892.

37. ——— "Über Echinoderes," 'Verh. d. deutschen Zool. Gesell.,' 4th Jahrssam., 1894, p. 46.

EXPLANATION OF PLATES 17—20.

Illustrating Mr. Cresswell Shearer's paper "On the Anatomy of Histriobdella Homari."

Lettering.

ac. Accessory glands of the male reproductive apparatus. *an.* Anal aperture. *ap. p.* Appendage of the posterior leg. *blc.* Blastocœlic cavity. *bl. m.* Muscular organs of jaws. *br.* Bridle piece of jaws. *brn.* brain. *cd. g.* 1–*cd. g.* 3. Ganglia of the caudal region. *cl.* Clasper. *cl. p.* Ciliated pit of the head. *cœ.* Cœlom. *cœ. ep.* Cœlomic epithelium. *com.* Nerve commissures. *f.* Fulcrum of jaws. *fol.* Follicle cells. *fgl.* Flagella of the nephridial canals. *g.* 1–*g.* 5. Ganglia of the ventral nerve-cord. *gl. cl.* Gland-cell of clasper. *gr. g.* Granule gland. *int.* Intestine. *int.* 2. Intestine, posterior part. *j.* Jaws. *j.* 1. Upper ramus of jaws. *j.* 2. Lower ramus of jaws. *l. a.* Anterior legs or feet. *l. p.* Posterior legs. *m.* Mouth. *m. d.* Dorsal longitudinal muscles. *m. d. p.* Median duct of the penis. *m. gl.* Salivary glands of the mouth. *m. ob.* Oblique muscles. *m. v.* Ventral longitudinal muscles. *n. c.* Ventral nerve-cord. *neph.* 1–*neph.* 4. Nephridia. *neph. c.* Nephridial canals. *neph. h.* Head of the nephridium. *neph. o.* Opening of the nephridial canal on the external surface. *neph. s.* Spaces on the course of the nephridial canals. *œs.* Œsophagus. *o. im.* Immature ova. *or. p.* Orifice of the penis sheath. *ov.* Ovary. *ovd.* Oviduct. *p.* Penis. *r.* Ramus of upper jaw. *sprm.* Spermatidia. *spc.* Spermatocyte. *st.* Stomach. *t.* 1. Median tentacle. *t.* 2 and *t.* 3. Lateral tentacles. *te.* Testis. *th.* Teeth. *v. def.* Vas deferens. *ves.* Vesicula seminalis. *vn. c.* Ventral nerve-cord. *vit.* Vitellarium or shell-gland.

PLATE 17.

Fig. 1.—Female Histriobdella with eggs. The largest egg shows the presence of a maturation amphiaster × 300.

Fig. 2.—Clasper extended.

Fig. 3.—Third and fourth nephridium in the female. This and all the subsequent figures of the nephridia have been drawn from living preparations impregnated with methyl-blue.; 2 mm. oil-immer., comp. ocs. 4 and 6. × 500 and × 1000.

Fig. 4.—Third nephridium in the male.

Fig. 5.—Third nephridium in the male.

Fig. 6.—Second nephridium in the male.

Fig. 7.—Male Histriobdella with claspers retracted. × 300.

PLATE 18.

Fig. 8.—Rupture of an egg through the body-wall in a living preparation by compression of the cover-glass. The first maturation amphiaster is seen outside the body-wall in the sea-water. × 300.

Fig. 9.—Male with claspers extended. × 300.

Fig. 10.—Third and fourth nephridium in the male. × 800.

Fig. 11.—Generative segment in the male. Taken from a living preparation, showing the reproductive organs.

Fig. 12.—Fourth nephridium in the male. × 800.

Fig. 13.—Fourth nephridium in the male. Segment contracted. × 800.

Fig. 14.—Second nephridium in the female. × 800.

PLATE 19.

Fig. 15.—Reconstruction of the nervous system in the male, showing the dorsal curve taken by the ventral nerve-cord in the region of the penis. Lateral view. × 300.

Fig. 16.—Young in egg-capsule.

Fig. 17.—Young in egg-capsule. Earlier stage than that shown in fig. 16.

Fig. 18.—Oöcyte with first maturation amphiaster. This, with the subsequent figures, 19, 20, 26, and 32, are all drawn from the same egg-cell. They show the changes of shape assumed by the egg in the movements of the animal. They were drawn at intervals of from ten to twenty minutes.

Fig. 19.—Oöcyte, same as that shown in fig. 18, drawn twenty minutes later.

Fig. 20.—Oöcyte, same as that of fig. 19, fifteen minutes later.

Fig 21.—Reconstruction of the nervous system in the female. The brain surface is measured from the ganglion cells and not from the fibrous core. Ventral view. × 300.

Fig. 22.—Sagittal section in the female showing the sac-like nature of the ovarian cavity.

Fig. 23.—A cell of the accessory gland of the male.

Fig. 24.—Transverse section in the male in the region of the penis. × 400.

Fig. 25.—Eggs attached to the membranes of the lobster "berry."

Fig. 26.—Oöcyte twenty minutes after that shown in fig. 20.

Fig. 27.—Spermatidia.

Fig. 28.—Reconstruction of the nervous system of the male. Ventral view. × 300.

Fig. 29.—Longitudinal section of the wall of the intestine in the posterior region.

Fig. 30.—Young, a short time after hatching. × 300.

Fig. 31.—Section through the generative region in the male showing the granule glands.

Fig. 32.—Oöcyte twenty minutes later than fig. 26.

PLATE 20.

Fig. 33.—Transverse section in the male through the region of the vesiculæ seminales.

Fig. 34.—The same. In a region a little posterior to the last.

Fig. 35.—Transverse section through the middle of the generative region in the male.

Fig. 36.—Chitinous jaws.

Fig. 37.—Horizontal section in the male.

Fig. 38.—Transverse section through the middle of the second segment.

Fig. 39.—Transverse section through the anterior region of the generative segment in the male, showing the divided nature of the anterior portion of the testis.

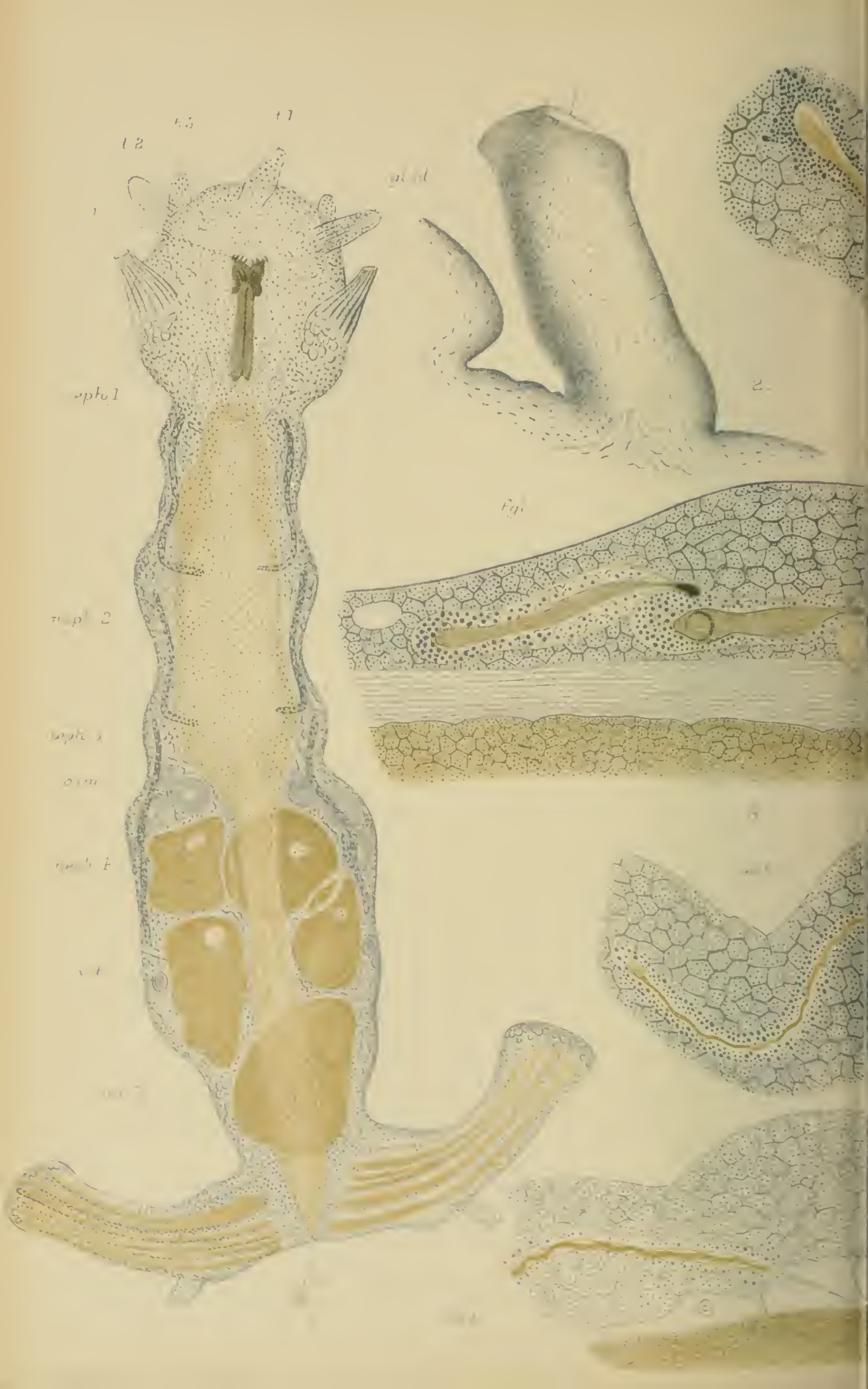
Fig. 40.—Transverse section through the posterior region of the generative segment in the male.

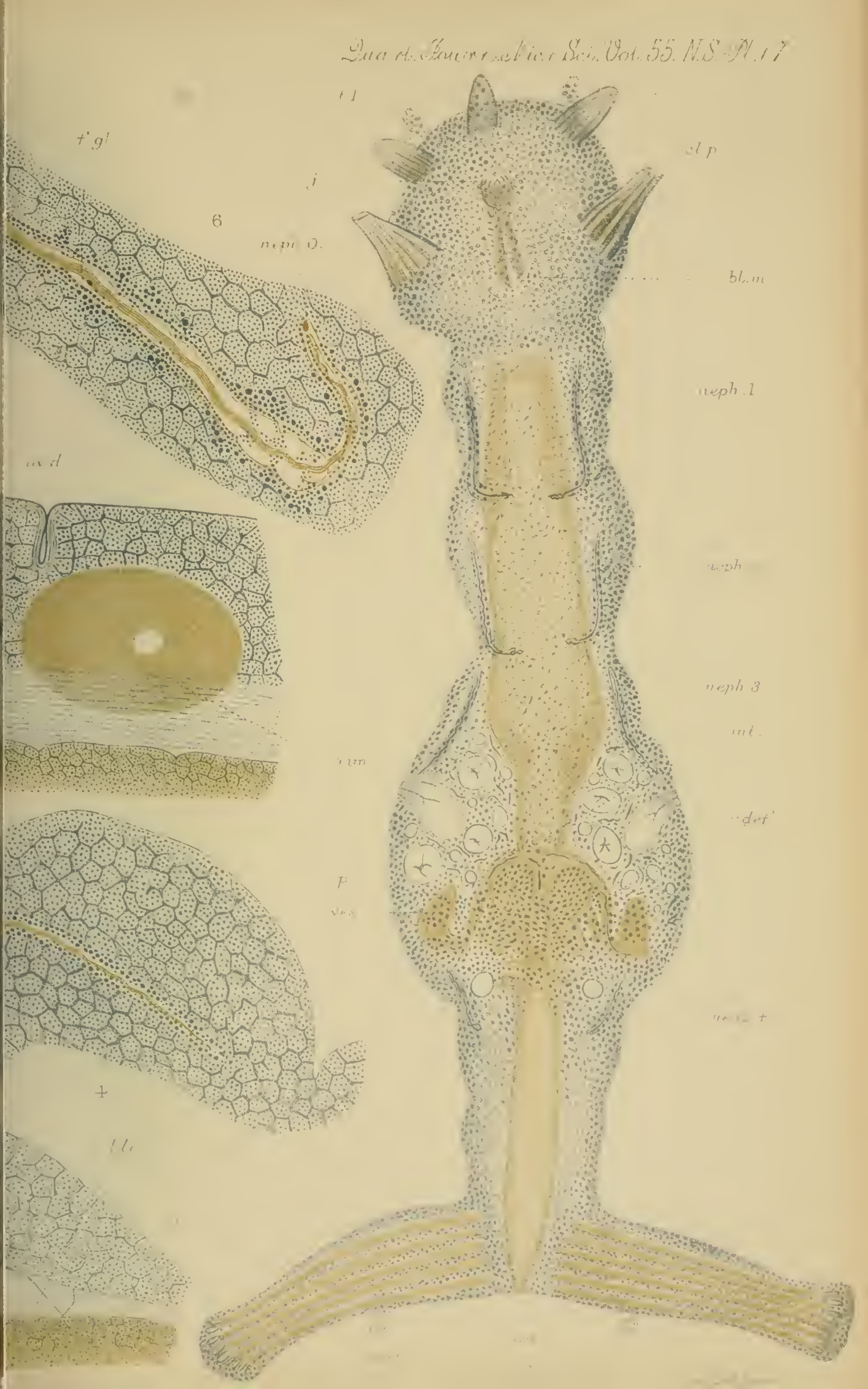
Fig. 41.—Transverse section through the dorsal region of the body-wall in the caudal segment, showing the insertion of the oblique muscle-fibres.

Fig. 42.—Horizontal section through the region of the first segment in the male, showing the head of the first nephridium.

Fig. 43.—Transverse section through the neck region.

Fig. 44.—Transverse section through the caudal region, showing the oblique muscles.

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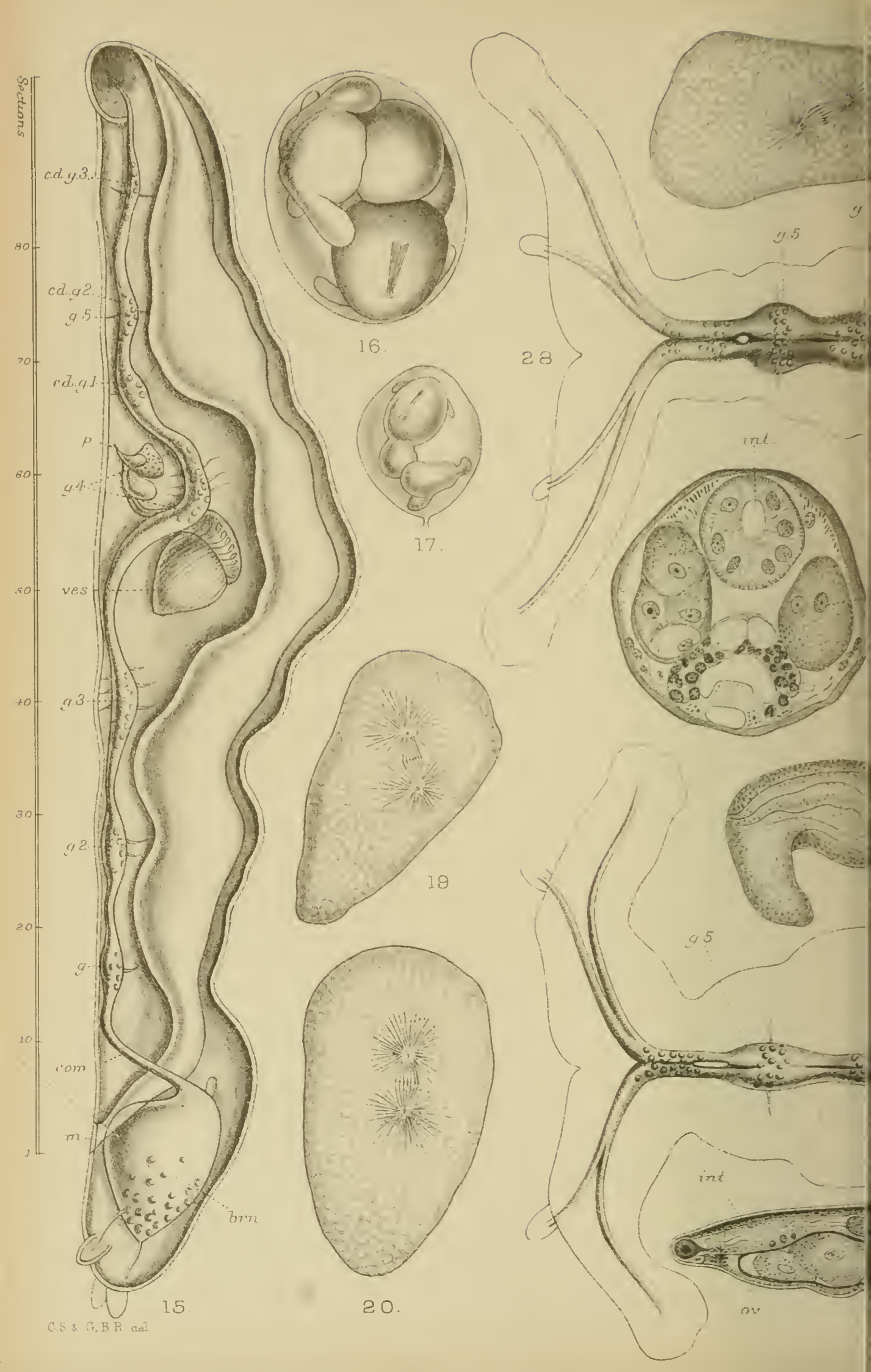
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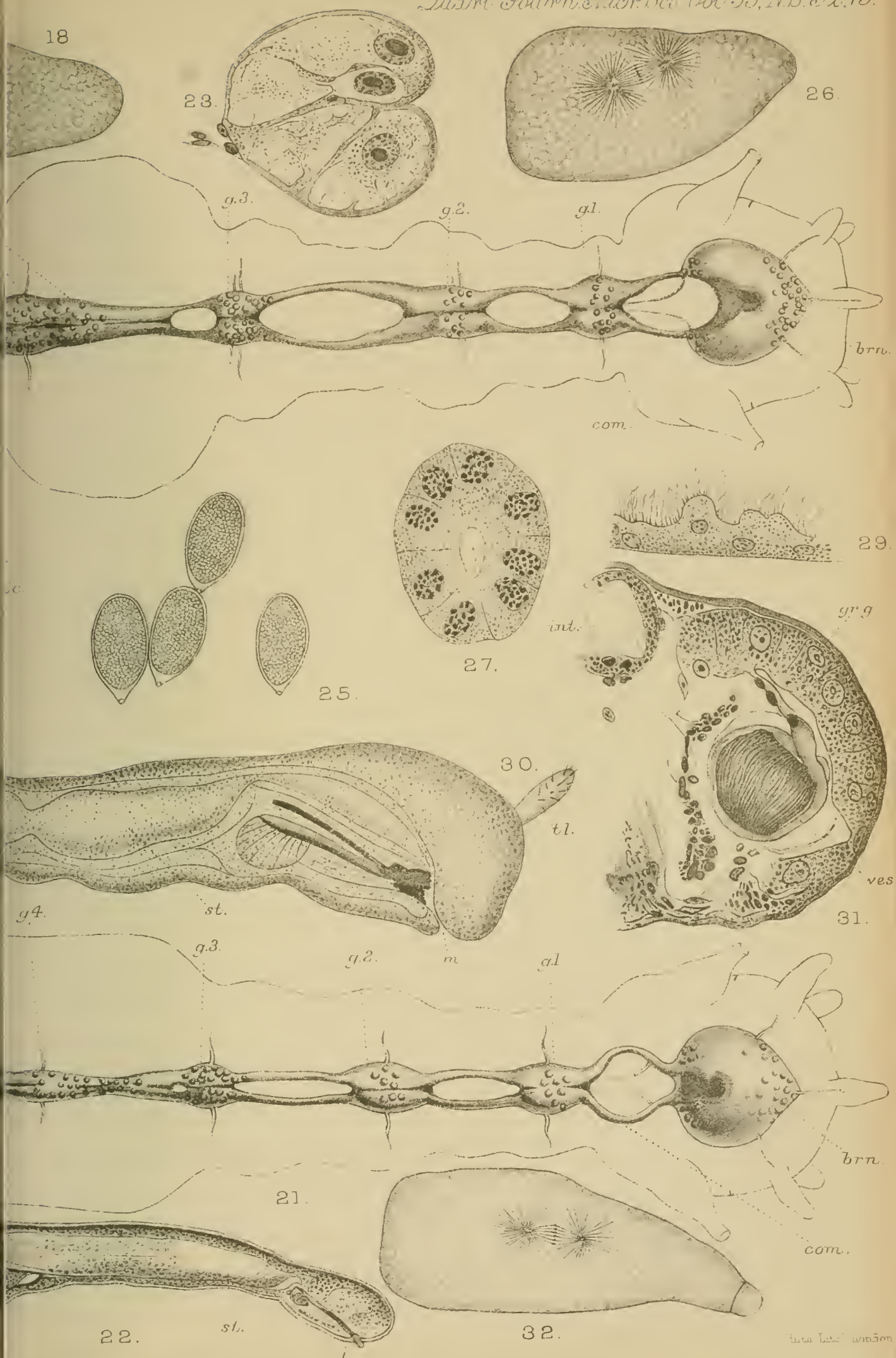
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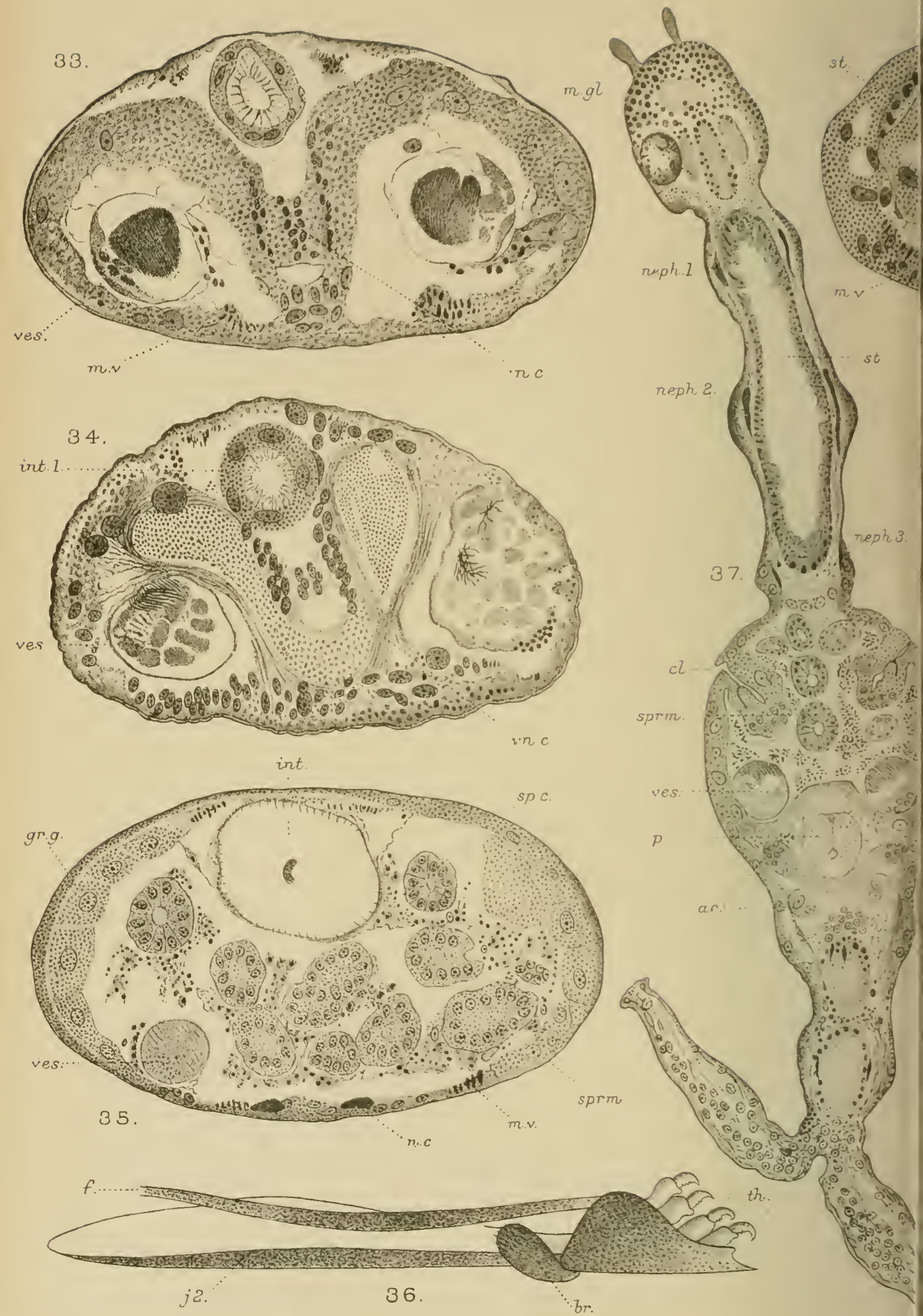
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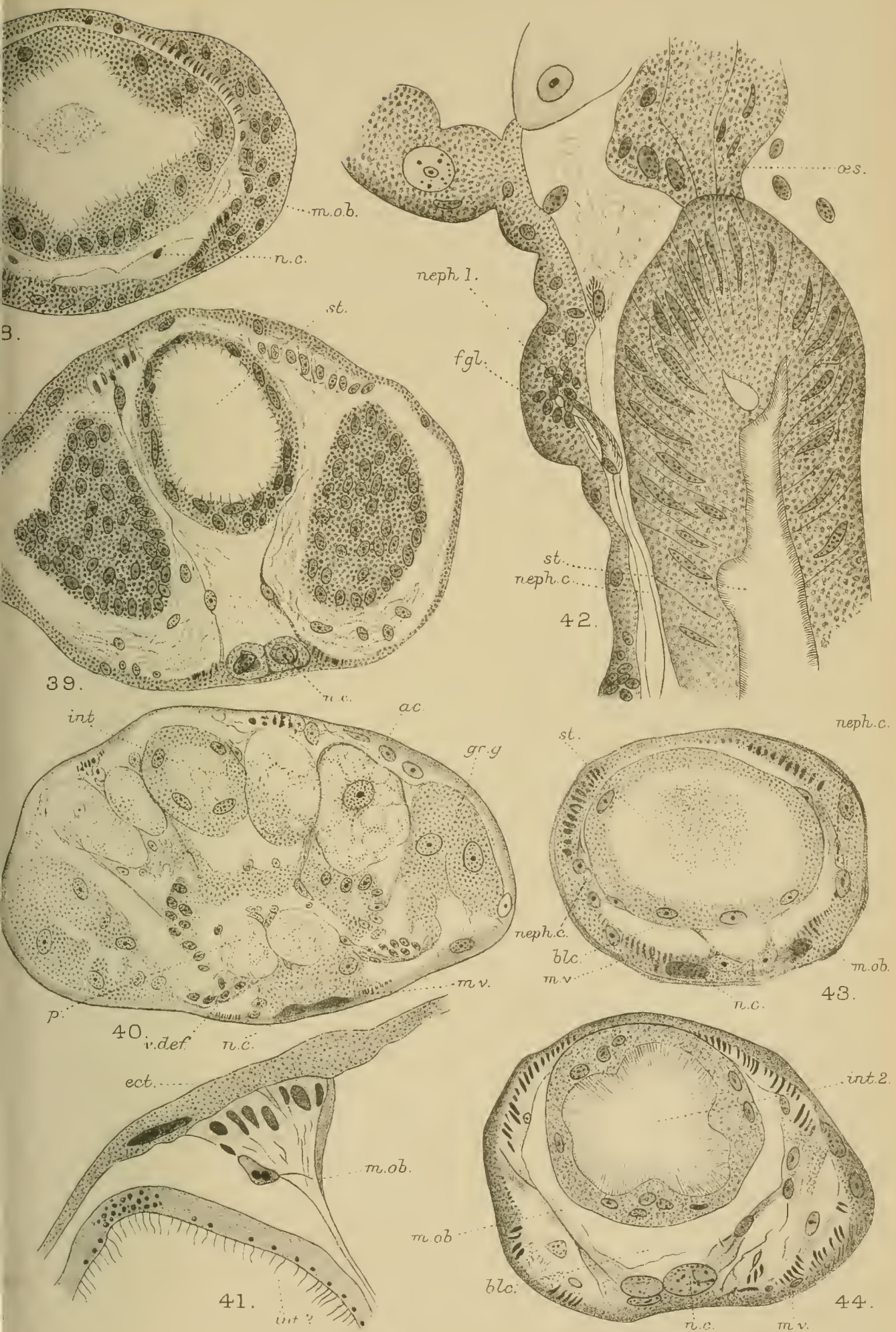

Huth Lith^r London.

On the Artificial Culture of Marine Plankton Organisms.

By

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and

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Contents.

[1] Owing to pressure on our space, this memoir could not be published when first in type. It has in the meantime been issued in the 'Journal of the Marine Biological Association,' vol. viii, No. 5.—E. R. L.

Introduction.

The observations to be recorded in this paper were commenced in March, 1905. They originated in an attempt to find a general method for rearing marine larval forms. Several investigators had previously succeeded in rearing echinoderms, molluscs, and polychætes from artificially fertilised eggs, under laboratory conditions, but the process was generally difficult and the results more or less uncertain. The most promising method seemed to be that adopted by Caswell Grave (**26**), who was able to rear his larvæ by feeding them on diatoms. Grave obtained his diatoms by placing sand, collected from the sea bottom, in aquaria, and using such diatoms as developed from this material. All the methods, however, suffered from the uncertainty of not

knowing what organisms were introduced into the aquaria in which the larvæ were to be reared, either in the original sea-water or along with the food supply.

It appeared, therefore, at an early stage of the work, worth while to make an attempt to carry out rearing experiments on a more definite and precise plan, to endeavour, in fact, to introduce the larvæ to be reared into sterile sea-water, and to feed them with pure cultures of a suitable food. This was the ideal to be aimed at. As a matter of fact it has seldom, if ever, been attained in practice; nevertheless, a considerable measure of success has been achieved by working upon these lines, and during the course of the work innumerable problems relating to the physical conditions under which plankton organisms can best flourish have presented themselves. Some account of the experiments made may be of interest to other workers, although many of the problems raised are not yet solved, notwithstanding the fact that some 1500 cultural experiments have been under observation. It is rather with a view of stimulating other work upon similar lines than of bringing forward conclusive results, that this paper is being published.

In the summer of 1907 Mr. E. W. Nelson became associated with the investigation, and since that date the experimental work has been carried out by him. The discussions in this paper of a more chemical character, particularly the section on alkalinity, are almost entirely the work of Mr. Nelson, and we have both had throughout the advantage of the constant advice and help of Mr. D. J. Matthews on all such matters.

I. Culture of Plankton Diatoms.

(A) Practical Culture Methods.

1. Miquel's Method.—Attention was first directed to the culture of plankton diatoms; and the methods, which had been elaborated by Miquel (11) for fresh-water diatoms and had been found by him to succeed with marine bottom diatoms, were tried.

The essential features of Miquel's method, as applied to marine diatoms, are as follows:

Two solutions are prepared:

Solution A.

Magnesium sulphate	10 grm.
Sodium chloride	10 ,,
Sodium sulphate	5 ,,
Ammonium nitrate	1 ,,
Potassium nitrate	2 ,,
Sodium nitrate	2 ,,
Potassium bromide	0·2 ,,
Potassium iodide	0·1 ,,
Water	100 ,,

Solution B.[1]

Sodium phosphate	4 grm.
Calcium chloride (dry)	4 ,,
Hydrochloric acid	2 c.c.[2]
Ferric chloride	2 c.c.[3]
Water	80 c.c.

Forty drops of solution A and ten to twenty drops of solution B are added to each 1000 c.c. of sea-water, and the sea-water is sterilised by keeping it at 70° C. for about twenty minutes.

According to Miquel it is also necessary to add "organic nutritive material in the form of bran, straw, or filaments of

[1] "The preparation of solution A presents no difficulty. Solution B should be made up as follows: To the sodium phosphate dissolved in 40 c.c. of water are added first the 2 c.c. of hydrochloric acid, then the 2 c.c. of hydrous ferric chloride, and then the 4 grm. of calcium chloride dissolved in 40 c.c. of water, taking care to shake the mixture, which I call phospho-ferro-calcic solution. The addition of this last solution to the maceration throws down a slight brownish flocculent precipitate, formed for the most part of ferric oxide, which should be carefully separated from the liquid used for cultivations."

[2] "Acid chlorhydrique pur à 22°." Presumably meaning degrees Baumé = sp. gr. 1·169.

[3] "Perchlorure de fer liquide à 45°." As above = sp. gr. 1·421.

weeds, such as Zostera. Macerations of these should be made up separately some time before they are required for use, and should be carefully filtered and sterilised. Organic matter must, however, be used very sparingly, or else putrefaction will set in and the cultures will be irrevocably lost." As a matter of fact we have found that such organic infusions are unnecessary when dealing with plankton diatoms, and it has not been our practice to employ them (cf., however, p. 392).

Miquel obtained cultures of single species of diatoms either by picking out individual diatoms under the microscope and introducing them into the prepared water, or by adding a small quantity of water containing a mixture of diatoms and other organisms to some prepared water, and subdividing this into a number of tubes. If the subdivision has been carried out sufficiently some of the tubes may contain one kind of diatom only, from which fresh cultures can be made. In this way, by repeated subdivision, cultures can be obtained which, by inoculating fresh quantities of prepared water from time to time, may, with care, be maintained indefinitely. Such cultures, however, must practically always contain bacteria, and Miquel distinguishes them from bacteria-free cultures, which he terms "cultures des diatomées a l'état de pureté absolue." The latter he found very difficult to obtain, but through repeated washing in sterile water, followed by fractional subdivision, he succeeded in getting some in which he could find no trace of bacteria by ordinary bacteriological methods (Miquel [**11**], p. 155; cf. also Richter [**16–18**]).

We propose to call any diatom culture which can be carried on practically indefinitely by inoculating fresh supplies of prepared water a "persistent" culture, the term "pure" culture being reserved for cultures which can be proved to contain not more than one organism. We are not satisfied that we have yet succeeded in obtaining cultures of the latter kind. For the most part our persistent cultures contain one species of diatom only, and are free from all organisms larger than small flagellates.

In our earlier experiments with plankton diatoms we

obtained persistent cultures, containing a single species of diatom, by both of the methods recommended by Miquel. We, however, have rarely succeeded by picking out single diatoms or chains of diatoms, for although we have passed the selected diatom through several changes of sterilised sea-water, the resulting cultures, even when the diatoms have multiplied to some extent, have generally shown evidence of contamination by harmful organisms, and have soon died down. Only in one of the earliest experiments, and in one more recent, has complete success resulted. In the first case a small chain of six or eight frustules of Skeletonema costatum, picked out in April, 1905, gave rise to a culture which still persists (November, 1909). Subcultures can still be obtained even from the original flask inoculated in April, 1905. In the second case a chain of eight or nine cells of Chætoceras densum, picked out from a Petri dish culture, has given a particularly good growth.

The method of dilution and subdivision has been more successful, and persistent cultures of a number of species have been obtained in this way.

A more ready method of obtaining the cultures is, we have found, to add one or two drops of plankton to, say, 250 c.c. of a suitable sterile culture medium, and to pour this into shallow glass dishes (Petri dishes). The dishes should be placed in a position as free as possible from vibration, and where they can be easily examined with a lens in situ. The temperature should be kept as constant as possible and the dishes exposed to light of moderate intensity, direct sunlight being avoided. In the course of a few days, colonies of diatoms of different species will be seen at different spots on the bottom of the Petri dishes. These can be picked out with a fine pipette and transferred to flasks containing fresh culture medium. The colonies should be picked out from the Petri dishes at as early a stage as possible, because if left too long some one organism, a diatom or a flagellate, may have multiplied so rapidly that the whole of the water in the dish becomes infected with it. In this case persistent cultures of

a single species would not be obtained. The above method is similar to one described by Miquel, excepting that he placed gelatinous silica at the bottom of the vessel. Some very successful persistent cultures were obtained from the following experiment, which will serve to illustrate the method : A sample of plankton, from a very fine-mesh bolting-silk tow-net, was diluted down with sterile sea-water, until a single drop examined under a $\frac{2}{3}$ in. objective contained on an average ten organisms, chiefly diatoms, of various species. Petri dishes (4 in.), containing 60 c.c. each of Miquel sea-water, were then inoculated with various numbers of drops of the diluted plankton. The two dishes, to which two and three drops respectively were added, gave the best results, and from these persistent cultures of several species of diatoms were obtained. Hence we may conclude that the most advantageous number of single cells or short chains of cells to be added to a 4 in. Petri dish, containing 60 c.c. culture medium, is about twenty to thirty.

We have succeeded in obtaining the following species of plankton diatoms in persistent cultures :

Asterionella japonica Cleve.
Biddulphia mobiliensis (Bail.) Grun.
Biddulphia regia (M. Schultze).[1]
Chætoceras densum Cleve.
Chætoceras decipiens Cleve.
Chætoceras constrictum Gran.
Cocconeis scutellum Ehr. var. minutissima Grun.
Coscinodiscus excentricus Ehr.[2]
Coscinodiscus Granii Gough.
Ditylium Brightwellii (West) Grun.
Lauderia borealis Gran.
Nitzschia closterium W. Sm.
Nitzschia closterium W. Sm. forma minutissima.
Nitzschia seriata Cleve.
Rhizosolenia stolterfothii H. Perag.

[1] See p. 413.
[2] See p. 412.

Skeletonema costatum (Grev.).

Streptotheca thamensis Shrubs.

Thalassiosira decipiens Grun.[1]

It is hardly necessary to add that in dealing with these cultures similar precautions to those used in bacteriological work must be taken, all vessels and instruments being carefully sterilised before they are brought into contact with the prepared sea-water. The cultures are best made in small, wide-mouthed flasks, which may be plugged with cotton-wool, or simply covered with watch-glasses. The flasks should be kept at as uniform a temperature as possible (from 12°–17° C.) and should be exposed to strong daylight, direct sunlight being avoided. A flask should not be more than half filled with culture fluid, so that the surface exposed to the air may be large in proportion to the volume of fluid.

Other Methods.—The addition of the solutions devised by Miquel to sea-water has in all cases given us good cultures of diatoms, and the method is certain in its action. We have, however, made numerous experiments by treating sea-water in other ways, with a view to finding out what are the best conditions under which plankton diatoms will grow, and of arriving at some explanation of the action of the different salts contained in Miquel's solutions.

2. Houghton Gill's Method.—H. Houghton Gill (**5**), a contemporary of Miquel, made use of a culture medium not essentially different from that employed by the latter. Unfortunately he died before publishing his work, but an account of his principal results is given by Van Heurck. In his final method Houghton Gill made use of four distinct solutions, as follows:

Solution 1.

Crystallised sodium phosphate	2 grm.
Calcium chloride	4 „
Syrup of iron chloride	0·5 „
Strong hydrochloric acid	1 „
Water	100 „

[1] See p. 412.

Solution 2.

Crystallised magnesium sulphate	4 grm.
Crystallised sodium sulphate	4 ,,
Crystallised potassium nitrate	4 ,,
Common salt (sodium chloride)	8 ,,
Potassium bromide	0·2 ,,
Potassium iodide	0·2 ,,
Water	100 ,,

Solution 3.

Crystallised sodium carbonate	4 grm.
Water	100 ,,

Solution 4.

Well-washed, precipitated calcium silicate	25 grm.
Water	75 ,,

All the salts employed must be chemically pure. Three c.c. of each of these liquids are added to 1000 c.c. of fresh water or sea-water (according to circumstances), and the whole sterilised. In his earlier work Houghton Gill added a sterilised infusion of grass or of diatoms, but it is not clear from the accounts whether this was still employed with the above solutions. We have obtained very good cultures with the above solutions, to which we did not add any organic infusion.

3. (A) Modification of Miquel's Method: "Miquel Sea-water."—Since several of the components in Miquel's formula for solution A (p. 363) are obviously unnecessary when sea-water is being used as the basis of the culture medium, we adopted for our own work the following modifications: After some preliminary experiments it was found, as would be expected from the composition of sea-water, that the only salts of value to the medium are the three nitrates KNO_3, $NaNO_3$, NH_4NO_3, and possibly KBr and KI. The omission of the two latter was soon found to make no

difference. Experiments also showed that the formula for solution A could, without any appreciable detriment to results, be further simplified to the one salt KNO_3 or $NaNO_3$, but not NH_4NO_3. At first the amount of KNO_3 dissolved in 100 c.c. distilled water, used to make the modified solution A, was the same as the sum of the weights of the nitrates in Miquel's own formula, viz. 5 grm. But later experiments showed that a considerably greater concentration of KNO_3 than this gave more lasting cultures; the strength of solution and amount to be added to a litre of sea-water in order to obtain the best results being 2 c.c. 2 M KNO_3.

In the case of solution B no modification has been adopted, but it has been found that small variations in the amounts of the ingredients used do not affect the results. A convenient method for measuring the right amount of $FeCl_3$ is to warm the salt until it just melts in its own water of crystallisation, and to pipette out 2 c.c. with a previously warmed pipette. No temperature corrections need be considered. Also 2 c.c. of the ordinary pure concentrated hydrochloric acid at room-temperature will suffice.

Our own formula for preparing Miquel sea-water is now:

Solution A.[1]

Potassium nitrate, 20·2 grm.
Distilled water, 100 ,, } = 2 M KNO_3.

Solution B.[2]

Sodium phosphate ($Na_2HPO_4 12H_2O$)	4 grm.
Calcium chloride ($CaCl_2 6H_2O$) . .	4 ,,
Ferric chloride (melted) . . .	2 c.c.
Hydrochloric acid (pure concentrated)	2 ,,
Distilled water	80 ,,

[1] This strength has only been used in the most recent experiments; and solution A in this paper, unless otherwise stated, means the 5 % solution of KNO_3.

[2] For preparing this solution see p. 364.

To each 1000 c.c. of sea-water[1] add 2 c.c. solution A and 1 c.c. solution B and sterilise by heating to 70° C. When cool, decant off the clear liquid from the precipitate, which will have formed when solution B is added to the sea-water.

As a rule our cultures were made in 60 c.c. of this medium contained in short-necked, wide-mouthed flasks of 125 c.c. capacity, so that the proportion of air-surface to volume of liquid was large.

The medium was found to give constantly satisfactory results. On inoculation from a persistent culture of such diatoms as Thalassiosira, Skeletonema, Chætoceras, etc., a growth visible to the eye is obtained in about ten days, and then multiplication takes place very rapidly. In from three weeks' to a month's time a very considerable growth will be seen making a brown, flocculent mass at the bottom and back of the vessel containing the culture.

In from two to four months the culture begins to show signs of exhaustion and the frustules lose colour, but they do not, as in the case of sterilised outside and tank-water, completely die off. A great number certainly do die, but some remain in a resting condition, and often, after a period of six months or so, these begin to multiply again and the culture regains its former vigour. This is probably due to the food-stuffs contained in the dead frustules going into solution again, possibly by means of bacterial action. This periodicity in cultures is interesting in that it resembles what takes place in the ocean. Cultures in this medium will persist indefinitely, so far as our experience goes. The oldest culture in our possession is one of Skeletonema costatum made at the very commencement of this work, dated April, 1905. Although the frustules in this culture are quite unrecognisable as any diatom now, on making a subculture in fresh Miquel a normal and healthy growth can always be obtained.

In old cultures the diatoms are nearly always found to be very much deformed, and often appear to be only a mass of

[1] "Miquel water" seems to succeed equally well, whether it is made by adding Miquel's solutions to "outside water" or to "tank-water."

broken-down chromatophores. Whether regeneration can be successfully obtained from a single chromatophore, which must presumably be contained within a cell-wall of some kind, has not been definitely decided, but results seem to point in this direction.

At the start of a culture a tendency to teratological forms is often exhibited, but when the growth is well advanced, the shape of the frustules is usually quite normal.

(B) English Channel Water ("Outside Water").—In a large number of our experiments sea-water brought in from outside the Plymouth breakwater, and therefore taken at some distance from the shore, has been used. This is referred to as "outside water." It has an average salinity of about 35·0 $^{o}/_{oo}$ and the temperature range for the year is from 8° to 16° C.

If a sample of "outside water" is inoculated from a persistent culture of a plankton diatom, a small growth is obtained in from five to fifteen days. But soon minute bottom forms of diatoms, other algæ, flagellates, infusoria, etc., appear, and the inoculated species is lost. The total growth of any form is never large. If the growth of these foreign forms is prevented by sterilising the water before inoculation, a considerably better growth of the plankton form is obtained. The water was, as a rule, sterilised by simply heating to 70° C., which temperature was found to be quite adequate. Boiling gave equally good results, but the former was preferred, as less concentration due to evaporation took place. Even under these conditions no permanent culture can be obtained, the diatoms soon beginning to lose colour and getting into an exhausted condition. Death takes place in from two to three months after the culture has been started, and in many cases considerably sooner. Long before inability to start new cultures, the test of death, has been established, the valves appear on examination quite colourless and practically empty.

Samples of outside water, taken at times when the quantity of plankton was widely different, gave no appreciable variation in the results obtained by culture methods. It is, however, doubtful whether differences in the amounts of growth

in cultures, proportional to the seasonal variation in the quantity of phytoplankton, would be sufficiently marked to be appreciable.

The total growth under cultural conditions, although small for a culture, is very much greater than any natural plankton that has come within our experience.

(c) Tank-Water.—"Tank-water," or water taken from the supply of sea-water circulating through the tanks of the Aquarium at Plymouth, shows some striking and interesting differences from "outside water." This water is pumped up from the sea, just below the Laboratory, into two large, covered-in, settling reservoirs, with a capacity of 50,000 gallons each. Pumping is only done at high-water spring tides, so as to get the least contaminated water, and no water is pumped that does not show a specific gravity, measured with a hydrometer, of $\rho^{17\cdot5} = 26\cdot00$ ($S = 34\cdot00$) or over. The water is allowed to settle for about a fortnight before being used for the general circulation.

The tanks themselves are made of slate and glass, and the pipes which convey the sea-water to them are of vulcanite, so that the water does not come in contact with metal, excepting in the pumps, which are of cast iron. The two settling reservoirs are used alternately for about a week each. From time to time, tide and water allowing, waste is replenished, and about twice a year each reservoir is emptied, cleaned out, and refilled. The aquarium takes about 20,000 gallons, and this is in circulation with one of the two 50,000 gallon reservoirs. An estimate of the amount of life in the tanks of the aquarium must be exceedingly rough, but the intensity of the larger forms of life is far greater than anything met with in natural waters. About 500 fish and 2000 invertebrates, including all forms as large as an Actinia equina, might be somewhere near the mark. So it will be seen that the accumulation of excretory products must be a by no means negligible factor. The flora of the tanks is very restricted, and is chiefly composed of minute forms of algæ. Minute naviculoid diatoms, Ectocarpus, Cladophora,

Enteromorpha, *Vaucheria*, and unicellular algæ are the commonest forms. The large seaweeds, such as *Fucus* and *Laminaria*, do not live long if introduced. Plankton diatoms, although a great number must be pumped up when the reservoirs are being filled, are not represented.

As in the case of outside water, a sample of "tank-water," inoculated from a persistent culture, will only give a very small growth, minute forms, etc., soon multiplying and choking out the plankton form. The ultimate growth of minute unicellular algæ other than diatoms is often considerable, and many quite unknown and unidentified forms have been obtained. The total growth of vegetable forms is always found to be greater than in the case of outside water.

In cultures of plankton diatoms made with sterilised tank-water, a very great improvement on outside sterilised water was always noted. The culture of the diatom used to inoculate this medium persists for a considerable period, and the colour of the frustules remains normal for two to three months.

(d) Animal-Charcoal Water.—The use of animal charcoal, as a means of purifying the water in small aquaria, has for a long time been known and practised by those who have kept such aquaria in inland places. At an early stage in our experiments, water from a tank, which was not in a satisfactory condition, was treated with some powdered animal charcoal and filtered. It was noticed that a good growth of diatoms took place in this water. Systematic experiments with the use of animal charcoal were then commenced, and these have resulted in a method of great value, both for the culture of diatoms and for the rearing of pelagic larvæ.

Animal charcoal is made by the carbonisation of bones,[1]

[1] Analysis of animal charcoal, from Thorpe's 'Dictionary of Applied Chemistry'—

Carbon	10·51
Ca., Mg. phosphates, Ca. fluoride, etc.	80·21
Calcium carbonate	8·30
Other mineral matter	0·98
	100·00

and is sold in two grades known as "pure" and "commercial." Our earlier experiments were all made with "pure" animal charcoal, but subsequently the "commercial" animal charcoal was largely used, and appears to give equally good, if not better results. In both cases the animal charcoal is used in the powdered form. Animal-charcoal water is prepared as follows:

(1) A quantity of sea-water is sterilised by heating it in a flask to 70° C., at which temperature it should be kept for about twenty minutes. At the same time some animal charcoal is heated sufficiently to sterilise but not to burn it, covered over, and allowed to cool. When both are quite cold the charcoal is added to the water (ca. 15 grm. to 1000 c.c.), and well shaken up in it several times. After an interval of half an hour or more the water is filtered through fine filter-cloth,[1] the whole filter having been first sterilised with boiling sea-water, and is received in a sterile flask. It is then ready for use.

(2) For many experiments, where larger quantities of water were required, the sea-water was not sterilised before being treated with animal charcoal. In this case, if the first part of the filtrate be rejected, the subsequent water will generally be practically sterile, and few, if any, extraneous organisms will develop in it.

(3) At a later date an automatic apparatus was set up in the Plymouth Laboratory, by which very considerable quantities of sea-water could be treated with animal charcoal, and subsequently filtered through a "Berkefeld" filter; water treated in this manner we call "Berkefeld water." Tank-water was always used in this apparatus, and was mixed with animal charcoal,[2] in a clean sulphuric acid carboy, by blowing air through with a pair of bellows. The mixture was allowed to settle for at least twenty-four hours, and then syphoned

[1] The filter-cloth used for this purpose is the same as is made for use in filter presses, and is known as extra-super swansdown. To prevent this becoming clogged another cloth, known as hydraulic twill, was, as a rule, used over it.

[2] Ca. 300 grm. to 20 litres of water.

over into an inverted bell-jar, with a tubulure at the bottom, into which the Berkefeld candle was fitted. Filtration under these conditions was found to be rather slow, so in order to increase its rate an apparatus was devised by which the pressure on the filter was considerably augmented.

This apparatus (see Fig. 1) consists of a glazed earthenware "tobacco jar," with two tubulures, one at the side, the other at the bottom, and a lid which can be screwed down tightly on to a rubber washer, by means of a triangular metal arrangement fitting into grooves above the lid.[1] The internal

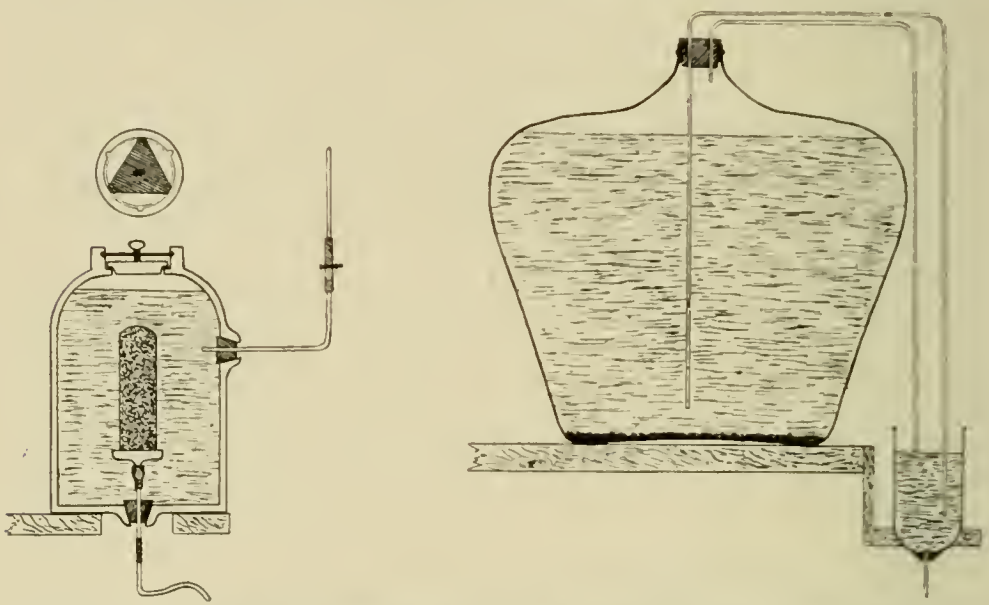

FIG. 1.—Diagram of apparatus for preparing sterile sea-water by filtration, without contact with metal.

dimensions of our jars are 11 in. by 6 in., and the diameter of the opening at the top is $3\frac{1}{4}$ in. The tubulures are coned, with the smaller diameter external, and make a good fit for a No. 8 rubber bung. When setting up this apparatus a bung, through which a short glass tube bent at right angles is passed, is fitted into the side tubulure. This tube is connected, by means of rubber pressure-tubing, to another glass

[1] These jars were made to our specification by Messrs. Price, Powell, and Company, Bristol. The clamps usually supplied with such jars are not strong enough to obtain a tight joint, but these are easily replaced by stronger ones.

tube leading down from the bottom of a small inverted bell-jar, placed some height above (in our case 14 ft., which gives a pressure of ca. 6 lb. to the square inch inside the jar). A screw pinch-cock on this connection serves as a tap. The carboy containing the treated water stands just above the bell-jar, and is fitted with a tightly fitting rubber bung, through which two tubes pass. One is an ordinary syphon, the other the only air-inlet into the carboy. This latter automatically keeps the level of the water in the bell-jar constant, by closing the air-inlet as soon as the water covers the end of the tube. When filtering water the modus operandi is as follows: The carboy is filled with tank water, treated, and allowed to settle as before. The Berkefeld candle,[1] bung, delivery tube, and connections (see fig. 1) are sterilised by boiling for half an hour, and fitted into place from within. (The delivery tube is shaped so that any drops of water, accidentally running down outside it, do not enter the vessel receiving the filtrate; and the jar should be large enough to allow the hand to fit the filter into place without much trouble.) The pinch-cock is closed, and the syphon from the carboy started, which will automatically stop if the bung has been properly fitted. This should be watched to avoid accidents. The pinch-cock is then opened until the water rises in the jar well above the top of the candle, but still leaving some air-space. The lid can now be fitted into place and screwed down. The tightness of this joint can be tested by pouring a little water into the crack round the lid, and observing if any bubbles are formed when the pinch-cock is opened. If all is right, no bubbles will be seen, and a good stream of water will flow out from the delivery tube. Our apparatus will filter about 20 litres an hour, and the filtrate is exceptionally bright and clear. The candle should be sterilised every three or four days that the apparatus is in use to avoid indirect contamination by growths of organisms through the substance of the filter.[2] The water while passing

[1] No. 5 porcelain mount, length 8 in., diameter 2 in.

[2] See Bulloch and Craw, 'Journ. of Hygiene,' vi, No. 3 (1906), p. 409.

through this apparatus only comes into contact with glass, earthenware, and rubber, the use of metal having been purposely avoided.

(E) Peroxide of Hydrogen Water.—As it seemed probable that the action of animal charcoal was due to contact oxidation with the oxygen occluded in the charcoal, experiments were made to determine whether a similar effect could be produced by the use of hydrogen peroxide (H_2O_2). This was used in two ways. In the first method a sufficient quantity of H_2O_2 was added to the sea-water to ensure complete sterilisation (1 c.c. of H_2O_2 of twenty vols. strength per 1000 c.c. of tank-water was found to be satisfactory), and the excess of H_2O_2 was decomposed by adding manganese dioxide. The water was then filtered through filter-cloth, and the filtrate appeared to remain quite sterile. Good cultures of Chætoceras constrictum, Biddulphia mobiliensis, and Skeletonema costatum were made in this water, which seemed to be as good as water treated by the animal charcoal method.

The second way of using the peroxide of hydrogen was to start with water sterilised by heating to 70° C. and to add to this H_2O_2, in small quantities at a time, until its presence could just be detected on testing the sea-water with permanganate of potash. In these circumstances, the first amounts of H_2O_2 are decomposed in the oxidation of organic substances in the water, and a very slight excess of H_2O_2 persists. For tank-water 1 c.c. of one vol. H_2O_2 per 1000 c.c. was found to give the best general effect. Cultures grown in water prepared in this way developed satisfactorily, being practically equal to those made in animal-charcoal water, but they became exhausted rather quickly.

The treatment of aquarium water with ozone was also tried, as this seems to offer a possibility of treating large quantities of water,[1] such as the whole bulk of water in an aquarium

[1] The use of ozonised air for the purification of fresh water for town water supplies has been adopted in some localities. (See Bridge, J. H., paper read before Franklin Institute, reprinted in 'English Mechanic,' 1907, pp. 369 and 392.)

circulation, without very considerable expense. Experiments on a small scale, which we were able to make, unfortunately only with imperfect apparatus, showed that water treated with ozonised oxygen gave distinctly better cultures than untreated water. Although the sea-water was not absolutely sterilised by the treatment to which we actually subjected it, a sample of water which was visibly clouded with bacteria became quite clear and bright.

(F) Cultures in these Media.—In order to make clear the different results which are obtained by using these different waters, we will describe the probable result which would be got from a series of flasks set up with the following media, and each inoculated with a persistent culture of a true plankton diatom, such as Thalassiosira, Skeletonema, or Chætoceras.

A. "Outside water" untreated.
   Small growth in from five to fifteen days, almost immediately swamped by growths of foreign forms; the latter, however, will never be large.

B. Ditto, sterilised.
   Slightly larger growth, very soon becoming exhausted.

C. "Tank-water" untreated.
   Same result as in A, but growths will be much larger and healthier, and will last longer.

D. "Tank-water" sterilised.
   A fair growth of the inoculated species, but the total growth will not be as great as in C; the diatoms will retain their normal appearance for some time.

E. "Outside water" + Miquel's solutions A and B, sterilised.
   Best culture in series, both in quantity and quality. The diatoms will remain normal and healthy for a very long period.

F. "Outside water" sterilised and treated with animal charcoal.
   Fair growth, especially at first; diatoms will soon grow pale and become exhausted; better than D.

G. "Tank water" sterilised and treated with animal charcoal.

As F, only growth will be slightly greater and will last considerably longer. Third best in series.

H. "Tank-water" treated with animal charcoal and filtered through Berkefeld filter.

This will usually be the second best culture in the series, but the difference between this and G will only be slight.

K. "Outside water" treated with H_2O_2.

This will most resemble F, but will not be quite so good.

L. "Tank-water" treated with H_2O_2.

A distinct improvement over K. This medium is rather variable, and in some cases the growth obtained has been quite equal to F, if not better.

B. Experiments with a View to Determining the Conditions which underlie the Successful Culture of Diatoms.

The attempt to make cultures of diatoms for use as food when rearing pelagic larvæ, led naturally to an effort to determine the best culture medium and the most favourable conditions for the rapid and continuous growth of diatoms. Before success can be attained in this direction exact knowledge as to the nature of the essential food-stuffs, and, in fact, as to the general physiology of the Diatomaceæ, is necessary.[1] Numerous experiments, extending over the last three years, have been carried out, with a view to obtaining such knowledge, and the results, though still by no means complete or conclusive, are perhaps worth recording.

A great difficulty which has to be met in carrying out such investigations on marine diatoms, is caused by the fact that when sea-water is used as a basis for the culture media, we

[1] For general references to literature see "Bibliography," especially Miquel (12), Richter (18).

are dealing with a solution of a very complex and very variable character, the exact nature of which it is extremely difficult to determine. The most direct method of research, namely, chemical analysis, has not proved of much service, owing to the uncertainty, and in many cases impossibility, of accurate determinations, in sea-water, of such minute quantities of substances as those upon which the growth of plankton diatoms has been found to depend.

We have had, therefore, to rely, for the most part, on the lengthy and tedious process of analysis by "trial and error," the experiments being largely conducted on lines suggested by Liebig's well-known "law of minimums" (Pfeffer, vol. i, p. 413). The ideal at which we aim is to find a culture medium with artificially prepared sea-water as its basis, such that the absence, or diminution in quantity, of any one of its constituents would have a profound effect upon the growth of diatoms in it. Whether the conditions regulating growth in such a medium would be at all comparable to the natural conditions of life in the sea is a question that would have to be decided by experiment, but in any case this could be made a starting point for much more definite research than has yet been attempted. Up to the present time we have not, unfortunately, succeeded in finding such a culture medium. Throughout the work we have had very great difficulty, in spite of much care and many precautions, in obtaining consistent results. It may even happen that in two flasks containing the same culture medium, inoculated with the same culture of diatom and standing side by side, under exactly identical conditions, as far as can be recognised, quite different degrees of growth will be observed. All experiments must therefore be frequently repeated before entire confidence can be felt in any conclusions which they seem to indicate.

It must be remembered, also, that in all the persistent cultures of diatoms that we have used, bacteria have probably been present, and this fact has probably had some influence on the result. Unfortunately our attempts to obtain absolutely pure cultures have not met with success.

Methods.—In carrying out the experiments to be described in this section the procedure has been as follows: All media have been prepared from sterile sea-water, and sterile vessels and instruments have always been used. The cultures have usually been made in 60 c.c. of liquid, in short-necked, wide-mouthed flasks of 125 c.c. capacity. When a number of cultures were to be compared, the flasks were kept standing in a row together in such a way as to keep the physical conditions as similar as possible. Control cultures in standard media were included in each series, so that results from different series could be compared by reference to the controls. The various media were inoculated from a persistent culture of a species of plankton diatom, which in the great majority of cases was Thalassiosira decipiens (p. 412). When preparing the different media the methods used were, as far as possible, identical, and although only about 60 c.c. was needed for a culture, a litre was made up, so that errors due to measuring very minute quantities might be avoided. The media were all freshly prepared for each comparative series of cultures, the same sample of sea-water being used, when the basis of any two or more was the same. Comparative estimates of the amount of growth in the different cultures were made by eye alone. Any difference between amounts of growth that has been described here as appreciable has always been accompanied by a marked difference in appearance to the eye on holding the cultures up to the light. A few drops from each culture were also, from time to time, examined microscopically, as a test of the quality and purity of the growth.

The Sea-water Employed.—The sea-water employed as a basis for the culture media has been either (1) "outside water" or (2) "tank-water." A general description of these will be found on pp. 372–374. An accurate chemical analysis of both types of water would probably make clear many difficult points, but, as already pointed out, no chemical methods of sufficient delicacy have yet been devised.

We have seen that if we compare "tank-water," i. e. water from the closed circulation of the Plymouth Aquarium, with

off-shore sea-water in situ, a most obvious difference is the much increased density of the larger forms of animal life in the former, combined with the almost complete absence of plant life. Hence the concentration of excretory products in the tank-water must be very much higher than in outside water. Other factors, such as increased bacterial action, artificial aëration, etc., in tank-water, must also be taken into account (cf. Vernon [58], Smith [56]). There seems to be direct evidence to show that the concentration of nitrates, possibly due to the action of nitrifying bacteria on the products of excretion, such as urea, ammonia, etc., is considerably higher in the tank-water, and the presence of soluble organic matter in concentrations never met with in the sea, can almost certainly be assumed. It is probably due to the presence of these nitrates and soluble organic substances that sterilised tank-water is a much better medium in which to grow diatoms than sterilised outside water (see p. 379).

The Constituents of Miquel's Solutions.—It has been already stated that no better medium for the culture of plankton diatoms has been found by us than the solutions recommended by Miquel, although these solutions may be modified and simplified in various ways with equally good results. The formulæ recommended by Houghton Gill give very similar cultures. The essential features of Miquel's and Houghton Gill's methods, when adapted to sea-water, are the same. Miquel's solution A and Gill's solution 2, can both be replaced by a solution of potassium nitrate (p. 369). Again, Miquel's solution B and Gill's solution 1 only differ in the proportionate amounts in which the various constituents are prescribed. The formulæ are :

	Miquel's sol. B.	H. Gill's sol. 1.
$Na_2HPO_4,12H_2O$	4 grm.	2 grm.
$CaCl_2$	4 „	4 „
$FeCl_3$ (syrupus)	2 c.c	0·5 „
HCl (concentrated)	2 „	1 „
Water	80 „	100 „
	Use 1 c.c. per 1000.	Use 3 c.c. per 1000.

The proportionate amounts added to equal volumes of sea-water are :

	Miquel's sol. B.	H. Gill's sol. 1.
Na_2HPO_4	10	12
$CaCl_2$	10	24
$FeCl_3$	5	3
HCl	5	6

Since cultures can be obtained with no appreciable difference by using media prepared by adding either of these solutions, together with Miquel's solution A, to sea-water, a considerable latitude in the proportions of the salts present is tolerated.

We must now consider what is the rôle of the various constituents in Miquel sea-water. The part played by any salt of a culture medium may be considered as being either, firstly, "nutritive," or secondly, "protective."[1] Under the first heading, any direct addition of food material must be included; under the second, any removal or neutralisation of harmful substances, such as toxins and possibly bacteria, and any more remote effects, which, although influencing growth, do not directly enter into the metabolism of the plant.

Our experiments have proved that solution A can be reduced to a simple solution of potassium nitrate[2] without detriment (cf. p. 369), and that the amount of growth is, within limits, roughly proportional to the amount of KNO_3 added, as the following experiment shows :—

Inoculated from persistent culture of Thalassiosira decipiens:

A. Normal Miquel sea-water.

Growth as usual.

B. Ditto, but only half amount of solution A.

[1] Loeb, 'The Dynamics of Living Matter,' New York, 1906, p. 77.

[2] For the sake of convenience the expression solution A will be used throughout the rest of this paper to indicate a simple solution of potassium nitrate (5 per cent.), and solution B to indicate Miquel's phospho-ferri-calcic solution. Unless otherwise stated the amounts of each added to 1000 c.c. sea-water will be normal, i.e. 2 c.c. solution A and 1 c.c. solution B.

Good growth at first, but exhausted sooner than A.

C. Ditto, but two and a half times amount of solution A.
Was slower than either A or B at start, but afterwards was better than A or B, and lasted longer.

D. Ditto, but five times amount of solution A.
As C, but in greater degree.

Considering the nature of the substance added, and its already well-known action in plant metabolism, these results, coupled with the fact that exhausted cultures can often be regenerated by the simple addition of nitrates (see below, p. 390), are quite consistent with the assumption that solution A is simply nutritive in action. The concentration of nitrates in natural sea-water is so low (Brandt [**47**]) that the amount available in a culture of untreated water very soon becomes completely exhausted, and it is this deficiency that solution A probably corrects.

Considering now the action of solution B, it must first be observed that increased concentration of nitrates alone will not explain the whole action of Miquel's solutions, for no increase in growth is obtained when nitrates or solution A only are added to sea-water. To illustrate this point an account of an actual experiment may be given :—

Inoculated with Thalassiosira decipiens:

A. Normal Miquel sea-water.
Good strong culture, in every way normal.

B. Outside water sterilised.
Small growth at first, very soon exhausted.

C. Ditto + solution A.
No improvement over B.

D. Ditto + solution B.
Fair growth. Great improvement on B and C, but exhausted considerably before A.

E. Tank-water sterilised.
Appreciably better than B, but growth not large.

F. Ditto + solution A.
Not even as good as E.

G. Ditto + solution B.

Next best in series to A; lasted longer than D, and had better colour.

To generalise, no improved culture is obtained with solution A alone, but a fair, though not very lasting, growth can result from using solution B only.

The action of solution B is to some extent obscured by the fact that, when this solution is added to the alkaline sea-water, a precipitate is formed. This precipitate is at first white, but, on heating or standing for some time, it becomes greenish-yellow. We are indebted to Mr. D. J. Matthews for the following analyses.

Ten litres of normal Miquel sea-water were prepared, and the precipitate was collected on a filter-paper, washed, and dried at 100° C.

Weight of dry precipitate from 10 litres = 0·2949 grm.

Analysis of Dry Precipitate.

P_2O_5	26·36 per cent.
Fe_2O_3	41·31 „
CaO	7·63 „
H_2O	24·86 „
	100·16 „

Or the precipitate from 1 litre of normal Miquel sea-water contains—

P_2O_5	·00777 grm.
Fe_2O_3	·01218 „
CaO	·00225 „

An analysis of 1 c.c. Miquel solution B, the amount added to 1 litre Miquel sea-water, gave—

P_2O_5	·00825 „
Fe_2O_3	·0105 „
CaO	·0145 „

Comparing these figures it seems probable that, when added to sea-water, all the iron in solution B is precipitated, and a certain amount also of the phosphate and calcium. The

additive effect on the sea-water is, therefore, a slightly increased concentration of phosphate and calcium.

An analysis of a sample of tank-water for phosphorus, before and after treatment with solution B (1 c.c. per 1000), gave the following figures:

Tank-water ·5 mgrm. P per litre = ·00163 grm. P_2O_5.

Tank-water + solution B (without precipitate) 1·5 mgrm. P per litre = ·00488 grm. P_2O_5.

It will be noticed that the figures from the different analyses do not agree very well. This is probably due to the fact that different samples were used for analysis in each case, and also to the fact that the solutions were made up in the ordinary way, without any special precautions, volumes, for instance, being measured in cylindrical glasses, pipettes, etc.

Cultures were tried in sea-water containing the normal amount of solution A, plus the normal constituents of solution B, less all the iron and less the amount of phosphate that would combine with the iron to form basic ferric phosphate (P_2O_5 $2Fe_2O_3 12H_2O$). This solution should have very nearly the same chemical composition as normal Miquel sea-water from which the precipitate has been removed. Successful cultures could not, however, be obtained in it. Neither could cultures be grown in sea-water to which had been added the normal amount of solution A and 1 mgrm. P (as sodium phosphate) per litre.

To ascertain the effects of the different constituents of solution B, experiments were carried out with separate solutions of these constituents, each of the same strength, as in Miquel's formula. Different combinations of these solutions were added, together with solution A, to sterilised sea-water, and the resulting media were inoculated in the usual way. It was found necessary to repeat these experiments a great number of times, as the results obtained were rather contradictory. To illustrate the methods used a list of the different media, and notes of the cultures obtained in them, are given below. These media were inoculated from cultures of Thalassiosira decipiens, and the cultures were kept

under observation for at least four months. Series were made as uniformly as possible, and controls in standard media were included in each. The strength of the various solutions used in these experiments was the same as in Miquel's formula.

A. Outside water + solution A + solution B (normal Miquel sea-water.

First control.

B. Outside water + solution A + Na_2HPO_4 solution + $FeCl_3$ solution + $CaCl_2$ solution.

Second control.

Good normal cultures were always obtained in these two controls.

C. Outside water + solution A + Na_2HPO_4 solution.

A very uncertain medium. Sometimes no growth has been recorded, and at other times a fair growth results, but these cultures are never equal to normal Miquel.

D. Outside water + solution A + $FeCl_3$ solution.

Occasionally a very small growth has been obtained, but at the best it is very poor.

E. Outside water + solution A + $CaCl_2$ solution.

About equal to D.

F. Outside water + solution A + Na_2HPO_4 solution + $FeCl_3$ solution.

Uncertain as C. No cultures have been obtained equal to the best in C.

G. Outside water + solution A + Na_2HPO_4 solution + $CaCl_2$ solution.

Some cultures very nearly equal to the controls have been obtained in this medium.

H. Outside water + solution A + $FeCl_3$ solution + $CaCl_2$ solution.

Poor, about equal to D.

Analysing the above results we see that—

(1) None of these modifications of solution B give results equal to solution B itself.

(2) The best result is obtained from the combination of the phosphate and calcium chloride solutions.

(3) Of the solutions used singly the phosphate is the best, the iron and calcium chloride being about equal.

(4) The addition of $FeCl_3$ to Na_2HPO_4, or the addition of $CaCl_2$ to $FeCl_3$, does not improve the medium to any extent.

Experiments were also made to determine whether the precipitate thrown down in sea-water by Miquel's solution B, itself had any influence on culture media. A quantity of this precipitate was prepared, filtered off, and then added to outside sea-water + solution A (nitrates). A small growth was obtained, which was a distinct improvement on the control without the precipitate, but exhaustion soon set in.

Further discussion of the mode of action of solution B, and as to whether that action is purely nutritive, or partly nutritive and partly protective, is better postponed until a later section, after the action of animal charcoal and other substances has been considered (see p. 405).

Animal Charcoal and Peroxide of Hydrogen.—The most successful culture medium for plankton diatoms, next to Miquel sea-water, is that prepared from animal charcoal (cf. p. 379). Animal charcoal water gives at first almost as good cultures of plankton diatoms as Miquel sea-water, but the tendency to paleness and exhaustion appears much sooner. The best cultures were obtained in "Berkefeld water," that is, tank-water from the Plymouth Aquarium treated with powdered commercial animal charcoal and filtered through a Berkefeld filter. Tank-water as a basis for animal charcoal water is very much better than outside water, probably on account of the higher concentration of nitrates, etc.

There is a very striking resemblance between the effect of animal charcoal and of Miquel's solution B upon sea-water used for diatom cultures, and the growths obtained by using tank-water + solution B and tank animal-charcoal water are very similar in character. If Miquel's solution A is added to animal-charcoal water there is a great improvement, both in

the colour and quantity of diatom growth, and in the case of Thalassiosira decipiens the chains are long and well formed. With animal-charcoal water + solution B, on the other hand, practically no growth was obtained.

It is possible that a certain amount of phosphate, and perhaps of calcium, from the animal charcoal, goes into solution and serves as a "nutritive" material for the diatoms. But we are inclined to think that its chief action is "protective," and due to its power of occluding gases, such gases being in a state of higher chemical activity than under normal conditions.[1]

As was explained in a previous section (p. 378), the possibility that the action of animal charcoal might have some sort of effect comparable to oxidation, led us to experiment with hydrogen peroxide. Fair growths of diatom could be obtained in sea-water prepared in the manner described, but they always showed a tendency to rather rapid exhaustion. As in the case of animal-charcoal water, tank-water proved a much better basis for treatment with H_2O_2 than outside water.

Reviving Exhausted Cultures.—Several experiments were carried out with water from old, exhausted cultures. The sediment was filtered off, the filtrate was sterilised by heat, and then treated by various methods.

In one typical experiment the following was the result :—

Water from an exhausted culture of Skeletonema costatum in Miquel sea-water, reinoculated with the same diatom :

A. Filtered and sterilised.
No growth obtained.

B. Ditto + solution A (nitrates only).
Good culture, but did not last very long; further addition of nitrates made no improvement.

C. Ditto + solution B.

[1] Against this view would seem to be the fact that when powdered cocoa-nut charcoal, which has a still higher power of occluding gases, was used in place of animal charcoal, very poor cultures were obtained.

No growth.

D. Ditto + solution A + solution B.

Very good growth, lasting considerably longer than B.

E. Ditto + animal charcoal.

No growth.

Exhausted cultures in animal charcoal water gave the same general results on treatment and reinoculation. In an old culture of Biddulphia mobiliensis in outside water + solution B only, which was in a very exhausted condition (nine months old), the addition of KNO_3 gave a very rapid regeneration, and the diatoms became of normal colour and form. This renewed growth, however, did not last very long, and a further addition of KNO_3 did not give any result. The addition of sodium phosphate also failed to stimulate growth. The same rapid regeneration, on the addition of potassium nitrate, has been obtained with almost every medium, but a second attempt has always failed.

Silica.—A very noticeable character of the true plankton species of marine diatoms is that their skeletons are very markedly less siliceous than the great majority of other forms. Their valves are only feebly marked, if at all, and they will not stand the vigorous treatment of cleaning with acids and heat that is commonly used in the case of fresh-water diatoms. In cultural forms this absence of silica is still more obvious, and no marking can usually be seen on even those forms, which, under natural conditions, are the most siliceous, e.g. Coscinodiscus excentricus. Deformed and distorted frustules are the rule in certain stages of growth in our cultures, and it is often very hard to make out more than the thinnest coating of silica. It is quite probable that this deformity can be accounted for simply by the absence of a strong siliceous skeleton. As a rule, the more rapid the growth the more teratological forms will be found. In untreated outside water little deformity will take place, but in normal Miquel, where very rapid growth takes place, the diatoms may assume almost any conceivable shape. The

form of the frustules tends to come back to the normal again, when the culture is well started, and in old stages the majority will be perfectly formed, although small and pale. It was found that the addition of silica (in early experiments as fragments of potassium silicate) was, as far as could be judged, immaterial, which fact led to the conclusion that a sufficiency dissolved out from the glass flasks in which the cultures were kept. During rapid growth, it is possible that the silica does not dissolve out fast enough to supply the demand, although it is also possible that diatoms, during rapid division, cannot absorb silica and form a perfect skeleton, even when the supply is abundant. Richter (**18**) has proved the necessity of either $CaSi_2O_5$ or $K_2Si_2O_5$ for the growth of Nitzschia palea, grown in pure cultures. We tried the addition of silica in various forms, and in one instance, in a culture of Coscinodiscus excentricus, to which a little precipitated calcium silicate had been added, the uniformity and markings of the valves were much more regular than in the control. The presence of a trace of pure, dialysed silica also, in one experiment, gave an improved regularity of form, but the quantity or rapidity of growth did not seem to be affected. No sign of regeneration could be obtained in exhausted cultures by the addition of silica.

Organic Infusions.—Miquel recommends the use in culture media of infusions of organic substances, such as bran, straw, diatom broth, etc., in addition to the saline solution. He does not make it quite clear if he ever dispensed with them at all. In his general directions he certainly states that the addition of both saline and organic nutrient material is necessary. As would be expected from the general metabolism of plants, the saline constituents are sufficient for growth. At the same time, excellent cultures have been obtained from dilute organic infusions, both with and without the addition of Miquel's solutions A and B. About a square inch of Ulva was boiled in 600 c.c. of sea-water for half-an-hour, cooled, and filtered. In this medium an excellent growth of Coscinodiscus excentricus in one case, and Biddulphia

mobiliensis in another, was obtained, the growth lasting for some considerable time.

Infusions, made in the same way from a small piece of fresh fish, gave the same results, and although growth was rather slower at first, the final result was, if anything, slightly better. As Miquel points out, these infusions must be made very dilute, otherwise growths of bacteria, moulds, etc., will completely swamp the diatoms. Karsten (**7**), in some interesting experiments, showed that Nitzschia palea (Kutz) W. Sm. could be made to alter completely its mode of nutrition. On placing this diatom in organic nutrient solutions, it lost all chlorophyll and became colourless, but in saline media the chlorophyll would not regenerate, and the nutrition change back from heterotrophic to autotrophic.[1]

Of course, with our infusions, it cannot be said that the diatoms were necessarily feeding on dissolved organic material, as some necessary, saline, nutritive materials could have dissolved out from the weed or fish. If the former is the case, it might explain the superiority of tank-water over outside water, since the tank-water must contain a much higher percentage of organic substances in solution. If an alternative mode of nutrition, autotrophic or mixotrophic, could be proved, especially in the case of the "bottom" forms of diatoms, a great many phenomena could be explained, but the evidence is as yet far too slight to warrant any such assumption.

Artificial Sea-water.—As we have explained in a previous section, the ideal aimed at in this part of our work has been to obtain strong growths of Diatomaceæ in purely artificially prepared solutions of simple salts. If this end could be satisfactorily attained the difficulties due to the unknown and variable composition of natural sea-water at once disappear. According to van 't Hoff (**35**) sea-water is a solution containing salts in the following molecular concentrations: NaCl 100·0, KCl 2·2, $MgCl_2$ 7·8, $MgSO_4$ 3·8, $CaCl_2$ 1·0 (varies).

[1] Cf. Zumstein, 'Zur Morphologie u. Physiologie d. Euglena gracilis,' Leipzig, 1899.

Using these molecular concentrations, a sea-water of any desired salinity can be prepared. The chlorine content of average Atlantic water is about Cl = 19·4, and samples of artificial sea-water were prepared with the same chlorine value, thus:

NaCl	26·75
KCl	·75
$MgCl_2$	3·42
$CaCl_2$	·51
$MgSO_4$	2·1
Double distilled water . . .	966·47
	1000·00

To make this solution comparable to natural sea-water, the "alkalinity" must be raised by the addition of an alkali such as Na_2CO_3. After the importance of "alkalinity" as a factor had come before our notice, 2·4 c.c. M/2 Na_2CO_3 was always added to the above solution in order to make the amount of base in equilibrium with CO_2 equivalent to the usual 40 mgrm. OH $^0/_{00}$.

The only success we attained with artificial sea-water as a basis for culture media was with four isolated cultures in one of our earlier experiments. Two of these were cultures of Coscinodiscus excentricus in artificial sea-water + Miquel's solutions A and B. The two cultures were identical except that one was in an ordinary bohemian glass flask and the other in a "resistance glass" flask. No difference between these two could be seen. The growth obtained in both was in every way equal to normal Miquel sea-water, and is still fair, although over two years old. The other two successful cultures were growths of the same diatom in the same media, plus a small quantity of weed infusion, made by boiling up a small piece of Ulva in artificial sea-water. These gave just as good results, but the addition of unknown factors from the weed detracts from their general interest. In spite of frequent attempts, over fifty in number, we have not been able to repeat this experiment, which may possibly be due to

some accidental impurity in the salts or distilled water from which the successful media were prepared.

Alkalinity.—Tornöe (**43**) and Dittmar (**33**) were the first to investigate the fact that sea-water showed on analysis an apparent excess of base over acid, which excess they termed "the alkalinity of sea-water." Dittmar defines the alkalinity of sea-water as "a measure of its potential carbonate of lime," but this definition and his supposition that this excess of base combines directly with dissolved CO_2 to form carbonates and, further, but only in very small proportion, bicarbonates, is liable to give a quite erroneous idea of the state of equilibrium actually occurring in the ocean. For, as Fox (**34**) has shown, "sea-water reacts in *situ* very nearly neutral, and actually just slightly more acid than distilled water." This is due to the fact that sea-water always contains a considerable quantity of dissolved CO_2.

If a salt solution with neutral reaction, that is, containing $H^{\cdot}$ and OH^{1} ions in concentrations equal to one another and the same as for pure water, be exposed to an atmosphere containing CO_2, a definite amount, depending on pressure, temperature, and salinity, would go into solution. This CO_2 would combine with water and form the very weak acid H_2CO_3, which would ionise with the formation of free $H^{\cdot}$ ions, thus:

$$H_2CO_3 \rightleftarrows H^{\cdot} + HCO'_3$$
$$(HCO'_3 \rightleftarrows H^{\cdot} + CO''_3)$$

The second stage of dissociation is so small as to be negligible. The concentration of $H^{\cdot}$ being now increased and OH^{1} decreased, the solution would have an acid reaction. The actual amount of CO_2 thus dissolved would always be small; for instance, a salt solution of strength Cl = 20·00 (average Atlantic water Cl = 19·4) will at 10° C. dissolve about ·3 c.c. CO_2 per litre from an atmosphere containing 3 $^{o}/_{oo}$ CO_2 (about normal). But the ocean is found to contain very much greater quantities than this, 60 c.c. or 200 times this amount being a not unusual figure for the total CO. The difference between this amount and the ·3 c.c. or so dis-

solved by the neutral salt solution, as above, is kept in equilibrium with the 3 $^{o}/_{oo}$ CO_2 of the atmosphere by the amount of "excess" base equivalent to the amount of acid neutralised when an acid such as HCl is added to sea-water in excess. If a solution identical with sea-water but absolutely free from CO_2 (a practical chemical impossibility) could be obtained, then there would be present an excess of base over acid, and consequently an excess of OH^1 ions over H. ions, and an alkaline reaction. On exposing such a solution to the atmosphere, CO_2 would go into solution, ionise, and the $H^{\cdot}$ ions thus set free would react with the OH^1 ions, due to the excess base, to form water. And this reaction would continue to take place, on more CO_2 dissolving, until all the excess OH^1 ions were neutralised, at which point the solution would react neutral. Now, as before with the neutral salt solution, a further small amount of CO_2 would go into solution, bringing the solution into equilibrium with the atmosphere, and the excess $H^{\cdot}$ ions thus formed would give an acid reaction. The final result would be a solution exactly identical with natural sea-water. The total CO_2 found in sea-water can be considered as existing in two parts: the larger part in equilibrium with free base, its amount depending on temperature, pressure, and alkalinity; the smaller in equilibrium with the partial pressure of CO_2 in the atmosphere, its amount depending on temperature, pressure, and salinity. Although sea-water in situ has an acid reaction, it still maintains the property of being able to neutralise a certain amount of any acid stronger than H_2CO_3, that is, any acid which, on dissociation, forms a higher concentration of $H^{\cdot}$ ions; for the stronger acid will turn out the H_2CO_3 in equilibrium with the "excess base" and CO_2 will be evolved.

In consideration of these points, a less confusing definition of the "alkalinity of sea-water" would perhaps be a measure of its potential capability of neutralising a strong acid[1] with the evolution of CO_2. This can be conveniently expressed, as is usual, in mgrm. OH $^{o}/_{oo}$.

[1] Such as HCl, with a high degree of ionization.

Some of our earlier experiments seemed to show that "alkalinity" was a factor of considerable importance for the successful growth of cultures of plankton diatoms ; so an attempt was made to analyse the varions samples of water both before and after treatment as culture media. The method adopted was a modification of that used by Tornöe and Dittmar. Solutions of NaOH and H_2SO_4 of strength $N/_{50}$, by intention, were made up and stored in five-litre "aspirator" bottles. Two accurately graduated burettes standing side by side were connected to these by tubes, so that they could be readily filled by gravity. All air inlets to burettes and stock bottles were fitted with tubes of soda lime. A standard solution of Na_2CO_3 of exactly known alkalinity, approximately that of average sea-water (40·00 mgrm. OH $^0/_{00}$), was prepared by diluting down from a $N/_{10}$ solution, all operations being performed by weighing. These standards were stored in stoppered bottles of the fairly insoluble dark green glass, but those that had been kept for any length of time were not trusted, fresh standards being prepared. On analysis these standards agreed with one another to well within ·1 mgrm. OH $^0/_{00}$. The water used for diluting the standards was distilled water from the laboratory still, re-distilled in all-glass apparatus with potassium bichromate and sulphuric acid.

When carrying out an analysis, equal volumes (about 100 c.c.) of sample and standard were measured out into Jena glass Erlenmeyer flasks with a Knudsen automatic pipette. The specific gravity of each was determined by weighing in a 25 c.c. pyknometer. Sample and standard were then titrated by running in acid from the burette and back titrating with alkali, using a 1 per cent. alcoholic solution of aurine as an indicator and keeping the liquid boiling. The acid to alkali equivalent was determined by titrating a pipetteful of double distilled water in the same manner. The mean of at least four readings was always used. Let N and n be number of burette divisions of alkali equivalent to standard and sample respectively, and D and d their density at the time of pipetting out.

Then if A is the alkalinity of the standard and X the required alkalinity of sample:

$$X = A \frac{Dn}{Nd}$$

Since all operations were carried out at the same room temperature, no corrections for temperature are necessary.

In spite of the greatest care consistent results could not be obtained by this method of analysis. A sample analysed against the same standard would sometimes give results varying as much as 0·5 mgrm. and occasionally 1·0 mgrm. OH $^{o}/_{oo}$. The work on indicators by Salm (**42**) and its application to this question has only recently come to our notice, and it is our intention to experiment on this in future research. The figures quoted below as the results of analyses have been rounded off as whole numbers, since their interest lies in their comparative rather than their absolute value. For convenience they are quoted as "alkalinities," although we are fully conscious that the methods used do not warrant this assumption, and that their actual chemical significance is still obscure.

The mean value for "outside water" was found to be fairly constant at 40·0 mgrm. OH $^{o}/_{oo}$, which figure agrees with results obtained by others for average ocean water. Samples from the aquarium tanks never gave as high figures as this, the average being approximately 37·5 mgrm. OH $^{o}/_{oo}$. From this it seems that the amount of base in equilibrium with CO_2 in tank-water is appreciably less than in outside water. A series of thirteen samples taken from seven miles beyond the Eddystone to well inside the Cattewater (an inner tidal harbour near Plymouth) showed a gradual lowering of the alkalinity from the normal 40, to 38 mgms. OH $^{o}/_{oo}$ as the water became more estuarine and polluted.

The addition of Miquel's solution B to sea-water was found, on analysis, to reduce the "alkalinity" by an amount equivalent to 10 mgrm. OH $^{o}/_{oo}$ or more. The 1 c.c. solution B added to a litre of sea-water in itself contains a certain amount of free acid, equivalent to less than 4 mgrm. OH $^{o}/_{oo}$.

But this reduction of alkalinity cannot be accounted for by the addition of free acid alone, because if only one quarter the amount of solution B is added, the alkalinity of the sample will be found to be, if anything, only very slightly higher. Also, if the various constituents of solution B are added as separate solutions, thus obviating any addition of free acid, a reduction equivalent to about 6 mgrm. $OH\,°/_{oo}$ is still obtained. The presence of ferric chloride in solution B gives a possible explanation of this phenomenon. If a solution of ferric chloride is added to a solution of a soluble carbonate, a reaction, which can be expressed by the following equation, takes place:

$$3\,R_2CO_3Aq. + Fe_2Cl_6Aq. = 6\,RCl.Aq. + Fe_2O_3Aq. + 3\,CO_2.$$

When the ferric chloride is added to sea-water, the final result will be that a certain amount of the "excess base," which was in equilibrium with CO_2, will then be in equilibrium with the chlorine, available on the precipitation of hydrated ferric oxide, with a consequent liberation of CO_2, and a reduction in "alkalinity" will, therefore, take place.

An analogy between the actions of Miquel's solution B and animal charcoal can be seen in the fact that water treated with animal charcoal also shows a reduced "alkalinity," the amount being very variable in different samples.

Sea-water treated with H_2O_2 also showed a lowering of the alkalinity, but in a much less degree when, as usual, minimal quantities were used.

Control experiments on double distilled water, which had been treated with these substances, were tried, but great difficulty was found in obtaining an end point with the indicator. As far as could be judged, distilled water treated with solution B (quantities as with sea-water) showed a negative "alkalinity," equivalent to about 8 mgrm. $OH°/_{oo}$, and in the case of animal charcoal a positive alkalinity equivalent to 6 mgrm. $OH°/_{oo}$, but the colour change was so slow that these results are only the roughest estimates. The possibility that the above results are due to some effect on the indicator, which entirely cloaks the true alkalinity, must always be taken into consideration.

Before any attempts at analysis had been made, the probability that considerable differences might be found in the alkalinity of the various media had presented itself. Improvement in the growth of diatom cultures was found to result from the purely empirical addition of $NaHCO_3$, this result being most marked in normal Miquel sea-water, outside water + solution B only, and Berkefeld water. No growth could be obtained in either "tank-water" or Miquel sea-water to which had been added 1 c.c. HCl (pure, concentrated) per litre, but on again raising the alkalinity of the latter by the addition of $NaHCO_3$ or KOH, good normal growths resulted. Richter (18) and H. Gill (5), also, both state that a weak alkaline reaction is necessary for the growth of diatoms.

In our most recent experiments, all the media have been analysed for alkalinity, and those given in detail below illustrate the importance of determining this factor. Cultures of Thalassiosira decipiens were made in the following media:

A. Tank-water. Control.

Poor growth, hardly normal. Later, good growth of minute forms, etc.

B. Tank-water, treated with cold commercial animal charcoal, and filtered.

Very good growth indeed.

C. Tank-water treated with cold, pure animal charcoal, and filtered.

Very poor growth, comparable to A without minute forms.

D. Tank-water treated with pure animal charcoal as in C, but the animal charcoal was added red-hot.

Fair growth, much superior to C, but not up to B.

The sample of pure animal charcoal used here had been previously found to give very poor results, and it was also quite contrary to our experience that any improvement in growth should be obtained by adding it hot. But if we examine the results of analysis of these media for alkalinity

a probable explanation presents itself. The following figures are only comparative:

A. 38 mgrm. OH $^{o}/_{oo}$ (used as standard).
B. 37 „ „ (higher than usual).
C. 16 „ „ (very low indeed).
D. 34 „ „

It will be seen that the amount of growth in each treated sample follows the alkalinity very closely.

Solutions of Na_2CO_3, $NaHCO_3$ and HCl were made up, so that 4 c.c. of any one contained an amount of acid or alkali equivalent to 10 mgrm. OH. From these a series of normal Miquel sea-waters of different alkalinities were prepared. Cultures of Thalassiosira decipiens were grown in these media.

A. Normal Miquel sea-water. Control. A = 32·7 mgrm. OH $^{o}/_{oo}$.
Perfectly normal growth.

B. Ditto + 4 c.c. Na_2CO_3 per litre. A = 41·7 mgrm. OH $^{o}/_{oo}$ (= + 9·0).[1]
No difference between this culture and A.

C. Ditto + 8 c.c. Na_2CO_3 per litre. A = 50·2 mgrm. OH $^{o}/_{oo}$ (= + 17·5).
Best culture in series in quality and quantity.

D. Ditto + 4 c.c. $NaHCO_3$ per litre. A = 42·4 mgrm. OH $^{o}/_{oo}$ (= + 9·7).
Slightly better than control.

E. Ditto + 8 c.c. $NaHCO_3$ per litre. A = 51·5 mgrm. OH $^{o}/_{oo}$ (= + 18·8).
As D.

F. Ditto + 4 c.c. HCl per litre. A = 22·2 mgrm. OH $^{o}/_{oo}$ (= − 10·5).
Fair growth, but never up to control; exhausted much sooner.

G. Ditto + 8 c.c. HCl per litre. A = 11·1 mgrm. OH $^{o}/_{oo}$ (= − 21·6).
Poorest in series.

[1] Figures in parentheses are difference in alkalinity from control, in mgrm. OH $^{o}/_{oo}$.

Except in the cases where the alkalinity was lowered by the addition of HCl, the results obtained from this series were not up to expectation. Nevertheless the majority showed a distinct improvement from increased "alkalinity," and in c, where the alkalinity had been raised 17·5 mgrm. OH $^{0}/_{00}$, this improvement was very marked.

Another point illustrated by cultural experiment is that in two samples of animal-charcoal water, one with "outside" and the other with "tank-water" as a basis, the amount of growth in the latter considerably exceeded that in the former, and at the same time it was found that, with the tank-water, the alkalinity had not been reduced to the same extent as in the case of the outside water.

How far apparently anomalous results, which have so frequently occurred in our experimental work, could be explained by unforeseen changes in "alkalinity," can only be answered by future research.

Salinity.—The salinity (or amount of salts dissolved in 1000 grm. sea-water) of the outside water used in these experiments only varied between small limits, S = 34·5 to 35·5 $^{0}/_{00}$. The salinity of "tank-water" is also fairly constant, the average being about S = 34·9 $^{0}/_{00}$; water is only pumped up into the reservoirs at high water, spring tides, and unless the salinity on analysis is well above S = 34·5 $^{0}/_{00}$ no water is taken. Experiments to show what effect salinity pure and simple had on the growth of diatoms were undertaken. Samples of sea-water of various salinities were prepared by diluting down "outside water" with double distilled water, and by concentrating "outside water" by slow evaporation. Two litres of "outside water," S = 34·9, were evaporated down to the bulk of one litre, giving a 50 $^{0}/_{0}$[1] concentration. Miquel solutions 4 c.c. A, 2 c.c. B, were now added, and the solution was divided into ten culture vessels, 20 c.c. in each. Double distilled water was added, 2 c.c. to the first, 4 c.c. to the second, 20 c.c. to the last, so that a series of media were obtained, varying in salinity from

[1] i.e. from every 100 c.c. sea-water 50 c.c. H_2O had been subtracted.

normal to nearly 50 °/₀ concentration, each containing the same amount of Miquel's nutrient solutions. These were inoculated from a mixed culture of Skeletonema costatum, Biddulphia mobiliensis, and Coscinodiscus excentricus. A good growth took place in all except the two with highest concentration. Of these two, the last remained practically sterile and the growth in the other was very poor. The limit of concentration, therefore, seems to lie between 35 and 40 °/₀. In the same way series of lowered salinities were prepared, and cultures of the same diatoms were grown in these. Dilution up to 100 °/₀ did not seem to make any difference at all in the quantity or quality of growth. In a series extending the dilution to 200 °/₀, even in the cultures of lowest salinity a fair quantity of growth took place. The range of salinities covered by the various series was S = 12 °/₀₀ to S = 60 °/₀₀, and within these limits no effect on growth could be observed, except in the very highest, where a distinct deterioration was noted.

An attempt to grow Coscinodiscus excentricus in tap-water + Miquel's solutions was tried, and it was thought that some slight multiplication took place, although it was certainly not at all considerable. Inoculating a culture of normal Miquel sea-water from this after six weeks gave no growth.

Light.—Of all the factors controlling the rate of growth of a culture, light seems to be by far the most important. Without light a culture soon dies off completely, showing marked signs of malnutrition very soon after having been placed in the dark, the brown pigment being the first to go and later the chlorophyll. A culture (Thalassiosira) placed in the dark for five months was found to be completely killed, the diatoms being quite colourless. In cultures kept in bulbous flasks or in any spherical vessel, the strongest and earliest growth always takes place at the side of the vessel away from the source of light, where the light will be found to be concentrated owing to the lens effect of a sphere of water. By

painting a flask black on the outside up to the water-line of the medium, a very marked diminution in the rate of growth was obtained. The total growth was not affected, but depends on the available quantity of food-stuffs present.

Experiments on the reaction of cultures to different rays of the spectrum, obtained by coloured glass, were tried, but no results obtained. Miquel obtained marked results with yellow light, but in our experiments, with plankton diatoms, satisfactory cultures could not be obtained under these conditions.

Temperature.—The highest temperature which diatoms and allied forms can stand was about uniform for all the species tested, and lay between 35°–40° C. Cultures of the following species, viz. Asterionella japonica, Nitzschia closterium, minute naviculoid diatom, Pleurococcus mucosus, Chilomonas sp., were slowly heated in a water bath, and at every rise of 5° C. from 15° C. to 45° C. a few drops of the culture were pipetted out and a fresh flask inoculated. In all the flasks cultures were obtained where the heating process had not been carried above 35° C., but none in those where the temperature had exceeded this.

In the earlier stages of experimentation the cultures of diatoms were kept in various places about the laboratory, and so were under quite different temperature conditions. Those placed in the warmer situations, i. e. near hot-water pipes, as a rule gave the most satisfactory results. In all the later work the cultures have been kept in one room, and an attempt has been made to keep the temperature of this room as nearly as possible constant at 15° C. A continuous record of its temperature has been kept by means of a recording thermograph, and no very great change of temperature has been noted. In a few isolated cases the temperature has dropped as low as 9° C., and in hot weather has risen just above 20° C., but these have only been for very short periods, the average temperature having kept remarkably constant. An apparatus in which flasks could be kept at different uniform temperatures from 10° to 25° C., by means of hot air, was used, but no really satisfactory result could be obtained. About 17° C. seemed

to give the maximum growth, and the cultures below this temperature were usually superior to those above.

General Conclusions.—The general conclusions to be drawn from the experiments described in this section, which were made with a view to determining the conditions that underlie the successful culture of diatoms, may now be discussed. Although the experiments have involved the making of some 750 different cultures, our conclusions on many of the questions raised are still indefinite, and much further work will be necessary before a satisfactory answer can be given to them.

If we wish to obtain the maximum quantity of healthy growth of a plankton diatom, the diatom must first be obtained as free as possible from all other organisms, if not in a "pure" culture, at least in a "persistent" culture. All culture media should be sterilised either by heat or filtration, and the experiments should be conducted under sterile conditions. Starting with normal sea-water as the basis for the culture medium, it seems to be first necessary to raise the concentration of the nitrates, and possibly also of the phosphates, in solution. But this simple addition of nutrient materials will not in itself suffice. Some other action, such as that exerted by Miquel's solution B, by animal charcoal, or by peroxide of hydrogen, seems to be imperative in nearly every case. The exact nature of this action we have not been able conclusively to determine. If the substances contained in solution B were purely nutritive in character, we should expect that, when alterations in the amounts of the different ingredients were made, or when any one of the ingredients was omitted altogether, the differences in the quantity of growth would show a direct relation to the kind of modification introduced. But our usual experience has been that solution B can be modified within certain limits, without producing any appreciable effect upon the resulting cultures, whilst, if these limits are exceeded, there is an almost complete inhibition of growth. In supplying a necessary increase of phosphates, both Miquel's solution B and

animal charcoal may, and probably do, act as "nutritive" substances; but, since the addition of phosphates alone does not yield cultures comparable with those produced by either of them, and since, excepting phosphates, there is no possible common nutritive substance in their composition, we are led to conclude that, in addition to any nutritive effect, they must exert some other action. This view is supported by the results obtained by using H_2O_2. This substance cannot be directly "nutritive," although it may be so indirectly, by oxidising into useful food-material substances which the diatoms are incapable of using in their metabolism, e.g. nitrites into nitrates. The absence of any increase in phosphates, when using H_2O_2, may possibly be the reason why better results were not obtained with this medium. The action, which, in addition to any nutritive value, we must assume that solution B, animal charcoal, and H_2O_2 can all effect, would appear to fall into the class of "protective" actions (p. 384). It is quite conceivable that, with different samples of sea-water, this "protective" action is not necessary in every case, and this would account for the anomalous results met with when using sea-water + nitrates + phosphates only, in which medium sometimes good cultures, but more often the reverse, are obtained. The effect of Miquel's solution B, animal charcoal, and H_2O_2 on the "alkalinity" of the sea-water, also points to some chemical change, which does not directly enter into the metabolism of the plants.

It may be pointed out that the action of such substances as finely powdered carbon, and ferric oxide precipitates, have been shown to produce a favourable effect on nutrient solutions used for the culture of certain higher plants, and it has been suggested that the beneficial action of these substances is the removal of toxic elements from the media (Breazeale [3]). Such removal of toxins would fall under our definition of "protective" action.

Of nutritive substances, other than those already mentioned, we have still to consider, (1) silica, and (2) dissolved oxygen and carbonic acid. Having regard to the conditions under

which our cultures have been grown, i.e. in glass flasks, the question of silica does not seem to enter into the problems which we have discussed. A few words must, however, be said as to the dissolved gases. Whipple (**62**) and Baldwin (**44**) have drawn attention to the observed relations, which are found in natural waters, between algal growths and the amounts of dissolved oxygen and carbonic acid. That these factors are of great importance cannot be doubted, but in our cultures it seems reasonable to suppose that the conditions of saturation of these gases are the same in all, since series of cultures in standard media, such as Miquel sea-water or Berkefeld water, can be set up with the certainty that, if not every one, at least a very high percentage, will give normal results.

Of the purely physical factors, light is by far the most important. Within limits, the rate of growth in a suitable medium seems to depend directly on the intensity of the light (Whipple [**60**]). Absence of light, as would be expected, soon completely kills the diatoms.

Temperature also seems to affect the rate of growth to a certain extent, but for those temperatures at which we have experimented it does not appear to alter the quantity of growth.

Salinity, apart from the quantities of available nutrient materials, can be varied within large limits without appreciable effect on the diatoms.

II. Mixed Cultures.

In what has been said up to the present, we have been dealing with persistent cultures containing a single species of diatom, which are comparatively, if not entirely, free from admixture of other organisms. The study of cultures which contain a considerable mixture of organisms is not without interest.

A number of experiments have been made on the following lines: About 10,000 c.c. of water, taken at some distance

from shore, was placed in a tall bell-jar fitted with a "plunger," which keeps the water in constant movement ('Journ. Mar. Biol. Assoc.,' vol. v, p. 176). The water was treated with Miquel's solutions in normal proportions, and a considerable quantity of plankton taken with a fine-meshed net (150 meshes to the inch) was added, say 10 or 20 c.c. of a moderately rich sample of tow-netting. The experiments were made during the spring and summer months, and the general course of events has been the same, with a certain amount of difference in detail according to the nature of the plankton present at the time.

During the first two days the water often became cloudy, owing to the rapid multiplication of small flagellate infusoria, though this was not always the case. Plankton copepods and other animals gradually died off, though some survived for as long as a week or ten days. The plankton diatoms, on the other hand, generally multiplied rapidly during the early days of the experiments, the first to become abundant in the body of the water being usually Skeletonema costatum, which at the end of a week might be so thick that a number of chains could be seen in every drop of water examined with the microscope. Along with the Skeletonema were found other plankton diatoms, such as Lauderia borealis, Chætoceras (two or three species), Biddulphia mobiliensis, Ditylium Brightwellii, and in nearly every case Thalassiosira decipiens. These latter diatoms were present in moderate numbers only, when the Skeletonema was at its height, but as the Skeletonema died down they increased in quantity. At the same time Nitzschia closterium commenced to appear, both amongst the precipitate on the bottom of the jar and in the general body of the water. Small green flagellates often began to get numerous also at this stage. The true plankton diatoms were at their height about a fortnight after the experiments were started. At this time a great many diatoms of all kinds were to be found amongst the precipitate at the bottom of the jar, Asterionella japonica and Coscinodiscus excentricus being often

numerous here. During the course of the next week, however, Nitzschia closterium rapidly increased in quantity, until not only the sides of the jar were coated with it, but the whole mass of the water became thick and opaque. By this time the plankton diatoms had all disappeared, with the exception of those which may survive for a considerable period amongst the precipitate at the bottom of the jar. Bottom diatoms (Navicula, etc.) had begun to grow on the sides of the jar, and small green and brown algæ (Pleurococcus mucosus, Ectocarpus, etc.) also appeared. Infusoria (Euplotes and other smaller forms) then became numerous, and as the Nitzschia and bottom diatoms increased on the glass, large numbers of Amœbæ made their appearance among them. The jars continued in this condition for many months, the algæ becoming more and more predominant.

From these experiments, as well as from instances of mixed cultures obtained in the course of our attempts to secure persistent cultures of single species of diatoms, it seems usual that, in a culture obtained by inoculating Miquel sea-water with plankton taken freshly from the sea, the true plankton diatoms are the first to develop in considerable numbers. Subsequently bottom diatoms and algæ of various kinds become abundant, and the true plankton forms die out.

A complete explanation of this sequence of events would probably be of a very complicated character, and we have practically no evidence from our experiments which bears very directly on the question. It would seem, however, that the early predominance of the plankton forms in the cultures would naturally follow from the fact that, in the plankton material used for inoculation, these plankton forms are numerous, whilst bottom diatoms and spores of algæ are rare. The subsequent very great predominance of such a species as Nitzschia closterium may be due simply to a very much more rapid growth rate, though it is difficult to avoid the impression that the organisms, which finally take possession of the cultures, are in some way directly inimical to those

which they supersede, not merely by robbing them of their food supply, but perhaps, also, by the production of toxic substances. This suggestion does not, however, give an adequate explanation of the essential facts concerning these organisms. We have to consider two sets of species—(1) the true plankton forms, which flourish in the open sea and can be grown quite easily in the laboratory, provided the cultures remain pure, and (2) what we may call "aquarium" or "bottom forms," which under experimental conditions invariably take possession, when present in mixed cultures, whilst the plankton forms are killed off. Why is it that, although species of the second class are always present in small numbers in plankton taken from the sea, they are there altogether outnumbered by the true plankton forms, whereas under conditions such as those of our experiments they invariably succeed in gaining the upper hand? What are the factors which determine the difference in behaviour of these two sets of organisms in the sea and in the culture vessels? The whole question offers a very fruitful field for further experiment. The evidence at present available is so slight that further discussion of it here is not likely to be of much service.

The details of two experiments which we have made bearing on the subject of mixed cultures may, however, be recorded.

A flask, containing about 1000 c.c. of sea-water treated with Miquel's solutions, was inoculated with approximately equal amounts of certain persistent cultures of diatoms, which we possessed at the time. The following diatoms were in this way introduced: Chætoceras constrictum, Biddulphia mobiliensis, Skeletonema costatum, Coscinodiscus excentricus, Streptotheca thamensis. The flagellate (Chilomonas sp.) was also introduced, since it was present in the culture of Coscinodiscus. The experiment was started on August 26th, 1907. On September 6th (11 days) Biddulphia, Coscinodiscus and Chætoceras were increasing rapidly and were very healthy,

Skeletonema was not so good, and no Streptotheca was found.

On October 2nd (37 days) Biddulphia was numerous and healthy, Coscinodiscus was healthy but not so numerous, Skeletonema was poor, and Chætoceras was not seen. Flagellates (Chilomonas) had become very numerous.

On October 31st (66 days) all the diatoms were in very poor condition, Coscinodiscus being slightly better than the others. The flagellates (Chilomonas) were extremely thick, giving the water a deep red colour.

Subsequently a small green alga (Pleurococcus mucosus) appeared, having probably been derived from the Coscinodiscus culture. This increased very greatly in quantity, whilst the flagellates become inconspicuous.

On July 28th, 1909 (1 year 11 months) some Coscinodiscus, which were still in a healthy condition, were seen in a sample examined from the flask. A great quantity of Pleurococcus, in a healthy condition, was also present, but no other oganisms were noted. On this date a subculture was made from the flask in normal outside Miquel. The subculture gave a considerable growth of Skeletonema, the cells being, however, of a very abnormal character, and a good many normal and healthy Coscinodiscus were found in each sample examined. The whole culture was crowded with Chilomonas in a very active state, which gave the whole contents of the flask a deep red-brown colour. Up to August 24th the green alga (Pleurococcus) had not become sufficiently abundant to be detected by the naked-eye appearance of the flask, though it could be seen in samples examined with the microscope.

In another experiment a flask of Miquel sea-water was inoculated (May 4th, 1908) from two cultures, one containing the green alga (Pleurococcus mucosus) and the other Thalassiosira decipiens. At first both did well, and on May 20th (16 days) there was a very good crop both of the diatom and the alga. Gradually, however, the alga became predominant, and on October 14th (163 days) only quite empty

frustules of Thalassiosira could be found, whilst the growth of Pleurococcus was abundant and healthy. The only case where a diatom was observed to flourish in the presence of this green alga was in a culture of Nitzschia, a bottom form. In this case a very abundant growth of the diatom was obtained, but the Pleurococcus did not multiply to any extent although it could always be found on microscopic examination.

III. Notes on Particular Species of Diatoms, on their Methods of Reproduction, and on other Algæ Occurring in Cultures.

A list has been already given (p. 367) of those species of diatoms which we have obtained in "persistent" cultures. Of these a species belonging to the genus Thalassiosira has been used for experimental work in the great majority of cases. We are not quite certain as to the identity of the species, but since it most resembles T. decipiens Grun. we have called it by that name, although it does not exactly conform to the published descriptions of that form. The most characteristic feature of this particular species is the eccentric markings on the valves, which are also seen on the valves of the diatom Coscinodiscus excentricus Ehr., and, as is typical of the genus, the frustules are united into chains by a delicate filament. Jörgensen (**50**, p. 96) describes the valves as "decidedly convex," Gran (**49**) as "flat," and both agree that there are marginal spines and a single asymmetrical spine. Our cultural forms are united together by a filament into chains, some of which are made up of 500 cells and more, but the distance between each is considerably smaller than that figured by Gran. The valves are quite flat and the marginal spines are often present, although this is not always the case. The odd, asymmetrical apiculus can nearly always be seen. The eccentric markings have only been observed in a few isolated cases, and are then usually very indistinct. In one culture these markings on the valves were very distinct,

and were also easily seen on the megafrustules (cf. below), which developed in it, but in none of the several generations of cultures started from this one have we been able to find any traces of marking at all. The genus seems to be in considerable confusion, and it is probable that the conflicting descriptions given by different observers are due to variations in what is really one species.

Persistent cultures of Coscinodiscus excentricus Ehr. have also been obtained, and it is interesting to note that this diatom sometimes forms chains, but they are rather exceptional. These chains are never as long as those commonly found with Thalassiosira, two or four cells only being the rule. The filament joining the valves is also finer and more easily broken. The two species are quite distinct, and cultures of them can be discriminated by a practised eye.

Two species of the genus Biddulphia are commonly met with in our cultures, namely Biddulphia mobiliensis (Bail.) Grun. and Biddulphia regia M. Schultze. These two forms are generally regarded as one species, but Ostenfeld (**54**) has recently shown that they are really distinct. We have obtained persistent cultures of both forms from several different samples of plankton, and the two species are easily recognisable, never merging into one another. When Petri dishes, inoculated from plankton (see p. 367), contain both species, the colonies can be easily distinguished with a small hand lens.

The most generally accepted theory of the reproduction of the Diatomaceæ is briefly that the cells divide by simple fission, but on account of the rigid character of the cell-walls each division necessitates a decrease in size of the new valve, since this must always be formed inside the old valve. So the frustules gradually get smaller and smaller as multiplication proceeds, thus necessitating some process by which the original size can be re-established. This takes place by the formation of what are known as auxospores, which ultimately form megafrustules, and these in turn multiply by division until the minimum limit of size has again been reached.

There are also several special processes of reproduction, but no occurrence of any of these has been noted in our work (cf. Miquel [**14**]).

The diatoms in our cultures multiply by simple fission, and although there is, in nearly every case, a considerable diminution in size when compared with specimens from the plankton, this diminution soon seems to reach a limit, where further decrease does not take place. In chains of Thalassiosira, several hundred cells in length, no difference in size between individuals could be made out. Auxospores are commonly formed with every species, but only in cultures of Coscinodiscus and Thalassiosira have megafrustules been found, and in these they are very exceptional. These megafrustules seem to divide once or twice and then die or form new auxospores. What exactly is the fate of these auxospores, which are often exceedingly numerous, we have not been able to make out. It seems that cultural conditions are not favourable to this mode of reproduction, and that the auxospores do not further the multiplication of the diatom at all. If this were not the case, stages of the formation of auxospores into frustules must have been seen in some at least of the very numerous samples examined. As it is, what has been seen to take place is, that the cell contents expand and force apart the valves of the diatom and emerge as a spherical body about three or four times the diameter of the parent cell. The chromatophores and diatomin then collect to one side, forming a compact cap against the cell-wall. Beyond this point no stages have been found, except in the case of the few cultures where megafrustules were formed. In these the chromatophores, etc., gradually formed into the shape of the diatom (Coscinodiscus); the siliceous coat with plain eccentric markings was easily seen inside the spore; and lastly, the cell-wall of the spore burst, leaving the megafrustule free. The megafrustule was measured and found to have a diameter three times that of the parent cell.

In the case of the diatom we have very largely used for feeding larvæ, etc., namely Nitzschia closterium, forma

minutissima, a great number of cultures have been made, all originating from the single drop from which the first persistent culture was obtained. The total amount of growth in all the various cultures has been enormous, and the number of generations must be quite inconceivable. No diminution in size has, however, been appreciable, and no sign of any method of re-establishment of size has been seen, although these cultures have been under constant observation for over two years. This seems to prove that the theory of gradual decrease in size with successive generations cannot be generally applied.

The following experiment on the rate of multiplication of Thalassiosira in normal Miquel sea-water was carried out. A single drop from a fresh and vigorous culture was kept under a microscope as a hanging-drop preparation in a moist chamber. The number of diatoms in this drop was counted from time to time and the results are given in the following table:

Day.	Number of frustules.	Geometric progression.
11th	59	63
14th	62	68
19th	85	85
27th	140	120
34th	170	160
41st	190	220

The curve obtained by plotting the number of diatoms against the number of days approximates the curve of an ordinary geometric progression, where the ratio is 2 and the periods are equal to sixteen days. To show this the figures read off from the curve at the same intervals as the diatoms are appended in the table. From this it will be seen that, after a start had been made and before exhaustion set in, the numbers obtained agree fairly closely with the assumption that every diatom divided once in a period of sixteen days. Probably in normal cultural conditions the rate of multiplication greatly exceeds this figure on account of better lighting, etc. (cf. Miquel **12**).

Besides diatoms, many other organisms appear in these cultures. We are indebted to Mr. G. S. West for the identification of a form of unicellular alga, which is very common and difficult to avoid when attempting to obtain persistent cultures of the Diatomaceæ, namely, Pleurococcus mucosus (Kutz.) Rabenh. This small green alga, if once introduced into a culture of a plankton diatom, will soon multiply at the expense of the latter with its ultimate extinction. It is very hardy, and cultures of it in almost every medium seem to last indefinitely. Multiplication beyond a certain point probably does not occur, but the cells retain their colour and normal shape, and will start active reproduction if suitable nutrient material is provided.

In cultures inoculated from plankton, many other forms of unicellular and filamentous algæ thrive. Several species belonging to the classes Rhodophyceæ and Myxophyceæ commonly occur, but we have not been able to identify them. The most usual filamentous forms of Chlorophyceæ are Enteromorpha, Vaucheria, Rhizoclonium, etc. It is interesting to note that it was the unintentional appearance of young plants of Laminaria digitata in some of our Petri dishes that led Mr. Drew (4) to cultivate this alga in Miquel sea-water and so discover its early life-history. Cultivations of marine algæ by these methods would without doubt yield many new species, and would also provide rich material for the study of their modes of reproduction.

Many forms of flagellates live either together with diatoms or alone. Among these is an unidentified species of Chilomonas, which we have obtained in persistent culture. It multiplies very rapidly, colouring the whole medium a deep red-brown. It flourishes in Miquel sea-water and its nutrition is evidently autotrophic. In one culture, in Miquel sea-water inoculated with plankton, a number of coccospheres developed, probably Coccosphæra atlantica Ostenf. Other flagellates and ciliated infusoria are very commonly met with, such as Bodo, Euplotes, Euglena, etc., which all seem to depend on the diatoms or other vegetable organisms for their food material.

IV. The Rearing of Marine Larvæ.

In the rearing of pelagic larval forms of marine animals,[1] the principle which we have followed has been to introduce into pure, sterile sea-water the larvæ to be reared, together with a pure culture of a suitable food. As far as practicable all other organisms have been excluded from the rearing vessels. It should be added that the food used in all successful experiments has been of a vegetable nature, and has continued to grow actively in the vessels. This is important from the point of view of oxygen supply. Under the above conditions, or rather under the nearest approach to them at which we have been able to arrive, no change of water has been found necessary.

Methods.—It will, perhaps, best make the matter plain if we first of all describe the actual procedure, which we now follow in the case of such an animal as Echinus esculentus or E. acutus. The water to be used is first of all prepared by treating water from the aquarium tanks with powdered animal charcoal, filtering it through a Berkefeld filter (p. 375), and collecting it in sterilised glass vessels. All instruments and pipettes are sterilised by baking in an oven, and a fresh sterile pipette is used for each operation during the progress of the work. Specimens of Echinus are then opened until a perfectly ripe female has been found, that is to say, one in which the eggs separate quite freely when a portion of the ovary is shaken in sea-water.

Pieces of ovary, taken from a little below the exposed surface, are then placed in sterile sea-water in a shallow glass dish, and shaken with forceps in order to get the eggs well separated, or a number of eggs from the centre of the ovary are drawn up with a pipette and placed in the water. A very small quantity of active sperm from a ripe male is then added, very little being sufficient to fertilise a large number of eggs. Excess of sperm should be avoided owing to its

[1] See "Bibliography," especially Grave (26), MacBride (28–30), Doncaster (25), etc.

liability to putrefy. After an interval of ten or fifteen minutes the water, containing the eggs, is filtered through bolting silk of 100 meshes per inch, which just allows single eggs to pass through, whilst keeping back clusters of eggs or other large material. The filtrate is divided amongst a number of tall narrow beakers containing sterile sea-water, and the beakers, after being covered with a glass plate, are placed where the temperature will be uniform and not rise much above 15° C. In the course of twenty-four hours the healthy larvæ will swim up to the surface and can be easily seen and removed from vessels of this shape. They are transferred by means of sterile pipettes to jars[1] of sterile sea-water, about fifty to seventy larvæ being put in each jar of 2000 c.c. sea-water. At the same time, a good pipetteful of a pure culture of diatom is added to each jar. The small diatom Nitzschia closterium, forma minutissima we have found most useful, as its size is suitable, and it grows well in animal-charcoal tank-water, floating throughout the body of the water, and so being in intimate admixture with the larvæ. The jars are placed in a moderate light and at as even a temperature as possible.[2] No further attention is necessary until the larvæ have metamorphosed. The metamorphosis takes place in from six to nine weeks after fertilisation. Larvæ may be taken out from time to time and examined to see if they are feeding well. If the diatoms do not grow sufficiently rapidly in the jar more should be added from the culture flasks. We are more often troubled, however, towards the end of an experiment, by an excessive abundance of diatoms. In this case the jar may either be put in a darker place, or some of the water may be drawn off and replaced by a fresh supply of sterile sea-water. Care should

[1] The vessels we use are ordinary green-glass sweet-jars, having a capacity of about 2000 c.c., which are kept covered with the glass stoppers provided with such jars, from which the cork band has been removed.

[2] In hot weather we often stand the jars in one of the tanks of circulating aquarium water, which maintains them at a very uniform temperature.

be taken to have a sufficient supply of food at the beginning of the experiment, so that the larvæ may be able to feed as soon as they are ready for food.

The method just described can be modified in various ways without detriment to the result. Sufficient sterilisation of the water may be effected by heating to 70° C. for fifteen minutes, after which it should be aërated by violent shaking. "Outside water" may be used instead of "tank-water," and may be treated with Miquel's solutions in the ordinary way, to ensure a satisfactory growth of the food-diatom.

With regard to the food organisms, we have tried to obtain as large a variety of these in pure culture as possible, and then to make trial of a number of them with each batch of larvæ on which we have experimented. If no suitable pure cultures are available, success can sometimes be obtained by adding a few drops of tow-netting, collected with a fine-meshed net (180 meshes per inch), directly to the treated sterile water containing the larvæ. In this case one depends on the chance of a suitable food-organism growing in the vessel, unaccompanied by any destructive organism. On several occasions a satisfactory result has been reached by proceeding in this way, and the method is generally worth a trial, seeing that the number of larvæ obtainable from an ordinary fertilisation is very large and many different experiments are easily made with them.

We will now give details of some of the results obtained by making use of the methods described, or of their modifications.

Echinus acutus.—The first successful experiment was made with this species. Eggs fertilised on June 13th, 1905, produced healthy larvæ, fifty to seventy-five of which were placed, three days later, in a glass jar containing 2000 c.c. of ouside sea-water, filtered through animal charcoal, to which modified Miquel solutions were added. They were fed on a diatom culture, containing a small species of Chætoceras, which did not form chains, a small diatom probably belonging to the genus Melosira, a small naviculoid diatom, two

minute flagellates, and a small green organism, probably one of the Pleurococcaceæ. The vessel stood in a shallow tank, through which a stream of aquarium water was flowing and the temperature was fairly constant at 15° or 16° C., though there is one record of 19° C. at the end of July. The first two young Echinus were seen on July 25th, forty-two days after fertilisation, and on August 1st twenty were counted. On August 5th (the fifty-third day) a careful search through the jar gave twenty-one young Echinus of normal size attached to the glass, six minute but fully formed Echinus, about twenty-three still in the Pluteus stage, roughly half of which were well advanced. On August 16th some of the water, which had not been changed since the beginning of the experiment, was replaced by "outside" water. On October 5th (sixteen weeks after fertilisation) twelve Echinus were still alive. Some pieces of red seaweed were placed in the jar, upon which the Echinus fixed themselves and fed. Several of these specimens lived for over a year, but sufficient attention was not given to finding suitable food for them after the metamorphosis, so that they did not grow very large.

Echinus esculentus.—Three successful experiments have been made with E. esculentus. In the first (eggs fertilised April 5th, 1907), "outside" water treated with animal charcoal and filtered through filter-cloth, but not otherwise sterilised, was used. A number of jars of 2000 c.c. capacity containing larvæ were set up, and, to the most of these, various diatom cultures then in our possession were added, none of which, however, gave a satisfactory result. In two jars, on the other hand, to which no culture was added, there was considerable growth of diatoms and of a flagellate, upon which the Plutei fed. The first young Echinus were recorded in both jars on June 8th (sixty-four days), but may have been present a few days earlier. Eventually from thirty to forty metamorphosed in one jar and about twelve in the other. The temperature varied from 10·5° C. to 12·5° C.

In the second experiment (eggs fertilised June 8th, 1908), made with similar water, the larvæ were fed a on pure culture of Nitzschia closterium var., and six had completely metamorphosed on July 26th (forty-eight days after fertilisation), two more subsequently coming through. The temperature was generally 15° to 16° or 17° C.

In the third experiment (eggs fertilised March 29th, 1909) aquarium tank-water treated with animal charcoal and then filtered through a Berkefeld filter was used. Plutei fed with a pure culture of a small flagellate (probably Chilomonas sp.) grew satisfactorily, and eight young Echinus were found on June 5th (sixty-eight days after fertilisation), which had probably metamorphosed some days earlier. Two other jars, in which Nitzschia closterium var. was used as food, were not successful, probably because the growth of diatoms became too thick towards the end of the experiment.

Echinus miliaris.—In the first experiment with this species animal-charcoal Berkefeld water was used, each jar containing, as usual, 2000 c.c. In one jar the Plutei, from eggs fertilised on August 27th, 1907, were fed on a pure culture of Nitzschia closterium, var. On October 4th, i. e. thirty-eight days after fertilisation, one Echinus has just metamorphosed. On October 29th about a dozen healthy-looking Echini were climbing about the jar, and many were still in a healthy condition on January 8th, 1908. Temperatures: September, 15° to 19° C.; October, 16° dropping to 13° C. towards end; November, 12° to 11° C.; December, 15° to 10° C.

To another jar containing larvæ from the same batch a few drops of fresh Plankton were added as food. The Plutei in this case fed on flagellates and Nitzschia which grew in the jar, and several metamorphosed.

In a second experiment with eggs fertilised on September 13th, 1907, the larvæ were fed with Nitzschia closterium, but although there were a few well-advanced plutei still living on January 8th, 1908, none completed the metamorphosis.

Cucumaria saxicola.—A female Cucumaria, one of a number in a dish containing "outside" water, laid eggs, which were fertilised, and segmented on May 12th, 1906. A number of these were placed in a flask in 800 c.c. of "outside" water, which had been sterilised by heating and then treated with animal charcoal and filtered. About 1 c.c. of fine plankton, containing diatoms, was added to the flask on May 12th. On May 25th some of the water was poured off and a new supply added. As the amount of food seemed small, some culture of a green alga (Pleurococcus mucosus [Kutz.] Rabenh.) was added, and this continued to grow well in the flask. The larvæ continued healthy and formed young Cucumaria, of which many were still alive on July 25th, 1907, i. e. fourteen months after fertilisation. Some of the water was changed in this flask on May 30th, 1906, June 18th, 1906, and September 15th, 1906, and July 25th, 1907. Although many of these Cucumaria remained quite healthy they did not grow to any great size. Probably the food which was suitable to the larvæ and early stages, ought to have been changed as the animals grew older.

Pomatoceras triqueter.—The larvæ of Pomatoceras are perhaps the easiest to rear, and give the most certain results of any with which we have experimented. They do well on the minute variety of Nitzschia closterium, but will feed upon almost any small diatom. Since the adults live in calcareous tubes attached to stones, and the tubes have to be broken open before the eggs can be obtained, it is not easy to get the latter free from infection of other organisms. If, therefore, the eggs are fertilised and placed in sterilised animal-charcoal water with only moderate precautions, sufficient growth of diatoms or other organisms will generally take place in the jar to feed the larvæ and bring them to the adult state. When once fixed to the glass the worms are very hardy and healthy, and a stream of ordinary aquarium water can be run through the jar. They then grow rapidly and attain a size equal to any found on the shore. The following experiment may be given in detail to illustrate the time

occupied in development. On August 29th, 1907, eggs of Pomatoceros triqueter were fertilised in animal-charcoal Berkefeld water, and some pure culture of Nitzschia closterium var. added. The larvæ fed well, and on October 1st (i.e. thirty-three days after fertilisation), a great number had fixed on the sides of the jar and made quite normal tubes. A constant stream of the ordinary aquarium water was then allowed to run through the jar, and the worms continued to grow and flourish, reaching a large size, and are still alive and healthy (November, 1909). A similar result was obtained from the same batch of eggs by feeding on a pure culture of a flagellate infusorian. Temperatures during these two experiments were between 15° and 19° C.

Chætopterus variopedatus.—Four experiments were made with this species. The food which gave most promise of success was the diatom Nitzschia closterium var. Larvæ from eggs fertilised on July 20th, 1908, fed on this material lived until October 30th, and reached an advanced stage. They did not, however, adopt the adult habit and form tubes. Two larvæ were also reared to an advanced stage by using flagellates, and, in later stages, the diatom Skeletonema costatum as food.

Sabellaria alveolata.—One experiment only was made with this species, on eggs fertilised on July 19th, 1908. The eggs were fertilised in "outside" water, and the larvæ subsequently transferred to jars containing animal-charcoal Berkefeld aquarium water. They were fed on a pure culture of Nitzschia closterium var., and kept healthy and active, and developed well until nearly the end of October, when, simultaneously with a sudden drop in temperature from 15° and 16° C. to 12° and 9 C., they sank to the bottom of the vessel, and in about three days were all dead. Temperatures: During July and August the temperature kept fairly constant at about 17° C., with a range from 15° to 19° C. During September it was generally about 15° C., and continued at about this level until the fall in the middle of October.

Archidoris tuberculata.—A good many trials have

been made to rear the larvæ of nudibrunchiate molluscs, but up to the present not much success has been achieved. The best experiment was one made with larvæ of Archidoris tuberculata. A number of veligers of this species hatched out on May 8th, 1906, from some spawn which had just been collected from the shore. Some of these were put in a flask containing 1000 c.c. of sterilised animal-charcoal water, and about 1 c.c. of fine plankton was added. On May 14th a few veligers were transferred to another flask of sterilised animal-charcoal water and some pure culture of the green alga, Pleurococcus mucosus, was added. Whereas the larvæ in the original flask did not live long, those provided with the green alga fed well and developed for some considerable time. A number of them were active and vigorous on July 4th, i. e. fifty-one days after hatching, and several were still swimming at the end of July. On August 15th none could be seen moving, but two of those which lay on the bottom, when examined with the microscope, showed no sign of decomposition. The animal was retracted in the shell, but the tissue looked healthy, and the eye-spots and otoliths could be seen. The growth in the flask seemed to be a quite pure culture of Pleurococcus. Larvæ were examined again on September 14th, and appeared much as in August, the tissue still showing no sign of disintegration. The flask was not again examined microscopically until July 25th of the following year (1907). No sign of the larvæ could then be seen, but the culture of Pleurococcus remained pure and healthy.

Subsequent experiments were made with spawn, which was deposited by the females in confinement. Although the spawn hatched and gave apparently healthy larvæ, these did not live for more than a few days.

Calanus finmarchicus.—A single experiment is perhaps worth recording, as showing that it ought to be possible to rear this species without great difficulty. On August 8th, 1905, to a flask containing 1000 c.c. of outside water (unsterilised) there was added ½ c.c. of Miquel's solution B

and ½ c.c. of a 1·5 per cent. solution of anhydrous sodium carbonate. A few Calanus finmarchicus and some decapod Zœas were put in, together with a quantity of a culture containing mixed diatoms. On September 8th all the Zœas were dead, but three Calanus were alive, and Nitzschia and a number of bottom diatoms were very plentiful. On September 17th the three large Calanus were alive and vigorous, and a considerable number of Nauplii were seen in the flask. By September 22nd two of the Nauplii had developed into young Calanus. These, however, did not live for more than a week or ten days, and the adults also died. The flask was abandoned on November 13th, the water in it not having been changed since the commencement of the experiment.

Echinus hybrid.—A successful experiment on crossing E. esculentus and E. acutus was carried out by Mr. W. De Morgan, who was working at the Plymouth Laboratory. We provided him with treated water and diatom cultures for food, and he followed our methods. We are indebted to him for allowing us to publish these results. Some eggs from a ripe E. esculentus were fertilised by active sperm from an E. acutus, in sterilised water, on March 29th, 1909. Healthy larvæ were obtained, and were transferred two days later to tank-water, which had been treated with animal charcoal and filtered through a Berkefeld filter. A culture of Nitzschia closterium var. was added as food, and the larvæ developed rapidly, feeding well. Several were completely metamorphosed on May 7th, or thirty-nine days after fertilisation, In all thirty young hybrids were obtained, and a number of these are still alive and feeding on red weeds.

Sacculina carcini.—Mr. Geoffrey Smith has recorded the fact ('Quart. Journ. Micr. Sci.,' vol. 51, 1907, p. 625) that he was able to rear the larvæ of Sacculina up to the Cypris stage, when they attached themselves to their host, Carcinus mænas. These larvæ were kept in aquarium tank-water treated with animal charcoal and filtered through a Berkefeld filter. In this case the question of food did not

arise, as the larvæ do not feed after hatching. It must be noted, however, that these larvæ had previously been reared by Müller and by Delage.

Summary of Method for Rearing Larvæ.—We have found that the best results in rearing marine larvæ have been attained by taking the following precautions :

(1) The eggs of the female selected must be really ripe, and the spermatozoa of the male active.

(2) The smallest quantity of sperm necessary to fertilise the eggs should be used.

(3) Sterile sea-water, treated in such a way that diatoms etc., will grow well in it, should be used. No frequent change of water is then necessary.

(4) All dishes, jars, instruments, and pipettes, should be carefully sterilised before use. Every possible effort should be made to prevent the introduction into the rearing-jars of any organisms other than the larvæ to be reared, and organisms on which they feed. The jars should be covered with loosely fitting glass covers.

(5) The eggs after fertilisation must be separated from all foreign matter, pieces of ovary, or testis, etc. As soon as the larvæ swim up they should be pipetted off into fresh vessels of treated water, so as to leave behind any unsegmented eggs, etc.

(6) The food organisms should be small in size, so that the larvæ can draw them into the mouth by ciliary currents. The food should distribute itself through the body of the liquid, and not settle too readily on the bottom of the vessel. (This is one of the great advantages of the diatom Nitzschia closterium, forma minutissima.)

(7) The food should be abundant early, so that the larvæ may commence feeding as soon as they are able to do so. The food, however, must not be allowed to get excessively thick in the water. It can be kept down by diminishing the light, or by changing some of the water.

(8) The temperature should be kept as constant as possible. Within limits the actual degree of temperature

is not so important as the avoidance of rapid changes of temperature.

(9) A good north light, not exposed to direct sunlight, is most suitable for the rearing-jars.

(10) In determining the amount of water to be used in any particular vessel, regard must be had to the amount of water surface exposed to the air, which should be large in proportion to the volume of the water.

(11) A change of food is generally required after the metamorphosis of the larvæ.

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Notes on the Free-Living Nematodes.

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With 11 Text-figures.

I.—The Hermaphrodite Species.

Contents.

Introduction.

Orley divided the Nematoda into three groups, roughly corresponding to differences of habitat found in the phylum. (1) Nematozoa embracing all parasitic forms, (2) Rhabditiformæ which live free in "decomposing organic substances

or in earth saturated with such substances"; and (3) Anguillulidæ, the rest of the free-living nematodes, found in soil or water. Such a classification, grounded on œcology, pays no attention to the facts of morphology, and is naturally out of place in zoological arrangement, which aims at expressing the relationship of animals by descent. The methods of life of an animal are, moreover, largely ruled by the mode of procuring nutriment which has been adopted. The first two groups of Orley are parasites and saprophytes respectively, but in the Anguillulidæ we have a heterogeneous collection of forms varying greatly in their habits of life. Little is known of their sources of nourishment save in the case of a very definite division (e.g. Tylenchus, Dorylaimus), which live on the juices of plants, and for that end are provided with a small protrusible spear for piercing tissues and suctorial pharynx for absorbing sap thus set free. The vast majority of this family, however, possess an unarmed buccal cavity; but in all the muscular pharynx is constantly at work, now dilated, now collapsed, constantly pumping fluid through the alimentary canal. There is no morphological distinction to be observed between such a free-living nematode as is found in the mud of a lake or amongst the algæ of the marine littoral and a Rhabditis or Diplogaster of the soil. But the latter class can be kept in a culture fluid which swarms with bacteria, in which individuals of the former class would speedily succumb. The tissues of a Rhabditis must be resistant to bacterial action and unharmed by the toxins which such organisms produce, and the worm is, in fact, capable of building up protoplasm from the bacteria themselves or from the products of their action. These are the most prominent physiological characteristics of the soil nematodes, Orley's Rhabditiformæ, and account for the peculiarities of their distribution, for they are apparently absent from dry soils and those with a small admixture of organic matter, and even in soils rich in humus are only detected in quantity by allowing some animal or vegetable substance to putrefy on the sample. Sufficient

attention has not been paid to the part which nematodes play in the economy of the soil,[1] but an investigation of this problem may well reveal results of as great interest as those which have been put on record by Maupas, working on the sexual organisation. In the present paper it is proposed to confine attention to the reproductive phenomena in certain hermaphrodite species, but it is hoped in a subsequent research to return to the nutrition and distribution of the class.

Cultures of free-living nematodes in connection with this work were first started at the Stazione Zoologica, Naples, in 1906, and continued at intervals in the next two years at the Zoological Laboratory, Cambridge, using for the most part Diplogaster linstowi. In 1909 I spent July to September at the Sutton Broad Laboratory, Norfolk, and procured from the neighbourhood the two forms, Rhabditis gurneyi and Diplogaster maupasi, the study of which enables me to amplify in one or two particulars Maupas' account of the free-living hermaphrodite species of nematodes. I wish here to express my sense of the value of the opportunities for research afforded by the Sutton Broad Laboratory, and to thank Mr. Robert Gurney for his great kindness to me while working there.

Summary of Sexual Phenomena in the Hermaphrodite Species.

Guido Schneider, in his 'Monographie der Nematoden' (1866), first discovered and put beyond doubt the existence of self-fertilising hermaphrodite species of free-living nematodes.

[1] The importance of the protozoan fauna of soil has but recently been realised. Like that of the nematodes their nutrition is composed of bacteria, and the place they take as a limiting factor in the increase of nitrifying forms has the closest possible bearing on the fertility of the soils they inhabit. It is, however, probable that these protozoa are more widely distributed in soil and so exercise a more important influence. (See E. J. Russell and H. B. Hutchinson, 'Journ. Agric. Sci.,' vol. iii, 1909, "The Effect of Partial Sterilisation of Soil in the Production of Plant Food," especially p. 141.)

In 1900 Maupas,[1] in a brilliant paper, drew attention to many striking features in the reproductive phenomena of such species. A full description of all prior work relating to hermaphroditism in the Nematoda is given by Maupas, and I shall here content myself with a short resumé of his own results, which later will be quoted more in extenso in connection with my own observations.

The species of the free-living nematodes Rhabditis and Diplogaster fall into one or other of three categories:

(1) Bisexual species, in which male and female individuals are produced in equal numbers.

(2) Hermaphrodite species, in which, besides the self-fertilising protandrous hermaphrodites which form the great mass of the species, there are occasional male individuals, perfectly developed apparently, but taking no part in reproduction.

(3) Parthenogenetic species, in which males have not been found.

It is reasonably supposed that each hermaphrodite species is derived from a bisexual form by the development of spermatozoa in the ovary of the female individuals, which thus become self-fertilising. The males are now useless, and have even to a large extent lost their sexual instinct. Their number dwindles in most cases to an almost imperceptible figure, but final disappearance does not appear to be reached in any species, and this persistence of apparently useless forms is one of the most curious facts recorded in biology.

The hermaphrodite species appear even more numerous than the bisexual. There is, indeed, some evidence that the conversion of females to hermaphrodites in the bisexual species is a present-day process, furnished by the examples of partial hermaphroditism described by Maupas. An intermediate condition is shown in some hermaphrodite species by the occasional occurrence of pure females, or in the production of

[1] E. Maupas, "Modes et Formes de Reproduction des Nématodes," 'Arch. de Zool. Exp. et Gen.,' Sér. 3, t. 8, 1900, pp. 463–624, Pls. xvi–xxvi.

spermatozoa in one half of the genital gland only, the other producing eggs alone. Maupas emphasises the significant fact that these species with an incipient hermaphroditism yield the highest proportion of males he was able to chronicle. This conclusion that the more complete development of hermaphroditism and the suppression of the male sex necessarily proceed closely together is discussed further below.

It is also highly characteristic of the hermaphrodite species in general that the sperm each individual produces only suffices for the fertilisation of a limited number of eggs, so that the period of fertility is followed by one even more prolonged, during which unfertilised eggs are laid, which do not develop. Such a phenomenon marks the hermaphroditism of the free-living nematodes as a character comparatively recently acquired and as yet not shaped by natural selection in anything like its final form.

Finally, a most interesting result was obtained by experiments with hermaphrodites which had exhausted their stock of spermatozoa and supplemental males of the same species. In the rare occasions in which fecundation took place the eggs which were afterwards laid produced males and females in equal numbers.[1]

Systematic Part.

Diplogaster M. Schultze.

This genus includes representatives both from soil and fresh water. But while the former possess a weakly developed bursa, which indicates the relationship of the genus to Rhabditis, the latter are without this character, and this fact, according to Bütschli, affords a natural distinction between the classes.

[1] A preliminary note published in 1908 ("Sexual Phenomena in the Free-living Nematodes," F. A. Potts, 'Proc. Camb. Phil. Soc.,' vol. xiv, Pt. IV, pp. 373–5) gave a general confirmation to Maupas' results, founded on observations on Diplogaster linstowi which was kept in cultures for over a year and then died out.

The soil-nematodes belonging to this genus differ widely among themselves, particularly in respect of such definite characters as the number and arrangement of the papillæ on the tail of the male. The typical number is nine or ten pairs, but D. gracilis Bütschli and others have eight, and D. robustus Maupas, eleven. The arrangement of the papillæ is more variable than their number, but in a small group of species, with which I am more specially concerned here, the relative positions are fairly constant and characteristic.

The arrangement of the papillæ follows the scheme given below. The numbers correspond to those given in the various diagrams (see Text-fig. 4).

(1) A pair of papillæ opposite the anterior end of the copulatory spicules. D. robustus Maupas possesses an extra pair, situated far in front of the spicules. In D. maupasi sp. n., as a frequent variation one of this pair may have been shifted forward to a markedly pre-spicular position.

(2, 3) Two pairs of papillæ opposite the posterior end of the copulatory spicules.

In D. robustus Maupas shows three pairs in this position.

(4) One pair slightly post-spicular.

(5, 6) Two pairs, the anterior situated about half-way between the root of the tail and the anus, and the posterior at the root of the tail.

(7–9) Three small pairs at the root of the tail, more ventral than the last-named.

Since, then, there is so much similarity between the members of the group, the species are best distinguished by differences in size, proportions and biology, to which they are remarkably constant.

Common Characters of the Group.—Buccal cavity surrounded by lips with short setæ. Within two[1] chitinous teeth. Vulva situated in middle of body.[2]

[1] Some species of Diplogaster, for instance D. fictor Bastian, possess only one buccal tooth.

[2] D. gracilis Bütschli has a "monohysterous" ♀ organ with the vulva a short distance anterior to the anus.

Male with bursa and nine (in one case eleven) pairs of papillæ arranged in manner described above. Spicules slender, with accessory piece.

Synopsis of Group.

(1) Bursa with nine pairs of papillæ: D. longicauda Claus. Bisexual species. Length of ♀ 1000–1300 μ; œsophagus fairly long (one sixth to one seventh of whole length); tail long (one third to one fourth of whole length). Germany.

D. linstowi sp. n. Hermaphrodite species. Length of hermaphrodite 1760 μ; œsophagus short (one ninth of whole length) and tail short (one-seventh). Oviparous at first, but soon became viviparous. Naples.

D. maupasi sp. n. Hermaphrodite species. Length of hermaphrodite 1024–1232 μ; œsophagus (one seventh to one eighth of whole length), tail short (one sixth to one seventh). Oviparous throughout life; 150–300 fertile eggs always laid at early stage of cleavage, and then about as many unfertilised eggs. Norfolk Broads.

(2) Bursa with eleven pairs of papillæ. D. robustus Maupas. Hermaphrodite species. Length of hermaphrodite 2488 μ; œsophagus short (one ninth body length); tail very short (one ninth body length). First oviparous, then viviparous, after laying 150–230 fertile eggs.

In addition to the summary diagnosis above the following characters are distinctive of the two new species.

Diplogaster maupasi sp. n. (Text-figs. 1, 4, 5, 6, 8).

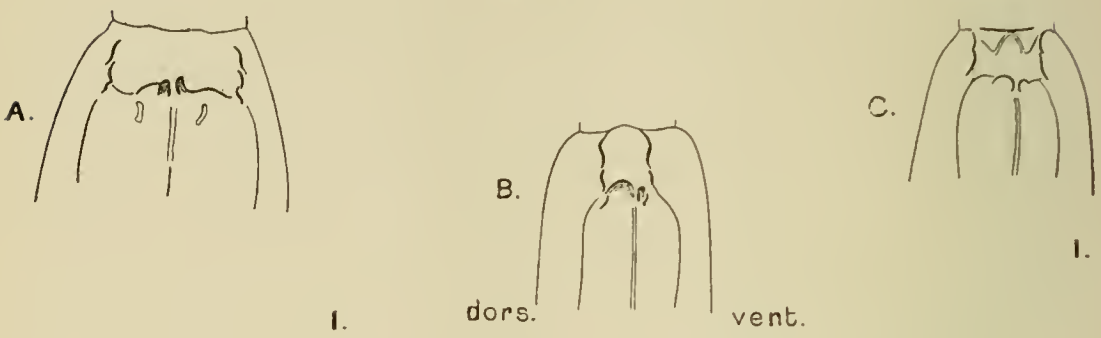
Typical measurements of old ♀:

Total length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.	Length of egg.
1232 μ	608 μ ($\frac{1}{2}$)	152 μ ($\frac{1}{8}$)	176 μ ($\frac{1}{7}$)	5·6 μ

Buccal cavity small, with three indistinct lips, each with a slender seta, often distinguished with difficulty. Hermaphrodite at first lays eggs at long intervals, more frequently later. Males often fairly common. Spicules short, slender,

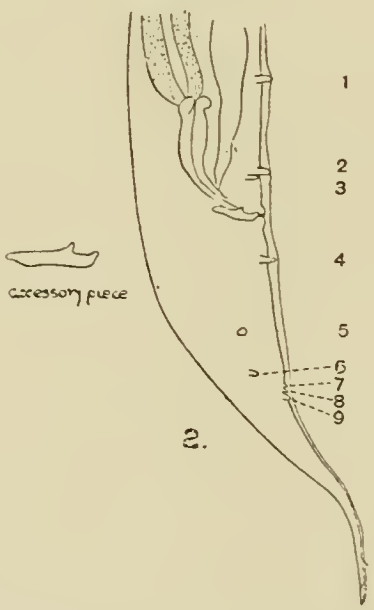
and almost colourless; accessory piece small, in lateral view generally a right-angled triangle, but frequent departures from this type by the rounding of the angles. Number and arrangement of the bursal papillæ strikingly variable.

Text-fig. 1.



It was at first thought that the shape of the buccal cavity was distinctive of species. The accompanying diagram of D. manpasi shows how greatly the state of contraction of the mouth affects the buccal cavity.

Text-fig. 2.



D. linstowi sp. n. (Text-fig. 2).

Typical measurements of old ♀ :

Total length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
1760 μ	840 μ ($\frac{1}{2}$)	200 μ ($\frac{1}{9}$)	240 μ ($\frac{1}{7}$)

Buccal cavity large, as broad as deep, with six papillar lips, each with a slight seta not easily seen.

Males with long and slender copulatory spicules and stout accessory piece, elongated and pointed distally (contrast triangular piece of D. manpasi).

Rhabditis Dujardin.

(1) R. gurneyi sp. n. (Text-figs. 9, 10).

Measurements:

	Length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
Old hermaphrodite	1456 μ	709 μ ($\frac{1}{2}$)	243 μ ($\frac{1}{6}$)	149 μ ($\frac{1}{9}$–$\frac{1}{10}$)

Diagnosis.—Hermaphrodite rather long and slender, tail short. Lips of buccal cavity indistinct, with very minute setæ; buccal cavity narrow and deep. First division of œsophagus thick. Vulva median. Hermaphrodite gland with alternating production of spermatozoa and ova. Spermatozoa of large size. Number of fertile eggs laid up to 800.

Male unknown; probably never produced.

Locality.—In peaty soil, Longmoor Point, Sutton Broad, Norfolk.

(2) R. sechellensis, sp.n. (Text-fig. 3).

Measurements:

	Length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
Old hermaphrodite	680 μ	344 μ ($\frac{1}{2}$)	128 μ ($\frac{1}{5}$)	120 μ ($\frac{1}{5}$–$\frac{1}{6}$)

A male measured 496 μ in total length.

Diagnosis.—Small Rhabditis of pale, transparent

appearance. Lips of buccal cavity indistinct, surmounted by minute setæ, only made out with greatest care. Buccal cavity narrow and deep. Tail of moderate length. In hermaphrodite vulva median. Number of eggs produced small

TEXT-FIG. 3.

(150 or less), mother dying before exhaustion of spermatozoa. Males rare, inert. Copulatory spicules short and thick, accessory spicule small and inconspicuous. Bursa supported by nine rays, arranged as in Text-fig. 3.

Locality.—Found in moss from Seychelles; brought back by Professor J. Stanley Gardiner.

Biology in Relation to Methods of Experiment.

To obtain soil-nematodes in large quantities, it is only necessary to place scraps of flesh on samples of rich soil or mould kept moist and warm, and wait till decay has set in. Though the normal nutriment of these animals is presumably associated with the decay of vegetable products rather than decomposing animal matter, the latter prove exceptionally attractive. When once putridity commences, five or six days more suffice for the appearance of very large numbers of rhabdites or diplogasters, generally belonging to one or two species. Before, however, the last remains have vanished, it is probable that other species will have appeared and become dominant, entirely replacing the first kinds, so that an alternation is obtained somewhat similar to the succession of Protozoa in putrefying broth. It seems that the soil contains scattered throughout it numerous encysted larvæ, for, as Maupas has pointed out, when insufficient nutriment is supplied to soil-nematodes, the young larvæ envelop themselves in a thick cuticle, and become rigid and immobile. They are capable, however, of violent contortions, as if for the purpose of freeing themselves from the cyst, and by these movements migrate easily through the soil. The cuticular protection enables them to live uninjured in a dry environment, so that soil, etc., which has been subjected for long periods to fairly high temperatures, will yet yield large numbers of nematodes when treated in the way described above. The power of encystment, and consequently of resisting prolonged desiccation, is confined to the larvæ. Adult worms at once die when a liquid culture in which they are contained is allowed to dry up, and the eggs of these forms are provided only with a thin cuticular envelope, and are incapable of resisting the vicissitudes to which the eggs of parasitic forms like Ascaris are successfully exposed. When, then, animal-matter putrefies on a sample of soil, it is the encysted larvæ which are attracted to its neighbourhood, where they emerge from their cysts and commence to feed

and grow rapidly. The rate of increase is very great: a single individual when once it has become mature will in five or six days give rise to one or two hundred, the eldest of which will be beginning to lay eggs. But a short interval then elapses between the migration of encysted larvæ toward the putrid meat and the appearance of the swarms of young worms of the second generation.

It is perfectly easy to keep free-living nematodes in drops of a nutrient fluid, and observe under the microscope every stage of their growth and reproduction. Each of these drop-cultures is contained in a solid watch-glass and secured against evaporation by a vaselined glass cover. Solutions of peptone were adopted as convenient culture media, and used almost exclusively in these experiments. The solutions were first allowed to putrefy till a cloudy growth of bacteria had developed throughout the liquid. So favourable an environment for growth does a peptone solution in this condition constitute, that in four days the eggs laid by a mature hermaphrodite nematode have themselves produced mature individuals. It is only in the presence of great numbers of bacteria, or the substances formed by them, that the nematodes thrive so well. In sterile solutions growth is suspended, and eggs are only laid at long intervals, for apparently nematodes find it difficult or impossible to assimilate peptones in an unaltered condition. It has not been discovered whether digestion takes place by the secretion of juices dissolving the protoplasm of the bacteria, or is merely confined to the absorption of soluble substances present in the culture fluid and prepared by the action of bacteria. If the second alternative be correct, then a parallel is established with the parasitic nematodes which nourish themselves on the dissolved and broken-down food of their host. An easily observable phenomenon of nematodes in culture is the rapid pumping action of the second œsophageal bulb and the rectum, and it may be argued from this that the nutriment obtained from the stream of fluid so constantly passing through the alimentary canal is in the form of easily abstracted soluble substances.

The insignificant development of glandular cells (which are found only in the œsophagus) may be cited against an intra-intestinal digestion of the bacteria, and whatever else its significance may be, the chitinous layer which lines the alimentary canal throughout must prevent an ingestion of bacteria by the endoderm cells themselves in such a way as Colpidium preys upon the bacteria of the soil.

Besides peptone solutions other culture media have been used in the course of experiment. It was found possible to raise two or three successive generations in a saturated solution of gelatin in water, and free-living nematodes matured from the egg in solutions of amides like tyrosin and leucin, but in these cases the growth was so much retarded and the production of fertile eggs so curtailed that only peptone solutions were used for extended experiments.

The temperature at which the cultures were kept varied from about 18° C. in the summer to 12° C. in the winter, though at one period it fell within three or four degrees of zero. The effect of a temperature approaching freezing-point was very marked, and showed itself in the almost entire suspension of growth. Sterility was not induced, but only a very few eggs were laid every day.

Experiments were also made to find the highest temperatures under which life and reproduction could continue. The cultures were placed in a water-bath which could be kept down to 25–30° C. Several individuals of the sixth generation were isolated with the temperature of the bath at 26° C., going up to 28° C. One of these laid forty-three eggs on September 8th. By September 11th these had developed into hermaphrodites of mature size, but although they lived for several days and were apparently in a quite healthy condition, they never produced mature eggs or spermatozoa. The ovary was distinctly seen with small nuclei, but there was no aggregation of yolk. Changes of this kind occurred in the other cultures.

In addition individuals just ready to lay eggs were isolated from the cultures at the temperature of the room and placed

in a bath at 26–28° C. Under these conditions the ovary continued to produce large-yolked eggs, and at first these were fertilised and laid, but after they had completed a few divisions they became disorganised. With eggs which later passed from the ovary into the uterus fertilisation did not apparently take effect. No egg-shell was formed, and the uterus became full of an amorphous, yolky mass.

It seems, then, that the limits of reproduction lie in Diplogaster manpasi between 19° C. and 25° C., though life may be continued at slightly higher temperatures. It was found impossible, however, to keep cultures at a constant temperature of over 30° C. The individual worms became rigid and after a short exposure died. It is seen that the free-living nematodes are most sensitive to increased temperature in the egg stage, when they can hardly endure high summer heat. The adult is also likely to succumb at temperatures which must be common in tropical countries at least. The encysted larvæ are probably the most resistant stage, and it must be supposed that these animals depend for their existence in periods of exceptional heat to their capacities for survival in this condition.

The Males of Hermaphrodite Species.

(1) Structure and Organisation.

The male sex in Rhabditis and Diplogaster, as in all nematodes, is sharply discriminated by the relation of the vas deferens to the alimentary canal, and by the well-defined secondary sexual characters, including a membranous bursa for adhesion to the female during copulation, and an arrangement of spicules for insertion into the vulva to facilitate the transference of the spermatozoa.

The males of hermaphrodite species occurring in such small numbers, and apparently taking no part in reproduction, might naturally be expected to show some marked signs of degeneracy in organs other than the reproductive system.

In the Cirripedes we have another clear case of the successful establishment of hermaphroditism in a group in which the sexes were originally separate. Here, too, in hermaphrodite species there is a survival of the male sex, but the individuals which represent it are so degenerate in form and structure as to be described as little more than a bag of spermatozoa, and so reduced in size as to well merit the title of "dwarf males."

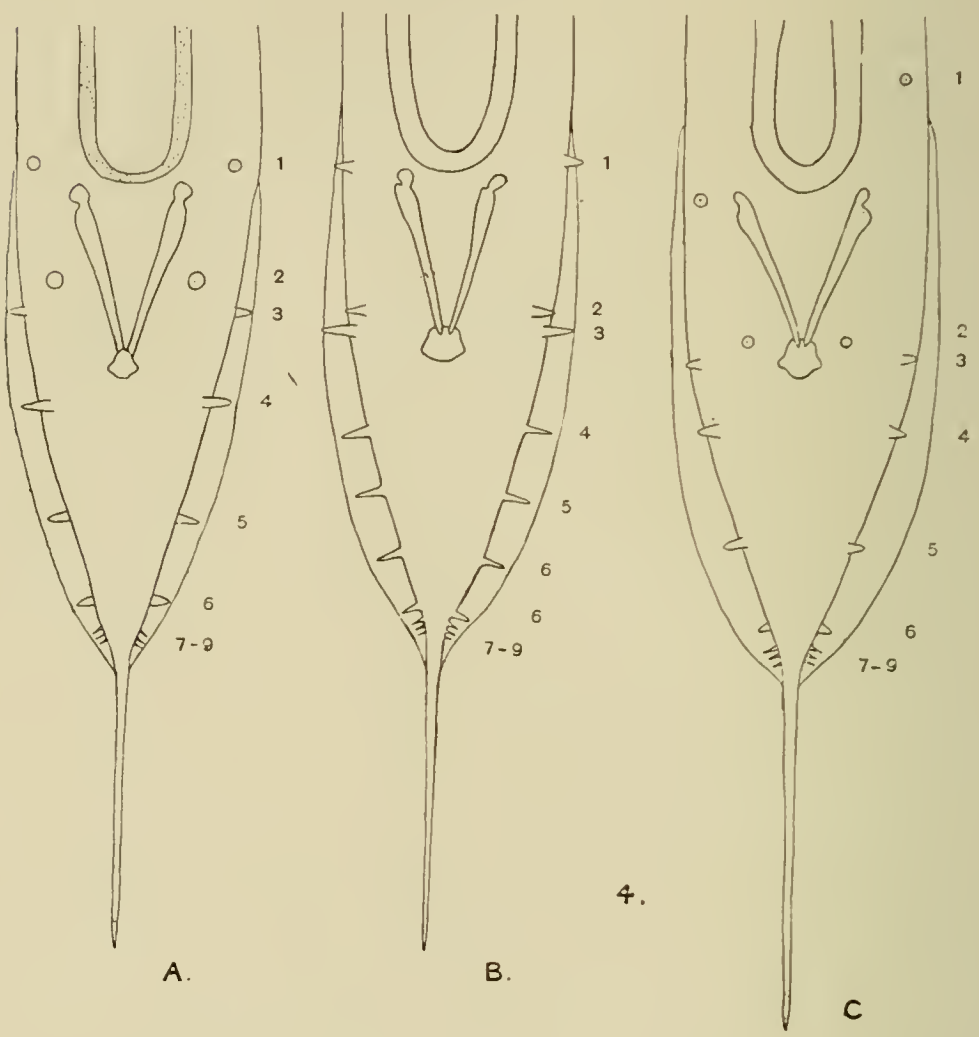
It is, however, a surprising fact that in no particular of structural organisation do the males of hermaphrodite species appear to fall behind those of bisexual nematodes. The conclusions which Maupas reached on this subjects are summed up in the following quotation :

"Ces mâles . . . n'offront rien de particulier et d'anormal. On ne remarque rien dans leur structure et dans leur organisation générale qui puisse les faire considérer comme des animaux mal venus ou mal constitués. Par leur taille, par les proportions de leur corps et par tous les details de leur organisation, ils répondent de tous points au type mâle ordinaire des Rhabditides dioique. Leur testicule luimême est constitué d'une façon absolument normale et, ses produits, les spermatozoïdes, sont palreur forme, leur volume et leur structure absolument identiques a ceux que la glande génitale des femelles produit pendant sa periode d'activité protérandrique."

My own observations show that there is no imperfection of development in the residual males of such species as I was able to study. The spermatozoa were always produced in vast quantities and exactly like those formed by the hermaphrodites. When liberated by pressure from the body of the male, they could be observed to put out amœboid processes like those which Ziegler figures taking up their position in the uterus of Diplogaster longicauda after fertilisation. This observation tends to show that the spermatozoa are physiologically active though the individual which carries them is prevented from playing its part in reproduction, possibly by a defect in nervous organisation.

The experiments of Maupas with Rhabditis elegans showed that on the rare occasions when males do fertilise hermaphrodities, the spermatozoa are perfectly efficacious in the production of embryos. The curious change in the sex-proportions of the offspring of such unions may, however, be

TEXT-FIG. 4.

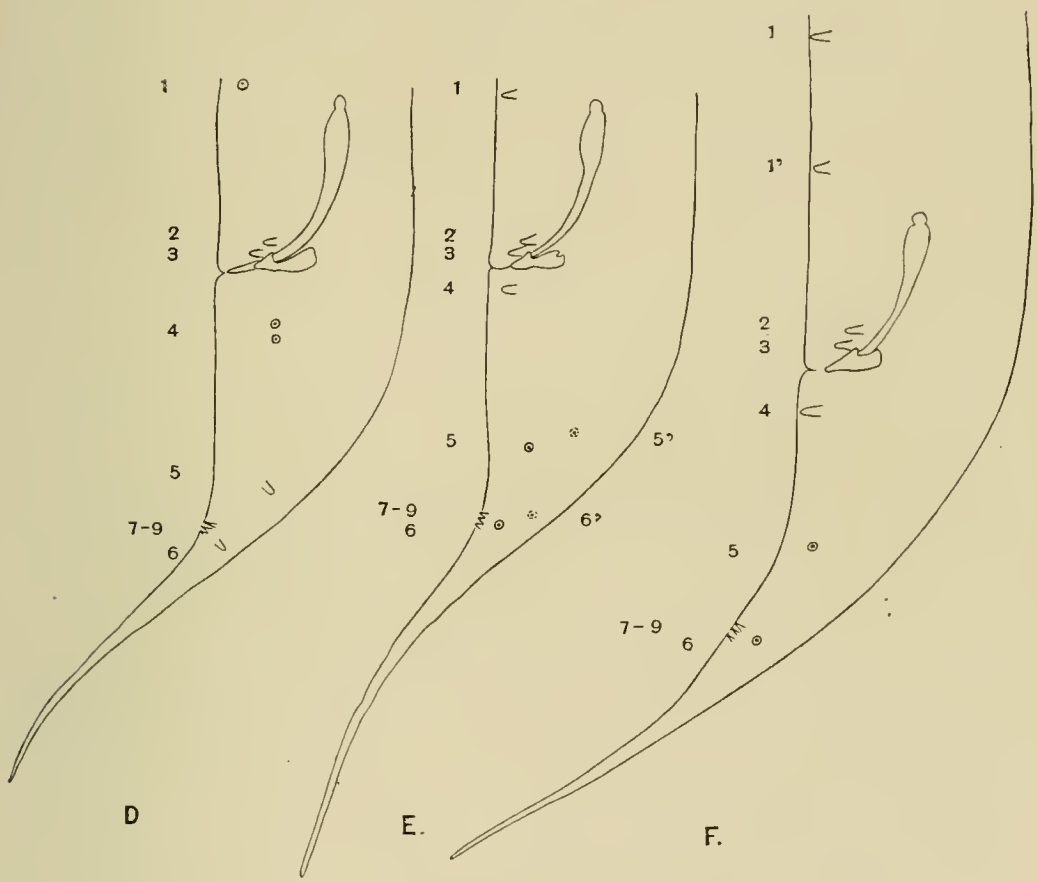


eventually traced back to some essential difference in the spermatozoa of males and hermaphrodites respectively, which might be revealed by a thorough examination of the spermatogenesis in the two cases.

But though there is no manifest imperfection of organisation in the males of hermaphrodite species, they appear to be

sometimes distinguished by extreme variability of the secondary sexual characters. In such specific characters as size and proportions of various parts the males are fairly constant, but the arrangement of the papillæ supporting the copulatory bursa and the shape of the accessory piece of the copulatory spicules show wide differences. When Diplogaster manpasi was first obtained from various

TEXT-FIG. 4.



samples of soil round Sutton Broad, the differences existing between the males found in separate cultures made me conclude that I was dealing with a number of nearly related species. It soon became clear that distinct types of male were not characteristic of each culture, but that even brothers from the same family often exhibited wide differences.

The typical arrangement of the bursal papillæ in Diplo-

gaster maupasi is shown in A, Text-figure 4. Departure from this type was found, however, in almost every other specimen examined. Below are given some of the clearest cases of variation observed in dealing with a comparatively small number (about forty) of males.

(1) There should be normally a pair of papillæ situated exactly opposite the anterior end of the copulatory spicules. One of the most frequent and easily demonstrated variations occurs when one of the pair (or very rarely both) is shifted forward a smaller or greater distance. So marked a case as fig. C was observed two or three times.

(2) A pair of papillæ (4–4′) occurs a short distance posterior to the anus. Only small variations in position were recorded here, but on one occasion a duplication of the papilla of one side was observed (fig. D). (The papilla of the other side was seen on altering the focus, so it was quite evident that the twin papillæ belonged to the same side.)

(3) In the position of papillæ 5 and 6 there is rather frequent variation; they are sometimes nearer together, sometimes further apart. Occasionally it may be seen (when the animal is lying on its back) that the papillæ of the two sides (5, 5′, and 6, 6′) have a tendency to alternate in position (fig. B shows this, but not very well). An example like fig. B was observed once, in which one of the papillæ, either 5 or 6, was duplicated on both sides, and the twin papillæ then shifted apart.

(4) The three small papillæ at the root of the tail (7–9) are rarely replaced by two.

It is only occasionally on examining these animals that a frontal view is obtained, showing the rays of the bursa on both sides. In side views it is often difficult to correctly observe the position of the papillæ. On this account only a few definite cases of variation are referred to above. They were observed in dealing with forty to fifty males.

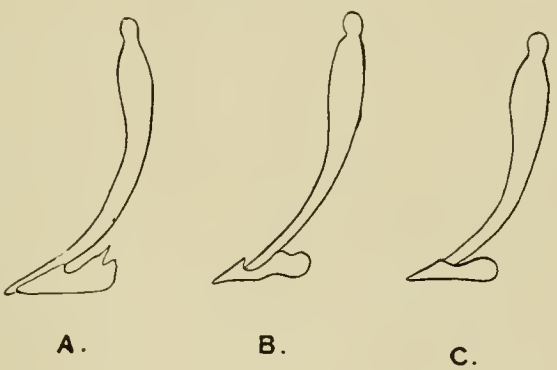
The accessory piece of the spicular apparatus varied in form in nearly every individual. Three types are figured. The first shows the most typical, in the shape of a right-

angled trangle, with an indentation at the anterior angle. In the other two the angles become more and more rounded.

In Rhabditis sechellensis variations in the secondary sexual characters are occasionally found, but are much less numerous than in Diplogaster manpasi. Such variability as was observed was manifested in (1) inequality of the copulatory spicules, and (2) occasional asymmetrical disposition of the rays of the bursa.

The only reference to analogous phenomena which occurs in Maupas' paper is found in his description of Rhabditis guignardi (p. 525). He obtained only two males, but in one of these the copulatory bursa possessed on each side nine

TEXT-FIG. 5.



supporting rays, in the other only seven. In the latter the remaining rays showed a disposition to fuse with each other, a phenomenon, it may be remarked in passing, which was responsible for the asymmetry of the bursal rays in R. sechellensis. The entire disappearance of two rays is a variation as great as any recorded above for Diplogaster manpasi.

The position and number of bursal papillæ or rays is looked upon as clearly diagnostic of species of Rhabditis or Diplogaster, and as far as I know no striking variation has ever been observed in the bisexual species. The connection of such a variability in the males with their disappearance from the economy of the species is no doubt significant, but it is impossible to offer any explanation of the facts.

(2) Proportions of Males in Hermaphrodite Species.

Another remarkable feature of the males of hermaphrodite species studied by Maupas is their extreme rarity. In only one out of eleven species investigated was he unable to find a male; but in others males were only discovered by organising cultures of very considerable size, containing several thousand mature worms. So while in the majority of species the males were less than 0·1 per cent. of the whole number of adults, the proportion of 4 per cent. to which they rise in Rhabditis marionis affords quite a striking contrast. In Diplogaster maupasi, one of the species obtained from the Norfolk Broads, the ratio of male to female is very much more notable than anything which Maupas records, and does occasionally approach, though remotely, that equality of the sexes which is characteristic of the majority of animal forms. In one large culture the males reached 10 per cent. of the whole (377 ♀, 38 ♂ ♂), and in batches of eggs laid by the same individual up to 30 per cent. (16 eggs, 11 ♀, 5 ♂ ♂; 29 eggs, 23 ♀, 6 ♂ ♂). These instances are, of course, specially favourable, and picked from amongst scores of cultures which did not yield a single male. It is very unlikely that a species will be discovered uniformly consisting of equal numbers of males and hermaphrodites. Southern[1] supposed that in Rhabditis brassicæ he had discovered such a species, but in a culture with which he kindly supplied me I have been only able to find males and females, but no hermaphrodites.

To illustrate the manner of occurrence of the males, I give here an analysis of cultures of Diplogaster maupasi carried on over twenty-five generations, from August, 1909, to January, 1910. The whole series of cultures commenced with a single individual. In every subsequent generation at least one hermaphrodite was isolated just before maturity to carry on the succession. When such an individual had commenced to lay eggs it was removed every day to another

[1] Rowland Southern, "On the Anatomy and Life-History of Rhabditis brassicæ n. sp.," 'Journ. Econ. Biol.,' vol iv, 1909, pp. 90–95.

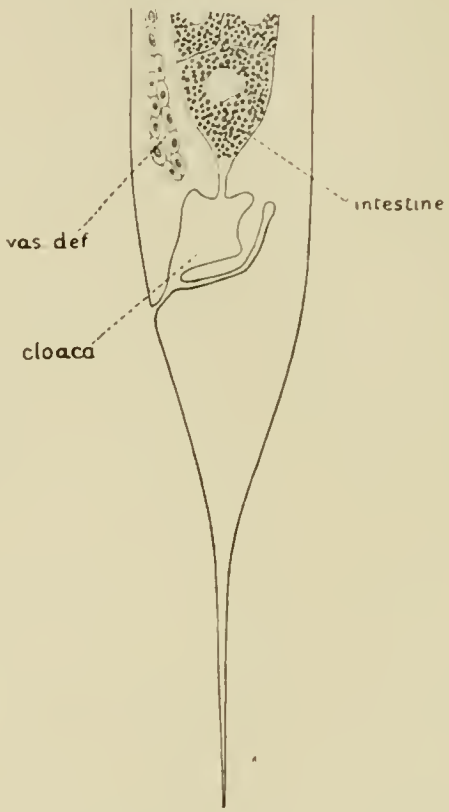
watch-glass, so that the batch of eggs laid during the preceding twenty-four hours was kept isolated. Each batch was carefully counted to compare with the actual number of individuals attaining adolescence, and in this way records of cultures which gave the actual sex-proportions were distinguished from others in which mortality before maturity obscured the true figures. In any drop culture which contained more than about thirty eggs the crowding which ensued was distinctly unfavourable to the chances of survival.

Precautions were adopted in these experiments to prevent absolutely an association of mature males and hermaphrodites, and so remove any suspicion of cross-fertilisation in the line of descent here followed out. To this end the individual destined to give rise to the next generation was separated before any male had become mature, or else the males themselves were removed from the culture before the last moult, when they were perfectly recognisable as males, but had not yet assumed the spicular apparatus necessary for internal fertilisation.

Both sexes become easily distinguishable a considerable time before maturity by the position of the developing gonad and its duct. In the majority of species of Rhabditis and Diplogaster, the vulva opens at the middle of the body of the female, and the gonad is paired, so that the immature hermaphrodite may be recognised by the symmetrical disposition of the clear ovarian rudiments round the middle point of the body. In the male the rudiment of the testis is situated in the posterior half of the body, so that with a little experience it is easy to distinguish a male, even among a ceaselessly twisting mass of other individuals, by the clear transparent testis running alongside the posterior part of the gut. Sperm-formation begins, it is true, before the last moult. But though the body of the male may contain mature spermatozoa, these can only be conveyed to the hermaphrodite individual by the co-operation of the copulatory spicules and bursa. A young male just before the last moult, at which

these latter are developed, is shown in Text-fig. 6. The proximal part of the vas deferens leading into the cloaca does not appear to be yet fully formed. The cloaca is spacious, and is produced on its dorsal surface into a pair of definite pouches in which the chitinous copulatory spicules are formed at the time of the last moult.

TEXT-FIG. 6.



The history of the cultures may be divided into alternating periods, which are distinguished respectively by the frequent occurrence of males and their entire absence. During the first six generations, while these experiments were being prosecuted in Norfolk, the percentage of males was often quite high in batches of twenty or thirty eggs, and the offspring of the majority of individuals contained at least one or two. In addition, the total number of eggs laid by each parent seldom exceeded 130 (150 in one case), and the spermatozoa were not exhausted before death. The seventh and eighth generations were reared away from a laboratory,

under conditions which made careful recording difficult. On removing the cultures to Cambridge a new kind of peptone[1] was used for the preparation of a culture-medium, and the behaviour of the nematodes altered considerably with this change. In five generations, from the ninth to the fourteenth, not a single male was produced. The interval elapsing between the arrivals at maturity of successive generations decreased from seven days to four, and the number of fertile eggs laid by each parent rose to between 150 and 300. In every case the life of the individual was prolonged under these more favourable (?) conditions, the period of fertile production being succeeded by another at least as long, during which sterile eggs were laid.

Later, in the fifteenth generation, the peptone used in Norfolk was again tried, and at once males appeared sparingly in the cultures. Later the individuals raised from certain batches of eggs showed a fairly high ratio (e.g. in the nineteenth generation [23] 19 ♀ 4 ♂♂), but in general males were rarer than in the early cultures of August. After another removal at Christmas, 1909, the second period of male production was terminated like the first. It may well be supposed that the alteration of conditions, slight or otherwise, which ensues on changing the place of experiment was directly responsible for the disappearance of the males.

It is not probable, however, that the proportions are controlled by nutrition, for though at first circumstances seemed to indicate that the use for a culture-medium of white peptone acted as a stimulus to male production, from the fifteenth generation onward four series of cultures were maintained, two in white peptone and two in brown (which is the more favourable medium for growth). As mentioned above, males first appeared in the former medium, but in the seventeenth generation they were also observed in brown peptone, and there was no sufficient difference in the figures to suggest which peptone was the better material for the production of males.

[1] In dark brown crystals completely soluble in water.

In the second table a fuller analysis of the experiments lasting over the first six generations is given. An attempt was made to isolate strains, constantly producing high proportions of males, by breeding from a large number of individuals of the same generation. Thus in the third generation a batch of 44 eggs produced 32 ♀ and 12 ♂ ♂ (about 28 per cent.) did not, with one exception, maintain those high proportions. One, however, though giving at first hermaphrodites only, laid a batch of 16 eggs of which 11 became ♀ and 5 ♂ ♂ (31 per cent.). Nearly all these hermaphrodites were kept for an examination of their progeny, but five individuals, whose records were kept separate, furnished strikingly retrograde results, though males occurred in every case but one. The male ratio was greater in a culture consisting of the offspring of three individuals, reaching 11 per cent. of the whole number. Further selection for the next generation proved equally indecisive.

In the third generation a control series was also established by taking sister individuals from a culture in which only hermaphrodites were represented. The total number of offspring of the five parents selected was 319, of which 302 were ♀ and 17 ♂ ♂. This is exactly comparable to the total of 262 ♀ and 15 ♂ ♂ produced by the five individuals from a culture with 28 per cent. of males. The individual details are closely similar in the two series.

A brief inspection will serve to show how extraordinarily irregular is the distribution of males in the progeny of any single worm. There is no rule that they should appear at stated intervals or restricting their production to a period or periods of maturity, but on the contrary the appearance of a few males from an early batch of eggs may be followed by a succession of hermaphrodites only and vice-versâ; the last eggs may produce males when there have been only hemaphrodites hitherto, or, again, males may occur in several successive batches.

TABLE I.

Note. The figures enclosed in circles represent the number of eggs laid in each batch: those to the right the individuals counted on arrival at maturity or before.

1st Generation. Offspring of a single isolated hermaphrodite. no males observed.

2nd	26 ♀	2 ♂♂.	
3rd	(44)	: 32 ♀	12 ♂♂.
4th	(25)	: 25 ♀.	
	(23)	: 9 ♀.	2 ♂♂.
	(12)	: ——	.
	(23)	: ——	.
	(16)	: 11 ♀.	5 ♂♂.
	(9)	: ——	.
Total	108	: 45 ♀	: 7 ♂♂.
5th	(36)	: 32 ♀	: 4 ♂♂.
	(33)	: 32 ♀	: 1 ♂♂.
	(30)	: 30 ♀.	
	(34)	: 34 ♀.	
	(17)	: 16 ♀	: 1 ♂♂.
Total	150	: 144 ♀	: 6 ♂♂.
6th	(18)	: 12 ♀	: 1 ♂♂.
	(46)	: ——	.
	(25)	: ——	.
	(38)	: ——	.
Total	127		
7th	(18)	: 14 ♀	: 1 ♂♂
	(16)	: ——	.
8th	(7)	: 7 ♀.	
9th	(40)	: ——.	
	(30)	: 21 ♀.	
	(30)	: ——.	
	(74)	: 26 ♀.	
10th	(10)	: 10 ♀.	
	(102)	: 26 ♀.	
	(117)	: 73 ♀.	
	(14)	: 11 ♀.	
Total	243	: 120	

(90)	: 55 ♀	3 ♂♂.
(44)	: ——	.
(10)	: ——	.
(42)	: 29 ♀	1 ♂♂.
(27)	: ——	.
—	: 51 ♀	2 ♂♂.
—	: 26 ♀.	
(35)	:	
(80)	: 31 ♀.	
(48)	: 37 ♀.	
(12)	: 12 ♀.	
175	: 80 ♀	

These first six generations were bred in the Sutton Broad Laboratory, Norfolk. For the first generation an infusion of Beef was used. Afterwards two or three varieties of Peptone (Dry, Albumen, Witte's) supplied by Harrington Bros., all of which had substantially the same value as a food stuff.

The 10th–19th generations were bred in the Zoological Laboratory at Cambridge.

TABLE I (Cont°)

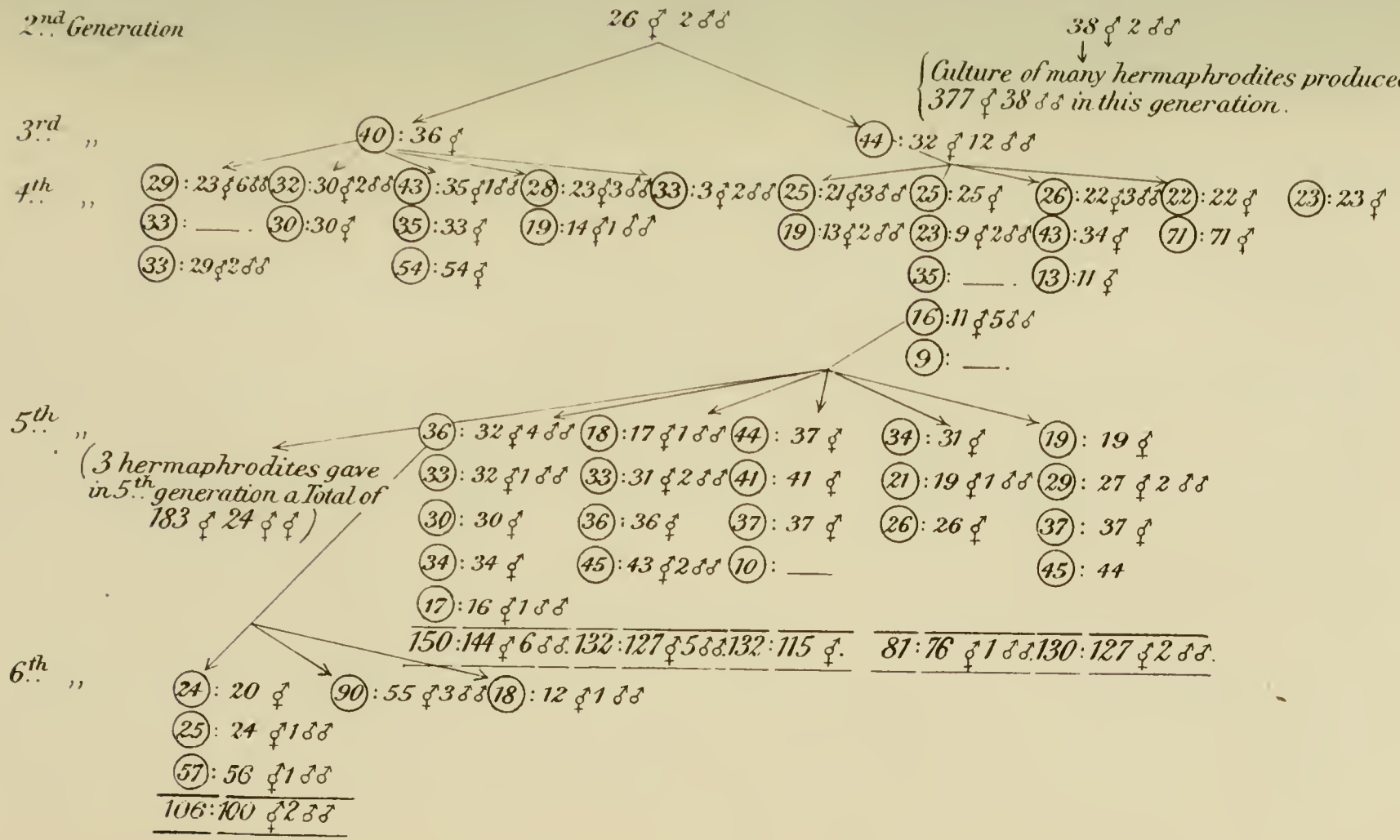
11th Generation.	(37)	:	35 ♂	(26)	:	19 ♂	
	(60)	:	25 ♂	(39)	:	25 ♂	
	(65)	:	—	(48)	:	48 ♂	
	(20)	:	—	(55)	:	—	
	182	:	60 ♂	168	:	92 ♂	
12th ,,	(18)	:	18 ♂	(14)	:	14 ♂	
	(56)	:	46 ♂	(35)	:	10 ♂	
	(38)	:	38 ♂	(29)	:	17 ♂	
	(66)	:	47 ♂	(50)	:	—	
	(68)	:	—	(65)	:	27 ♂	
	246	:	149 ♂	193	:	68 ♂	
13th ,,	(40)	:	28 ♂	(18)	:	18 ♂	
	(65)	:	11 ♂	(50)	:	6 ♂	
	(24)	:	8 ♂	(26)	:	16 ♂	
	(56)	:	9 ♂	(49)	:	40 ♂	
	(65)	:	12 ♂	(110)	:	75 ♂	
	250	:	68 ♂	(4)	:	4 ♂	
14th ,,	(14)	:	14 ♂	257	:	159 ♂	
	(25)	:	6 ♂	(28)	:	28 ♂	
	(32)	:	31 ♂ 1 ♂♂	(32)	:	12 ♂	
	(36)	:	36 ♂	(35)	:	28 ♂	
	(34)	:	—	(40)	:	39 ♂	
	(2)	:	2 ♂	(41)	:	34 ♂	
	143	:	89 ♂ 1 ♂♂	(16)	:	12 ♂	
				192	:	153 ♂	

	Brown Peptone.			White Peptone.			Brown Peptone.			White Peptone.
15th gen.	(42)	:	32 ♂	(27)	:	20 ♂				
	(26)	:	26 ♂	(21)	:	20 ♂	(20)	:	20 ♂	The one selected here was apparently entirely sterile.
	(72)	:	40 ♂	(45)	:	30 ♂	(18)	:	18 ♂	
	(92)	:	70 ♂	(40)	:	31 ♂	(50)	:	50 ♂	
	(31)	:	31 ♂	(44)	:	36 ♂ 1 ♂♂	(81)	:	52 ♂	
	(22)	:	22 ♂	(41)	:	36 ♂	(30)	:	30 ♂	
	285	:	221 ♂	(24)	:	24 ♂	(32)	:	30 ♂	
				242	:	197 ♂ 1 ♂♂	231	:	200 ♂	

TABLE I (Contd)

	Brown Peptone.	White Peptone.	Brown Peptone.	White Peptone.
16th Generation.	(26) : 24 ♀♂	(38) : 35 ♀ 3 ♂♂	(24) : 24 ♀♂	(13) : 6 ♀♂
	(94) : 22 ♀♂	(38) : 8 ♀♂	(100) : 42 ♀♂	(49) : —.
	(62) : 54 ♀♂	(34) : 19 ♀ 1 ♂♂	(39) : —	(41) : 13 ♀♂
	(41) : 36 ♀♂	(39) : 39 ♀♂	(67) : 35 ♀♂	(56) : 56 ♀♂
	(26) : 26 ♀♂	(29) : 29 ♀♂	(27) : 19 ♀♂	(38) : 38 ♀♂
	249 : 162 ♀♂	(56) :	(41) : 2 ♀♂	(20) : 20 ♀♂
		234 : 130 ♀ 4 ♂♂	292 : 122 ♀♂	217 : 133 ♀♂
17th ,,	(24) : 24 ♀♂	(38) : —. —	: 33 ♀♂	(52) : —.
	(55) : 34 ♀♂	(64) : 51 ♀ —	: 20 ♀♂	(54) : —.
	(60) : 35 ♀♂	(50) : 50 ♀♂ —	: 31 ♀♂	(39) : 39 ♀♂
	(48) : —.	(46) : —. —	: 26 ♀♂	(15) : —.
	187 : 93 ♀♂	(29) : —. —	: 15 ♀ 3 ♂♂	(2) : 1 ♀♂
		(27) : 23 ♀♂	: 125 ♀ 3 ♂♂	162 : 40 ♀♂
		254 : 124 ♀♂		
18th ,,	(39) : 36 ♀♂	(33) : 33 ♀♂	(30) : 24 ♀♂	(25) : 23 ♀♂ 2 ♂♂
	(20) : 20 ♀♂	(16) : 12 ♀♂	(19) : 17 ♀♂	(15) : —.
	(26) : 17 ♀♂	(11) : 11 ♀♂	(96) : 40 ♀♂	(2) : —.
	(52) : 28 ♀ 1 ♂♂	(25) : —	(30) : 14 1 ♂♂	42 : 23 ♀♂ 2 ♂♂
	(13) : 11 ♀♂	(29) : 28 ♀ 1 ♂♂	(2) : 2 ♀♂	
	150 : 112 ♀ 1 ♂♂	(12) : 12 ♀♂	177 : 97 ♀♂ 1 ♂♂	
		126 : 96 ♀♂ 1 ♂♂		
19th ,,	(24) : 19 ♀♂	(26) : 26 ♀♂	(17) : 13 ♀♂	(23) : 19 ♀♂ 4 ♂♂
	(22) : 10 ♀♂	(15) : 15 ♀♂		(8) : 8 ♀♂
	(16) : 16 ♀♂	(11) : —.		(24) : 23 ♀♂ 1 ♂♂
	(16) : 8 ♀♂	(38) : 19 ♀♂		(76) : 74 ♀♂
	(58) : 51 ♀♂	(88) : 88 ♀♂		(14) : 13 ♀♂ 1 ♂♂
	136 : 104 ♀♂	(21) : 21 ♀♂		145 : 137 ♀♂ 6 ♂♂
		199 : 169 ♀♂		

TABLE 2.


2nd Generation
26 ♀ 2 ♂♂
38 ♀ 2 ♂♂
Culture of many hermaphrodites produced 377 ♀ 38 ♂♂ in this generation.
3rd ,,
40 : 36 ♀
44 : 32 ♀ 12 ♂♂
4th ,,
29 : 23 ♀ 6 ♂♂
32 : 30 ♀ 2 ♂♂
43 : 35 ♀ 1 ♂♂
28 : 23 ♀ 3 ♂♂
33 : 3 ♀ 2 ♂♂
25 : 21 ♀ 3 ♂♂
25 : 25 ♀
26 : 22 ♀ 3 ♂♂
22 : 22 ♀
23 : 23 ♀
33 : ——
30 : 30 ♀
35 : 33 ♀
19 : 14 ♀ 1 ♂♂
19 : 13 ♀ 2 ♂♂
23 : 9 ♀ 2 ♂♂
43 : 34 ♀
71 : 71 ♀
33 : 29 ♀ 2 ♂♂
54 : 54 ♀
35 : ——
13 : 11 ♀
16 : 11 ♀ 5 ♂♂
9 : ——
5th ,,
(3 hermaphrodites gave in 5th generation a Total of 183 ♀ 24 ♀♀)
36 : 32 ♀ 4 ♂♂
18 : 17 ♀ 1 ♂♂
44 : 37 ♀
34 : 31 ♀
19 : 19 ♀
33 : 32 ♀ 1 ♂♂
33 : 31 ♀ 2 ♂♂
41 : 41 ♀
21 : 19 ♀ 1 ♂♂
29 : 27 ♀ 2 ♂♂
30 : 30 ♀
36 : 36 ♀
37 : 37 ♀
26 : 26 ♀
37 : 37 ♀
34 : 34 ♀
45 : 43 ♀ 2 ♂♂
10 : ——
45 : 44
17 : 16 ♀ 1 ♂♂
150 : 144 ♀ 6 ♂♂
132 : 127 ♀ 5 ♂♂
132 : 115 ♀.
81 : 76 ♀ 1 ♂♂
130 : 127 ♀ 2 ♂♂.
6th ,,
24 : 20 ♀
90 : 55 ♀ 3 ♂♂
18 : 12 ♀ 1 ♂♂
25 : 24 ♀ 1 ♂♂
57 : 56 ♀ 1 ♂♂
106 : 100 ♀ 2 ♂♂

Sexual Instincts of the Males.

Maupas' conclusion that the residual males could not take any part in the production of offspring is expressed in the following words: "Mais si ces animaux examinés dans leur structure et leur morphologie, représentent des mâles vrais et complets, il n'en plus de même lorsqu'on les étudie au point de vue de leurs facultés et de leur activité sexuelles . . . ces mâles ont a peu près totalement perdu tout instinct et tout appétit sexuels. . . . Nous trouvons en présence d'une decadence psychique non concomitante avec une regression morphologique."

This conclusion is supported by the inert behaviour of the males, the fact that they are never seen in copulation with hermaphrodites, but principally by the results of a fairly full series of experiments which Maupas made with males and hermaphrodites which had exhausted their own stock of spermatozoa. These conclusively showed that the males have almost, but not quite, lost their sexual instinct. One species alone stands as an exception. In Rhabditis marionis at various times cultures containing in the aggregate 28 hermaphrodites and 42 males were kept under observation. Since all the spermatozoa of the hermaphrodites were exhausted, any production of developing eggs was plainly due to the intervention of the male, and thus a measure of the activity of this sex was obtained. Fertile eggs were laid by 13 individuals to the total number of 150–200, and all these produced hermaphrodites. This species is one of those for which Maupas described a partially developed hermaphroditism, and the author himself regarded it as specially significant that in such a form the male should be less degenerate.

The most complete series of experiments was made with Rhabditis elegans. Here, in twelve cultures, a total of 139 hermaphrodites with their own sperm exhausted and males were associated. Only six of the hermaphrodites were actually fertilised, a proportion which illustrates exceedingly

well the sexual inactivity of the males. The chief point of interest lies in the constitution of the offspring of these six individuals. The young produced numbered 274, and of these 147 were hermaphrodites and 127 males. So numerical equality of the sexes is secured in this species by cross-fertilisation, a result in striking contrast to that obtained when R. marionis was the subject of investigation. No permanent effect was produced on the heredity of sex, for when 38 of the hermaphrodites obtained by fertilisation by males were employed as parents for the next generation, 2964 individuals were produced, of which only 7 were males, but the rest hermaphrodites.

Further evidence of the psychical decadence of the males was secured in other species. Though nearly 100 males were employed belonging to five species only a single successful case of re-fecundation was observed, and in this (Rhabditis duthiersi) the fertilised eggs gave 70 hermaphrodites and 1 male.

The Hermaphrodites in Hermaphrodite Species.

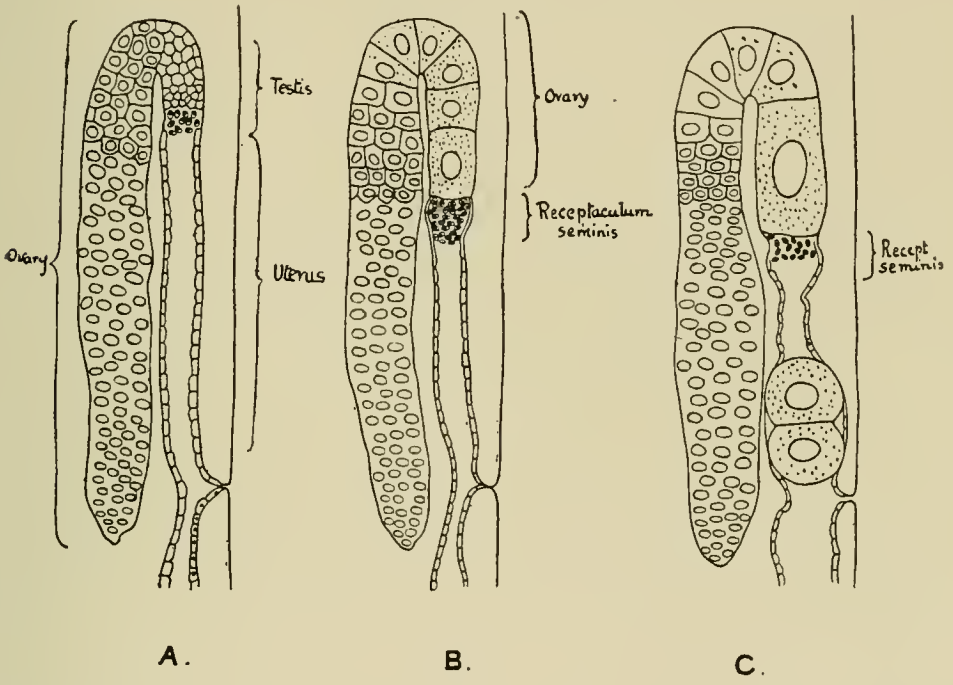
(1) The Hermaphrodite Glands in Rhabditis and Diplogaster.

In Rhabditis sechellensis the structure and development of the reproductive glands exactly correspond to the description which Maupas gives of R. elegans and R. dolichura. Though no new details can be given, it will be convenient to summarise the changes which the hermaphrodite gland goes through before oviposition commences in any of the above three species. The three diagrams which illustrate the description are partly after my own drawings for R. sechellensis, but closely follow Maupas' sketches of R. dolichura in Plate XXI, figs. 7A, 7B, and C.

The hermaphrodite organ is double, its two divisions being of equal development, and joining at the short and indefinite common vagina. Each division is U-shaped, and consists of a uterus, which extends from the vagina to within a short

distance of the bend of the tube, and an ovo-testis, occupying the proximal part of the ventral limb and the whole of the dorsal limb. In individuals examined some hours before the first egg is laid the whole of the ovo-testis appears to consist of cellular elements of nearly equal size, which possess definite boundaries near the bend, but merge into a syncytium distally. The anterior testicular region is indicated by the more regular polygonal form of a comparatively narrow belt of spermatocytes which succeed the uterus. The young egg-

TEXT-FIG. 7.



cells which come next are all of small size, and can hardly be distinguished from the male cells. Text-fig. 7, A represents a stage where the testis has begun to function, and several spermatozoa have been formed in the anterior part of the testis.

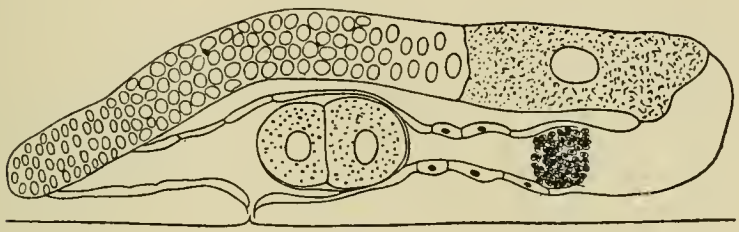
In the second stage (B) sperm formation is in full activity, or may even be completed by the conversion of all the spermatocytes into spermatozoa. The female part of the gland now begins to show functional activity by the growth of the oöcytes most anteriorly situated. The width of the

gonadial tube is so small in comparison with the size of the egg that the growing oöcytes are arranged in a linear series. The oöcyte nearest maturity is just posterior to the sperm-forming region, and behind it is a line of developing egg-cells showing the stages of growth from the scarcely differentiated oögonia. The spermatozoa as fully formed are small circular discs, capable of amœboid movements when effecting fertilisation. They remain in the region of the gland where they were formed, so that what was testis in the first stage becomes receptaculum seminis in the second. In its formation, since the spermatozoa occupy a much smaller bulk than the spermatocytes, the receptaculum seminis shortens considerably; its epithelium is of course the investing layer of the testis. The spermatozoa are now so disposed that the ripe ovum can pass out of the ovary and through the receptaculum seminis without its motion being impeded. During its passage a single spermatozoan fuses with the egg-cell and brings about fertilisation. The fertilised egg immediately becomes enveloped by a cuticular shell, and lies for some time in the uterus undergoing segmentation before it is finally ejected to the exterior by the pressure of eggs from behind (Text-fig. 7, c.). The formation of ripe eggs after the first is perfectly regular, and fertilisation occurs in every case. Since, then, the whole quantity of spermatozoa is formed before the first egg is ready for fertilisation, it follows that a limit is set to the number of fertile eggs it is possible to produce, and as a matter of fact this limit is reached at a comparatively early point in maturity. When the receptaculum seminis is completely emptied of its spermatozoa eggs still continue to be laid at a uniform rate, though they never develop to larvæ.

In Diplogaster maupasi (Text-fig. 8) events follow a very similar course. There is, indeed, one difference in detail during the early periods of egg-laying which may be briefly mentioned. The proximal limb of the gonad is shorter, the distal longer than usual. The former is entirely occupied by the uterus and testicular region, and the ovary is confined to

the distal limb. Possibly in accordance with this shortening there is no linear succession of eggs increasing regularly in size in the anterior part of the gland, but each egg grows and reaches its full size before the one next in order begins to differentiate itself in size from the other oögonia. After an egg has passed out of the ovary and been fertilised, a period of some length elapses before the next finishes its growth in the ovary and travels through the receptaculum in its turn. It is only in the early stages, however, that oviposition is a slow process, for as the period of maturity advances, the zone of egg-maturation increases in length, and oögonia are able to start their growth long before the ovum in front is

Text-fig. 8.



ready to be fertilised. The deliberate character of egg-production in D. maupasi is responsible for the fact that few individuals are seen with more than a single pair of eggs contained in their uteri.

Rhabditis gurneyi.—When this species was first examined large numbers of adult individuals were obtained from cultures of decaying flesh. Amongst these a few were seen which, judging by their size, had only just attained maturity, but whose uteri and vaginæ were occupied by disorganised eggs, as in hermaphrodites, which have exhausted their stock of spermatozoa. It was at first supposed that this was such a species as Rhabditis marionis (cf. Maupas, p. 512), in which a small number of females producing eggs only occur together with the hermaphrodites. When, however, young immature worms were isolated, they were often

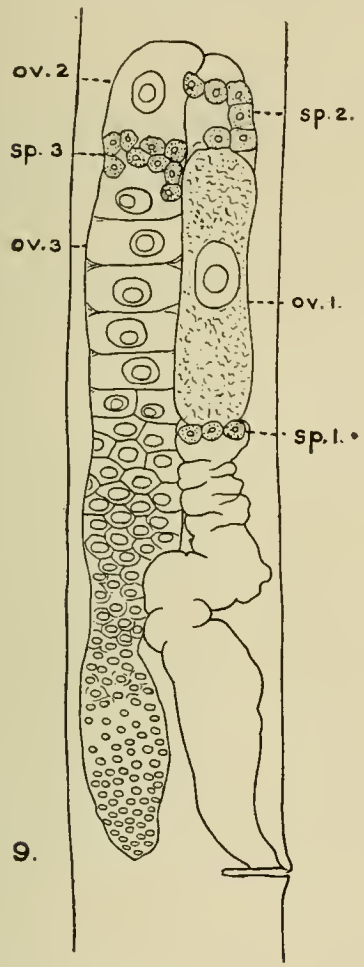
seen to a stage, sometimes extending over several days, during which eggs passed into the uterus and degenerated. Later, however, the amorphous egg material was expelled and its place taken by fertile eggs which continued to be produced in large numbers. In this species, one could easily see, the hermaphroditism was not protandrous, but the formation of spermatozoa was sometimes delayed till a number of eggs had ripened. In some cases, it is true, fertile eggs are produced from the first onset of maturity, and at first sight there is nothing to distinguish such forms from the typical protandrous hermaphrodite found in other species. But beside such an introductory period of infertility, there may be later interruptions of egg-production, which indicate a failure of the stock of spermatozoa. Frequently this is but temporary, and the worm begins again to lay fertile eggs. So short sometimes is the duration of sterility that it is indicated only by the ejection of one or two disorganised eggs, and very often only one gonad contains a supply of spermatozoa while they are lacking in the other.

It is, then, suggested by the culture observations, and fully borne out by examination of the glands under high powers of the microscope, that eggs and spermatozoa come to maturity more or less alternately throughout the period of reproductive activity.

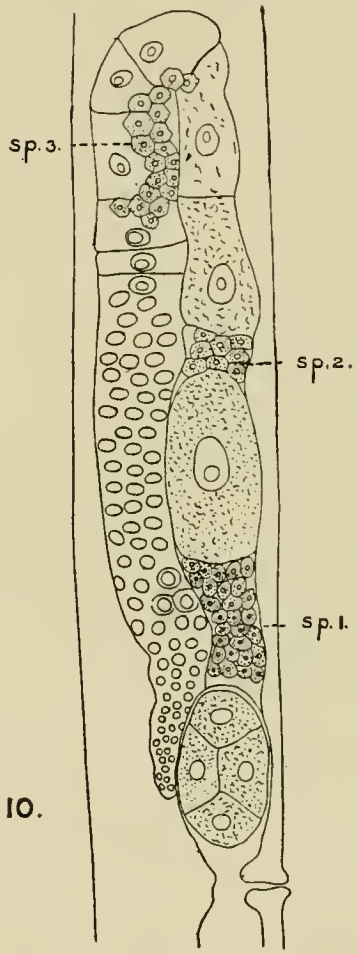
Structure of the Gland.—In the general form of the reproductive glands of R. gurneyi there is no departure from that described above for other species of the genus. At various periods of development the arrangement of the histological elements differs rather widely from the typical protandrous gland. Text-fig. 9 shows part of the reproductive organ of a hermaphrodite which has just attained maturity. It will be seen that reproductive activity commenced with the formation of a very small number of spermatozoa (*sp.*[1]). And after the maturation of a single egg (*ov.*[1]) a more numerous succession of spermatozoa (*sp.*[2] and *sp.*[3]) was produced, only briefly interrupted by the appearance of another single egg (*ov.*[2]) which has not yet reached the limit of its

growth. After this, a prolonged period of egg-formation appears likely, for posterior to the spermatozoa there is a single row of developing egg-cells (*ov.*[3]) gradually diminishing in size and quantity of yolk, till in the middle of the limb the ovary becomes an undifferentiated syncytium. In this gonad

TEXT-FIG. 9.



TEXT-FIG. 10.



we have at one time the evidences of three alternations of male and female activity within a very limited period.

In the second individual figured (Text-fig. 10) maturity is rather further advanced. The results of the early activity of the gonad are large numbers of spermatozoa and a few eggs. A series of developing ova now promise a long period of female productivity. There is an interesting departure

from the appearance of developing sperm-cells and egg-cells in successive belts, for here cells lying side by side may give rise respectively to spermatozoa and eggs. In one case the sperm-cells seem to have been actually formed at the expense of the ovum. The early maturation of the spermatozoa will be noticed here, which terminates while young egg-cells forming from a mother-cell of the same age have only completed the first stages of their growth.

(2) The Fertility of the Soil-nematodes.

The hermaphrodite species of Rhabditis and Diplogaster are distinguished from the bi-sexual, as Maupas points out, by their lesser fertility, a character which indicates the incompleteness of the hermaphroditism. In eleven of the twelve species investigated by Maupas the number of fertile eggs laid by a single hermaphrodite individual varied between 200 and 250, while in the twelfth (Rhabditis guignardi) the limit of production rose to 500 or 520. Maupas states that the female of a bi-sexual species is, on the other hand, capable of laying 700 to 800 fertile eggs. The low fertility of the hermaphrodites is due to the insufficiency of the supply of spermatozoa, for if to the number of fertilised eggs be added that of the unfertilised eggs laid when the male gametes are exhausted, it may be seen that a hermaphrodite produces as many eggs as the female in a bi-sexual species. Individuals producing 200–250 fertilised eggs will afterwards lay two or three times as many unfertilised,[1] so that the total equals the figure given for the bi-sexual species.

Fertility, then, in these hermaphrodites is entirely controlled by sperm-production, and probably the actual number of spermatozoa formed in an individual is given or very closely indicated by counting the eggs laid which develop into larvæ. In these experiments the eggs laid by each parent were counted every twenty-four hours from the beginning of maturity onwards, and the mother then removed to a fresh drop of peptone. Usually after about six days of active ovi-

[1] Maupas. loc. cit., p. 587.

position the spermatozoa become exhausted, but it is difficult to observe exactly when the limit has been reached, because the first laid unfertilised eggs undergo a kind of incipient parthogenetic development. Such eggs possess a shell like fertilised eggs and they complete a few divisions, but the blastomeres are more regular and equal than in normal segmentation; the egg-substance appears greatly shrunk, so that a wide space occurs between it and the egg-shell.

An examination of the table of descent of Diplogaster maupasi will show how widely the fertility varies in a single species even under apparently uniform conditions. A few entries may be specially quoted here for comparison, each pair of individuals being taken from the same generation of nearly related strains and supplied with the same nourishment:

(1) 12th generation October 20th–25th, 257 eggs.
12th generation October 18th–22nd, 153 eggs.

(2) 14th generation October 25th–31st, 143 eggs.
15th generation November 1st–5th, 285 eggs.
In this case a parent with low fertility gave in the next generation exceptionally prolific offspring.

(3) 14th generation October 25th–31st, 192 eggs.
15th generation November 1st–6th, 229 eggs.

Other cases fall within the wide limits indicated above, so that it may be concluded that under favourable conditions a hermaphrodite individual of D. maupasi will lay 140–290 eggs. It is not pretended that such figures as these prove that it is impossible to select strains characterised by high and low fertility respectively, but as far as my observations go, there is a fluctuating variability, not governed by the laws of descent nor always directly traceable to minor changes in the environment.

The influence of external conditions is, however, very great, and especially is this the case with nutrition. In peptone solutions of every kind, the number of eggs laid depends upon the development of bacteria in the culture-medium. When the peptone is fairly sterile the nematode only lays eggs at long intervals, and eventually dies when only a score

or so of eggs have been expelled from the uterus. In such a case of course the diminution in fertility is due to the small amount of nourishment supplied to the ovary, which is only enabled to produce a limited number of eggs. When a cloudy film of bacteria is seen at the bottom of the culture-drop the conditions are exceptionally favourable for the growth of the nematodes, and fertile eggs are laid rapidly till the spermatozoa are exhausted. If, instead of peptone, a saturated solution of gelatin be used as a culture-medium, a very different effect is produced. For the first day or so after a worm is moved from a peptone solution into gelatin the rate of egg-production is fairly maintained, but afterwards it sinks very low indeed, though the life of the parent and the period of fertility is much longer than that of individuals in peptone. Thus, for instance, for two hermaphrodites of the same generation bred in peptone but kept during maturity in peptone and gelatin respectively, the following figures were obtained:

(1) Peptone.	(2) Gelatin.
Sept. 2nd–4th, 28 eggs.	Sept. 2nd–4th, 19 eggs.
,, 4th–5th, 32 ,,	,, 4th–15th, 17 ,,
,, 5th–6th, 21 ,,	
,, 6th–7th, 20 ,,	
Total for 5 days 101 eggs	Total for 13 days 36 eggs

When a second generation of Diplogaster maupasi is raised in gelatin, when about twenty fertile eggs have been produced the uterus contains sterile disorganised ova. It appears from this that the effect of the substitution of gelatin as a foodstuff is not merely to curtail the formation of eggs in the ovary, but also to very considerably limit the number of spermatozoa produced.

Though under favourable conditions the average fertility varies between two and three hundred in the majority of species now known, there are undoubtedly some which normally produce a very much smaller number of offspring. In the summer of 1907 I had under observation a species of

Rhabditis from the neighbourhood of Cambridge which I cannot adequately describe from the notes taken at the time It was remarkable for the very small proportion of fertilised eggs laid by each individual. In one family six hermaphrodites were selected before maturity, and their fertility compared. In each case the separate numbers represent the eggs laid in a day, and those in brackets the total of fertile eggs :

A 7, 8, 1, 1, 7=[24] B 9, 10, 4, 4=[27] C 9, 9, 3, 2=[22]
D 7, 4, 12, 8, 2=[33] E 9, 7, 7, 5, 3=[31] F 7, 6, 1, 1=[15]

These cultures were carried on in July. Others, began later in August, gave rather higher numbers, e.g. :

A 1, 7, 13 (and 2 unfertilised eggs), 12, 1=[34]
B 14, 3, 8, 6, 5=[36]
C 15, 17, 11=[43]
D 8, 10, 8, 5, 2=[33]

In A of this second series it will be noticed that the succession of fertilised eggs was interrupted temporarily, but whether this was due to a retarded production of spermatozoa, as in Rhabditis gurneyi, or to some other cause, was not discovered. It is much to be regretted that no trustworthy observations on the occurrence of males were made, for a species like this in which the hermaphroditism is of such an apparently recent and inefficient type, should, according to Maupas' conclusions, possess a very large proportion of males, which was not, however, observed. It is hoped that the species may be rediscovered and this point investigated again.

Rhabditis coronata Cobb, which was investigated by Maupas (pp. 537–541) and shown to be a protandrous hermaphrodite, is probably a similar form with very low fertility. No figures are given of the total of eggs laid, but it is mentioned that an isolated hermaphrodite only laid six eggs in twenty-four hours, and that in general eggs were laid very slowly. An interesting feature shown in Maupas' drawing of the species (Pl. XXI, fig. 8) is the small size of the ovarian part of the gland, which might well account for a restricted

egg-production. In the Cambridge species of Rhabditis, on the other hand, the early sterility was certainly due to the extremely small number of spermatozoa. The length of the ovary was proportionately as great as in other species of the genus.

Rhabditis gurneyi, in contrast to the two species last discussed, is a free-living hermaphrodite nematode which has departed from the protandrous hermaphroditism, which we regard as the earliest development from the bisexual state. In consequence it far surpasses others of its kind in fertility. The spermatozoa are of unusual size, and possibly because of the difficulty of providing sufficient space to store a sufficient number at once, they are produced alternately with eggs throughout a great part of the period of reproductive activity. As a result of this adaptation each individual is capable of laying as many eggs as a bisexual female, which frequently has its supply of spermatozoa replenished by copulation.

It must be remembered that in many cases the hermaphrodites of this species only produce unfertilised eggs in the initial period of oviposition which represent a total loss to the organism. When once this critical period has been passed, and a sufficient supply of spermatozoa established, fertile eggs are produced at the rate of 60–80 each day, or distinctly faster than in the case of Diplogaster maupasi and others.

For figures to illustrate the fertility of Rhabditis gurneyi the following case is given. From the offspring of a single individual six immature hermaphrodites were selected. When maturity was reached the eggs laid every twenty-four hours were counted, and the parent removed to a fresh culture drop in the manner described above for Diplogaster maupasi. The dates in each case mark the period over which oviposition continued.

(A) September 6th–17th, 525 fertile eggs.

(B) „ 7th–17th, 686 „

The figures here are not complete, for the culture dried up while the parent was still laying fertile eggs. When 343 had

been produced a prolonged failure of spermatozoa, lasting twenty hours, occurred in one of the glands, so that 16 unfertilised eggs were laid with egg-shell, and the uterus beside blocked by disorganised egg material, while the other produced 40 fertilised eggs. After this interval developing eggs were counted to the number of 300.

(C) September 7th–12th, 168 fertile eggs.
(D) „ 9th–20th, 730 „
(E) „ 7th–17th, 362 „
(F) „ 7th–10th, 81 „

Out of the six individuals two laid about 700 eggs each, and though the figures obtained from the others show a high variability, this is partly to be explained by the very marked influence which even a slightly unfavourable change in the conditions can exert on sperm production. In cultures where several individuals are crowded together, it is noticeable that very few eggs are laid, and that the uterus of the worms speedily becomes crammed with disorganised eggs, showing that the sterility is caused by the failure of the male, not the female gametes.

In conclusion, it must be stated that the hermaphrodite species are apparently as successful as the bisexual species in the struggle for existence, for they are found in equal, or sometimes in greater abundance in nature. Evidently, though the means of dispersal of the species is limited by their generally low fertility, an advantage which more than counterbalances is secured by the self-fertilising capabilities of each individual.

(3) Partial Hermaphroditism.

It is here proposed to examine the description of certain species which are said to form a genuine link between the bisexual and hermaphrodite species. The species which Maupas deals with are Rhabditis marionis, R. duthiersi, and R. viguieri.

(1) R. marionis.—A single hermaphrodite kept under observation was found to lay only 129 fertile eggs, while

other individuals of the same species produced about 250 before their spermatozoa became exhausted. A closer examination of a similar hermaphrodite led to the discovery that spermatozoa were only produced in one genital gland; from the other only unfertilised eggs were traced. In half its reproductive system the animal was hermaphrodite, in the other female. A few individuals were also noticed in which both genital glands apparently gave rise to eggs alone and never sperm. The species is thus constituted of—(1) pure females (occurring very rarely); (2) individuals with one ovary and one ovo-testis; and (3) full hermaphrodites forming the majority of the society. No mention is made of any variation in fertility among this latter class, but we are led to believe that all individuals fall into one or other of three sharply marked categories, according to the condition of their gonads. In the light of the results recorded above for other species this seems so remarkable that I think this case should if possible be re-examined.

(2) Rhabditis dnthiersi.—Three hermaphrodites were observed, each producing fertilised and sterile eggs simultaneously, and it is suggested that these were possibly semi-hermaphrodites of the type described as occurring in R. marionis. It may, however, be pointed out that in R. gurneyi individuals are found with a similar appearance when the formation of spermatozoa is retarded and does not commence simultaneously on the two sides.

(3) Rhabditis vignieri.—In this species the proportion of males was the largest met with by Maupas (though falling far short of some of the records for Diplogaster maupasi). Males formed 4 per cent. to 5 per cent. of the total in large cultures, and it is almost certain that the proportion would have been larger if single individuals had been selected for cultures.

Of the other individuals some were females, which, when isolated, never produced offspring, but when united with males laid fertile eggs. The larvæ from such unions, it is to be regretted, were not kept. Hermaphrodite forms were in

a substantial majority, and it may be useful to quote Maupas' words as to the relative frequency of the three kinds of forms : " Les females non-hermaphrodites mais simplement unisexuées sont également très fréquentes. Il ne suffisait, en effet, de placer sous le microscope une dizaine de femelles prises au hasard pour en recontrer une ou deux unisexuées. Les femelles simplement unisexuées y sont même plus nombreuses que les mâles qui les fécondent sans difficulté. En résumé, chez cette espèce les mâles encore relativement nombreux paraissent avoir conservé leur instinct sexual intact."

It is evident that this species, could it be re-discovered, would form a most interesting subject of study. A precise investigation of the comparative frequency of females and hermaphrodites, and in particular of the relative effects of self- and cross-fertilisation on the sexual constitution of the offspring, would prove of the utmost value.[1]

(4) The Nature of Hermaphroditism in the Nematoda.

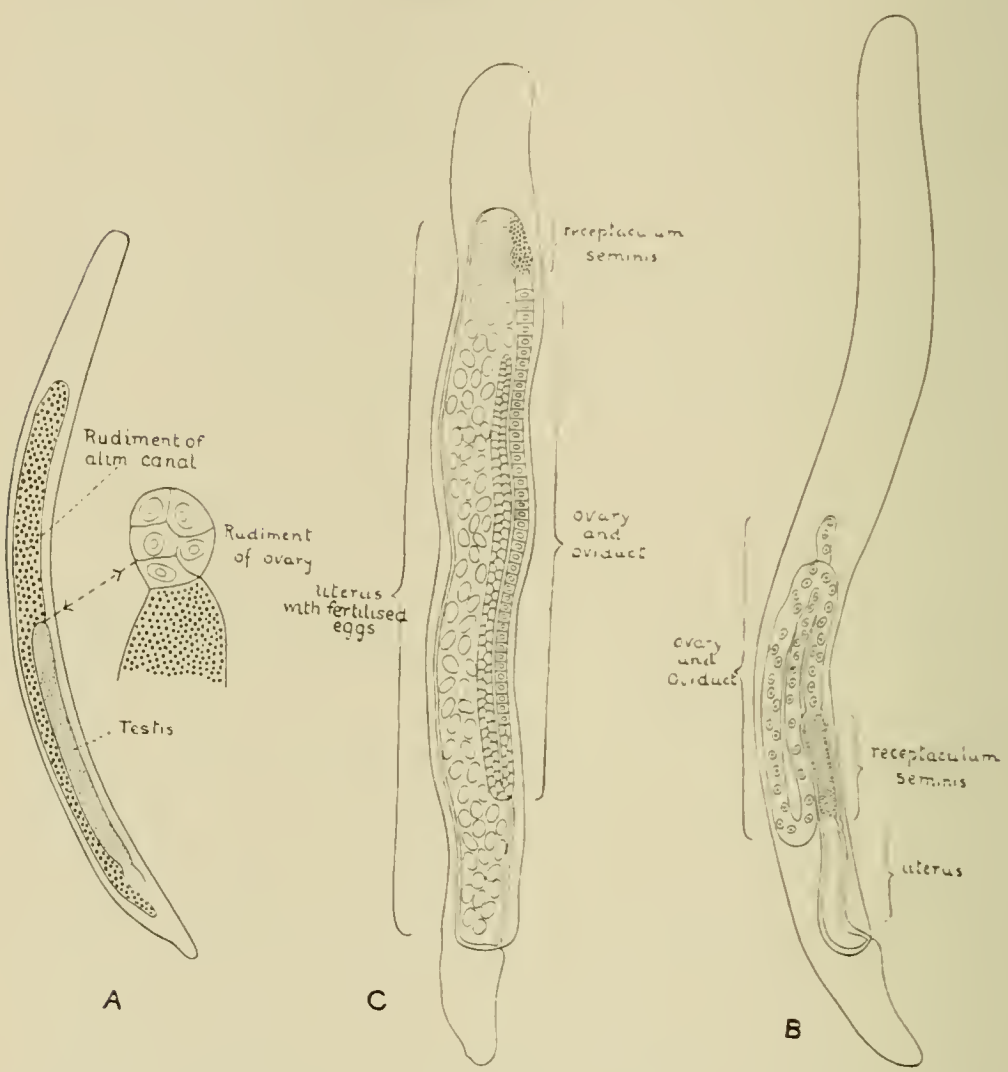
The evidence that the hermaphrodites described by Maupas and myself represent the females of bisexual species, in which a part of the gonad has been given over to the formation of spermatozoa, is, indeed, overwhelmingly strong. Hermaphrodites and females are identical in general anatomy, and the arrangement and form of their gonads are strikingly similar. Then, too, there exist a series of species showing the development of hermaphroditism from small beginnings in species where the ratio of fertilised eggs to unfertilised is very small, until in Rhabditis gurneyi the number of spermatozoa is almost equal to that of the eggs they are required to fertilise. Lastly, there are, apparently, species like Rhabditis viguieri which have not yet decided between bisexuality and hermaphroditism, and present an assemblage of pure

[1] In Diplogaster maupasi, though careful watch was kept only one hermaphrodite was found which failed to develop spermatozoa (see Table I, fifteenth generation).

females, males and hermaphrodites, in cultures probably derived from nearly related individuals.

Similarly, the various species may be arranged in gradation, to show the suppression of the male sex. In Diplogaster maupasi the males occur occasionally in such proportions as

TEXT-FIG. 11.



to recall their original numerical equality with the female sex. But this species, in the majority of cultures and most others, at all times produces males in exceedingly small numbers. Finally, in Rhabditis gurneyi the male has possibly entirely disappeared, though of this it is difficult to adduce positive proof.

There are, however, some indications that it is not the female alone which is capable of developing a hermaphrodite gonad. In Rhabditis elegans Maupas records (pp. 491–492, Pl. XVII, fig. 2) the occurrence of large egg-like cells in the testis. A similar phenomenon has frequently been recorded as characteristic of the normal male gonad in Crustacea (Orchestia), and in other Crustacea the appearance of eggs in the testis, without doubt to be attributed to the indirect action of parasites, is so definitely associated with the development of female secondary sexual characters as to indicate a change to hermaphroditism. In Rhabditis elegans the phenomenon is very slightly manifested, but there are indications that a very much more complete change is imposed on the male of Bradynema rigidum, a nematode parasitic in the body-cavity of the beetle Aphodius fimetarius.[1] This animal is so adapted for its parasitic life that mouth and anus have disappeared, and the alimentary canal, in the larva represented only by a single column of cells, has left not the slightest trace in the adult. In the autumn immense numbers of larvæ (up to ·51 mm. in length) are found in company with one or two adults in each host. These larvæ may be divided equally into females, whose genital glands, paired and situated in the middle of the body, have only attained to a rudimentary development, and males (Text-fig. 11, A), in which the testis, situated posteriorly in the body, often contains mature spermatozoa. When in this stage the larvæ bore through the walls of the alimentary canal and disappear. No intermediates are known between these forms and the adults 3–5½ mm. in length, with a single exception to be described later. In the adult condition there is only one class of individual with a long and vastly convoluted gonad opening to the exterior in the very posterior position which is occupied by the anus, in nematodes with a functional alimentary canal. It is this circumstance which led zur Strassen to derive the adults from the male larvæ, for if they are developed from female larvæ there must have occurred a

[1] Zur Strassen, 'Zeitschr. f. wiss. Zool.,' t. 54, 1892, pp. 656–747.

shifting of the gonad during growth from a median to a posterior situation, and the conversion of a double rudiment into a single mature organ. In the most advanced male larvæ the gonad is completely occupied by a brownish mass of spermatozoa save for an apical cluster of indifferent cells (inset to Text-fig. 11, A), and zur Strassen supposes that when the larvæ begin to grow rapidly these cells proliferate and form an ovary. In a single example of ·75 mm. length (Text-fig. 11, B) the testis was represented by a receptaculum seminis full of spermatozoa, and this was succeeded by an ovary still slightly developed and only posteriorly situated. In the adult (Text-fig. 11, C) the growth in size of the gonad has been so enormous that the whole of the body-cavity is occupied by it. The ovary and oviduct together form a narrow tube running twice the length of the body. Then succeed the receptaculum seminis, and lastly, the uterus, with a diameter nearly equal to that of the animal itself, runs from near the anterior end to the genital aperture. The great difference between this and the intermediate stage has been effected by the growth of the uterus with the fertilisation of the eggs.

Though in the absence of other intermediate forms it is impossible to produce clear proof that events take their course as indicated above, yet it is probable that the female sex, though represented by larvæ, disappear without functioning, while in the males, after the spermatozoa have been formed, ova are produced in large quantities by the residual cells of the gonad. The evidence for the derivation of hermaphroditism in Rhabditis and Diplogaster from the female, and in Bradynema from the male, is in both cases of essentially the same nature, and depends on—

(1) The recognition both in the original sex and the hermaphrodite derived from it, of a constant pattern of reproductive organ.

(2) The discovery that the gonad of one sex is capable of developing the gametes of the other sex.

If zur Strassen's explanation is accepted, then in the limits

of the Nematoda it is found that now the female, now the male, carries the characters of the other sex in a latent state, and when these are wakened to activity secondary hermaphroditism is developed. In Mendelian terminology either sex may be heterozygous. Moreover if the cytological phenomenon described by Maupas (p. 491) for Rhabditis elegans really shows that the male in that species is heterozygous, we are then forced to the hypothesis that both sexes are heterozygous in one and the same species, and at the same time. The phenomena of cytology and heredity as at present known in other groups, e.g. the Insecta, are capable of such diverse interpretations that it is impossible to say whether such a case as this suggested above is anomalous or no.

(5) Self-fertilisation in Animals.

Among hermaphrodite animals authentic cases of self-fertilisation are by no means common. In the Trematoda the rule of cross-fertilisation may occasionally be departed from, but only possibly in cases where the spermatozoa discharged into the body-cavity of the host find their way back into the female aperture of the same individual. Very little is known about the methods of fertilisation in the Cestoda. The evidence for self-fertilisation rests upon two observations, one by Leuckart of a penis inserted in the vagina of the same proglottis, and the other by Pagenstecher of similar relations between penis and vagina of adjacent proglottides.[1]

In the Mollusca it is easier to prove by the isolation of individuals the possibility of reproduction without cross-

[1] In the Rhabdocœl Turbellaria self-fertilisation is a very widely spread phenomenon and often the usual method of reproduction. Its existence has been put beyond doubt by the observations of individuals raised from the egg, but such experiments have not apparently been continued over several consecutive generations. In some forms the penis effects self-impregnation, in others there is no copulatory organ or female aperture and the spermatozoa migrate through the body tissue to the ovary (see Bresslau, 'Verh. deutsch. zool. Gesell.,' 1903, p. 126, and especially 'Sekera Zool. Anz.,' Bd. xxx, 1906, pp. 142–153). It must be noticed that in the three chief cases, the Turbellaria, the Nematoda,

fertilisation. A. H. Cooke quotes two cases in the Cambridge Natural History, volume "Mollusca." In both Arion ater and Linnæa auricularia, individuals isolated from birth produced fertile spawn, although in somewhat limited quantities.

In the Annelids a case has recently been described by Pierantoni[1] in Protodrilus. Ova are developed in the anterior segments, spermatozoa in the posterior, and a large proportion of the former are fertilised while still in the body-cavity. There is, however, a second method of reproduction, when by the rupture of the body-wall of the hermaphrodite the whole number of the eggs is discharged into the sea. At the same time certain male individuals commonly occurring in the species emit their spermatozoa, which unite with such eggs of the hermaphrodite as have escaped self-fertilisation.

In the Crustacea hermaphroditism is largely developed in two groups, the Isopoda and the Cirripedia. In the former, the production of the spermatozoa in each individual precedes that of the ova, and the absorption of surplus spermatozoa by phagocytes may preclude the possibility of self-fertilisation (e.g. Danalia[2]). In the cirripedes adjacent individuals normally cross-fertilise; a single case of self-fertilisation was recorded in Pollicipes (Gruvel). In the curious parasitic group, the Rhizocephala, both Sacculina and Peltogaster, invariably practise self-fertilisation.[3]

Great interest attaches to the restriction of sperm-production accompanying the condition in this group. A small part and the Rhizocephala, the self-fertilisation which they practise is evidently a secondary and adaptive phenomenon. In the first two cases it has been developed as a means by which the actual existence of the race may be safeguarded, for both classes of creatures are liable to sudden extinction by the desiccation of the pool or moist soil, where they respectively live, and it is a manifest necessity that an isolated survivor should be capable of independent reproduction when conditions again become favourable.

[1] 'Fauna u. Flora Golfes von Neapel,' t. 31, "Protodrilus," 1908, pp. 117–119.

[2] G. W. Smith, 'Fauna u. Flora Golfes Neapel,' Mon. 29, "Rhizocephala," 1906, p. 101.

[3] G. W. Smith, loc. cit., pp. 21–24.

of the testis only is used for the formation of spermatozoa, and to prevent squandering of the slender stock the maturation of the spermatozoa is completed punctually just after a brood of eggs enters the mantle-cavity.

Both the Rhizocephala and the Nematoda, the two best cases of self-fertilisation, show one advantage obtained by the animal which adopts this method of reproduction, and that is the need for a reduced number of spermatozoa. In Sacculina the economy has been effected by a special change, to be looked upon in the light of an adaptation, but in Rhabditis and Diplogaster, as we have seen, the small and markedly insufficient quantity of spermatozoa shows a recent entrance into the hermaphrodite condition, and only because every spermatozoon fertilises an egg do these forms succeed in maintaining themselves.

In the Tunicata, a group in which hermaphroditism has established itself completely, the ova ripen before the spermatozoa, and cross-fertilisation appears to be general. In Ciona ripe ova and spermatozoa are found in the ducts at the same time, and Castle[1] found that if the products from the same individual are mixed, as a rule fertilisation did not occur. This result is so significant that it is not surprising that the experiment should have been repeated. Morgan[2] found some variation in the degree of self-sterility, but generally endorsed Castle's results. In experiments which I carried out at Naples on the same tunicate in the early part of 1906 (and in which every care was taken to avoid contamination with foreign sperm), the eggs of an individual were found to be as fertile with their own spermatozoa as with those of other individuals, yielding in both cases nearly 100 per cent. of embryos. The pathological development which Castle found characteristic of self-fertilised embryos did not occur in my experiments. In conclusion, it seems possible

[1] Castle, W. E., "The Early Embryology of Ciona intestinalis," 'Bull. Mus. Comp. Zool.,' xxvii. 1896.

[2] Morgan, T. H., 'Journ. Exp. Zool.,' i, 1904, p. 137. 'Biol. Bull.,' viii. 1905.

that the American form of Ciona intestinalis differs markedly, at least in its physiology, from the Mediterranean type species, and that, as is illustrated in plants, species which differ but little from each other in external appearance may be respectively easily capable of self-fertilisation and entirely restricted to cross-fertilisation.

The free-living nematodes easily lend themselves to an investigation of the effects of continued self-fertilisation. Maupas organised cultures for this purpose, taking great care that the eight hermaphrodites chosen in each generation as the parents of the next should in no case have come into contact with mature males. With Rhabditis elegans, the period of experiment lasted from the beginning of December to the end of June, and in these seven months fifty-two consecutive generations were reared. During the whole of this time no decline in vigour or productivity could be ascribed to the continuance of self-fertilisation. It is true that immediately afterwards the race became extinct owing to the onset of sterility, but the cause of this may well be traced to a sudden rise of temperature in the month of June (Maupas, p. 493). That this is the true explanation is indicated by the fact that Rhabditis duthiersi, another hermaphrodite species, which had only been isolated from the possibility of cross-fertilisation for a few weeks, became sterile at exactly the same time when its cultures were subjected to the same conditions.

In my own researches Diplogaster maupasi has existed in cultures with no possibility of a cross through twenty-five generations, and that with not the slightest deterioration of the strain. It is hoped that under temperature conditions more equable than those of Maupas' laboratory at Algiers it will be possible to prove that self-fertilisation may continue through a longer period and larger number of generations than was the case in R. elegans.[1]

[1] The cultures have now (June 21st. 1910) been carried over forty-six generations without cross-fertilisation with no observable diminution in fertility.

Summary of Results.

In the preliminary summary on page 436, a short statement is given of Maupas' results alone. In the present paper these are completely confirmed where the material allowed, and in some of the following details the study of hermaphroditism in Rhabditis and Diplogaster has been pursued further.

(1) In one hermaphrodite species, Diplogaster manpasi, the residual males are much more numerous than in any other yet studied, and in small cultures may reach 30 per cent. of the whole number of individuals.

(2) The male secondary sexual chararacters, i.e. bursal papillæ and accessory copulatory spicule, show great variability.

(3) The production of males is cyclical, periods (each lasting a few generations) when males are frequent alternating with others in which only hermaphrodites are produced.

(4) Attempts to affect the sex-ratio artificially proved unsuccessful. It was also found impossible to increase the proportion of males by selection from favourable cultures. No rule could be discovered governing the constant fluctuations of production.

(5) Even when males were most common there was no tendency to find female or partially hermaphrodite individuals, and the males were sexually inactive. This contrasts with the conclusions reached by Maupas on Rhabditis.

(6) The number of fertile eggs laid by D. manpasi is subject to wide variation.

(7) In Rhabditis gurneyi a far greater number of fertile eggs may be produced by single individuals than in any other hermaphrodite species. The fertility is probably as great as the average bisexual species.

(8) The formation of spermatozoa is not confined to the anterior end of the gonad as in other species, but may occur in any part and at any time throughout maturity. Frequently a number of sterile eggs were laid at the onset of maturity owing to the retarded production of the spermatozoa.

(9) No males have been observed in this species, so that they are either excessively rare or extinct. R. gurneyi, then, represents a much more complete and sufficient type of hermaphroditism than has hitherto been recorded in the free-living nematodes.

(10) Self-fertilisation has formed the exclusive means of propagation throughout twenty-five[1] generations of Diplogaster manpasi without any deterioration in the character of the stock.

[1] Now forty-six. (See note on preceding page.)

Observations on Trypanoplasma congeri.

Part I.—The Division of the Active Form.

By

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With Plate 21, and 1 text-figure.

Contents.

General Introduction.

In the 'Zoologischer Anzeiger,' Bd. xxxv, Nos. 14 and 15, Mr. Elmhirst and I published a short note on a trypanoplasma parasitic in the stomach of the conger eel (Conger niger). Up to the present, as far as I am aware, no satisfactory account has been given of the division of any trypanoplasma, and the only point in connection with this process, on which previous workers have been unanimous, seems to be the extreme rarity of dividing forms. By what I must now regard as rather a fortunate accident, the second conger which I chanced to examine was so heavily infected

that frequently two dividing forms have been found in the same field. As such infections, however, seem extremely rare, I have decided to publish my observations on the division of the active form of Trypanoplasma congeri at once, reserving the notes we have at present made on the changes into the resting form for a later paper. In the later paper we hope also to deal more fully with the general literature of the group. I shall only mention in this paper the previous accounts of intestinal trypanoplasma, and, in a later section of the paper, I shall refer to the descriptions of division given for this genus by Keysselitz and Friedrich. The first description of an intestinal trypanoplasma was given by Lèger in 1905 for a form, Trypanoplasma intestinalis, which he found in the stomach of Box boops.

The second intestinal trypanoplasma, hitherto described Trypanoplasma ventriculi, was found by Keysselitz in the stomach of Cyclopterus lumpus, and is figured on p. 37 of his paper on Generations- und Wirtswechsel von Trypanoplasma borreli.

I do not propose to enter into any details as to the conditions under which Trypanoplasma congeri occurs in this paper, as these notes will be reserved for our later paper. The active form of the parasite is, however, always found in sections of the conger's stomach in the mucus lining the surface of the wall, and it never seems to spread into the deep glandular pits. Up to the present no sign of the active trypanoplasma has been found in any part of the intestine or rectum, and, in fact, if active trypanoplasma are mounted in the intestinal juice they almost immediately become agglomerated by their posterior extremities, and have disappeared entirely at the end of a couple of hours. Up to the present forty-seven congers have been examined, and of these only ten have been found to be infected. The parasite has been found in small numbers in some congers in which the stomach and intestine were full of food, but the only really heavy infections have been obtained from fasting congers.

I should like to take this opportunity of thanking Mr. Elmhirst, the director of the Marine Station at Millport, for his assistance in getting material, and Miss Robertson for help in the drawing of the figures.

Methods.

The stages figured in this paper were all obtained on wet smears from the stomach wall, fixed either in Flemming or corrosive acetic. Both of these methods gave excellent results. The films were stained in Giemsa, Twort, iron-hæmatoxylin and eosin, and Mayer's acid hæmalum and eosin. All these stains gave satisfactory results, but the figures were all drawn from preparations made either with hæmalum and eosin, or iron-hæmatoxylin and eosin.

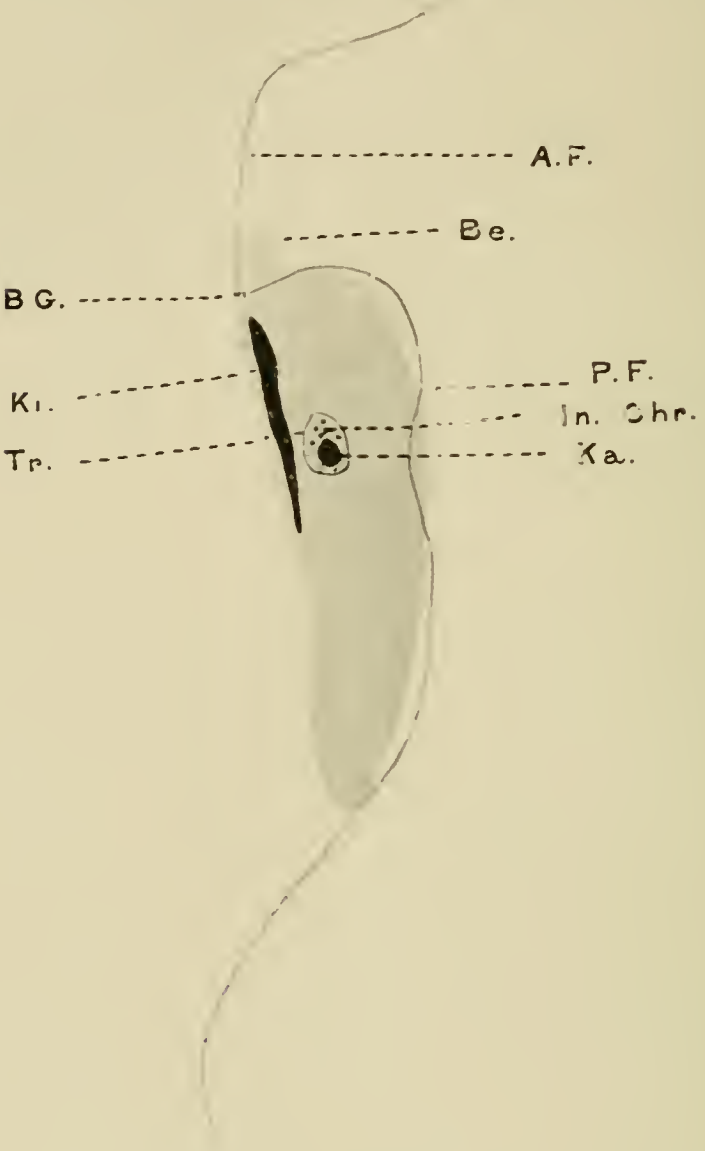
Morphology of the Active Form.

As there seems to be a certain amount of discrepancy amongst different authors in regard to the nomenclature of the various structures in trypanosomes and trypanoplasmas, I have indicated in the following diagram the nomenclature I have decided to adopt. It is practically that used by Minchin in his paper on the structure of Trypanosoma lewisi in relation to microscopical technique ('Quart. Journ. Micros. Sci.,' vol. 53, 1909, p. 799).

The normal active Trypanoplasma congeri has rather an elongate body, measuring roughly 18 μ by 2·7 μ. The two flagella arise apparently from a single basal granule near the anterior end of the kinetonucleus; the anterior flagellum passes up the mobile beak to end freely, while the posterior flagellum passes transversely across the body of the animal, and running down in connection with the narrow undulating membrane, projects freely for a distance of about 10 μ beyond the animal's posterior end. As regards the basal granule, most previous observers seem to have been of the opinion that each flagellum in Trypanoplasma arises from a

separate basal granule, although it is evident that they do not regard the matter as absolutely certain, e.g. Minchin, in his

TEXT-FIG. 1.—Active form of Trypanoplasma congeri.



A. f. Anterior flagellum. *Be.* Beak. *B. g.* Basal granule. *In. chr.* Intra-nuclear chromatin. *Ka.* Karyosome. *Ki.* Kinetonucleus. *P. f.* Posterior flagellum. *Tr.* Trophonucleus.

paper on the blood-parasites of fish, remarks as regards Trypanoplasma keysselitzi, p. 28, "In front of the

kinetonucleus are situated the two minute blepharoplasts, from which the flagella arise. I believe them to be always two in number, but in iron-hæmatoxylin preparations they are so minute and often so close together that it is impossible to resolve them as two granules, and they may appear as a single dot."

In the active Trypanoplasma congeri the two flagella always appear to me to arise from a single basal granule, and from what I have seen of the dividing and resting forms I am certain that if the flagella do not arise from a single basal granule, the connection between the two granules must be so intimate that the flagella always behave as though they arose from a single point. Passing down the side of the animal under the membrane a row of very faintly staining rounded granules are frequently seen; these may correspond to the structures described in Trypanophis, or possibly to the far more strongly staining granules seen in some forms of Trichomonas. The trophonucleus in the elongate form of Trypanoplasma congeri lies about one third of the animal's length from the anterior end, and usually consists of a conspicuous membrane containing a darkly staining elliptical karyosome, which is usually surmounted at its anterior end by a cap of chromatin granules. In some cases, however, the karyosome is central and the granules are arranged round it. These appearances recall Schaudinn's figure of the chromosomes in the resting nucleus of Trypanomorpha and Leger's description of the chromosomes of Trypanoplasma intestinalis. It will, however, I think, become abundantly clear from the behaviour of the dividing trophonucleus described below that it is impossible to regard the chromatin granules of Trypanoplasma congeri as chromosomes.

The kinetonucleus is usually a very darkly staining carrot-shaped structure lying laterally near the animal's anterior end, the narrow posterior end of the kinetonucleus passing down the animal's body to end in the region of the trophonucleus. In some cases the kinetonucleus presents an almost

segmented appearance, and apparently this appearance has in many cases been taken as an early indication of division, though I believe this interpretation to be erroneous.

Division.

All of the preparations here figured are taken from films of the stomach of a fasting conger which had been kept in the tanks at Millport for four months, and was killed at 5.30 p.m., November 27th. In the early stage of division (Pl. 21, fig. 2) the body of the animal becomes slightly shorter and thicker. The basal granule of the flagella divides, and this is followed by a splitting, first of the anterior flagellum along its whole length, and then of the posterior flagellum with its membrane. The trophonucleus and its contained karyosome become larger, and I believe that the intra-nuclear chromatin granules (? the "chromosomes" of Schaudinn) at this stage become condensed on to the karyosome. The kinetonucleus at this stage becomes slightly thicker, but shows no distinct indication of division. In the next stage (Pl. 21, fig. 3) the flagella have split along their whole length, and it is important to note that, in marked distinction to the state of affairs found by Friedrich in Trypanoplasma helicis, I have never been able to find the slightest evidence of the growth of new flagella in any stage of division. The trophonucleus now assumes a spindle shape, and the karyosome divides; the two halves, however, remaining connected by a rod, which persists until a very late stage of division. It might have been expected that some sign of the so-called chromosomes would be found at this stage lying around the dumb-bell-shaped karyosome in the spindle-shaped nucleus, but no trace of them has been detected. It is, of course, possible that this may be due to faulty technique, but so many of these dividing stages have been found lying near resting forms with nuclei clearly showing these granules that I believe this hypothesis is untenable. The relation of the axis of the trophonucleus

spindle to the longitudinal axis of the animal's body seems in these early stages to be rather variable, but in the later stages the long axis of the spindle seems always to be arranged in direction transverse to the animal's original longitudinal axis. The kinetonucleus now becomes very much enlarged, and gradually (Pl. 21, figs. 4–7) pushes out a posterior limb, which comes to lie at right angles across the dumb-bell-shaped trophonucleus. This relation seems very characteristic of this stage of division, which is a very common one on these films. It is rather interesting to note that the stages of division up to this point in the films from this particular conger are very common, the latter stages being comparatively rare. As these films were taken from various points all over the surface of the stomach, this would seem to point either to a cyclical epidemic of division in this parasite or (a view which seems to me rather more improbable) to an extremely short duration for the later as compared with the earlier stages of division. The basal granules have now moved some distance apart, and as the animal shortens and thickens the membranes and flagella become shifted round till in the later stages they pass down the opposite sides of the body. The trophonucleus now is completely dumb-bell shaped, the handle of the dumb-bell being formed by the strand connecting the two karyosomes. In its early stages the dividing trophonucleus has presented a very superficial resemblance, in outline, at any rate, to the mitotic spindles found in the metazoan cell, but in the succeeding stages, in which the new trophonuclei have become definitely rounded, and their connection is limited to the bar joining the two karyosomes, this resemblance is completely lost. In Pl. 21, fig. 8, a late stage of division is figured in which the two products of division are still connected with each other by a narrowing band of protoplasm, through which, even at this stage, the kinetonuclei and trophonuclei are still connected. In Pl. 21, fig. 9, a form is shown which has evidently just divided. It is characterised firstly by its small size and rounded shape, secondly by the length of the kinetonucleus, and thirdly by the remains of

the strand of the karyosome which had connected the two trophonuclei, and which has not yet been withdrawn.

Finally, the kinetonucleus becomes shortened and denser, the last remains of the karyosome strand are absorbed, and the animal elongates and regains its normal aspect.

Conclusions.

I have thought that it might be of some interest to compare shortly the above account of division of Trypanoplasma congeri with that given by previous workers for other species of Trypanoplasma. As far as I am aware, the only accounts of division in a trypanoplasma hitherto published are those by Keysselitz, in his paper, "Generations- und Wirtswechsel im Trypanoplasma borreli" (1906), and by Friedrich, in his paper, "Über Bau und Naturgeschichte der Trypanoplasma helicis" (1909). Keysselitz gives on page 28 of his paper five figures of dividing active forms from the blood of the fish, i.e. figs. 12, 14, 22, 23, 24. From these figures it would appear that the process of division in Trypanoplasma congeri shows some difference from that of Trypanoplasma borreli, though, as his series of division seems far from complete, it is quite possible that these differences may be more apparent than real.

(1) As regards the behaviour of the flagella, Keysselitz seems inclined to believe that one of the products of the division keeps the old flagella, and that the other at a comparatively late stage grows out new flagella.

(2) In T. borreli, according to Keysselitz, the trophonucleus divides, showing an internal division centre derived from the karyosome and eight chromosomes, at a stage at which there is no sign of division in the flagella, blepharoplast, or cell body.

(3) The kinetonucleus is said to divide transversely.

The difficult feature in this account of division seems to me the extraordinary amount of variability in the time factor for all these processes; in fact, Keysselitz himself states on page 31: "Den Verlauf der Teilung habe ich bisher in allen

seinen einzelnen Phasen im Leben nicht verfolgen können. Wie ich schon oben angegeben habe, trifft man relativ selten sich vermehrende Individuen an. Vorzugsweise sind es Tiere, bei denen die Teilung des chromatischen Apparates und des Plasmas, sowie die Bildung der lokomotorischen Organellen bereits beendet sind und die nur noch mit ihrer hinteren Enden zusammenhangen, eine Phase, die zeitlich längste im Laufe der Teilung zu sein scheint." It is particularly over this last point, however, that a great deal of caution should be exercised. In well-infected smears it is an exceedingly common occurrence to find two trypanoplasma lying in a position which suggests division, but unless there is some absolutely distinctive feature, e.g. as regards the structure of the nuclei, which can be definitely connected with a corresponding structure in an undoubted dividing form, I feel that it is always most hazardous to interpret these appearances as division stages. On the other hand, the differences between the division of Trypanoplasma helicis, as described by Friedrich, and that of Trypanoplasma congeri, seem to be of an absolutely fundamental character. In the first place the karyosome, which is so characteristic a feature of the trophonucleus of most trypanoplasmas, is entirely absent in Trypanoplasma helicis, and in correlation with this fact the division of the trophonucleus appears to consist in a simple constriction of the large vacuolar trophonucleus with its scattered chromatin granules (p. 387). The division of the kinetonucleus is said to be longitudinal (p. 385), but the figures of this process seem hardly convincing. The behaviour of the flagella, again, seems to be very complicated, since it is said on p. 390: "Nachem die für die neue Zelle notwendigen Teile entwickelt sind oder der Anlage nach vorhanden sind, rücken die Kerne und Blepharoplasten anseinander." "Dasselbe geschicht mit den Geisselursprungsstellen, die alsdann im die Nähe des Blepharoplasten verlagert werden. Dabei bildet sich die der alten undulierenden Membran zunächst gelegene Geisselanlage sur vorderen Geissel eines neues Tieres aus, während

die der ursprünglichen vorderen Geissel benachbarte zur undulierende Membran des neuen Tieres wird."

It would be seen from the above that there is hardly a single point of agreement between the division of Trypanoplasma congeri and Trypanoplasma helicis, and it would seem almost doubtful whether the two forms can be profitably united in the single genus. It would, I feel, be premature to enter here into a discussion on the comparative morphology of Trypanoplasma congeri and the trypanosomes proper until the rather complicated changes leading up to the resting-stage in the former have been more fully worked out. This I hope to do in a succeeding paper.

Results.

In the division of the active elongate Trypanoplasma congeri the following features are to be noted :

(1) The basal granule divides. This is followed immediately by a splitting of the anterior flagellum, and later, by the splitting of the posterior flagellum and membrane.

(2) The trophonucleus in the first stage enlarges, the intra-nuclear chromatin condensing on the karyosome. The trophonucleus assumes first a spindle and later a dumb-bell shape, which persists to quite a late stage in division. The karyosome appears to act as an internal division centre, and no trace of individual chromosomes can be seen at any stage of division.

(3) The kinetonucleus increases in size and divides by a simple transverse constriction. From its behaviour during division it is, I think, abundantly clear that, at any rate as far as Trypanoplasma congeri is concerned, the kinetonucleus cannot be regarded as a centrosome.

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EXPLANATION OF PLATE 21,

Illustrating Mr. C. H. Martin's paper on "Observations on Trypanoplasma congeri," Part I.

[All the figures were drawn with the camera lucida at table level under a Zeiss 1·5 mm. apochromat. and 18 compensating ocular. For the nomenclature of the structures compare text-figure.]

Fig. 1.—Normal active Trypanoplasma congeri showing flagella, single basal granule, kinetonucleus and trophonucleus with its karyosome and intra-nuclear chromatin granules. A row of faintly marked cytoplasmic granules may be seen passing under the membrane. Flemming, iron-hæmatoxylin, and eosin.

Fig. 2.—Early stage of division. The whole body of the animal is shorter and stouter. The basal granule has divided, the anterior flagellum is split along about a quarter of its length, and the beginning

of the splitting of the posterior flagellum is shown. The kinetonucleus is slightly thicker and the trophonucleus is distinctly enlarged. The intra-nuclear chromatin granules have probably condensed upon the karyosome, which no longer presents the hard outline characteristic of the resting nucleus.

Fig. 3.—The flagella have now split along their whole length. The karyosome has become drawn out into the characteristic dumb-bell-shape within the nuclear membrane. Corrosive acetic, iron-hæmatoxylin, and eosin.

Fig. 4.—The body of the animal has become still shorter. The kinetonucleus is becoming enlarged and losing its intense capacity for nuclear stain. The dividing trophonucleus is almost parallel to the longitudinal axis of the animal's body. Corrosive acetic, iron-hæmatoxylin, and eosin.

Fig. 5.—The body of the animal has become still more deformed. The basal granules with their flagella have shifted apart. The kinetonucleus has become thickened and has now lost its intense capacity for nuclear stains, its lower border is crossed by the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 6.—The basal granules with their flagella now lie at opposite sides of the dividing animal. The lower limb of the enlarged kinetonucleus has adopted its characteristic position at right angles to the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 7.—A slightly later stage than the previous figure, showing the characteristic relations of the enlarged kinetonucleus and the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 8.—A late stage of division. The two products of division are still united by a broad band of cytoplasm, through which the kinetonucleus and trophonuclei still retain their connection. Flemming, hæmalum, and eosin.

Fig. 9.—A recently divided form showing the characteristic rounded shape, the elongate kinetonucleus, and the unabsorbed strand which had connected the trophonuclei. The full length of the flagella are not shown. Corrosive acetic, hæmalum, and eosin.

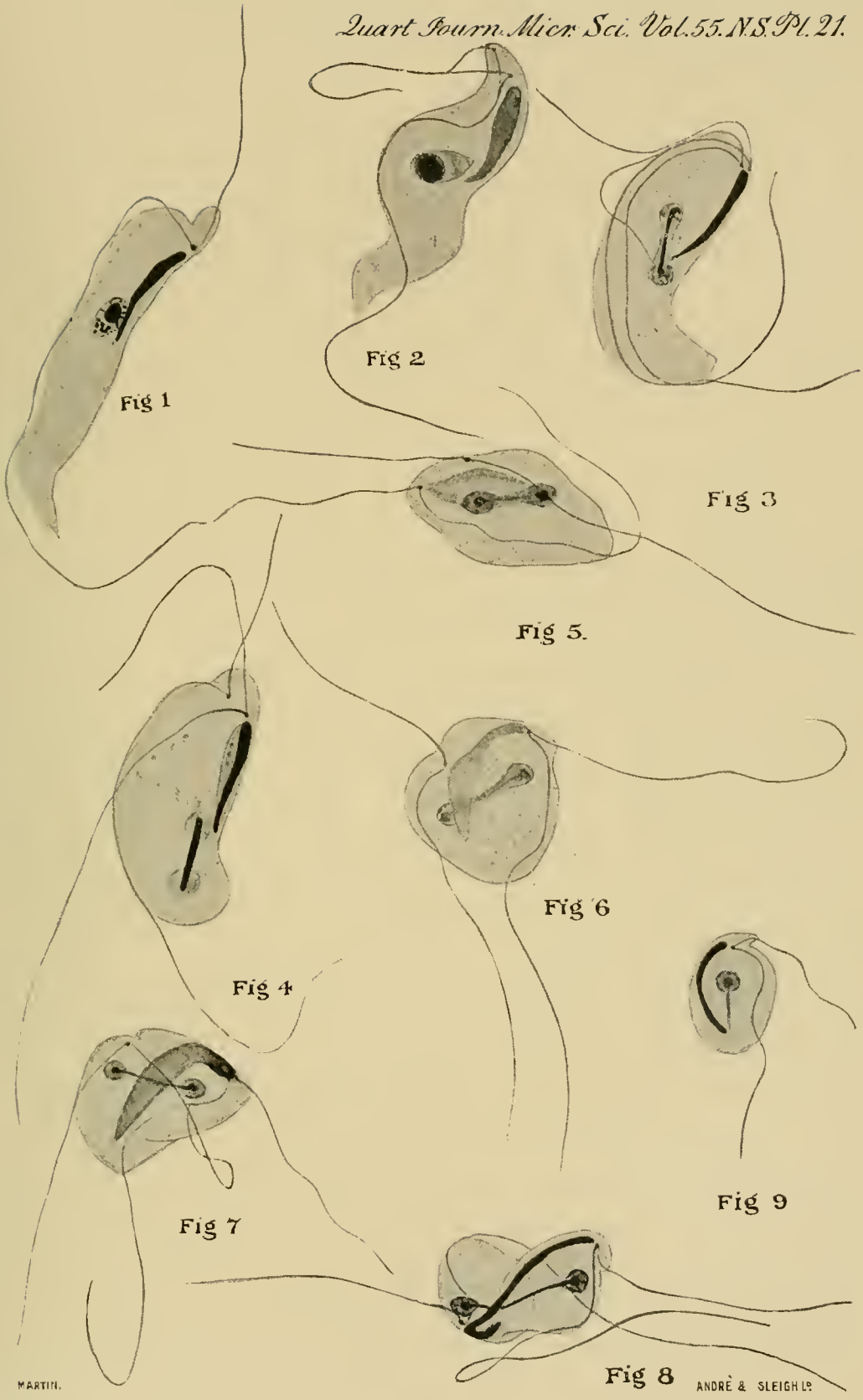


Fig 1
Fig 2
Fig 3
Fig 5.
Fig 4
Fig 6
Fig 7
Fig 9
Fig 8
MARTIN.
ANDRÈ & SLEIGH Lº

The Development of Aplysia punctata.

By

A. M. Carr Saunders and Margaret Poole.

With Plate 22 and 20 Text-figures.

This work was begun by one of us in the spring of 1909, at the Zoological Station at Naples, when holding the Oxford biological scholarship.[1] Owing to various reasons, the chief of which was ill-health, little more was done there than to collect material. It has been completed with assistance in reconstruction of sections and illustrations at Oxford in the department of Comparative Anatomy during the winter 1909–10. We must here express our gratitude to Prof. Bourne for the opportunity he has afforded us, and the encouragement he has given us to complete the work.

The bionomics of Aplysia have been described with great care by Carazzi and Mazzarelli. The former deals at length with the deposition of the eggs and their early development, while the latter, in his monograph on Aplysia (**14**), describes the general bionomics of the genus. The three common species found at Naples are punctata, limacina, and depilans. Carazzi (**5**), in his work on the cell-lineage of Aplysia, made observations on all three, and found little difference between them. Our results refer entirely to Aplysia punctata.

[1] I wish to take this opportunity of expressing my thanks to the staff at Naples for their continual kindness during the time I was there, and especially to Professor Meyer and Professor Eisig for their valuable advice with regard to methods.—A.M.C.S.

A number of stages of A. limacina were also examined, but the difference is insignificant.

Carazzi states that Aplysia punctata disappears in May to reappear again in the winter. We were able, however, to obtain this species in large numbers until the middle of June. No difficulty was experienced in keeping Aplysia in the aquarium, and they laid eggs in great quantities. The eggs develop normally, and equally well if kept in jars or in the tanks with circulation, provided only that the water be changed every two days or so. Early in the summer the eggs were at times attacked by bacteria, but if enough spawn was kept it was always possible to have some at the stage required in a healthy condition. Later in the year the eggs were attacked by algæ, and the embryos destroyed long before the free-swimming stage was reached. This was a more serious trouble than the bacteria, but the difficulty can be avoided by keeping the spawn in filtered water in the dark, where the algæ do not develop. The rate of development varies with the temperature of the water. This is described by Carazzi for the different species. In April some fifteen days elapsed between the deposition of the eggs of A. punctata and the emergence of the free-swimming larvæ from the capsules. It is possible to keep the larvæ in jars for some time, but even though they be kept in circulating water, they always die within a short time without exhibiting any change of structure. Mazzarelli states that he kept some larvæ of Bulla striata alive for twenty days, which is far longer than we ever succeeded in keeping Aplysia larvæ, but even these showed no change during that period. No one has yet raised any Opisthobranch larvæ through the metamorphosis, and there is therefore a large gap in our knowledge of the embryology of the group, for not only in the free-swimming larva are certain adult organs, such as the heart and pericardium and the gonads and genital ducts, entirely undeveloped, but the interpretation of some organs in the larva also must remain doubtful until the further development is known. Our failure to rear the larvæ of Aplysia beyond the free-swimming stage renders the present

work very incomplete, and it is therefore the intention of one of us to attempt to continue it and follow the metamorphosis. There would seem to be some hope of success if the methods of prepared sea-water and special feeding were used, such as have been employed so satisfactorily at Plymouth in rearing Echinoderms.

The living embryos are very opaque, and little can be seen of their organisation. As was the case with the work done previously on the cell-lineage, our observations were all made from preserved material. The eggs are enclosed in gelatinous capsules, and these are suspended in a long thread of jelly. Carazzi made the following calculations:—there are on an average seven eggs in each capsule in A. punctata and fifty in A. limacina; the whole thread, or "nest," as he calls it, will therefore contain on an average 80,000 eggs in the former species and 2,000,000 in the latter; this last number may at times be as high as 3,000,000. All the eggs develop with the exception of a few, which are not fertilised or are abnormal from some other cause. In the later stages, when movement is active and the muscles fully developed, the embryos will contract very considerably on the addition of the fixing agent, and this renders them difficult to interpret. To avoid this a 2 per cent. solution of cocaine in sea-water was used, which narcotises them in a few minutes and makes it easy to obtain preparations of fully-extended embryos. It is troublesome, and takes much time to extract the embryos alive from their capsules, and the great majority get injured in the process. Most fixing agents do not harden the jelly, and it is therefore equally difficult to extract the embryos when fixed by most of the common means. Formol, however, has the effect of hardening the jelly, and it is on this account extremely useful. Alone it makes a good fixing agent, but subsequent staining is rendered easier if it is used in combination with some other fixative. At the suggestion of Prof. Meyer a solution of formol and picric was used, made up in the following way:—ten parts 40 per cent. formol, ten parts 1 per cent. picric, eighty parts sea-water. This proved to be by

far the best of all the fixing agents which were tried, though for special purposes others were used, as, for example, Hermann's fluid to show up the liver.

When a thread of spawn was laid it was taken and suspended in the tank or jar by means of a string. When the embryos had reached a stage which it was desirable to preserve, a piece an inch or so in length was cut off the end of the thread, divided into a number of fragments a few millimetres long, and put in the picric and formol solution for about half an hour. At the end of that time it was easy to break the capsules with a needle and extract the embryos, the greater number of them entirely uninjured. Various stains were used, but paracarmine gave the best results for whole preparations; sections were stained on the slide with borax-carmine, followed by picro-indigo-carmine.

The eggs of Aplysia are small, being less than 100 μ in diameter, and this makes orientation before section-cutting practically impossible. In the end, therefore, it was found more convenient to embed large numbers close together which could all be cut at the same time, for in this way one could be certain of getting a few embryos cut in the plane that was desired. In order to embed a large number of eggs in a small area of paraffin the following method was employed—a watchglass was filled with paraffin and allowed to cool; a small round hole, reaching at least half way through the paraffin, was then made; the embryos were transferred into this by means of a fine pipette, and as much xylol as possible drawn off. The watch glass was then placed for half an hour on a stand on the warm bath, for half an hour on the bath itself, and finally inside the bath until the paraffin melted completely, when it was cooled.

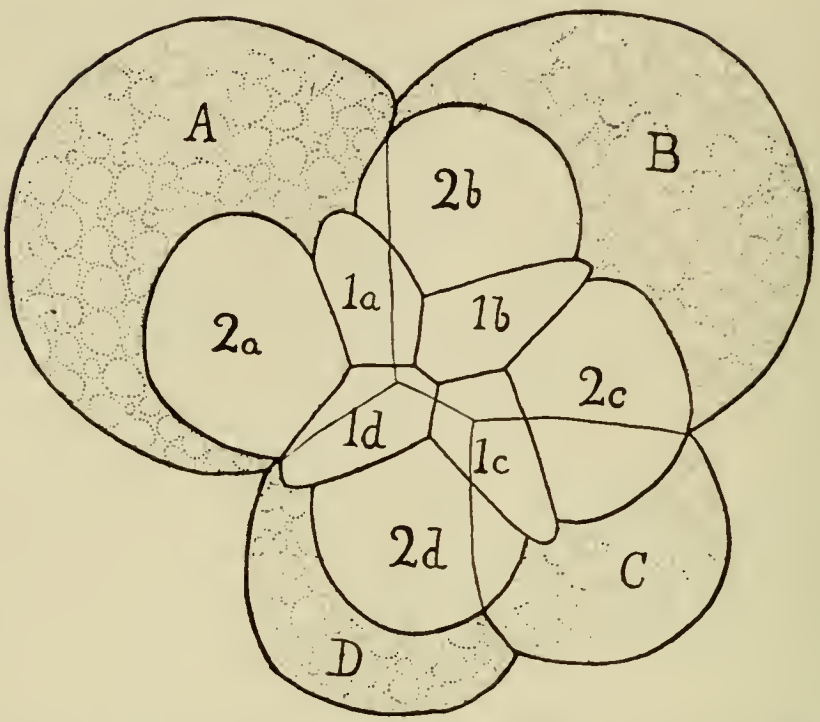
More points remain undecided in the ontogeny of Molluscs than perhaps in any other group in the Animal Kingdom. The cell lineage has been worked out in numerous cases among the various groups, but our knowledge of those stages which follow upon the end of segmentation is very incomplete.

This work was undertaken to throw light, if possible, on the origin of kidney, heart and pericardium, about which the most diverse statements have been made. It will suffice here to point out that at least six different types of excretory organs have been described in Molluscs, and that the origin and homology of all of them is disputed; while as regards the cœlom, opinions differ equally widely. The bearing of our results on these questions will be discussed at the end of this paper. Owing, however, to the fact already mentioned, namely, that it has not yet been found possible to rear the larvæ through the metamorphosis, they remain for the present inconclusive.

For the purpose in view, Aplysia was chosen for two reasons, firstly because it is easy to obtain material at any period of the year, and secondly, because a very careful and complete account of the cell lineage has been given by Carazzi. It was hoped that by beginning at the point where Carazzi left off, it would be possible to follow the development of the organs, and definitely to ascertain from which cells they arose. To the excellent account of the cell lineage referred to we have nothing to add; every cell has been followed up in it to a time when there are more than one hundred, and the history of the endoderm and mesoderm has been traced further. His last description is of an embryo consisting of two hundred and fifty cells, with the velum already developed.

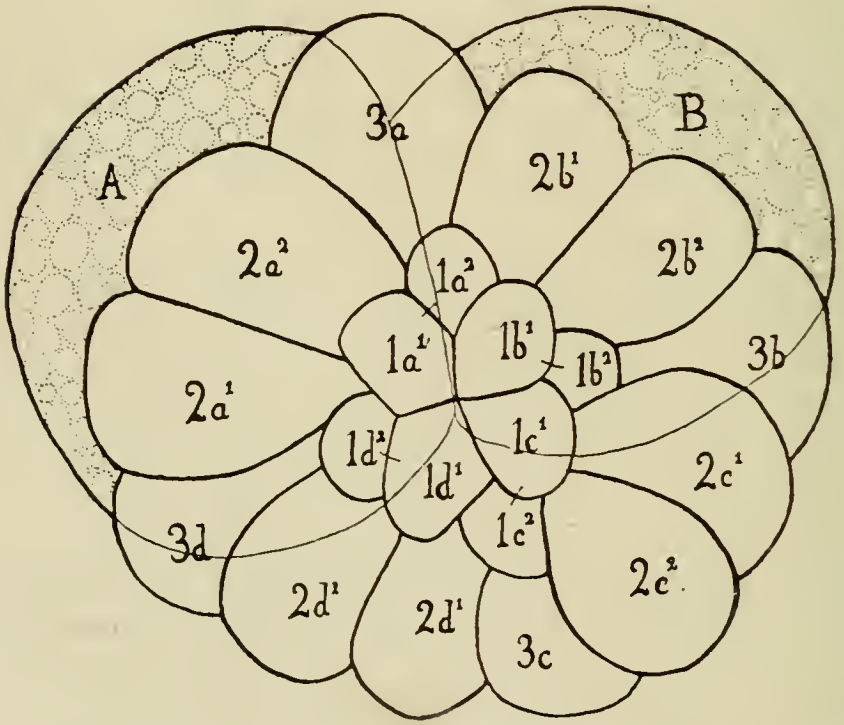
The development as described by Carazzi may be summarised as follows: Segmentation is spiral, dexiotropic and unequal, the endomeres *A* and *B* being far larger than *C* and *D*. The great size of these cells makes the cleavage look at first very irregular, but as a matter of fact their destinies show no exceptions to the scheme which has come to be recognised as normal in eggs the segmentation of which is of the spiral type. The first three quartettes give rise to all the ectoderm, 4 *d* entirely to mesoderm. There is no larval mesoderm arising from the ectoderm as has been described in some forms. The endoderm is derived from 3*A*, 3*B*, 3*C* and

TEXT-FIG. 1.



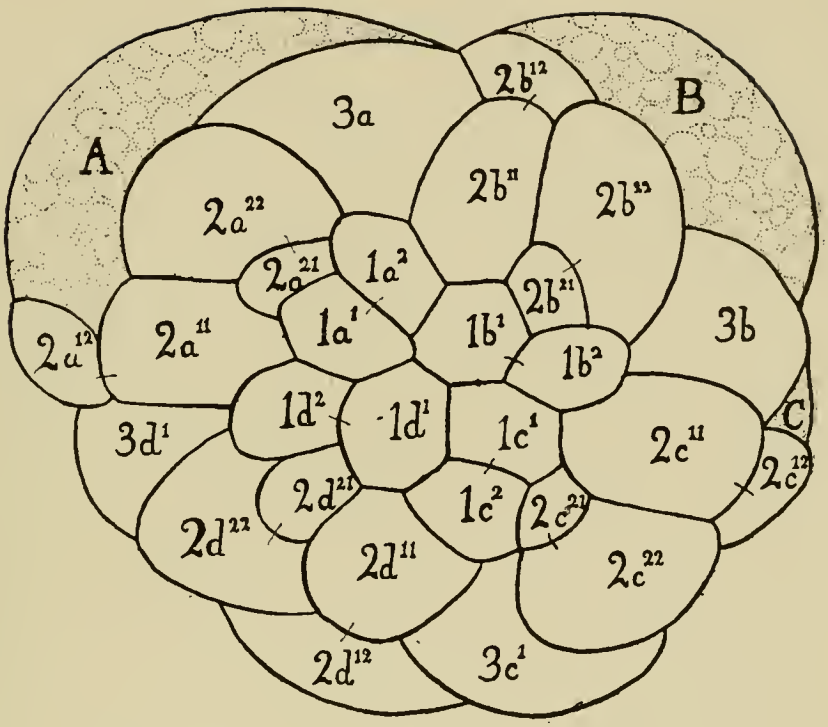
Surface view from the animal pole of an egg in the 12-cell stage.

TEXT-FIG. 2.



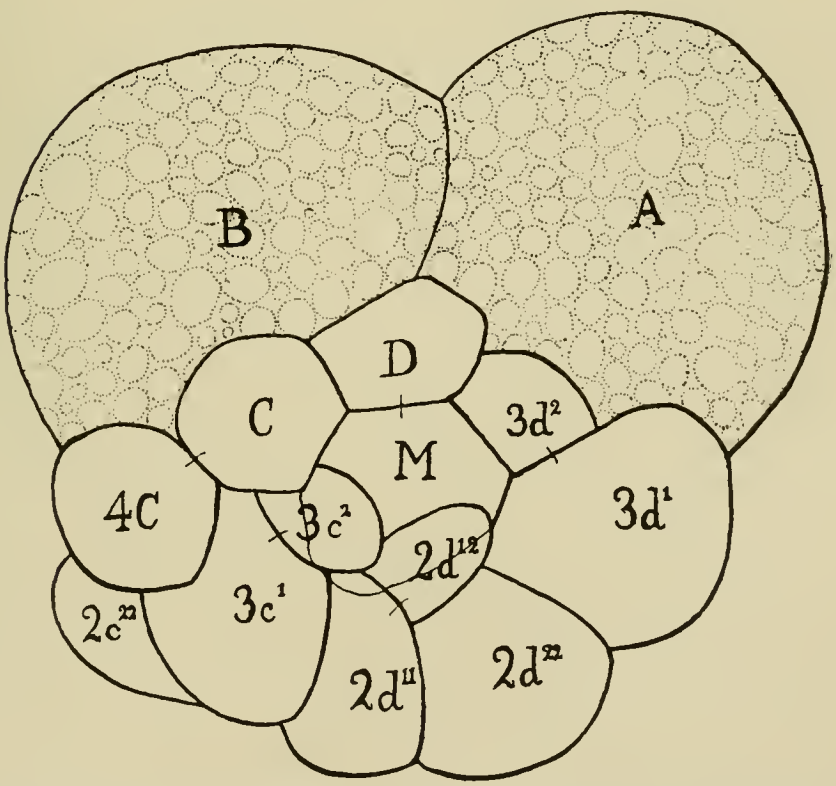
Surface view from the animal pole of an egg in the 24-cell stage.

TEXT-FIG. 3.



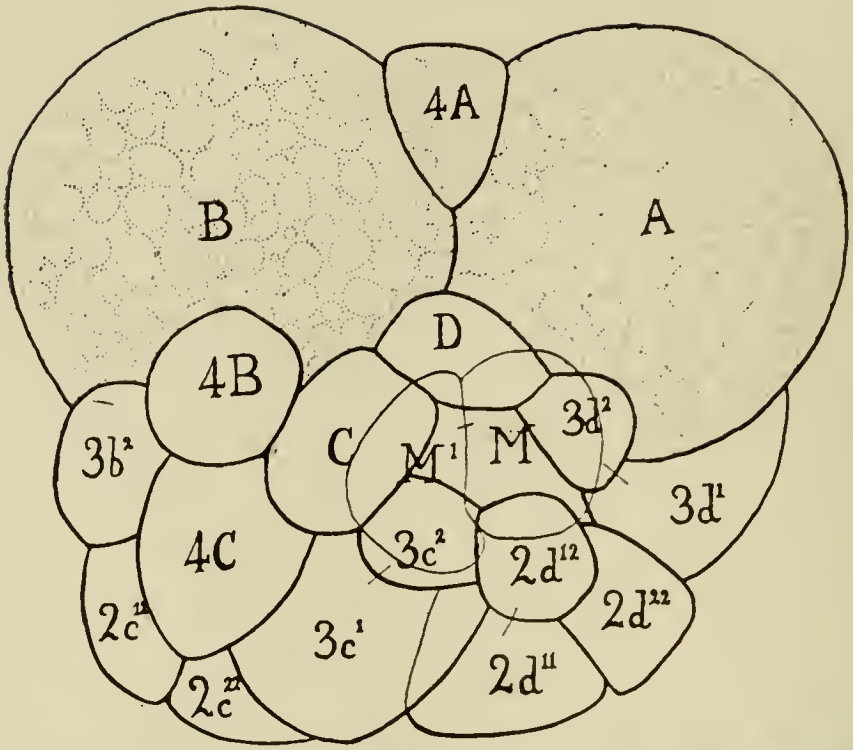
Surface view from the animal pole of a later stage; the apical ectoderm is now formed from 8 cells of the first quartette of micromeres, 16 of the second, and 6 of the third; $2a^{21}$, $2b^{21}$, $2c^{21}$, and $2d^{21}$ are the tip cells of the apical cross.

TEXT-FIG. 4.



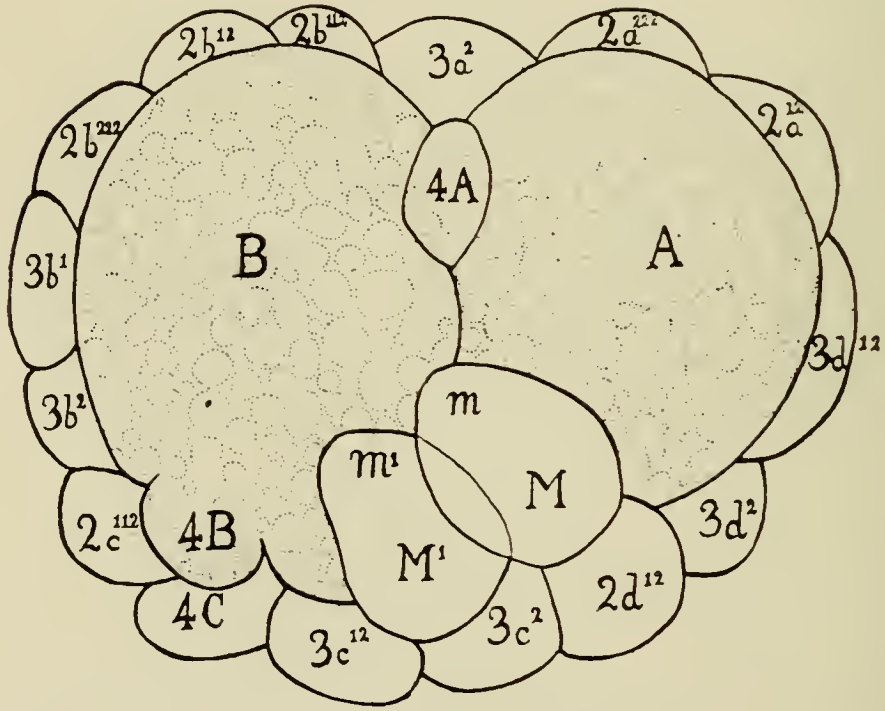
Egg seen from the vegetative pole at a stage corresponding to Text-fig. 2. The mesoteloblast *M* is already formed by the division of *D*.

TEXT-FIG. 5.



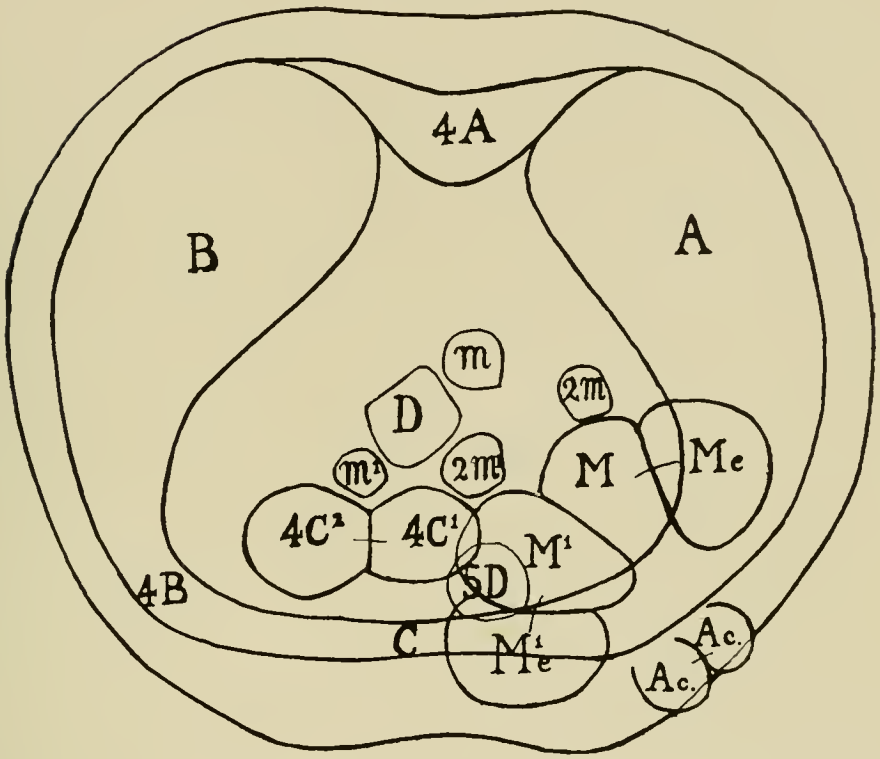
Egg seen from the vegetative pole at a later stage. M has divided to give rise to the paired mesoteloblasts M and M^1.

TEXT-FIG. 6.



Optical section of an egg at a somewhat later stage at the level of the mesoblasts, seen from the vegetative pole. The two cells, M and M^1, have each given rise to a small anterior mesoderm cell, m and m^1.

TEXT-FIG. 7.[1]



Diagrammatic optical sagittal section, seen from the vegetative pole of an egg in the 170-cell stage. The derivatives m, m^1, $2m$, $2m^1$, of the mesoteloblasts M, M^1, are seen spreading in an anterior direction from the region of the anal cells *Ac*. The macromeres *A* and *B* have diverged from one another to form a segmentation cavity.

[1] This and the preceding six text-figures have been modified from Carazzi's drawings. The notation of the various blastomeres throughout the segmentation follows the system, now almost universally adopted for the description of cell-lineages, of Wilson ("The Cell-lineage of Nereis," 'Journ. Morph.,' vi), slightly modified by Conklin ("The Embryology of Crepidula," 'Journ. Morph.,' xiii). *A*, *B*, *C*, *D* are the macromeres from which the successive quartettes of micromeres are divided off; the quartettes being distinguished by the co-efficients 1, 2, 3. Thus the first quartette will consist of $1a$–$1d$, and its derivatives will be $1a^1$, $1a^2$, $1d^1$, $1d^2$; while the descendants of the latter generation will be $1a^{1\cdot1}$, $1a^{1\cdot2}$, $1a^{2\cdot1}$, $1a^{2\cdot2}$–$1d^{1\cdot1}$, $1d^{1\cdot2}$, $1d^{2\cdot1}$, $1d^{2\cdot2}$. By the division of *D* $4d$ is formed, which, since it contains the material for the production of the mesoderm, is designated by the letter *M*. This later divides to form the mesoteloblast *M* and M^1, and from these, after the separation of a mesentoblast from each, small cells m and m^1, etc., are budded off

$4D$; gastrulation is of the epibolic type, and the blastopore is formed at the vegetative pole. It narrows to a slit-like opening, diminishing in size by the continual growth of the anterior and lateral parts of the ectodermal sheath, but does not close completely, but persists as the mouth. At the end of segmentation, owing to the large size of the endomeres A and B the embryo becomes somewhat heart-shaped. Between the large endomeres a small space appears, the segmentation cavity, which is more or less triangular in shape in optical section, the broadest end being towards the posterior end of the embryo. Two ectoderm cells, $2d^{22221}$ and $2d^{22222}$, increase greatly in size and come to project from the surface. These are known as the anal cells. During segmentation there is a shifting of the embryonic axis, and these cells come, in consequence, to mark the posterior end of the larvæ. At the opposite end the velum is formed as a simple ring in the region of the B quartette. By the time the cilia are developed the embryos begin to rotate within their capsules. At this stage there are about two hundred and fifty cells.

Text-fig. 7 represents an embryo with about one hundred and seventy cells, seen in optical section, from the vegetative pole. The blastopore is now reduced to a narrow slit, and posteriorly the anal cells project from the surface. Anteriorly the polar bodies were present still adhering to the embryo, but are not represented. Internally the two large endomeres diverge from one another to leave the segmentation cavity between them, while the derivatives of the much smaller endomeres C and D are shown. The mesomeres, which are at this stage eight in number, stretch across from the anal cells towards the position occupied by the blastopore, which is not represented.

anteriorly to give rise to the mesodermal bands. After the formation of the three quartettes of micromeres a fourth generation is produced by A, B, C; this consists of $4A$, $4B$, $4C$, which go towards the formation of the endoderm. The above lineage is given in tabular form by Robert (**26**), to which the reader is referred for the detailed analysis of the later segmentation stages.

We propose first to describe in some detail the earliest stage which we have investigated, and then to follow the development of the various organs separately up to the time when the larva becomes free swimming.

Structure of the Embryo at the End of Segmentation.

Pl. 22, fig. 1 is an external view of an embryo shortly before rotation begins, and fig. 2 in the same plate shows the cells which have sunk below the surface at the same stage. The total number of cells is more than 300. The ectoderm forms a thin and uniform layer covering the surface of the embryo. Round the anterior end there is a ring of somewhat larger cells derived from the *B* quartette, which bear long cilia and form the velum. At the opposite pole are the two anal cells $2d^{22221}$ and $2d^{22222}$, which are very prominent and project markedly from the surface, thus forming a convenient means of orientating the embryo with certainty. Near their bases are small nuclei which are sometimes difficult to see, and were not noticed by Blochmann (**2**). Their cytoplasm is very much vacuolated. They presumably function as temporary excretory organs. They are characteristic of Opisthobranch larvæ, though in some cases, as in Fiona, described by Casteel (**6**), they are small and but little differentiated from the other ectoderm cells. It is well known that in certain other Gastropod larvæ ectoderm cells of considerable size are found projecting from the surface; Glaser (**8**) has described them in Fasciolaria, where they occur singly or in groups of two or three together. These would appear to be comparable to the anal cells of Aplysia, but in Fasciolaria their position is variable, being almost anywhere on the surface. In the region of the A quartette there is a slight projection, the cells being somewhat enlarged. This is the rudiment of the foot. Between the foot and velum is the blastopore on the ventral surface, and round it the ectoderm cells are beginning to sink in. Though the blastopore is at this time very small, we have always found it perfectly distinct, and in this we agree with Carazzi in contradiction to Mazzarelli and

Blochmann, who assert that it closes and then reopens to form the mouth. The stomodæum does not at this stage communicate with the space between the endomeres; it is a blind sac lined by ectodermal stomatoblasts and œsophagoblasts. The former, according to Carazzi, are derived from $3a^1$ and $3b^1$, and the latter from $3a^2$ and $3b^2$; together they number between twenty-five and thirty. On the dorsal surface of the embryo and posterior is the shell-gland. It consists of a deep and narrow invagination, formed by a large number of cells, which are slightly differentiated from the adjacent ectoderm by their more elongated shape and rounder nuclei.

On each side of the embryo, just ventral to a line joining the anus and the mouth, a small ectodermal invagination, *ot.*, is seen to be in process of formation. These are the pair of otocysts. Fig. 3 shows the same structures at a slightly later stage. A little anterior to the anal cells on the right side are four large ectoderm cells identified by Carazzi as $3c^{1111}$, $3c^{1112}$, $3c^{1211}$ and $3c^{1212}$. These cells are at this time clearly in the ectodermal layer, but they soon sink below the surface and give rise to the secondary kidney. Their nuclei are of great size, and generally each contains one prominent darkly staining plasmosome.

The greater part of the interior of the embryo is occupied by the two large endomeres *A* and *B* (not lettered in the plate). They diverge somewhat from one another, and thus enclose between them an irregularly triangular segmentation-cavity (marked *st.* in figs. 2 and 3). The broad end abuts upon the shell-gland posteriorly, while the narrow end reaches to the bottom of the still blindly-ending stomodæum. The nuclei of the endomeres are large and oval in shape, lying to the inner side of the cells near the segmentation cavity. The cytoplasm is heavily laden with large yolk-granules, and some of the yolk is often found in the nuclei also, causing the latter to stain very deeply with plasma stains. A large vacuole is generally present in each of the endomeres, which is very conspicuous in the living embryo, and persists for a long time.

At this period there are about twelve other endoderm cells, the derivatives of $4a$ lying close against the stomodæum in the anterior end of the segmentation cavity, those of $4b$ at the opposite end rather dorsal to the shell-gland, and C and D, with their descendants, also lying at the posterior end against the wall of the shell-gland. The latter are already beginning to form a fairly definite row, which will become the posterior well of the stomach. At this stage the greater part of the cavity, which will be the stomach, is bounded only by the endomeres A and B, but this soon ceases to be the case. A and B gradually take less and less part in the formation of the wall and give rise to the left liver.

There are between fifteen and twenty mesoderm cells in the embryo at this stage. They form an irregular band, which arises at the posterior end near the anal cells, and stretches forwards to the blastopore. The band lies chiefly on the right side, but certain cells are already beginning to pass dorsally and ventrally into the foot.

We here see that it is possible to speak of a mesoderm band in Aplysia, though it is never clearly defined and soon breaks up. The conditions are very much like those described in Fiona and Umbrella, though the great size of the endomeres in Aplysia has forced the mesoderm chiefly on to the right side.

There is no secondary mesoderm either in this or later stages. It is present, however, in Fiona. Unfortunately, Heymons worked on Umbrella at a time when the existence of secondary, or ecto-mesoderm, was not recognised, so that its presence or absence in that form is unknown.

Development of the Organs.

The embryo rapidly assumes the appearance of the free-swimming larva, and from the beginning of rotation onwards there is but slight alteration in shape and very little increase in size until just before the embryo emerges from the capsule.

The Velum.—The velum is, as we have seen, originally a

simple ring of cilia round the anterior end of the body (Pl. 22, fig. 4). As the anterior end, however, grows out within the velar area, and then becomes flattened and expanded laterally, the circular shape of the ciliated band is soon lost, and the latter comes to surround the widely extended anterior prolongation of the body (Pl. 22, fig. 6). The velum then becomes notched in the mid-dorsal line and bilobed, but the latter characteristic is not so well marked as is generally the case in Opisthobranch larvæ.

In the free-swimming veliger the full extension of the velum is reached, but it can always be contracted completely within the shell. The cilia are long and prominent. Inside the circle of these cilia-bearing cells is a second row of cells of rather larger size with three or four cilia each, and in the middle, a cell with a single long and prominent flagellum.

The Foot.—The rudiment of the foot is at first broad and blunt, projecting from the ventral surface of the embryo between the blastopore and the anal cells. There is no sign of a division into two, as has been described in early stages of Patella. Between the stages represented in Pl. 22, figs. 4 and 5, the foot has undergone considerable change in shape, becoming elongated in an antero-posterior direction and flattened dorso-ventrally, and the operculum has been secreted on the lower surface. In the free-swimming larva it is still longer and covered with short cilia, and the operculum is capable of closing the opening of the shell completely when the animal is retracted.

The Shell-gland.—In Pl. 22, figs. 2 and 3, the shell-gland is invaginated to form a narrow pit. It soon afterwards becomes everted, and fig. 4 of the plate shows the posterior end of the embryo covered with a thin shell. The cells that were invaginated now form a cap, which secretes the shell, the edge of the former becoming the edge of the mantle. The mantle-cavity in the free-swimming larva is fairly deep, and into it on the right side open both the anus and the secondary kidney.

The Shell.—This is secreted directly the shell-gland is

everted. It is at first very thin and transparent, and even at its fullest development in the free-swimming larva never becomes thick or resistant enough to interfere with section-cutting. It grows at once into its ultimate exogastric form, and is always perfectly symmetrical. In the free-swimming larva it is marked by a number of fine lines, forming an irregular network.

The Anal Cells.—These have been already described (*Ac.* in Pl. 22). As development proceeds they decrease in size, this reduction being probably correlated with the growth of the secondary kidney, which takes on the function of excretion. In the free-swimming larva they are still prominent features, though they are neither figured nor described in this stage by Mazzarelli. They presumably disappear towards the end of the larval period.

The Otocysts.—These arise as ectodermal invaginations of about six cells, one on each side of the rudiment of the foot (*ot.* in Pl. 22). Later some ten or twelve cells sink well below the surface and form closed vesicles of some size, which are very obvious in the living larva lying at the base of the foot, below and to the sides of the œsophagus. At first these vesicles seem to be empty, but towards the end of embryonic life a large spherical otolith is very conspicuous inside each.

The Nervous System.—We have seen no trace of the nervous system before a stage corresponding to Pl. 22, fig. 8. In such an embryo there are visible rudiments of both cerebral and pedal ganglia (*c.g.* and *p.g.*). Our preparations do not make the mode of origin of the nervous system very clear. It would appear to arise as a cell-proliferation from the ectoderm, as there is no evidence of an ectodermal invagination to form the ganglia, as occurs in some forms, Dentalium for example. When the ganglia first appear, they take the form of slight thickenings in close contact with the ectoderm. The cerebral ganglia lie just above the mouth, the pedal ganglia to the outer and ventral sides of the otocysts, and slightly anterior to them. The ganglia become more definite and larger, but in the free-swimming larva they are still near

the surface. The two cerebral ganglia are close together and are united by a broad commissure. Mazzarelli states that cerebro-pedal and pedal commissures are present. We have been unable to discover these. The velum and foot are at this stage full of connective tissue, and it would be difficult to trace a fine commissure if it did exist. Visceral ganglia are absent at this stage of development.

The Secondary Kidney.—In making use of the term "secondary kidney" we are following the nomenclature of Mazzarelli. In his study of the free-swimming larvæ of Opisthobranchs he gives this name to the unpaired right kidney, which he has shown to be characteristic of all these larvæ. The term "primitive kidney" he reserves (and we follow him in doing so) for the smaller paired kidneys, the nephrocysts of Trinchese, which lie anteriorly to the "secondary kidney" at the base of the velum. It is necessary to make this clear, since owing to the nomenclature used in Carazzi's recent work confusion may arise. In the earliest stage with which we deal we have already described four large ectoderm cells, and have said that they would give rise to the secondary kidney. Now Carazzi mentions these cells and identifies them as $3c^{1111}$, $3c^{1112}$, $3c^{1211}$, and $3c^{1212}$. We have no doubt that these four cells are the same as those which we describe and figure. But Carazzi in his table of the cell lineage marks these cells as giving rise to the "reni primitivi." In the text all he says with reference to the fate of these cells is: "Una parolo devo aggiungere sul desterio delle grandi cellule $3c^{1111}$ $3c^{1112}$; esse constituiranno uno dei primi organi emissionali, civè il rene primitivo." It is quite impossible from this to understand whether, as one would incline to think from the quotation cited, Carazzi calls primitive kidney what we call secondary kidney, or whether, from the fact that in the table of cell lineage he says these cells give rise to "reni primitivi" (in the plural), he has not made the mistake of thinking that the cells in question are the rudiment of what we call primitive kidneys, and not of what we call secondary kidneys.

These four cells become differentiated while in the ectodermal layer on the right side of the embryo and slightly in front of the anal cells. Even in the earliest stages, which we have examined, their nuclei are clearly to be distinguished from all the other nuclei in the embryo, not only by their much larger size, but by the presence in almost every case, of a conspicuous deeply-staining plasmosome. Text-fig. 15 is a section of a stage where these cells have just begun to sink below the surface. Of the two cells shown one is still in the outer ectodermal layer, while the other has already sunk below. This process has gone further in Text-fig. 16 and the four cells are covered by a thin ectodermal layer. They continue to sink in further, and gradually give rise to a compact pear-shaped organ, the apex of which is directed towards the surface. Text-fig. 17 is a section taken at a stage when the organ is first becoming definite. We have never seen any of the four cells in the process of division; but the kidney in the free-swimming larva consists of eight cells, and therefore each original cell must divide once. The cytoplasm is at first finely vacuolated, but as development proceeds the small vacuoles become confluent, and form in the external part of the kidney several large cavities, the narrow ends of which converge to a point where they open into the mantle cavity. Here two small ectodermal cells form a short duct (Text-fig. 19). In the living larva drops of coloured liquid are seen to be contained within the vacuoles, but as a rule they are dissolved out by the reagents used in the course of preservation. The whole organ is clothed with a thin mesodermal epithelium.

This single excretory organ has long been known in Opisthobranch larvæ, but the most diverse statements have been made both as to its origin and function. With regard to the latter point, there can be no doubt that it is an excretory organ since it is easy in the living larvæ of some Opisthobranchs to observe the process of excretion. The view put forward by Lacaze-Duthiers and Pruvot (**13**) that it was an "anal eye" must be held to be one of the most curious and unwarranted of zoological speculations. Two investigators,

Casteel working with Fiona and Heymons with Umbrella, have come to the same conclusions as we have regarding the origin of this organ. In Umbrella it is originally paired. There are large ectodermal cells, $3c^{11}$ and $3d^{11}$, on either side of the embryo which divide and sink below the surface, one cell in each group remaining especially large. The cells on the left later disappear, while the right group forms the kidney. In Fiona the secondary kidney is unpaired from the beginning, as it is in Aplysia. It consists, however, of a single cell, $3c^{1111}$. This closely resembles the cells which form the organ which we have described in Aplysia, the cytoplasm being much vacuolated, and the nucleus large and containing nucleoli. In this case there are also other ectoderm cells near by, which seem to function in the same way. Clearly, we are dealing with a very similar organ in these three forms, but in Aplysia it is better developed, forming a definite organ with a duct and an enveloping epithelium.

The ectodermal origin of this kidney was first recognised by Lacaze-Duthiers and Pruvot. Mazzarelli is the only recent writer who upholds the view that it is mesodermal. He has worked on Aplysia and a number of other Opisthobranchs, and has come to the same conclusion for them all. We find his observations difficult to reconcile with our own. The organ in question, according to his account, is derived from two mesodermal cells at the aboral pole, which represent a paired rudiment of the kidney, as in Umbrella; in the course of torsion, however, both cells get pushed round on to the right side and form the single unpaired structure. They divide, become surrounded by other smaller mesodermal cells, and finally come to communicate with the exterior by an ectodermal invagination. It would seem that he took for the rudiment of the kidney two of the large mesoderm cells, which lie, at the stage he describes, on either side of the aboral pole; the large ectodermal cells, still lying at the surface, he has apparently overlooked. But why at a later stage he should describe two cells, when

there are never less than four present, it is not easy to explain.

The Primitive Kidneys.—These organs, described by Trinchese as nephrocysts, consist each of a large, much-vacuolated cell with a small nucleus, lying one on either side of the body at the base of the velum. In the living embryos they are very obvious on account of the brightly-coloured oily globules which they contain (Pl. 22, *K* 1, in figs. 4 to 9). They appear to be characteristic of Opisthobranch larvæ, and presumably constitute temporary excretory organs. Mazzarelli ascribes to these cells a mesodermal origin, and when first seen they certainly appear to lie well inside the body in both Fiona, according to Casteel, and Aplysia. All previous attempts to trace them back to their origin in segmentation have failed, and in spite of employing various methods of preserving and staining we have been equally unsuccessful. We consider it probable, however, that they are of ectodermal origin, of the same nature as the anal cells, which sink below the surface and lose their excretory function at a time when the secondary kidney is developed to take it on.

The Alimentary Canal.—We left the segmentation cavity at a stage when it was largely bounded by the two large endomeres, *A* and *B*. Pl. 22, fig. 3 represents a slightly later stage. The number of endomeres has increased, not by the division of the large blastomeres, *A* and *B*, but of the smaller endoderm cells. In the anterior region of the segmentation cavity the two large endomeres do not, therefore, contribute to form its boundary to such an extent as before. The cells which give rise to the wall of the cavity in this region are chiefly derived from 4*a* and *D*. The posterior wall of the cavity is still more complete, and its constituent cells are the derivations of 4*b*, *C*, and *D*. This corresponds very closely with the condition of things in Fiona, where the posterior wall is formed by 5*B*, 5*b*, 4*c*, 5*C*, 5*c*, 4*D*, and 5*A*. Umbrella agrees very nearly in this respect with Aplysia and Fiona.

At a slightly later stage (Pl. 22, fig. 4) the stomodæum

breaks through and comes into communication with the segmentation cavity. When this occurs, the anterior wall of the latter is still in part formed by the two endomeres, *A* and *B*. This, however, soon ceases to be the case. At this stage the intestine grows out as a tube-like evagination from the right posterior portion of the stomach, and reaches the surface just behind the anal cells. The anus is formed at once, and there is but a very slight ectodermal invagination, forming only the lip of the aperture. In this respect Aplysia agrees exactly with Umbrella. In this form the intestine arises from the derivatives of *5c* and *5d*; and this is probably the case also in Aplysia.

The œsophagus is long and narrow, and almost entirely ectodermal. From an early period it is ciliated, in the free-swimming larva the cilia being very long and numerous, and in sections filling up almost the whole lumen of the tube. Mazzarelli describes a cuticle which lines the cavity of the œsophagus of Opisthobranch larvæ; our preparations of Aplysia certainly do not show this structure.

The stomach wall is formed by rather small, clearly defined ciliated cells, constituting a columnar epithelium. In the embryonic stages the cilia are all alike throughout the lining of the cavity, but in the free-swimming larva, in the posterior region they are replaced by stiff hair-like structures, which Mazzarelli calls "bastoncelli." They are probably fused cilia, and serve as a staining apparatus. The fact that they are not developed until the larva becomes free-swimming supports this view, for until that stage is reached the embryo feeds upon the yolk stored in the liver, and does not take in food through the mouth. No epithelium can be seen covering the wall of the stomach externally. The intestine is a simple ciliated tube, and also appears to lack an epithelial investment. At first (Pl. 22, fig. 4) it arose from the right ventral posterior region of the stomach, but in the course of the torsion, which affects the whole of this part of the body, it becomes carried up on to the right side (Pl. 22, figs. 6 and 7); and finally, in the free-swimming larva it is seen to pass from the

dorsal surface of the stomach, slightly to the left of the middle line. The anus opens into the mantle cavity a short distance below the secondary kidney.

It will here be convenient to say a few words about the torsion which the embryo undergoes in the course of its development. This involves two processes, perfectly distinct from one another, though in Aplysia they take place simultaneously, one being the oro-anal flexion, so characteristic of molluscan organisation, and the other the rotation of the anus and adjacent organs through more than 120°, round an axis coinciding with the antero-posterior axis of the embryo. By the former process the anus is carried forward to open anteriorly, and the intestine to lie ventral and parallel to the œsophagus; while by the latter, torsion, properly so-called, the intestine, liver, kidney, and cœlom are carried from the ventral surface up on to the right side of the body. This movement is marked externally by the change in position of the anal cells (Pl. 22, figs. 4, 5, 6, 7, 8). The shell alone appears not to be affected by the torsion, for before this process is complete it has in miniature assumed its final shape, which it retains, while the organs inside it are being twisted in the manner described.

The Liver.—The endomeres *A* and *B* which form the left liver remain very large, and for a long time do not divide. At a stage corresponding to fig. 5 the nuclei divide, and thenceforward multiply slowly; but for some time no corresponding cytoplasmic divisions are to be distinguished. The nuclei are at first large and filled with yolk, scattered amongst irregular fragments of chromatin. Later they become reduced in size after repeated divisions and lose their yolk contents. The endomeres are full of yolk-granules, and their cytoplasmic structure is thereby entirely obscured. Of the two, *B* is approximately dorsal and *A* ventral. The effect of torsion is to move *B* more over to the left and *A* slightly to the right. They remain perfectly distinct from one another for some time, but eventually become fused together on the left side to form a single organ, the left liver. We have spoken

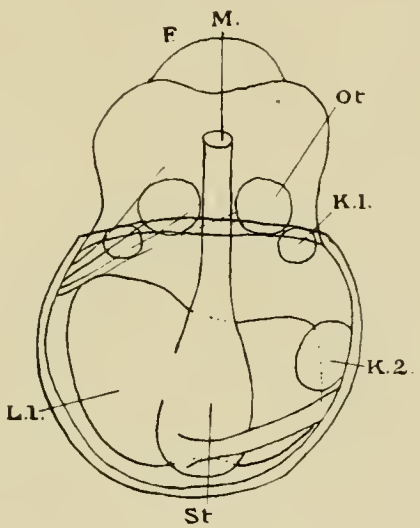
of the two large irregular spaces generally to be seen in the two endomeres. These coalesce and form a cavity within the liver, which communicates with the stomach by an irregular gap in the wall of the latter on the right side and rather ventrally. Text-fig. 20 is a section taken through this gap. On the left side is seen the cavity of the stomach communicating with the cavity of the liver; on the right side is the right liver, to be described later on. The nuclei have divided and are reduced in size. The size and shape of the liver in the free-swimming larva can be made out from Pl. 22, figs. 8 and 9. It may roughly be considered to consist of two lobes, of which the left is derived from *B* and the right from *A*. The left lobe is much the largest; it covers the left wall of the stomach, projects anteriorly to it, and rises dorsally. The right lobe lies ventrally, projecting beyond the stomach on the right side. The liver is clothed externally by a fine epithelium of flattened cells. During the whole of the embryonic period the yolk is being consumed, and when the larva emerges from its capsule it is entirely used up. The liver now takes on a new function, presumably one of secretion and digestion, for we have often observed algæ and other food material in the cavity, and drops of secretion are at times to be seen in the cells.

The right liver is formed in an entirely different manner. At a time between the stages represented in Pl. 22, figs. 4 and 5, certain cells of the right anterior wall of the stomach, rather nearer the dorsal than the ventral surface, become pushed out to form a flat, knob-like process (*R. l.*). This gradually takes on a rounder shape, and the contained cavity increases in size, while it becomes constricted off from the stomach to form a definite organ, which is the right liver. In the free-swimming larva it is nearly round, and communicates with the stomach only by a small and somewhat irregular aperture. When fully formed it lies almost entirely dorsal to the stomach, the smaller retractor muscle passes above it and the intestine below and to the right. The cells composing it are of large size, the cytoplasm consisting of a

network enclosing uniform small vacuoles which never contain yolk. The nuclei are not very different in appearance from those of the stomach wall, but are usually larger in size. Externally it is clothed with a thin epithelium. The right liver undergoes no apparent change when the larva emerges from its capsule. It is presumably always secretory in function.

Our account of the development of the liver differs from that of Mazzarelli, for he states that the right and left liver arise each from one of the two large endomeres, and this we

TEXT-FIG. 8.



Diagrammatic view of an embryo at a stage when the large retractor muscle is first formed; seen from the dorsal surface. *F.* Foot. *K* 1. Primitive kidney. *K* 2. Secondary kidney. *L.l.* Left liver. *M.* mouth. *Ot.* Otocyst. *St.* Stomach.

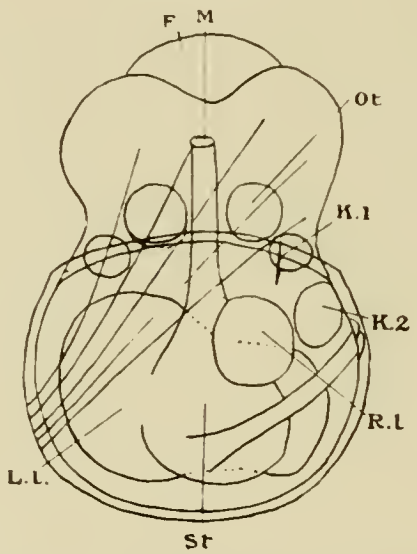
have shown to be incorrect. The peculiar mode of formation of the left liver is evidently correlated with the great size of the endomeres. All the yolk is contained within them, whereas, in such a form as Fiona, certain cells of the anterior portion of the stomach wall derived from $4b^2$ are heavily laden with yolk and become gradually evaginated to form the liver. This mode of formation of the liver in Fiona is, in fact, not unlike that of the right liver in Aplysia, but the latter, as we have pointed out, never contains yolk.

The large liver is very characteristic of Opisthobranch and

Pteropod larvæ, and serves to distinguish them from other Molluscan embryos. There may be present only a single liver, as in Fiona, or there may be also a smaller right lobe, as in Aplysia.

Muscles.—These are two in number, one large and conspicuous and the other much smaller. The former makes its appearance at a stage slightly earlier than that represented in Pl. 22, fig. 6, when it consists of two or three fibres only, which arise from scattered mesoderm cells descended from 4*d*.

Text-fig. 9.

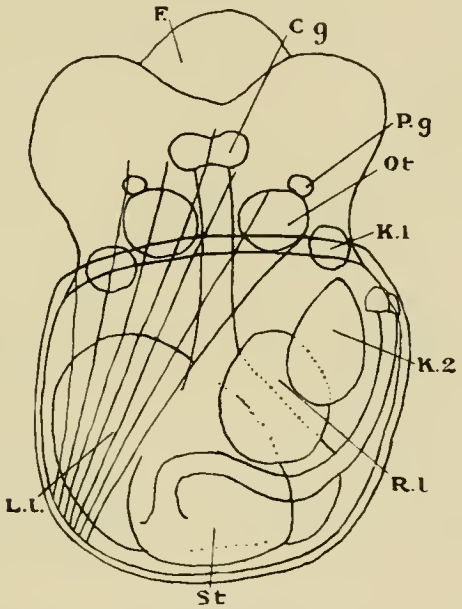


Similar view of an embryo at a slightly later stage. *R.l.* Right liver; other lettering as in Text-fig. 8.

There is no larval mesoderm in Aplysia derived from the quartettes of ectomeres, which in many cases give rise to the larval musculature. The fibres are at first attached to the body-wall dorsally and to the left of the middle line at about the level of the mantle-cavity (Text-fig. 8). At a later stage they are found to have increased in number and to be attached further back and rather more ventrally (Text-fig. 9), while in the free-swimming larva their attachment to the body-wall is posterior, but slightly to the left of the middle line and still nearer the ventral surface (Text-fig. 10).

The fibres are by that time fairly numerous, each one consisting of a single spindle-shaped cell, showing longitudinal striations. They pass dorsally to the left liver and are distributed to the velum and foot, and some are attached to the œsophagus near the mouth. The smaller retractor muscle appears later in development and always consists of three or four fibres only. They are similar to those of the larger muscle, and are no doubt of the same origin. This muscle is attached posteriorly to the dorsal body-wall on the right side,

TEXT-FIG. 10.



Similar view of a free-swimming larva. *C.g.* Cerebral ganglion. *P.g.* Pedal ganglion. Other lettering as in Text-figs. 8 and 9.

and thence appears to pass into the velum. As Mazzarelli has noticed, there are no other muscular elements of any kind to be seen in the larva.

The change in position of the large retractor muscle noticed above is to be attributed to the fact that the left side of the embryo, or more correctly, a particular zone in the left side, grows more quickly than the corresponding zone on the right side. This excess of growth on one side is a familiar feature in discussions of the question of torsion. It was first brought to notice by Bütschli. There is little evidence of its

occurrence, but it is most interesting to note that Casteel has stated that in Fiona a portion of the left anterior wall of the stomach can be observed to grow more quickly than the corresponding portion in the right. It is not necessary for us to discuss the theories of gastropod torsion, but we may point out that the excess of growth on one side is merely the ontogenetic cause of torsion. What the original phylogenetic cause of torsion may have been we do not know, and possibly never will know. It is not only possible, but it is probable, that the phylogenetic cause was something totally different from the actual ontogenetic cause. In the more modified members of a group it often happens that certain of the older features in the organisation of the larva get thrown back in development. To some extent this seems to have happened in Aplysia with regard to the torsion, organs seem, that is to say, to develop already twisted. We have indicated the manner in which this occurs with regard to the development of the shell. It must also happen when the visceral loop is developed, for there is no sign of it in the larva when torsion is complete. It must therefore develop already twisted.

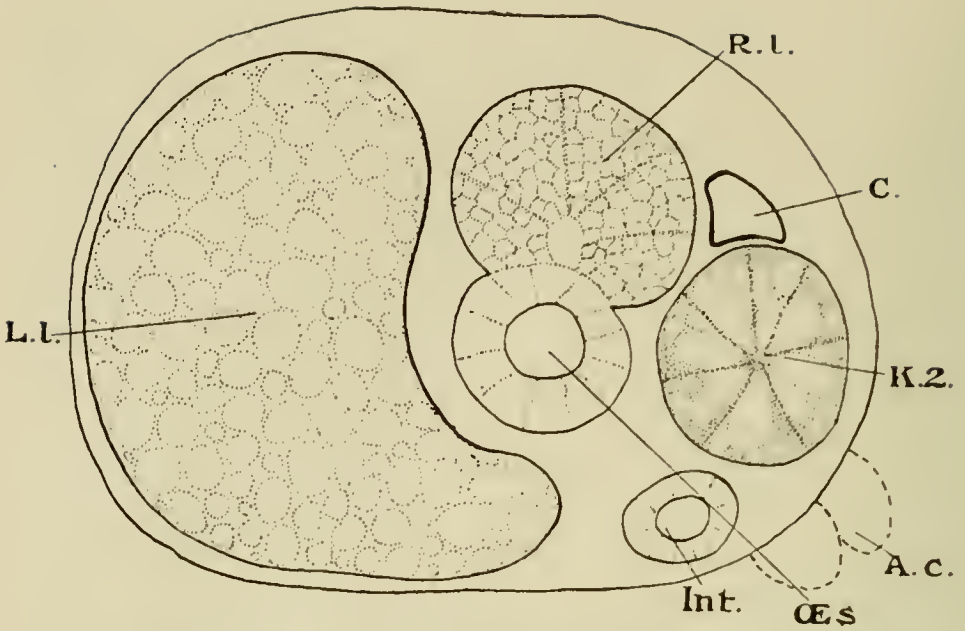
The Cœlom.—The mesoderm, as we have seen, appears at first in the form of an ill-defined band, but this arrangement is quickly lost. In Pl. 22, fig. 3 the cells are becoming irregularly scattered, and a little later are to be found everywhere lying between the large yolk-laden endomeres and the ectoderm. In Pl. 22, fig. 2 two small mesodermal cells are seen posteriorly, close to the anal cells; these are e and e^1 of Carazzi, which, he suggests, may possibly give rise to the rudiment of the genital organ. It is probable that these cells become involved in the formation of the cœlom, and that from the wall of the latter at a much later stage the germ-cells arise, but as it is quite impossible to follow these two cells through the larval development, their destiny must remain purely conjectural. As the foot and velum grow out, mesoderm cells pass into them, and there constitute a loose connective tissue; they also form thin epithelial investments

to the right and left liver and to the kidneys, and later give rise to the muscles. When the ectoderm cells of the secondary kidney have just sunk below the surface, and before they have become grouped together to form a definite organ, an irregular aggregation of mesoderm cells appears in this region, just anterior to the anal cells (Text-fig. 16). In Text-fig. 17, slightly later, these cells have formed a definite little mass close beside the now clearly developed secondary kidney, and in the next stage (Text-fig. 18) they are seen to bound a narrow slit-like cavity. This is the cœlom. In Text-fig. 19 it has begun to extend anteriorly and dorsally so as to cover the dorsal wall of the secondary kidney and the right and antero-dorsal surface of the right liver. In the last stage, before the emergence of the embryo from the capsule (Pl. 22, fig. 7), the cœlom forms two lateral sacs (coloured red in the figure), that on the right being the larger, connected with one another by two transverse passages—one lying in front of, and the other behind, the right liver, which thus projects dorsally between them. In the free-swimming larva (Pl. 22, figs. 8 and 9) this subdivision of the cœlom has disappeared by the union of the anterior and posterior passages on the dorsal surface of the right liver. The body-cavity now consists of a considerable thin-walled sac, lying on the dorsal side of the larva and covering the stomach, intestine, the right, and a great part of the left liver, and the posterior half of the secondary kidney. Its ventral extension, however, is nowhere very great.

Text-figs. 11–14 show the development of the cœlom in diagrammatic transverse sections through the region of the right liver and the secondary kidney.

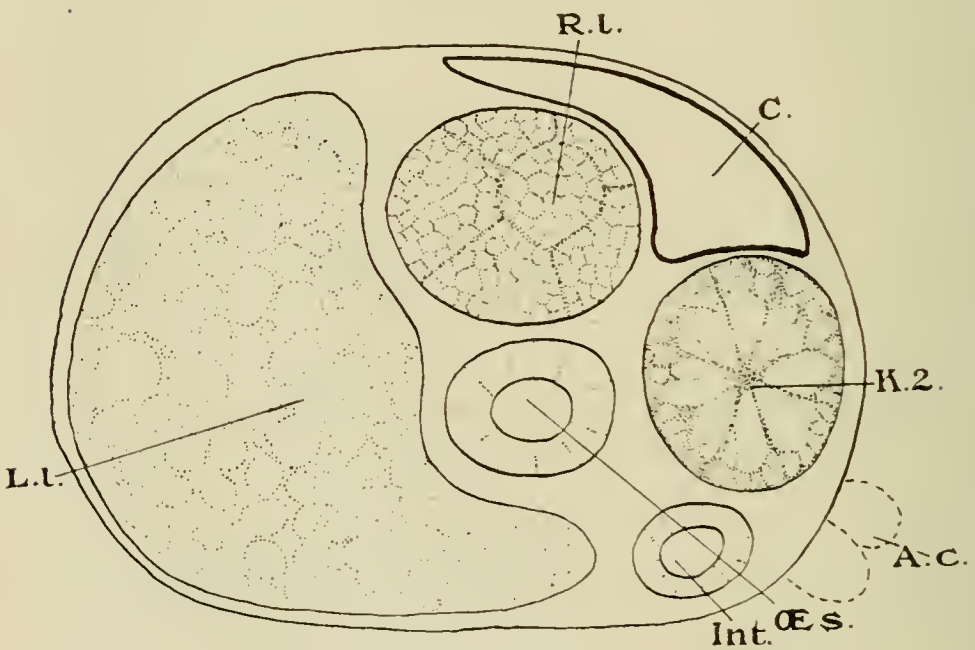
Hitherto the existence of the cœlom in Opisthobranch larvæ has passed unnoticed. Mazzarelli, it is true, mentions a pericardium, which he describes and figures as a small oval sac, but in Aplysia, as we have shown, the cœlom is of considerable extent and irregular shape. It would seem that Mazzarelli only observed the cœlom in whole preparations, which would account for his describing it as a small sac, for

Text-fig. 11.



A.c. Anal cells. *C.* Cœlom. *Int.* Intestine. *K2.* Secondary kidney. *L.l.* Left liver. *Œs.* Œsophagus. *R.l.* right liver.

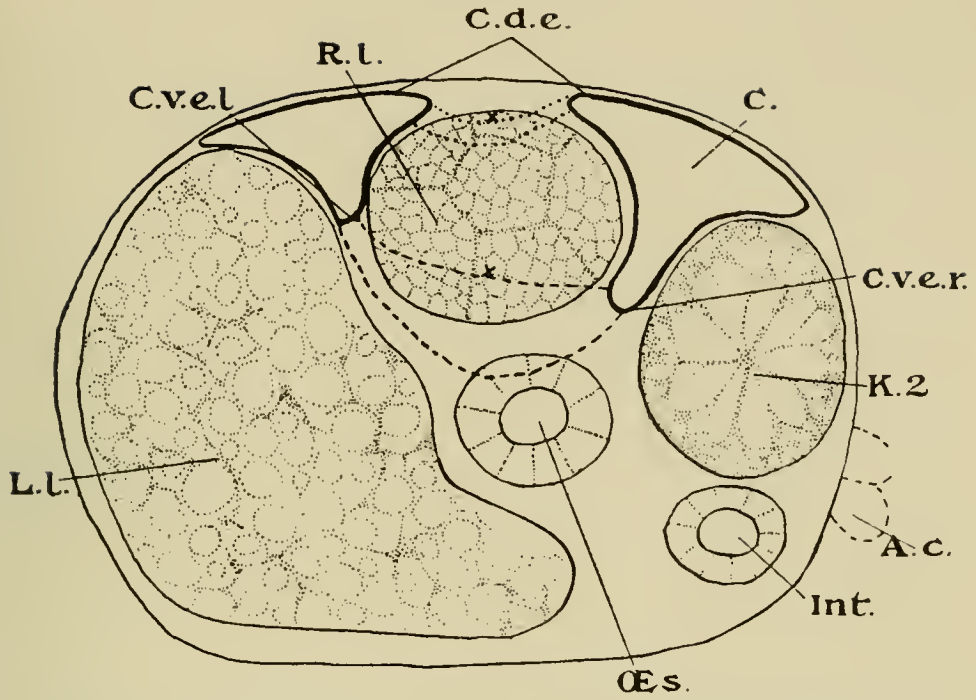
Text-fig. 12.



Lettering as in Text-fig. 11.

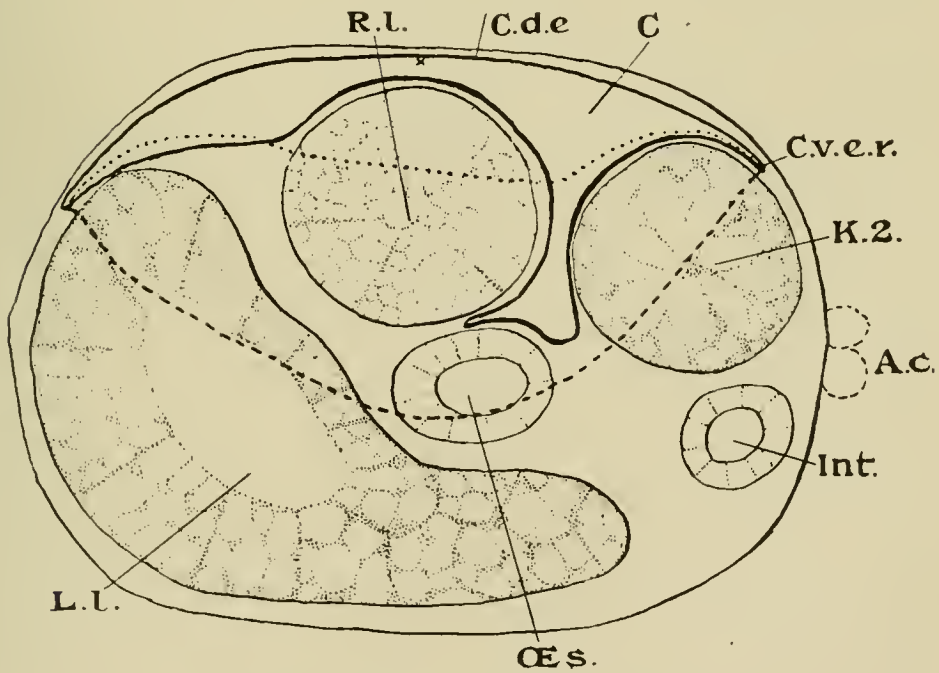
Text-figs. 11–14 are diagrammatic transverse sections through the region of the right liver and the secondary kidney, in order to show the development of the cœlom, which is lettered C.

Text-fig. 13.[1]



C.d.e. Dorsal extension of cœlom. *C.v.e.l.* Ventral extension of cœlom on left side. *C v.e.r.* Ventral extension of cœlom on right side. Remaining letters as in Text-fig. 11.

Text-fig. 14.



Lettering as in Text-figs. 11 and 13.

[1] The thick broken line indicates the posterior and the thin broken line the anterior extension of the cœlom; the × marks the spot at which the anterior and posterior portions unite dorsally to the right liver.

only the deeper central portion is rendered visible by this method, and does appear somewhat as he figures it, while the processes which spread and pass among the organs can only be reconstructed from sections.

General Considerations.

Although a large amount of work has been done on molluscan embryology, there still remain a number of important questions about which there is no general agreement. One reason for this is that, of the many cases investigated, only a few stages of each are as a rule known. In the majority of cases where the cell lineage has been worked out there is no account of the later stages; and conversely where these stages are well known, the cell lineage has not been traced. Such is the case with Paludina, about the later stages of which form more has been written than about any other molluscan genus. No satisfactory conclusion is likely to be reached until our knowledge of the earlier stages of Paludina is more complete. It was with the object of completing the account of the development in a single genus, in which the cell lineage is known, that we undertook this work. As yet it is not complete for reasons that have been mentioned. But the results that we have so far obtained make it necessary that we should consider their bearing upon certain theoretical points in connection with molluscan ontogeny.

The most important facts in our description of the development of Aplysia are the large extent of the cœlom and the ectodermal origin of the kidneys. And the first of these points bears directly on the question of the relation of Annelids to Molluscs. The resemblance of the trochophore to the veliger larva has been long recognised; there cannot, indeed, be shown to be any essential difference between them. But there are two points in the development of both trochophore and veliger which concern us here—the type of segmentation and the development of the mesoderm. That form of segmentation which is known as spiral cleavage

is typical in both Annelids and Molluscs. Whenever in these groups it does not occur, it is easy to account for its disappearance; in Nassa, for example, and in the Cephalopoda the form of cleavage is clearly correlated with the large amount of yolk present in the egg. There is often a strikingly close resemblance between the cell lineages that have been worked out in the two phyla, but too great stress should not be laid on this point, since the resemblances between the cleavage patterns may be taken to indicate a similarity of physical and mechanical conditions in the egg, rather than of any close phylogenetic relationship. However that may be, Carazzi's work on the cell lineage of Aplysia only adds another to the already long list of remarkable parallels in this respect between the two groups.

Our own work is more directly concerned with the origin and fate of the mesoderm in Annelids and Molluscs. In all Annelids in which the cell lineage has been investigated, the cell known as 4*d* gives rise to the most important part of the mesoderm, and sometimes to all the mesoderm, as in Aplysia. In some other forms the so-called larval mesoderm derived from the ectoderm contributes to a greater or less extent to the structure of the larva. In Annelids the subsequent history of the mesoderm is well known; and it is interesting to find that in many Molluscs well-developed mesodermal bands are found, in all ways comparable to those in Annelids. As might be expected, the best examples of mesodermal bands occur in the more primitive groups of Molluscs. Kowalevsky has described them in Chiton polii, and Heath in Ischnochiton. Among the Solenogastres also we find in Dondersia and Proneomenia that the bands are unusually distinct, and Patten figured them clearly in Patella. But in the more modified groups of the Mollusca one could hardly speak of mesoderm bands except on the analogy of the less specialised forms. This is the case in Aplysia, where the bands are never clearly defined and soon break up into scattered cells.

Turning to the development of the cœlom, it is here that we find the first essential difference between Annelids and

Molluscs. For in the latter phylum there is never any trace of segmentation, whereas in the former, as is well known, the bands become split up into blocks, in each of which a cœlomic cavity is formed. A further difference is the reduction of the cœlom in Molluscs, but this is by no means so great as is usually supposed. Though the evidence is as yet scanty owing to the small amount of work that has been done on the later stages on development, nevertheless there is reason to believe that the cœlom is, at a late period in the metamorphosis, of considerable size, and that even in the adults of some of the more primitive groups it remains large. Kowalevsky long ago described the development of the cœlom in Chiton polii, and some of his figures, which show the cœlom surrounding the gut, would pass well for a transverse section of an Annelid larva at a late stage. It is very probable that a similarly extensive development of the cœlom will be found in the Solenogastres, where, as we have seen, the mesoderm bands are of considerable size. Among the Aspidobranchia, as the most primitive of the Gastropoda, we might expect a larger body cavity than in the more specialised forms, if one regards the extensive cœlom as a primitive factor preserved from an Annelid ancestor; but unfortunately nothing is known about the later larval stages. In a recent account of the structure of the Neritidæ, Bourne, however, has lately described a very large cœlom, more extensive than has been described in any other gastropod. To find a parallel to it we must refer, he says, to the Cephalopoda. There are a number of descriptions of the development of the Pectinibranchia, in which the cœlom is extensive; all the authors who have worked on Paludina agree upon this. Other cases are Vermetus, where Salensky describes a somatopleur and a splanchnopleur, though unfortunately he gives no figures; and Bithynia, described and figure by Erlanger. Among Pulmonates and Lamellibranchs, as one might expect in such specialised forms, we find no evidence of the existence of a large cœlom, this structure being reduced in every case to a small sac-like pericardium and a reno-pericardial duct. These

examples will, however, serve to show that a well-developed cœlom is of frequent occurrence in the Mollusca, and that it is probable that when the later stages in other Molluscan groups have been more thoroughly examined, a large cœlom like that which we have described in Aplysia will be found to be a normal feature in the organisation of the Molluscan larvæ. But it is to be observed that the cœlom in Aplysia is developed at a stage when in both Annelids and Molluscs the mesoderm bands are still intact and a cavity has not yet been developed.

In very few forms among the Mollusca has the development of the cœlom been traced from the segmentation period onwards. Among the forms which have been worked out, Aplysia and Physa follow what we may call the normal Annelid type, that is to say, the mesoderm, all of it in Aplysia, and the greater part of it in Physa, is developed from $4d$, and from it the cœlom arises. In the others there is a departure from this type of development; there are Dreissensia, Limax, and Cyclas, which have been described by Meisenheimer, and Paludina, according to Otto and Tönniger. In the first three cases the cell lineage is known, and $4d$ develops in the usual way and gives rise to bands, which split up and form mesenchymatous tissue, and thus seems to correspond to the larval mesoderm described by Lillie in the Unionidæ, where it arises from $2a^2$, but is believed to give rise to the adductor muscle. The cœlom is stated to arise, not from the descendants of $4d$, but from cells which proliferate from the "ectoderm" at a comparatively late stage when segmentation is complete. The same is said to be the case with Paludina; the cell lineage is not known in this form, but it is distinctly stated that there are no pole mesoderm cells.

Such a marked departure from the typical mode of development was hardly to be looked for; it is to be noticed that it occurs in widely separated members of the phylum, and further, that there are no peculiar bionomic conditions common to them. So far as our knowledge goes, it would seem to be an alternative mode of development, which may occur anywhere in the Mollusca. At first sight it might

seem to involve serious difficulties as to the homology of the organs formed by these very different processes, but it would be clearly absurd to argue from this want of resemblance in the method by which the cells giving rise to the cœlom are segregated during development that the cœlom and its derivatives are therefore not homologous throughout the Mollusca. Evidently the heart, pericardium, and kidneys of adult molluscs are all homologous. It might thus seem that the evidence of embryology was worthless in this case; but these two modes of development are not so different as might seem at first sight. For, although a superficial examination of Molluscan cell lineages leads one to expect that mesodermal structures are always formed from the descendants of 4*d* at a parallel stage in development, closer inspection shows that this is by no means invariably the case. The period at which the mesoderm becomes segregated from the other embryonic elements varies considerably; it takes place in Planorbis marginatus when there are only twenty-four cells present; in Planorbis trivolvis when there are forty-nine; and in Trochus magnus when there are 145. Statements have also been made that in Tethys and Teredo 4*d* does not give rise to mesoderm at all. Differences in the mode of segregation are thus to be found in closely allied genera, and we cannot lay down any hard and fast rule to govern developmental processes even in the same phylum. All that we are justified in saying in the present state of our knowledge is that there are certain definite organ-forming substances present in the egg before segmentation begins which are homologous throughout the group. As this process takes place these may be separated into definite cells or groups of cells, from which the corresponding organs, or complex of organs, are subsequently developed; but this is by no means necessarily the case. The factors for the formation of certain organs, as, for example, the cœlom and related structures above mentioned, instead of being aggregated at an early stage into a single cell, may be localised in many different cells with a totally different destiny, and only at a

comparatively late stage become finally segregated out, in the present case by proliferation.

That the way in which organ-forming substances present in the egg are finally separated from one another is quite immaterial in affecting the homologies of the organs to which they give rise is very clearly demonstrated by certain experimental work, as, for example, that of Wilson on the egg of Nereis. In this case the cleavage pattern was totally changed by subjecting the egg to pressure, and yet the larva produced was normal.

These facts, taken together with what we know of the movements of the cytoplasm before and during segmentation in Cynthia, Dentalium, Cerebratulus, etc., show that the organ-forming substances often shift their position, and are segregated at different periods. Meisenheimer's results have demonstrated a remarkable instance of this, but provide no evidence concerning the homologies of the organs.

Before we discuss the larval excretory organs in Aplysia, we may briefly describe the types found among Molluscs.

I. Flame-Cells:

a. The flame is borne by one cell only; the duct is intra-cellular.

(1) Organ consists of two cells: Lamellibranchs.

(2) Organ consists of four cells: Fresh-water Pulmonates and Basommatophora.

β. The flames are borne by more than one cell; the duct is inter-cellular: Terrestrial Pulmonates; Stylommatophora and Paludina.

II. Ectoderm Cells which enlarge, become vacuolated and project from the surface.

a. Position variable, but near the base of the velum: Marine Prosobranchs.

β. Position definite, slightly anterior to the anus: Opisthobranchs.

III. Nephrocysts (primitive kidneys). A single cell some distance beneath the surface, and without a duct: Opisthobranchs.

IV. Secondary kidneys. Several large vacuolated ectoderm cells opening to the exterior by a short duct: Opisthobranchs.

The origin of Type I has been differently described by several investigators, and though there seems to be much evidence of its arising from the ectoderm, yet a mesodermal origin has been ascribed to it by Erlanger in Paludina, Stauffacher in Cyclas, and Rabl and Holmes in Planorbis. If the ectodermal origin of these larval excretory organs should be proved beyond dispute, we should fairly be able to compare them with the Annelid nephridia. Nevertheless, if these organs are taken as representing ancestral nephridia, and thus indicating a relationship between the Annelid and Molluscan phyla, it is remarkable that they have never been found in the primitive groups of the latter, the Amphineura and the Aspidobranchia, and yet are present in the highly specialised Pulmonates. The second type of larval excretory organ is of no special significance. It has obviously been developed to meet some special need during the early stages of ontogeny. Type III may possibly be of a similar nature to the preceding, but it certainly stands apart from the others, and until we know whether it is ectodermal or mesodermal, as has been asserted by Mazzarelli, it is impossible to compare it with any other form of excretory organ. The fourth type of kidney, which we have called the secondary kidney of Opisthobranchs, offers some difficult problems both as regards its homology and its ultimate destiny. The position it occupies is very similar to that of the definitive kidney in the adult. Mazzarelli, when he originally described it as arising from the mesoderm, believed it to be the rudiment of that organ. This idea he has abandoned in his later work on the free-swimming larvæ of Opisthobranchs, and is inclined to believe that the organ disappears in the metamorphosis, and is in no way connected with the adult organ. He produces no evidence for this view, though, as will appear later, we think it has much justification. Casteel thinks it probable that the secondary kidney persists through the metamorphosis and

becomes the kidney of the adult in spite of its ectodermal origin; he supports his view by referring to Meisenheimer's account of the "ectodermal" origin of the common rudiment of heart, kidney, pericardium, and gonad in Dreissensia and other forms. Heymons considered the kidney to be merely a larval organ; he further compared it to the external ectodermal kidneys of Prosobranchs, our Type II. This suggested homology seems to us very far fetched. To begin with, these Prosobranch kidneys are variable in position in the same species, while the Opisthobranch kidneys are derived from almost the same cell in the three forms, Aplysia, Fiona, and Umbrella. Further, the Prosobranch kidney is an external protruding organ, while the Opisthobranch kidney sinks well below the surface epithelium.

Our own conclusion is that the secondary kidney of Opisthobranchs cannot be homologised with any of the other various molluscan kidneys. We have already given our reason for believing that it cannot be homologised with our Type II. The fundamental difference between it and our Type I is that cilia are absent. It is possible that in such advanced forms as the Opisthobranchs the cilia might have been lost and the nephridium reduced to some such condition as that which we find in the secondary kidney. But the posterior position of the organ makes it unlikely that it has anything to do with the Annelid nephridium, the representative of which in Molluscs is always found close up under the velum. In the embryo of terrestrial Pulmonates, also, the nephridium is preserved, although they are more modified than the Opisthobranchs.

The position of the secondary kidney suggests at first sight that it is the rudiment of the definitive kidney. But in all those cases in which the origin of the definitive kidney is known for certain, and in which it has been traced from the embryo to the adult, it has been found to arise as an evagination from the cœlomic epithelium, which joins an ectodermal invagination and so reaches the exterior. A communication between the cœlom and the kidney is present

from the earliest stage in the formation of the latter, and persists as the reno-pericardial aperture in the adult of all Molluscs with the exception of Nautilus. Now in Aplysia there is never any connection between the cœlom and the secondary kidney; there is no reno-pericardial duct in connection with it; the two organs, both in origin and later development, are perfectly distinct. We consider it therefore probable that the secondary kidney of Aplysia is a larval organ which degenerates and disappears during the metamorphosis, and that the definitive kidney arises as an evagination of the cœlomic epithelium as it does in Physa.

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[1] For a complete bibliography of the whole subject the reader is referred to Mazzarelli's monograph (**14**) for list of literature up to 1892, and to Carazzi's paper (**5**) for more recent work up to 1906.

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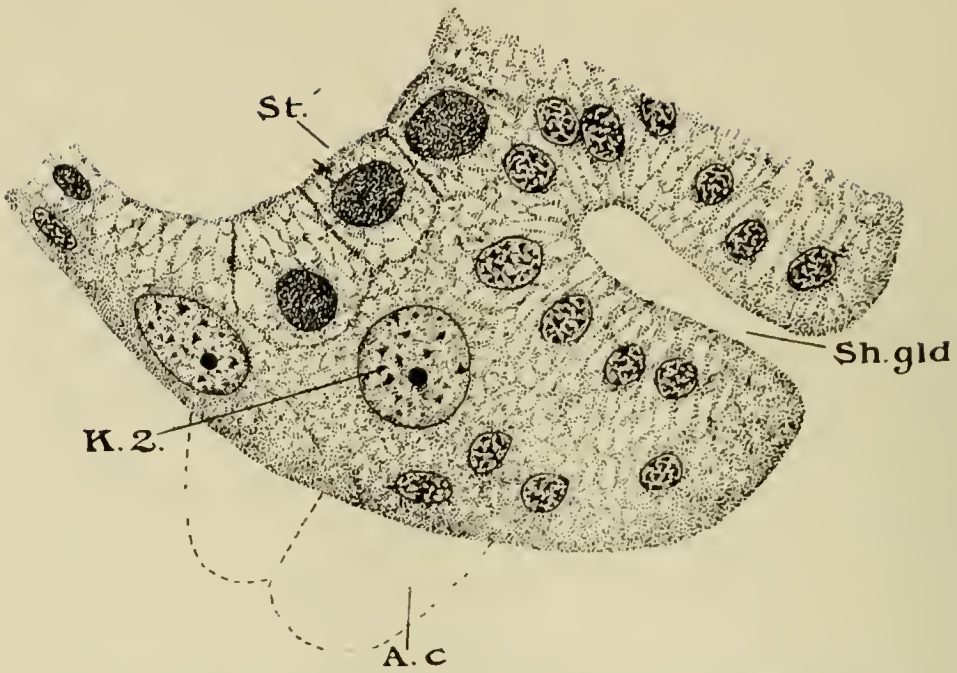
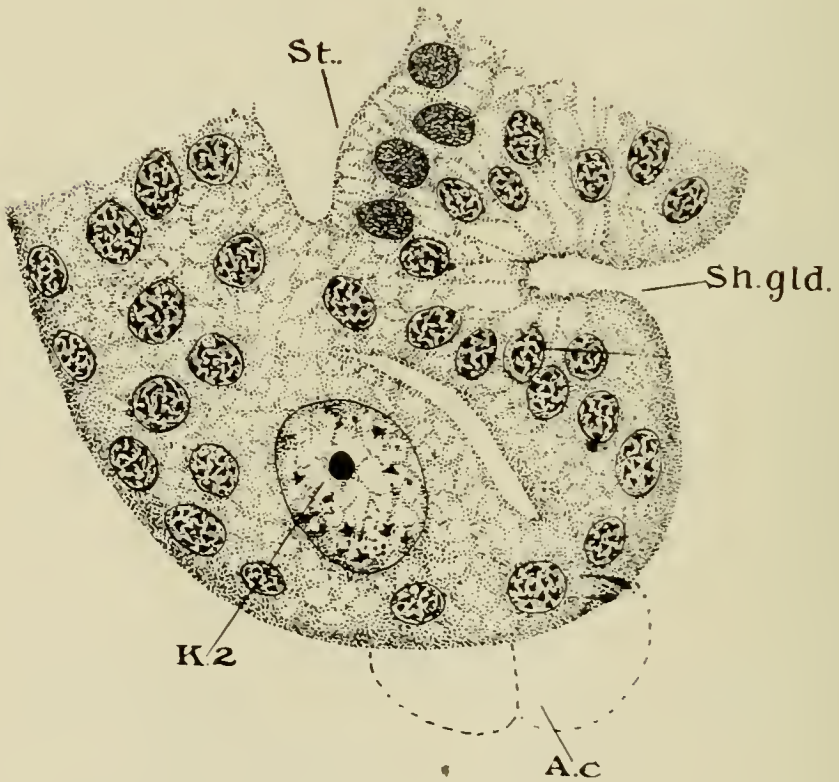
TEXT-FIG. 15.[1]



Section through the right posterior region of an embryo in the same stage as figs. 1 and 2 of Pl. 22, showing the ectoderm cells, K. 2, which will form the secondary kidney, still lying on the surface.

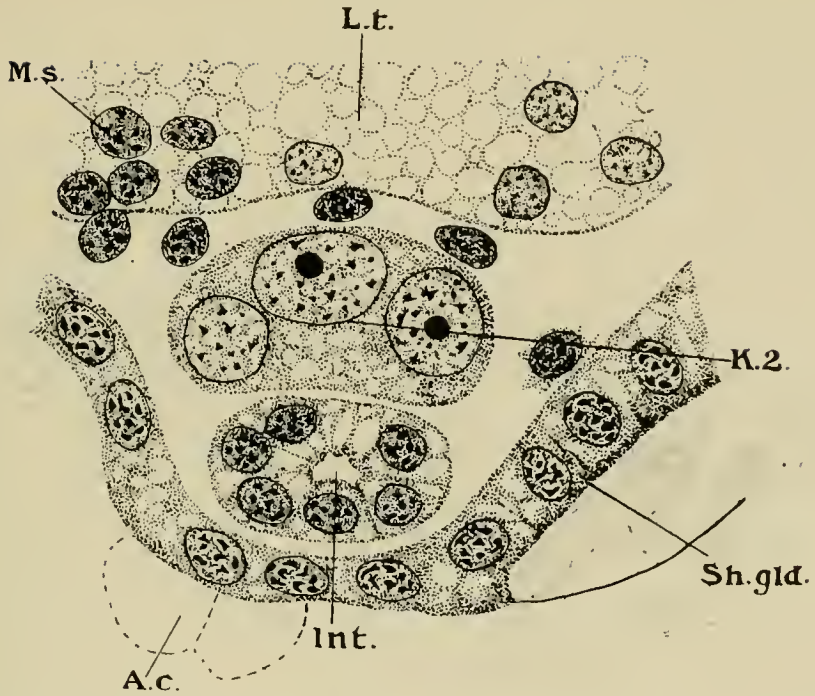
TEXT-FIG. 16.



Section through the same region of an embryo a few hours older.

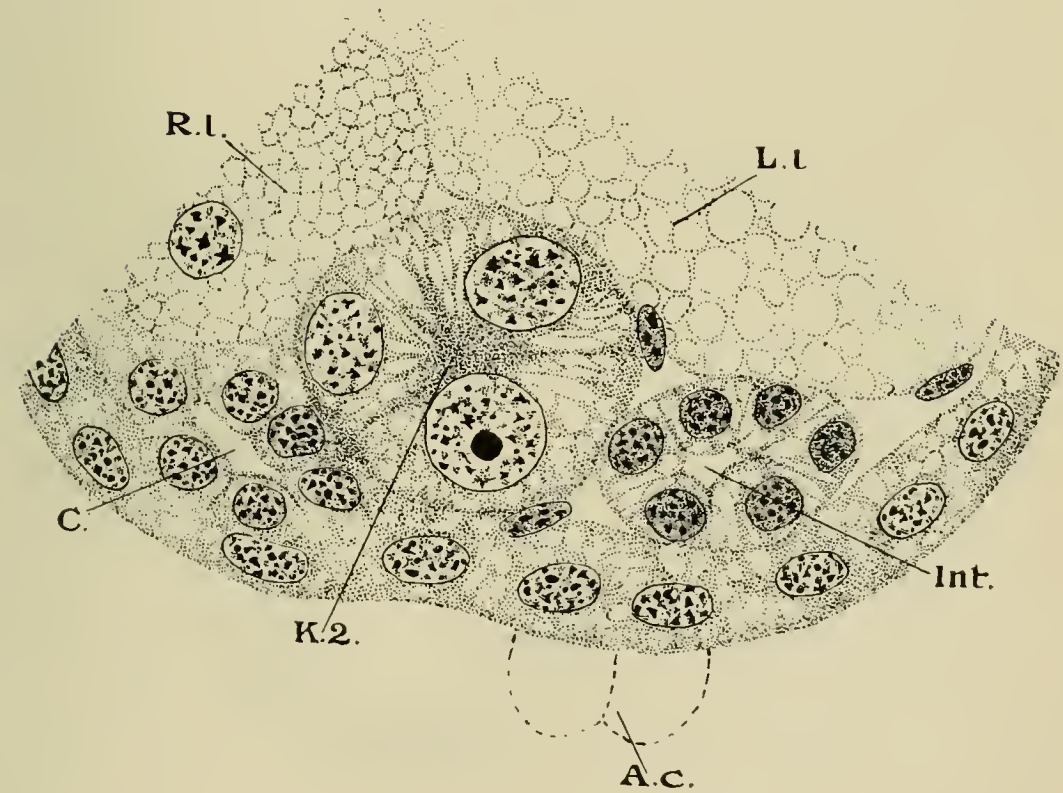
[1] Text-figs. 15–20 are drawn from sections 3 μ thick, with an Abbe camera with $\frac{1}{12}$ oil-immersion and a compensating eye-piece. Zeiss, No. 8. except Text-fig. 20, which is much less highly magnified. N.B.—For signification of the lettering of figs. 15 to 20 see the explanation of the same letters in Pl. 22, given on p. 539.

Text-fig. 17.



Section through the same region of an embryo in the same stage as in fig. 4 of Pl. 22, showing the first signs of the accumulation of mesoderm cells in which the cœlom is formed at a later stage.

Text-fig. 18.

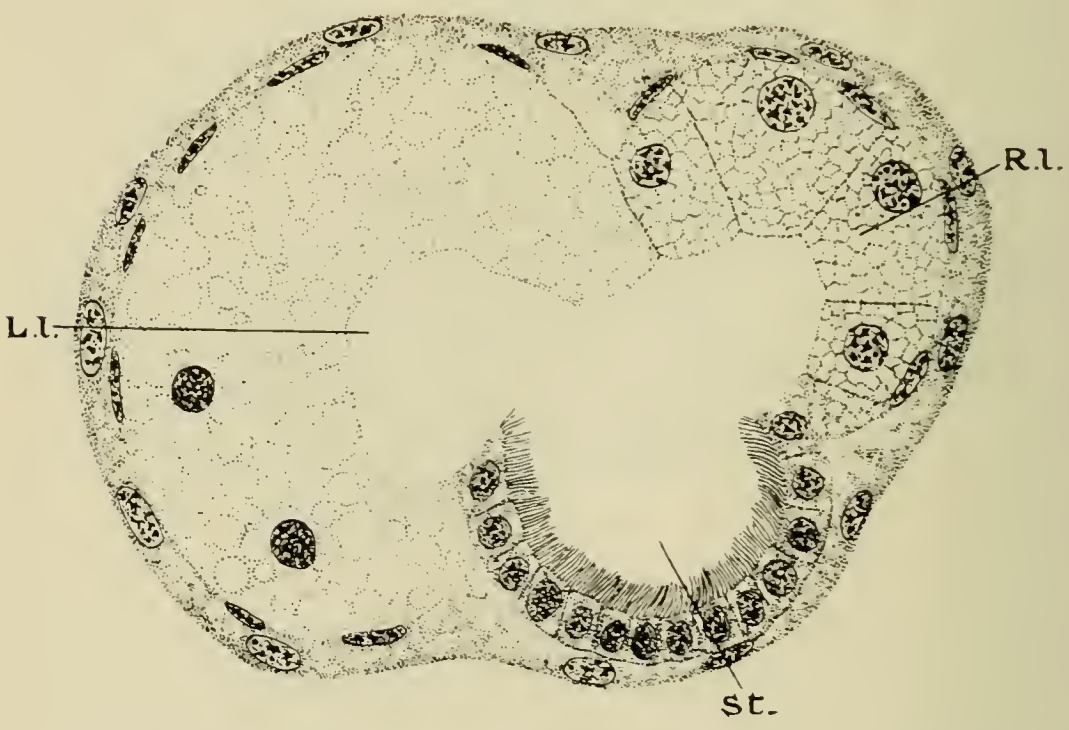


Section through approximately the same region of a slightly older embryo. The cœlom is now a definite cavity bounded by the mesoderm cells. The secondary kidney is here shown cut across its long axis.

Text-fig. 19.

Section through the same region of an older embryo, but taken almost at right angles to the section drawn in Text-fig. 18. The cœlom has increased in size and is beginning to extend dorsally between the body-wall and the stomach. The secondary kidney is cut longitudinally to show its vacuolated structure and the duct opening into the mantle-cavity.

Text-fig. 20.



Transverse section through an embryo at a stage corresponding to fig. 6, showing the openings of the right and left livers into the stomach.

EXPLANATION OF PLATE 22,

Illustrating Mr. A. M. Carr Saunders and Miss Margaret Poole's paper on "The Development of Aplysia punctata."

Explanation of Abbreviations in Figures.

A. Anus. *A.c.* Anal cells. *C.* Cœlom. *C.c.* Cerebral commissure. *C.d.e.* Dorsal extension of cœlom. *C.g.* Cerebral ganglion. *C.v.e.l.* Ventral extension of cœlom on left side. *C.v.e.r.* Ventral extension of cœlom on right side. *F.* Foot. *Int.* Intestine. *K.*1. Primitive kidney. *K.*2. Secondary kidney. *L.l.* Left liver. *M.* Mouth. *M.c.* Mantle cavity. *Ms.* Mesoderm. *Œs.* Œsophagus. *O.* Otolith. *Ot.* Otocysts. *P.g.* Pedal ganglion. *R.l.* Right liver. *Sh.gld.* Shell-gland. *St.* Stomach. *V.* Velum.

N.B.—This explanation of lettering applies to Text-figs. 15 to 20 (pp. 536–538), as well as to the figures on Pl. 22.

Fig. 1.—Embryo at the stage immediately before the beginning of rotation, seen from the right side.

Fig. 2.—Same embryo seen in diagrammatic optical section; the ectoderm is represented as peeled off from the right half of the embryo and thus seen in section.

Fig. 3.—Slightly older embryo represented as in fig. 2.

Fig. 4.—Slightly diagrammatic view of an embryo about twenty-four hours older than that shown in fig. 3, seen from the right side.

Fig. 5.—Similar view of older embryo, showing first appearance of the cœlom (coloured red).

Fig. 6.—Similar view of still later stage. The embryo has now assumed its characteristic veliger form.

Fig. 7.—Similar view of an embryo a few hours before its emergence from the capsule.

Fig 8.—Similar view of a free-swimming larva.

Fig. 9.—Free-swimming larva seen from the dorsal surface.

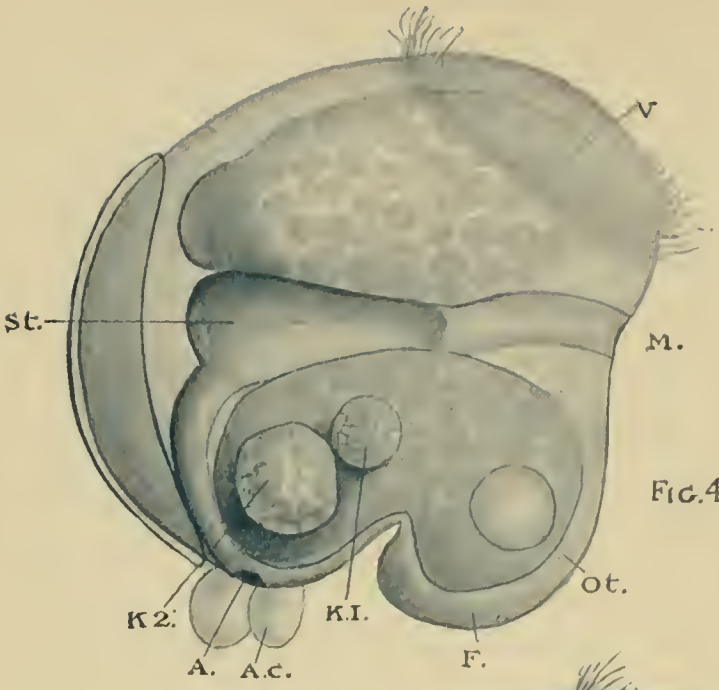
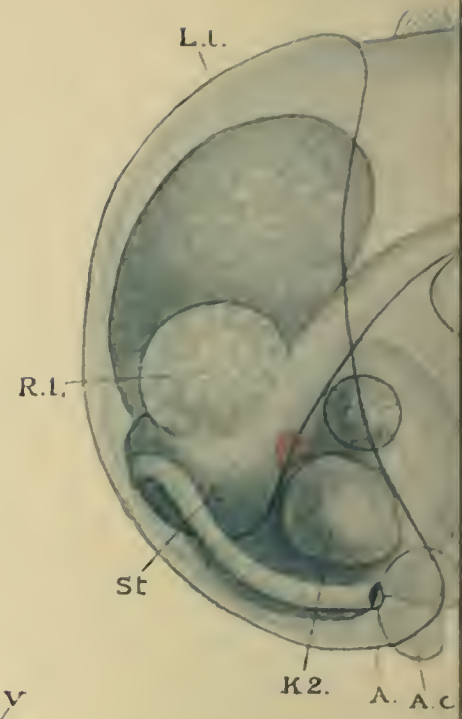


Fig.4.



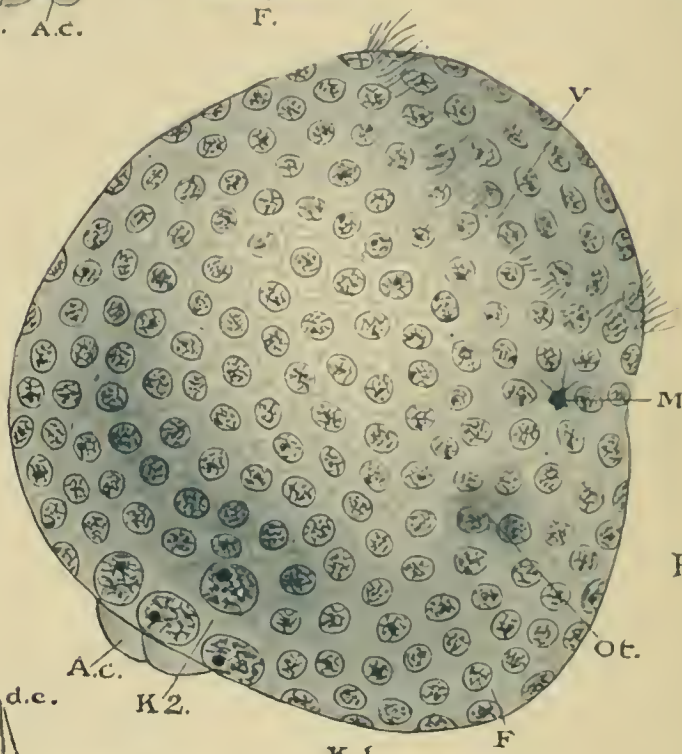
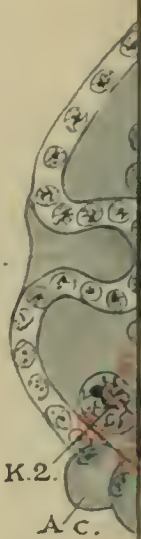


Fig.1.



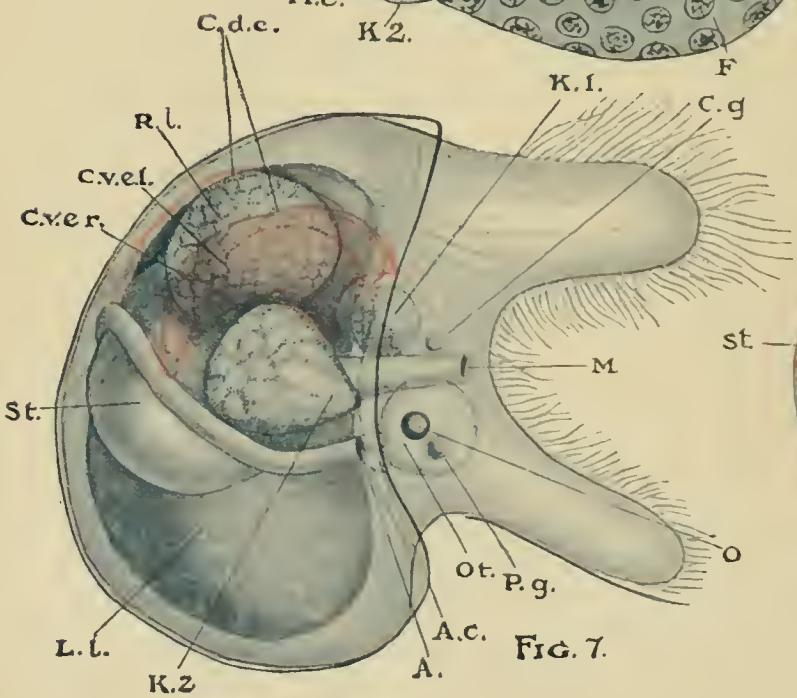
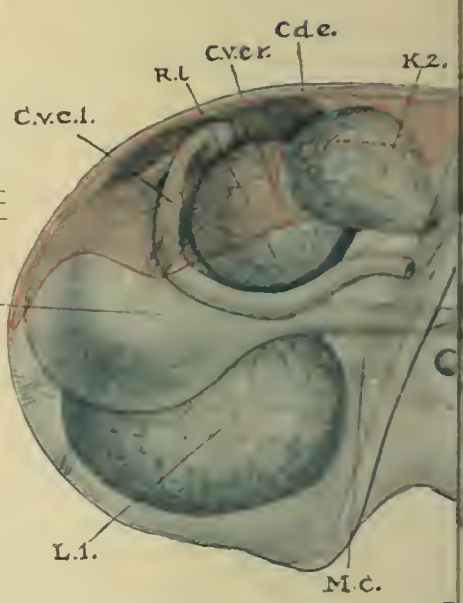


Fig. 7.



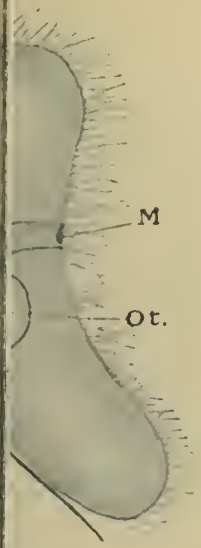


Fig 5.

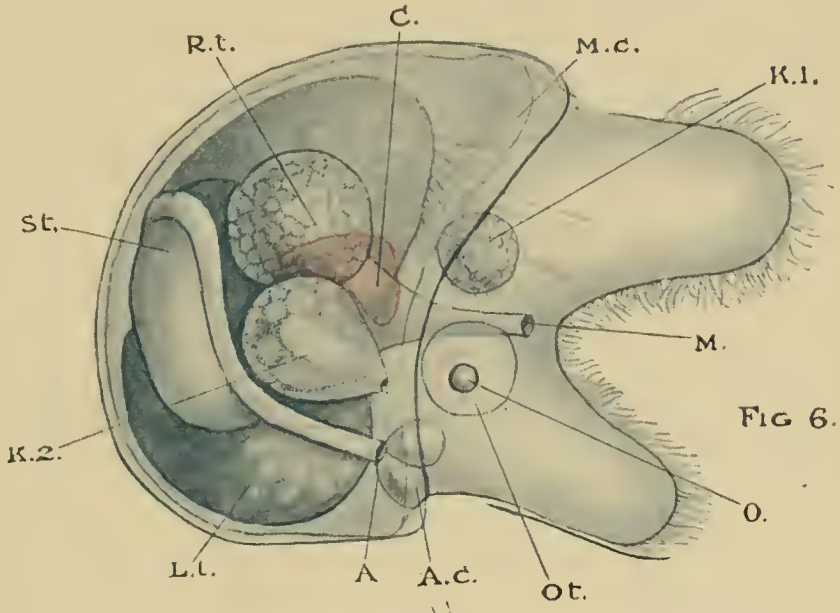


Fig 6.

Fig. 2.

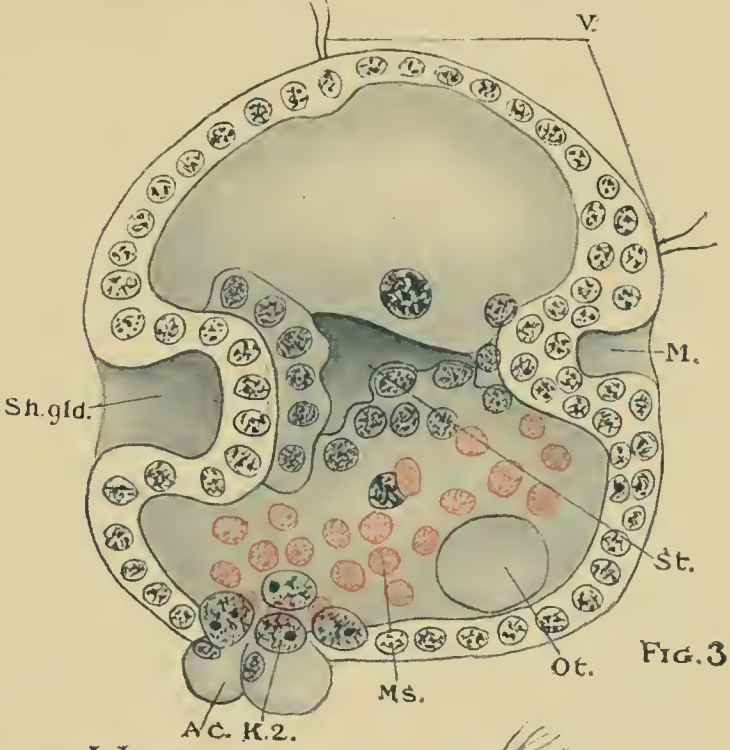
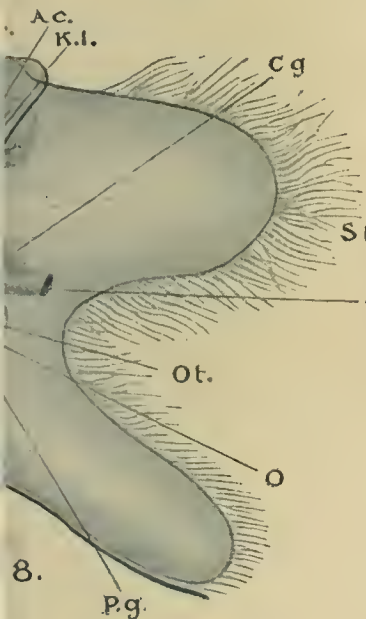


Fig. 3.



8.

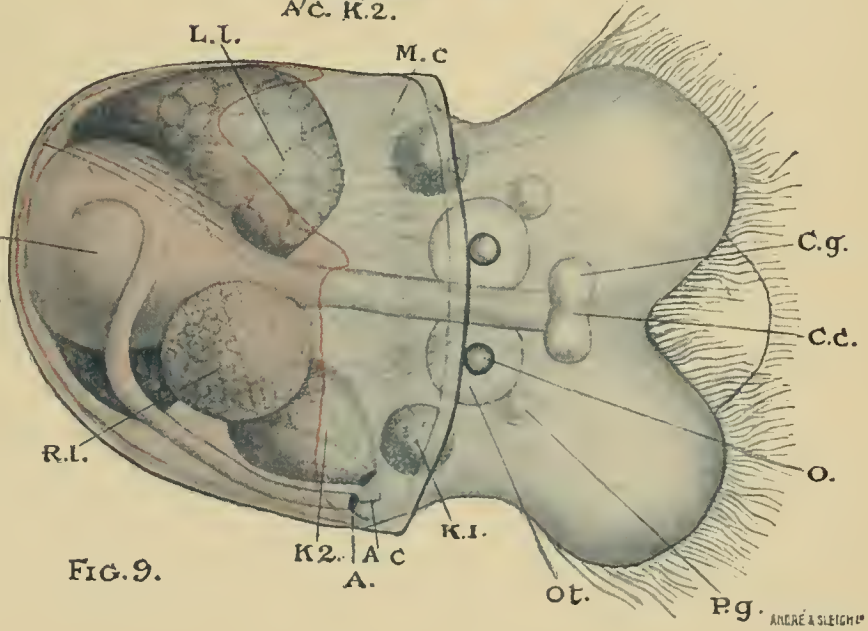


Fig. 9.

The Relation between Light and Pigment-Formation in Crenilabrus and Hippolyte.

By

F. W. Gamble, F.R.S.,

Mason Professor of Zoology, University of Birmingham.

With Plate 23.

Contents.

Introductory.

This paper is a continuation of the series hitherto published in conjunction with Professor Keeble (1900–1905), and contains a further instalment of experimental results of a research upon the colour-physiology of the prawn Hippolyte varians, and the wrasse Crenilabrus melops. The work was carried out by the author during the last three years, in part at the Plymouth Laboratory of the Marine Biological Association, in part at the Millport Marine Station, and also at Manchester University. To the directors of these laboratories and to the staff of the Plymouth and Millport Stations, the special thanks of the author are due for the unstinted help which they have always been ready to give. His former colleague, Professor Hickson, has given the author ever-ready assistance and much helpful criticism.

I. The Influence of Surroundings (Algal Backgrounds) on the Colour of Crenilabrus Melops.

(1) Introductory.

The immediate object with which this experiment was undertaken was to ascertain whether in the young stage of the fish there was a sensitive period at all comparable to that which is possessed by Hippolyte. I had thought that by exposing the young fish to backgrounds of diversely coloured weeds it would be possible to obtain some light as to the origin of the colour varieties which the wrasse exhibits.

As in the case of Crustacea these colour varieties may be classed under two heads: First, the individual colour forms, which show a series of more or less marked vertical bars on a variable body colour, and second, the colour phases exhibited by any given individual.

The Labridæ offer an exceedingly rich field of research for

experiment on both these lines. It is well known that the ballan wrasse varies through a series of monochrome, barred and spotted types of coloration from deep red to blue, and it has been ascertained by Holt that a given individual is capable of passing through colour phases from a spotted to a uniform livery with accompanying changes of colour. It is also asserted (Noé and Dissard), that these colour varieties are associated with the substratum over which the fish range. Thus Gourret, in his beautifully illustrated memoir on the wrasses of Marseilles, describes the varieties of several species, associated respectively with Zostera and with Nullipore-grounds, and the seasonal changes which they undergo.

The existence of a close relation between the coloration of many animals and that of their surroundings is a well-established conclusion. In the particular and striking case of Hippolyte varians, the development of this relation has been shown by Professor Keeble and the author to take place rapidly if young transparent animals are placed with algæ in a strong light. Thus an experiment conducted in bright sunshine at Tregastel showed that out of fifteen colourless or pale red lined Hippolyte, eleven became red after two days' association with red-brown weed; and that eight out of twelve became green on green weed in the same time. Analogous but much slower changes have been established by entomologists for sensitive geometrical moths during their early larval stages. Except for this group, however, the amount of experimental evidence on the factors that determine this colour sympathy is very limited. Certain insects excepted, Crustacea are apparently the only class in which the action of the environment has been tested; and even here the light-factors that determine the development and distribution of one or more pigments so as to produce an effect in harmony with the coloration of the environment, are quite unknown.

With a view of determining these factors I undertook in 1907 a series of experiments with one of the wrasses, the common gold sinny (Crenilabrus melops).

Young specimens of ballan wrasse were unfortunately not available at Plymouth, but this species would be an even more suitable one for such an investigation.

(2) Pigments and Colour Changes.

In the case of Crenilabrus melops the coloration is of a barred type. The head is marked with streaks of colour associated with the brain and with the lateral line organs on the operculum and jaws. The tail is usually marked by a central black spot, and the greenish or yellowish trunk is traversed by six or more vertical dark brown bars which extend from the dorsal fin to the anal, but do not cover the cœlomic region. This species in its young state is the most abundant of the wrasses in Plymouth Sound.

The arrangement of the pigment is as follows: Four colouring matters contribute to this result—blue, black, yellow, and red. Contrary to the statement by Krukenberg that the blue colouring of wrasses is due to a special pigment but is an optical colour merely, I find that in Crenilabrus melops a blue substance is associated with the skeleton in such a way as to give the young animal a transparent pale blue tone when the chromatophores are contracted. The nature of this substance, which, so far as I know, has not been previously recorded, is probably not pigmental, nor has it yet been determined. The green skeleton of Belone and the "vivianite" associated with some old red sandstone fishes possibly contain allied substances. Around the blood-vessels there is also a diffused blue substance, which is most easily noticeable in the fins and the peritoneum, and forms a blue line along the aorta. The yellow and red pigments form a network derived from yellow or orange chromatophores scattered over the back and flanks and along the fin-rays. The combination of this yellow network with the underlying diffused blue pigment and the blue skeleton gives a green tinge to the young fish, whilst the expansion of red pigment gives a ruddy colouring; when both red and

yellow chromatophores are expanded a dull yellowish-brown ground colour results (Plate 23).

The vertical stripes are due to the development of black and red chromatophores along six somewhat irregular bands, beginning just in front of the dorsal fin and ending at the base of the tail fin. These bands of chromatophores are of considerable interest. They occur in their most marked form in the superb cross-striping of coral reef Labroids and other families, but they also appear under stimulation as a series of evanescent banded markings on the skin of unstriped fish. The common Crenilabrus rupestris shows this very well. When at rest it is of a nearly uniform brown or dull reddish colour, but on being handled or when transferred to contrasting surroundings the body is seen to be overspread in a wave-like manner by bars of a deeper colour, which may continue to come and go in blushes. Again, Holt has recorded the appearance and disappearance of dark transverse bars in the common ballan wrasse (Labrus maculatus). In this case the fish had exactly the same property of expanding and contracting the metameric tracts of chromatophores without altering the body colouring. Sometimes, indeed, the bands disappeared almost entirely and the fish became of a uniform green colour. Crenilabrus rupestris has, at least in its younger stages, the same property. It may, and usually does, exhibit a banded appearance, but the bands may be extinguished and the body assume an almost uniform green tinge.[1]

The presence of these bars of colour is by no means wholly dependent on the nature of the surroundings. In Crenilabrus melops they tend to appear under conditions that favour expansion of pigments, but they also appear instantly if a fish is transferred from white to dark vessels. Tactile stimuli are especially effective in bringing about alternate flushing and pallor along these tracts. It is clear that they are more or less metamerically arranged tracts along which

[1] Since this was written the observations of Townsend (1909) and of Tate Regan (1909) have revealed an unexpectedly wide range of rapid colour-changes in tropical fishes.

nerve impulses act on the chromatophores. The phenomena, in fact, recall the pilo-motor or goose-skin reflex in man. Recent physiological researches (Van Rynberk) have shown in certain Pleuronectids that the ganglia of the sympathetic system supply each a definite transverse band-like region on the upper side, and that these regions overlap one another to the half of their width. Stimulation of these regions by induction currents produces contraction of the chromatophores. Section of the spinal nerves and of the rami communicantes of the sympathetic, leave the regions in question dark and their chromatophores permanently expanded.

This power of localised colour change is still very imperfectly understood. The development of the affected regions has not been undertaken, nor is the heightened coloration of the breeding season as yet in any way explained. It is therefore of some interest to note that in the case of the uniformly coloured adult Ctenolabrus rupestris I have been able to observe the banded pattern appearing in the post-larval stage 10 mm. long. The pattern at that stage differs but slightly from the livery of "melops," but the difference is that in the former the pigmentation arises in the form of these transverse bars separated by clear areas, whereas in melops, so far as I have observed the species, the barred pattern is inter-connected by diffused chromatophores. The subsequent monochrome pattern of "rupestris" is evidently derived from this earlier-barred one by development of interstitial pigment: but the presence of the bars even in the adult is revealed at the moment when under stimulation the skin becomes traversed by dark segmental bars alternating with areas of pallor.

(3) The Influence of Daylight reflected from the Algal Backgrounds.

Experiments on this problem were carried out as follows: The young wrasses, obtained by hand-netting over Laminaria fringes, were placed in clear glass vessels, and these, in turn,

were immersed in bell-jars filled respectively with Laminaria saccharina, Nitophyllum, and Ulva. Similar batches of wrasses were also placed in dark-bottomed and porcelain vessels, and in complete darkness. A double circulation was maintained, and the weeds were renewed twice a week. The young fish were fed with tow-nettings and with amphipods. The bell-jars stood on the south side of the laboratory, and received diffuse daylight on all sides.

Table I, pp. 574–575, gives the result of this experiment, which lasted for about three weeks.

The light reflected from the weed backgrounds is a most important factor in the case, and in previous experiments has not received sufficient attention. Different as the three weeds are to the naked eye, their spectroscopic examination reveals little diversity; indeed, the important differences in the light reflected from their surfaces (or transmitted through them) is the preponderance of one or more of the parts of the spectrum they transmit in common. Thus the green Ulva (in more than one layer) transmits from red to green, the green being somewhat more vivid than the red, but with no great difference of intensity. The red Nitophyllum also reflects red to green, but whereas the red is bright the green is exceedingly dim. Laminaria also transmits from red to green, but here the whole spectrum is very faint.

The results show that brown weed backgrounds produce the same effect on the coloration of young Crenilabrus melops as does a black background. The fish may undergo temporary flushing and pallor under the conditions of examination, and there is a tendency for the dark bands to lose their distinctness, but the result (Pl. 23, fig. 1) is decisive. The amount of red pigment is greater than in similar specimens exposed to light reflected from red or green weed. The reflected light is more dim and is diffused over the whole spectrum in the case of black backgrounds than in that of the brown weed, and it is probably this difference which explains a tendency to greenness in some of the records.

The results with green and red weeds en masse are some-

what surprising. The fish in both cases become green or greenish, with brown bands. There is no well-marked differential result, such as we shall find in dealing with transmitted light. The yellow pigment is well developed and well expanded; the red pigment, however, showed more expansion in green backgrounds than in red. This coloration is one intermediate between a white and a black background result. In the case of red weed the effective rays are the red or the red-orange, and so far from these encouraging the develop ent and expansion of the red pigment they seem to have the contrary effect, for from August 14th to 20th the records all run green, and though there is a subsequent period of darkening the red colour is not noticeable. The inference, therefore, is that in the case of red weed the red end of the spectrum is concerned in the formation and expansion of yellow pigment. In the case of green weed the results are so similar as to leave the specific action of the green rays uncertain. The red and orange rays, both of green and of red weed, appear to act alike, while the bright green rays of Ulva or the dull green of Nitophyllum does not exert any very definite action.

(4) Influence of Light transmitted through Algæ. (Table II, p. 576, and Pl. 23, figs. 2 and 3.)

When, however, the experiment of transmitting light through a thin layer of algal tissue is made, the results are not only more definite but also help to interpret the former experiment.

Table II gives the records obtained by exposing young wrasses of the same species as those employed for the preceding work, to light transmitted through two fronds of green, brown, and red weed respectively.

For this purpose two rectangular museum jars were employed. The inner one contained the fish, and was separated on three sides by a chink about 1 cm. wide from the outer, the space being filled with water and fronds of the

weed. The whole stood in strong diffused light and had a double circulation.

These experiments give a much more definite result. In green weed surroundings three of the fish lost in a week their initial greenness, and, together with the remainder, became entirely brown. Not only so; out of four, three showed considerable amounts of red pigment, and, as we shall see, contrasted very markedly with the other experimental batches (fig. 3).

The brown weed experiments gave a curious result. The fish were initially green and so remained, but in the red weed, out of five specimens, two of which had been originally greenish, three were now green and the remaining two showed only tinges of brown, although at first they had been barred with that colour.

In this experiment, therefore, green and red weed acted quite differently from each other; the green light-filter encouraged the brown colour and red pigment, whereas red encouraged green colour and yellow pigment. Brown surroundings resembled red ones in maintaining the green tint. The contrast between this result with transmitted light and the former with reflected light is so striking and puzzling that I, at this point, undertook the experiments (referred to on pp. 552–561) on Hippolyte with a view to clearing up the discrepancy.

The explanation, I believe, is to be sought in the spectroscopic analysis of the light transmitted or reflected by thin and by thick masses of the respective weeds. Thus Ulva, two fronds in thickness, transmits red to green, but as the thickness is increased it transmits orange, yellow, and green only. Single fronds of Nitophyllum transmit red to green, but several transmit almost pure red with a trace of green. Brown weeds transmit from red to green, the general intensity being low. Taking, therefore, the red as the purest screen, the remarkable feature about it is that along with the greater purity and intensity of the red light there is in the fish submitted to its action a green result due to the combina-

tion of many and well-expanded yellow pigmented chromatophores with an underlying blue pigment. The light, it is true, is not monochromatic, but in the succeeding section it will be seen that a similar result obtains even when monochromatic light is used.

Here, then, the result is that in strong red-orange light yellow pigment is well developed, but that red pigment is not. Turning to this green-weed experiment we have the converse conditions and result. In these fish a brown colour and red pigment are strongly developed (Table II).

Considering that the contrast of green weed to red weed lies in the extension and greater brightness of the green part of the spectrum, the inference is that the development of red pigment is due to the green light and that the strong red light encourages the formation of yellow. The two together give a brown coloration.

Brown weed in a thin film transmits from red to light blue, but only the red end is of fair intensity. Under a brown screen the fish maintain their green colour and the contracted condition of the red chromatophores.

(5) Note on Coloration of Larval Gobies.

Before passing on to these experiments on Hippolyte, I may interpolate a short statement of results obtained by subjecting certain larval fish to varying illumination. It is to be expected that the discovery of sensitive and responsive species will prove a difficult matter, and these notes may serve to help future workers in their choice of material.

During the past summer I obtained the eggs of two species of Gobius (G. paganellus and G. minutus) with a view to determining the rate and direction of pigmentary response to cultural conditions. The larval chromatophores are either black or of that "yellow" colour which is only seen by reflected light. Specimens developed and hatched in darkness showed normal pigmentation. On green backgrounds (obtained as explained on pp. 561, 562) the appearance of the larvæ after a week's exposure and again after a fortnight

was not perceptibly different from that of those kept on red backgrounds, nor from control specimens kept in clear vessels uniformly illuminated. Larvæ of Lepidogaster gonanii and of L. bimaculatus were equally intractable. There can be no doubt that long-continued experiments are necessary owing to the slow pigmentary changes in these animals.

(6) Summary.

(Crenilabrus melops.)

Darkness (for five days) produces extreme contraction of all chromatophores.

Black and white backgrounds in white light (three weeks' exposure) gave respectively the usual dark brown and the light (green) colouring associated with this illumination.

Brown-weed background acts like black. Green and red algal backgrounds produce a greenish tint intermediate between the effects of black and white backgrounds.

Weed light-filters produce an entirely different effect from the weed backgrounds (Table II). Daylight transmitted through green weed induced brown coloration and considerable amount of new red pigment. Daylight transmitted through red weed produced green coloration and yellow pigment. Brown weed is too opaque for any differential effect to show itself.

These results, then, go to show that the action of algal backgrounds is complicated by the impurity of the colours transmitted or reflected from them. The nearer these approach to the complexity of white light the more does the background resemble a black or a white one, i. e. general contraction or expansion results. The purer the colour or the more intense the particular part of the spectrum, the greater is the development of pigment of complementary colour. These results, however, were too few to establish the relation, and I therefore undertook the following experiments on a more convenient subject, Hippolyte varians.

II. The Colour-physiology of Hippolyte Varians.

Previous analysis (1904) of the factors that determine the wide range of sympathetic coloration in Hippolyte varians has revealed :

(1) That at the time of birth the chromatophore system is constant in organisation, and offers such slight variations in the amount of the only true pigment (red) as to suggest that the colour of the parent does not influence that of the offspring.

(2) That the next known (adolescent) stage (4·5 mm. long) presents three colour patterns : Red-lined pattern (by far the most common), barred pattern (rare), and monochrome pattern.

(3) That these young, transparent, adolescent animals become green on green weed, or red on red weed within forty-eight hours if placed amongst a mass of weed strongly illuminated by direct sunlight.

(4) That having assumed the tint of their surroundings the young animals can be persuaded to change it without difficulty, but that in later stages this elasticity is lost and colour-change is only effected slowly or not at all.

(5) That the adolescent colour-pattern may become the adult one if the environment is kept constant, but that lined and barred patterns are in all probability transformed into a monochrome by the filling up of the interstices upon exposure to a more uniformly coloured background. These results place the chief efficacy of colour-development in Hippolyte upon external factors. The eye and nervous system control the response to background, but do not determine it. Inheritance provides paths along which pigment develops, but does not settle the colour or pattern. The young animal appears plastic, but the old one is a creature of habit.

The need for a more careful analysis of these responsible external factors and of their continued working has led to the following results. The variability of broods born of similarly and of diversely coloured parents and the prolonged action of

monochromatic light are the two chief problems dealt with here. The conclusion is drawn that when monochromatic light is made to fall upon all sides of the experimental animals, so as to obviate a strong background effect, the result is a pigmentation complementary to the colour of the incident light and also to that obtained in Hippolyte by the use of coloured backgrounds and white light.

(1) Methods.

Although none of the methods employed for rearing the larvæ of Hippolyte were thoroughly successful, the record of the attempts made on this very difficult problem may be of assistance to other workers. A means of obtaining a satisfactory solution is one of the most pressing needs of experimental biology.

The vessels used consisted of large bell-jars, supplied with an air- or water-current or stirred by a glass plunger. Seasoned vessels as well as sterilised ones were used; filtered, "outside," and tank-water were respectively employed; diatoms (Nitschia) and algal cultures (Phæocystis mixed with other green flagellates) were used as food. The vessels were shaded, exposed to diffuse light, and kept in darkness; the backgrounds were translucent, absorbent, and reflecting; the incident light used was monochromatic (red and green) as well as white light. The temperature was kept down to 16° C. by a water-jacket, and in other cases allowed to rise to 18° C. or over, but in spite of all these variations of treatment the larvæ only survived about ten days. It is possible that some means of removing the first sickly specimens would be a great improvement, and it is, of course, also likely that a better diet could be found. The larvæ, however, readily eat green flagellates and seemed to digest them.

The monochromatic screens used in the case of larvæ consisted of selected pieces of coloured glass (ruby or green) combined with coloured gelatine films. These were placed over the inverted bell-jars, the sides of which were converted into absorbing or reflecting backgrounds.

A continuous air-current was led into the water, and the coloured screen was cut so that its halves embraced the air-tube, which was blackened at this point. The junctions of the screen with the bell-jars consisted of black velveteen so as to cut out any oblique white rays, but it was found that great care is needed to avoid liquefaction of the gelatine films. A trial was made with Schott's coloured glass, but except the red the samples submitted were not monochromatic.

In order to observe the prolonged effect of monochromatic light, and to obviate the dominant influence of the background, fluid screens were constructed. To insure a fairly strong light the screen was made of one cell only, and not, as in the case of Landolt's original design, of two or more. A double glass vessel was employed consisting of two beakers or of two large cuvettes, the inner one standing on glass supports, so that its rim just cleared that of the outer vessel. The inner vessel was then provided with young, transparent Hippolyte in filtered water, and finely divided Ceramium was used as food. The space between the two was then filled with the colour filter until the level exceeded that of the water in the inner vessel, the top inch or so of which was rendered opaque. A cover of glass, or of glass and gelatine, was then placed over the double vessel, and the whole was then transferred to a shallow aquarium in a strong light. In one case a circulation of tank-water was maintained in the inner vessel. The main point of the apparatus is to provide a means of flooding the animals (which remain in mid-water attached to their weed) with transmitted coloured light, and thus largely to avoid the affect of light reflected from an absorbent or reflecting background, such as has been generally employed in previous experiments. The surfaces on which the vessels stood were either slate or dull white brick, but there was always a layer of the fluid, some 2 cm. in thickness, btween the bottoms as well as between the sides of the two vessels.

The coloured solutions employed consisted of the following: For red a strong solution of erythrosin in distilled water, the strength being increased until a 2 cm. layer cuts out all the

orange. Weak lithium carmine solution in a 2 mm. layer was used in 1909. For green a 60 per cent. solution of copper chloride with a trace ($\frac{1}{20}$ of the volume employed) of 6 per cent. potassium chromate gave a good result in 1·5 and 2 cm. thickness. For blue, ammoniacal solution of copper sulphate was used, a concentrated solution to which strong ammonia was added until the precipitate was thrown down and could be filtered off. Unfortunately this blue screen, probably owing to the ammonia exhaled, is very toxic.

The light employed was direct, or direct and diffuse, daylight. In the former case the vessels stood for more than half their depth in a tank placed on the south side of the Plymouth Laboratory. In the latter the vessels were placed about 10 ft. from the south window on the slate base of the table tanks. In 1909 the vessels stood opposite a north window on a glass shelf, and were illuminated from below by a mirror as well as from above and laterally. The temperature maintained by a flow of water around the outer vessel was 16·5° to 17·5° C. even in direct light; that of the inner vessel with a continuous water-current was 16° to 17° C. Other experimental batches were maintained in clear glass, and under white or black background influence as well as in darkness.

(2) Variability of the Larval Pigment.

The chromatophores of Hippolyte varians at the time of hatching usually contain a single granular pigment of a red (scarlet) colour. No true yellow pigment is present, but there is a substance in the chromatophores that is yellow by reflected light and brownish by transmitted light. This is very constant in all broods. A variable amount of diffuse blue pigment is associated with the red.

Previous investigations (Gamble and Keeble) have shown that "the progeny of females (in Hippolyte varians) with much red pigment have more of this substance in each chromatophore than have those derived from green parents in which red pigment is less abundant.

The question is of some importance since the initial amount

of this substance might conceivably influence the subsequent colour-history. It seemed, therefore, advisable to obtain more records of this varying proportion of red pigment, and also to determine the conditions which favour or inhibit its development.

The results obtained are shown in the adjoining Table, and are derived from a study of the offspring of some twenty parents. Larvæ from those Hippolyte which are pink, red, or brown, possess a fair amount of the red pigment in their chromatophores at the time of hatching (the greatest amount in the samples examined being in the red-lined female broods). Larvæ from colourless (extremely pale pink) female varians are devoid of red pigment; whilst larvæ of green parents occupy an intermediate position, some batches being coloured like those of brown or pink parents, but not so deeply; others from equally good green parents exhibit no red; and others, again, exhibit, unlike all the preceding cases, an inconstancy, and show traces of the red pigment in only 36 per cent. or so of the offspring.

Hippolyte varians.

Number and colour of female parent.	Amount of red pigment in the just-hatched larvæ.
2. Red-lined forms . . .	Much; constant.
3. Pink	Fair amount: constant.
1. Pink	Traces only in some, fair in rest.
3. Brown	Fair amount: constant.
6. Green	Fair amount; constant.
2. Green	Traces in 36 % of larvæ examined.
2. Green	Absent.
1. Almost colourless (pale pink) .	Absent.

These results are of interest in several ways. They confirm on the whole the earlier conclusion that excessively red colour in the parent is associated generally with excess in the early larvæ; but they also show that the offspring of green female varians show two types of coloration, namely, that with some red pigment and that with none; and further that the two

types may be combined in a single brood in the proportion of 36 per cent. dominant or pigmented forms.

This result at once suggests that green in the parent is of twofold origin; and the facts of earlier experiments support the suggestion. It has been shown (Gamble and Keeble) that green is both an independent stable colour-form and also a colour assumed by brown specimens on a transfer to green weed. Further experiments are necessary to decide whether the green parents with recessive red colouring are of the former type, and those with more dominant red pigment are of the latter colour-history. The new points that emerge are the absence of red pigment in certain broods, and its presence in only a percentage of others.

Repeated attempts were made to experiment with broods from an isolated parent under diverse conditions of light, food, and temperature, but without much success after the first week or ten days. The chief results obtained were (1) that zœæ developed and hatched in darkness (from brown parents that became green under these conditions) possess the normal pigmentation, thus showing that light is not essential to pigment development, and also confirming the suggestion just made, that it is those green parents which had been previously brown that give rise to larvæ with red pigment; and (2) that there is a steady increase in the amount of red pigment in broods of green parents. For example, the tint of zœæ of green parents approximated after a few days to the colouring of the larvæ of red parents. It is, therefore, doubtful whether the initial differences in pigmentation between the broods of similarly or diversely coloured parents are of any moment in determining the ultimate coloration.

III. (1) The Influence of Transmitted Monochromatic Light on the Formation of Pigments in Hippolyte Varians.

Previous work on the influence of monochromatic light (1900, p. 619, 1904, p. 356) upon Crustacea concerned itself

chiefly with short exposures made upon an absorbing or reflecting background. The results showed that the light acted irrespective of its colour according to the nature of the background, almost as though it were white light of low intensity. Moreover, experiments with coloured backgrounds of weed, against which young, transparent, almost colourless Hippolyte were exposed to direct sunlight, showed (1905, see Tables) that in two days, eleven out of fifteen prawns became red on red weed, and eight out of twelve became green on green weed. The coloured backgrounds, when flooded with white light, produced sympathetic colouring. The red was a mixture of red and yellow, the former predominating, the green a mixture of the same two pigments but yellow predominating. In both cases a diffuse blue pigment occurs also.

This result appeared to lend some support to the view of Wiener (1895) (which has since undergone elaboration [Bachmetjew, 1903]), and to suggest that the dominant rays of the background evoked especially that pigment or that group which agreed in colour with the reflected light.

In order to ascertain more fully the effect of monochromatic light, I determined to eliminate, as far as possible, this dominant influence of background, and to ascertain the result of exposure to incident light of one colour. So far as I am aware, the experiment in this form has not hitherto been undertaken. The starting-point for this experiment was furnished by young transparent Hippolyte varians taken by netting over Zostera beds and Laminaria-fringes. These fall into two groups: typical faintly red-lined forms provided with red and yellow chromatophores along the gut and nerve-cord, and with red ones at segmental intervals in the integument; and more uniformly coloured specimens with similar pigments, but with chromatophores more evenly distributed. In both cases the amount of pigment is not enough to give the specimens a decided tinge. They are similar to those used for the weed background experiments quoted above, and are figured on Pl. 23, fig. 4.

The vessels which were used are described above (p. 554),

and the conditions of the experiment were such as to flood the animals with monochromatic light on all sides. The weed chiefly used for food was the natural food-plant, Ceramium; a little fine green weed was used in one of the red light experiments. The vessel was surrounded on three sides by the fluid colour screen and rested on a faintly reflecting surface, so there was no strong background effect. The light employed was direct and diffused sunlight, and the effects of heat and of ultra-violet rays were largely obviated by the conditions of the experiment.

The results of the experiment are given in Table III, pp. 577, 578, and show that whilst the Hippolyte, in white light, developed into brown forms containing both red and yellow pigment in about equal proportions, those in red light passed through a brown stage, but ultimately (three weeks) became green, some remaining, however, reddish-yellow in 1909, whilst the survivors in green light became bright carmine. In other words the ultimate colour in this experiment is the complement of that of the incident light.

The details of the end-result show clearly that the green Hippolyte produced in red light and the crimson Hippolyte produced in green light are peculiar and distinctive. The former possess yellow pigment in a maximally expanded state, and such little red as they possess is of a vermilion tint. Moreover, the yellow is of a distinctly greenish tinge and is accompanied by either very little diffuse blue or none. Thus the green colour in these experimental specimens under red light is largely due to an increase in the amount and quality of the yellow pigment accompanied by contraction of the formerly dominant red pigment (Pl. 23, figs. 6 and 9).

The crimson Hippolyte produced in green light is no less distinctive (Pl. 23, fig. 5). In contrast to the usual type of red forms, the yellow pigment has completely disappeared and the chromatophores are entirely filled with a deep carmine pigment suffused with a bluish tinge. The general deep carmine colour was new to me. Moreover, the chromatophores on the surface of the eye-stalks were abnormally developed.

In view of this very decided complementary colour-change the regrettable mortality that occurred in vessels exposed to green light in 1908 does not seriously diminish the value of this result, though larger numbers would add to its cogency. These were obtained in 1909. The experiments of 1908 and 1909 are compared on Table V and with the other experiments of this paper on Table IV.

(2) The Influence of White Backgrounds and of Monochromatic Backgrounds in White Light.

The effect of short exposures to the influence of white (porcelain) and of black (cloth or paint on glass) backgrounds on the colouring of young and old Hippolyte has been fully treated in a previous memoir.[1] It was there shown that whatever the quality or quantity of the light employed (within the experimental limits), the background effect dominated, producing contraction if white and expansion if black. It occurred to me, however, to see whether the same results would follow a long exposure made with young specimens in which the pigments were rapidly developing.

The results of a month's trial are of considerable interest. The Hippolyte on black surfaces simply followed the usual procedure under such conditions, and developed maximal amounts of red and yellow pigments, which gave them a deep reddish tint. On the white background, however, after a first phase of transparency, they began to develop red pigment along the nerve-cord, and finally became uniformly marked with a ventral red stripe, whilst over the rest of the body the pigments were reduced to microscopic dots or disappeared. This remarkably adaptive result was obtained in diffuse light, the top of the deep porcelain vessel being covered with muslin, through which a stream of water was maintained from a tank above (Table IV, p. 579).

In 1909 these background effects were extended so as to include the results of red and green. The vessels employed were large museum jars, painted, except for a large rectangular

[1] (1904), p. 353.

window, with several coats of pure paint. Spectroscopic tests showed that the red was pure and that the green paint reflected only a trace of blue in addition to the whole of the green light. These vessels were kept under a water-circulation and faced a south light. Finely divided pieces of Ceramium were employed as food. The Hippolyte used were small, almost colourless specimens, similar to those employed in the other experiments on coloured light.

The results of exposure to these monochromatic backgrounds was very decisive (Table IV, p. 579). Upon the green one the development of pigment was arrested. The Hippolyte assumed a semi-nocturnal (green) tint, and remained with the red pigment contracted throughout the experiment. This green colour is, however, not retained if the background is changed. Under these circumstances the animals revert to the pale red-lined colour variety which they exhibited initially. Upon the red background, on the other hand, the red and yellow pigments had considerably developed, and after a month's exposure gave a bright orange-red tint to the specimens, and this persisted after change to other backgrounds. It would be of interest to know whether Minckiewicz (1907–8), who has also obtained results of this kind with Hippolyte, tested the permanent or transient nature of the induced colouring.

IV. The Food of Hippolyte as a Possible Source of Pigment.

The relation of Hippolyte varians to the algæ of its choice is a distinctive one. The peculiar features of this species, the range and cryptic character of its variable coloration, its choice of, and tenacity of hold upon its weed, its distribution, and its food are all bound up with the presence of these plants. It is possible that Idothea and some Amphipods are equally intimately related to their habitat, but among macrurous Decapods Hippolyte varians is probably unique in this dependence upon its algal environment.

In former papers on the subject, the relation existing between the pigments of Hippolyte and the coloration of its surroundings was explained as due to light effects, as if the weed backgrounds in virtue of their disposition, of their luminous character, and colour, acted as stimuli to the chromatophores of the prawn. However, before we accept that explanation, the influence of two other factors must be considered: First, the effect of darkness on pigment-formation, and second, the source of these pigments, whether derivative or not. The first factor—darkness—is discussed on pp. 577–579, and it is there shown that the red (vermilion) pigment does not require the stimulus of light for its development, and that it increases in amount when the Hippolyte are kept in darkness. The yellow pigment, however, is more dependent on light for its formation and increase, diminishing in amount in specimens kept in darkness, especially if little or no food is supplied to them. The crimson pigment and the diffuse blue colouring matter are not at present investigated from this point of view. There is evidence, however, that light is essential to the production of all varieties of Hippolyte, except the reddish-brown ones. The other factor—the source of pigment itself—is less known than are the conditions which determine each particular tint. The colouring matter of the food is one possible source, and this has to be briefly considered, since, if proved, it would simplify the problem of sympathetic coloration. That the sub-hypodermal colours of caterpillars and beetle larvæ are due to diffusion of fatty pigments from the food-contents of the gut is a conclusion reached both by Poulton and Towers, though the physiological details of this remarkable process have never been ascertained. But the hypodermal colours of these animals are of an entirely different nature from those of Hippolyte, and appear to be determined by enzymes, elaborated by this layer acting upon the "primary" cuticle or retained within the hypodermal cells. In Hippolyte and in Crustacea generally (as in the insect larvæ), the first formed pigments are developed independently of the plant-food present in the

mother, and it would be of great interest to know how they were formed.

In order to test the influence of food-pigments on the development of pigment in Hippolyte, the following experiment was carried out at Millport, N.B. A series of double glass vessels were prepared, the Hippolyte being placed in the inner chamber and a mass of weed in the outer one. Two series of pressure-bottles, one in diffused light, the other in darkness, were set up for isolated specimens. The food employed was chosen from the following: The natural alga chopped up into fine pieces so as not to act as a massive background; etiolated Laminaria, also subdivided; the muscle of Hyas, the colourless ovary of Hyas, and the scarlet, mature ovary of the same crab. The specimens of Hippolyte employed were 5–7 mm. in length, colourless, and tending on a black background to assume a faint brown-lined colour pattern.

TABLE A.—Feeding Experiment.

Colour of Hippolyte after exposure to contrasted colours in food and surroundings, Millport, 1909. Colourless foods employed are crabs' muscle, etiolated Laminaria, and colourless ovary of Hyas. The Hippolyte used were from 6–8 mm. long and colourless to the naked eye. Experiment lasted seven to ten days.

Colour and nature of surroundings.	Colours of foods employed.			
	Colourless.	Red (scarlet ovary).	Brown (algæ).	Green (algæ).
Darkness	Pale brown-lined	Reddish-brown	—	Reddish or colourless, 1 green.
Green algæ	Green	Green	2 green, 1 brown	—
Red algæ	Pinkish, Greyish	—	—	—
Brown algæ	2 brown, 1 grey	—	—	—
Parti-coloured yellowish oilcloth	Black-lined	Brown-lined	Brown-lined	Brown-lined.

The results are shown on Table A, and at once bring out the fact that colourless muscle, white or red, ova are greedily taken up, but that the background is the dominating factor in the resultant coloration in daylight. Thus against a background of green weed Hippolyte fed with colourless food, with red ovary, and with fine brown weed became green. In darkness, however, the amount of pigment in the food has a rough relation to the resulting colouring that will need further experimental testing, but there is no good evidence that the colour of the food determines that of the prawn.

V. Analysis of the Coloured Light Experiments.

(1) In green light, and amongst red weed, Hippolyte develops crimson and deep, not superficial, colouring.

The presence in the experimental vessels of a fair quantity of finely branched red weed (Ceramium) would, under the action of diffused, strong green light act as a black background, and this, as we know, in the presence of white light, encourages the formation of vermilion and yellow pigments, and these are most notably absent.

The crimson effect in green light cannot, therefore, be merely due to dim light acting on a dark background. It must be due to a distinctive factor not present in the other experiments, and that factor can only be the green rays. In the presence of these rays, not only is the crimson pigment developed, but the vermilion and yellow pigments are dismissed. Whether a similar result would follow if a colourless food were used is of course a subject for further research.

The most striking feature of this crimson colouring obtained during exposure to green light, is the fact that it is complementary in colour to that of the incident light. This relation may have a considerable significance. In an earlier paper (1905) it was pointed out that strongly insolated Hippolyte showed mobile fat in their chromatophores, and as this fat disappeared in specimens transferred to darkness there was some ground for the inference that the production of this

fat was associated with the presence of light. If that were so the assumption of a complementary colouring would be obviously the best means of absorbing the maximum amount of coloured light, and of obtaining any other benefit which light might confer upon metabolism. Under the conditions of deep water, where the green or green-blue rays have filtered down from the surface, such a colouring would be the most efficient absorbing pigmentation, and it is well known that in hauls made from the deeper water of the English Channel the Hippolyte are uniformly of a crimson colour.

The facts as to these crimson Hippolyte produced in green light would be most comprehensively explained by saying that the red Ceramium acted merely as an excitement to coloration, but that the carmine pigmentation is produced under the direct stimulus of the green light employed.

Red light (2)	Green weed	Green coloration	Superficial and deep chromatophores.
	Red weed	Yellow or brownish-yellow	

The action of red light is less easily analysed. The constant effect associated with it, is the production of yellow pigment and the maximal expansion of that pigment into networks producing a grand colour. Then, according to the absorbent or reflecting nature of the background (i.e. green weed or red weed), we have a green or a brownish tint, in the latter case accompanied by a development of scarlet chromatophores both at the surface and along the lines of the alimentary tract and of the nerve-cord.

In the case of red light, therefore, it would seem that the direct action of the rays lies in the production of yellow pigment, and that the nature of the background, indirectly modified by the further action of the red light, modifies this yellow coloration less or more. If the background be red, the action of the rays is intensified, and a red background is thus instituted. Probably this is the factor that gives the scarlet chromatophores, for, as will be seen subsequently, that is the effect of a red background in white light; the resultant colour is then brownish-yellow; but where, as in

the case of green weed, the background is of a less luminous character, the red colour contracts in the Hippolyte and the resultant coloration is then green, owing, in some cases, to the presence of diffuse blue mingling with the yellow network, and in the longest experiment to an apparent change in the pigment from yellow to green. The most important and most clear influence of red light, however, is the spread of the yellow pigment.

These results are so strikingly dissonant from those obtained by subjecting Hippolyte to green or to red backgrounds that an explanation is clearly called for. They differ not only in being totally opposed to the sympathetic colouring so characteristic of the latter, but also in being slowly acquired. It may fairly be asked, if red light reflected from red surroundings gives red Hippolyte, why does red light diffused give green or yellow ones? The same contradictory relation obtains between the action of green surroundings and diffused green light.

In answer to this objection attention may be drawn to the double nature of the light affecting Hippolyte under natural conditions. There is the light reflected from the background and there is also the general diffuse light.

The rapid sympathetic background colour-relations obtained experimentally have been made in strong daylight, and as the depth of water is increased or as the red end of the spectrum is cut off the conditions of the experiment are materially altered. A strongly coloured background becomes black in every light except that of its own colour, and in the presence of it we should expect the usual black background effect (brown, i.e. red and yellow pigments) to be produced in Hippolyte in any light except that with which it agreed in colour. But whilst this background effect is an undoubted factor, its potency is determined by another factor, namely, the definite action of diffused monochromatic light. The action of many rays has yet to be determined, but from the foregoing account a case has been made out for the action of green and of red light. This action, though slow, is very

precise, and it would certainly help to account for the crimson and yellow colouring found in deep-water and shallow-water Hippolyte respectively.

The results, then, of these two factors, the action of diffused coloured light and that of backgrounds in white or monochromatic light, are not contradictory. They are the two factors which, so far as we yet know, are associated in the production of pigmentation in Hippolyte. The green specimens on Zostera are green, not only because they are on a green background in bright or fairly bright light, but because at or near the surface of the sea the red rays are most potent, and their action is to produce that network of yellow pigment in Hippolyte, which is the basis not only of green tints but of those yellowish tints that this animal assumes on the etiolated parts of Zostera, and of the brown specimens on various brown weeds so characteristic of the Plymouth littoral flora. The diffuse red light, on penetrating to more densely absorbing backgrounds, such as coarser brown weeds, is checked in its action upon Hippolyte by the tendency for such backgrounds to produce red pigment in them. Hence the absence in such cases of that more precise colour-relation to the incident light. The brown pigmentation contains many red and yellow chromatophores, but the red is scarlet and not the crimson of the deeper zones.

Passing out of the range of the action of the red rays, the characteristic zone of the Florideæ is encountered, and it is in this zone that the green rays are more potent. Their effect in producing crimson pigmentation is seen in parti-coloured specimens of the red-lined variety and in occasional pink specimens of the Laminarian zone, but it is not until a fair depth is encountered that their action is made clear by the dominance of this peculiar carmine pigment, which has hitherto been confused with the vermilion or scarlet one under the confusing term "red." No doubt there are similar effects of yellow, orange, and blue rays to be analysed before a full analysis of the coloration of Hippolyte can be given. The main conclusion derived from these experiments is that

Influence of Light on the Colours of Lepidopterous Pupæ. (After Poulton, Petersen, etc.)[1]

Light.	Colour of background.	Spectrum of background.	Resulting colouring.		
			Vanessa Io.	Pieris rapæ.	P. brassicæ.
None	—	—	Irregular (dark and light)	—	Irregular.
White	Red	Red	Darkest)	Dark (Poulton), Light (Peterson)	Dark green.
—	Orange	Red to yellow	Light (green)	Green	Green.
—	Yellow	Red to green	Very light (green)	—	—
—	Light green	Red to green (red to yellow largely absorbed in some experiments)	Light (green)	Light (green)	—
—	Dark green	General absorption least in green	Dark (brown)	—	Dark.
—	Blue	General absorption least in blue	Dark	—	—
Red (pure)	White	—	Light (green)	—	Green.
—	Light wood	—	Ditto	—	—
—	Orange	—	—	—	Intermediate.
Red (red and some yellow)	White	—	Light (green)	Green	—
—	Light wood	—	—	—	—
—	Dark	—	Unknown	—	—
Yellow (red to green)	Light	—	Green	—	Light.
—	Dark	—	—	—	Darker.
Green (pure), green glass	Plain wood	—	Green	—	—
	White	—	Light (green)	—	—
	Dark	—	—	Dark	—
	Green	—	Darkish (V. urticæ)	—	—
Green (some red, yellow, and green)	White	—	Darkish	—	—
	Red	—	Ditto	—	—
	Orange	—	2 light, 3 dark	—	—
	Blue	—	Dark	—	—
Blue. (General absorption least in blue; some red, yellow green and blue rays are transmitted)	Light	—	Ditto	—	Dark.
	Dark	—	Ditto	—	Ditto.

[1] The references to these papers are given fully in Bachmetjew's work quoted on p. 582. See especially 'Trans. Entomol. Soc. London,' 1892.

the pigments developed in Hippolyte, when kept in diffused monochromatic light, are not the same as those which appear in specimens kept in daylight on a background reflecting these rays. On a red background in white light, Hippolyte becomes reddish-orange; in pure red light it becomes yellowish or green. On a green background in white light Hippolyte becomes pale green. In pure green light it becomes crimson. On backgrounds of weeds, young colourless specimens speedily acquire the corresponding tint. Monochromatic light, then, when saturated, has an entirely different effect from the same light diluted with daylight. As we pass from the surface to the deeper waters of the sea this dilution becomes less marked. The "background effect," so potent in producing the more littoral colour varieties, becomes less overwhelming as the red and yellow rays are absorbed by the surface waters. Further down, in British coastal waters, the blue end of the spectrum is said to be absorbed, so that at eight fathoms the dominant light rays are greenish or bluish-green (Oltmanns[1]). Consequently the effect of saturated monochromatic light is most probably felt in the region below the eight-fathom line.

If this distinction between the effects of coloured backgrounds in white light and of diffused monochromatic light on pigment production is well founded, it should be supported by analogous results in other animals. Fortunately the work by Poulton and others upon Lepidopterous pupæ give a closely comparative result. As will be seen from the appended table extracted from their papers, the effects of monochromatic light are very different according as to whether the dominant rays are or are not diluted by white light. Although these experiments have not been made with a view to excluding background results so completely as those given in this paper, yet the distinction between the effect of red light, for example, when concentrated and when diluted, is quite analogous in the case of larval pigmentation in insects to its effect on pigment-production in Crustacea. As a pure

[1] 'Jahrbuch. Wiss. Botanik.,' 1892, p. 420.

concentrated light, both red and green rays act like orange-yellow ones in suppressing pigment. When diluted, however, with white light, red rays produce pigment and pure green rays do likewise. As a background in daylight, therefore, the monochromatic rays act in one way ; as a pure incident light they act in an opposite fashion. This apparently contradictory result is therefore supported by the evidence from experiments on two widely different groups of animals, Crustacea and Insecta.

What exactly, then, are the factors that determine the extraordinary close sympathetic colour-rendering of the environment in the pigmentation of these animals? First of all in both groups, light is not essential to the production of pigment. Poulton's results, as well as my own, show that dark-kept animals become dark coloured, though somewhat irregularly. In the case of Hippolyte darkness does not induce the formation of all the pigments. Red (vermilion), the dominant one, and yellow to a less extent (giving a brown coloration), are the only colours formed in the absence of light. In the insect larvæ, brown pigment is likewise formed in darkness, and develops as a sheath upon the green sub-epidermal layer. The action of light, then, in both groups is rather directive or inhibitory than effective. In the case of insects, the orange-yellow rays are apparently those which, when reflected from backgrounds, inhibit this brown pigment and allow the subjacent green pigment to confer its full value on the colour of the larva or pupa. In Crustacea the case is different; the action of these rays upon them is at present quite unknown. The colours are pigmentary, contained in chromatophores and not "hypodermal" as in insects, but the production of the well-known green, brown, and reddish varieties of Hippolyte is due mainly to manipulations of a reddish-yellow coloration which is formed in the absence of definite stimulation.

The light reflected from natural algal backgrounds is of a mixed character, but with some yellow, some green, and varying amounts of red in it. All we have to imagine is

that in the production of a green Hippolyte on Ulva the yellow and blue pigments are encouraged, the red discouraged. We do know that this effect occurs in pure red light, but in this case few red rays are reflected. We are driven to the conclusion that in daylight the yellow of Ulva directs the expansion and development of yellow pigment, and the green the expansion and development of blue pigment. In other words, we have here Wiener's effect or conclusion confirmed. But when the water deepens, the red (vermilion) pigment, no longer inhibited by light rays, develops more strongly, and yellow and brown, and even blackish, Hippolyte occur in response to the diffused background of brown weeds, the light from which contains chiefly red and yellow-green rays. At this depth the incident light has lost some of its red and yellow rays, and is of a more bluish-green colour. From this depth onwards the action of diffused light becomes more and more apparent, that of the background less so. In the dominantly green water the crimson and diffuse blue pigments of Hippolyte develop to the exclusion and repression of the red and yellow ones, thus giving the various shades of carmine, purple and violet, that characterise Hippolyte taken in deeper water and in deep, shady crevices near the shore. In a greater depth than that to which light extends, Hippolyte varians is not found. Indeed, it does not appear to extend beyond the range of some ten fathoms. In deeper water the genus is represented by Spirontocaris, the colour problems of which have not yet been investigated.

If we accept this conclusion, that carmine, purple, violet, are colour effects, related directly to the diffuse green light in which many animals of deeper water live, an explanation may be found for the prevalence of these colours in many other groups. For example, carmine is a tint acquired by some fish, Crustacea, many echinids, starfish, and corals. Violet or purple is an even more characteristic pigment of the deep-sea fauna. This purplish tint is complementary to green, and the relation has given rise to much speculation, but, so far as I am aware, the above experiments with

Hippolyte give the first indication that the purplish colour is actually developed in a few weeks when the animal is exposed to green light.

The significance of the scarlet colouring, so characteristic of abyssal Crustacea and of certain more shallow-water forms, e. g. Hemimysis lamornæ, is still obscure, but the observations made above as to the development of red (vermilion) pigment in young specimens kept in darkness may throw some light upon the subject. With regard to Hippolyte varians, the facts so far ascertained are these :

The red pigment is the first to appear. It arises in the larva, even if this is reared in darkness, and the amount at the time of hatching is roughly proportional to that in the mother. In adolescent specimens subjected to darkness the scarlet pigment increases in amount.

VI. Summary of Results.

Crenilabrus melops.

(1) The colouring of young specimens is due in part to the blue endo-skeleton and in part to chromatophores.

(2) On backgrounds of weeds these fish assume varied coloration. On brown weed they become brown, on green weeds green, on red weed green.

(3) In light transmitted through weeds, Crenilabrus assumes a colour, the complement of that which is most strongly represented in the incident light. Thus, in light mainly green, a brownish red colour (due largely to red pigment) develops. In light mainly red, a green colour (due largely to yellow pigment) develops.

Hippolyte varians.

(1) In any brood the amount of larval pigment (which is always red) is constant, and is correlated with the amount of red pigment present in the female parent in all colour-varieties except green.

(2) A given green Hippolyte throws one of three kinds

of young; red, colourless, or a mixed brood, containing red and colourless individuals in the proportion of nearly 3 : 1.

(3) This result suggests what is probable on other grounds —that green Hippolyte are of two, and possibly of three kinds: (1) Brown forms that have become green; (2) green forms that have undergone no change of colour; and (3) a cross between these two. In the absence of knowledge of the male parentage of the broods, the last suggestion needs confirmation.

(4) Light is not essential to the production of red pigment in the larva. Darkness does not prevent the continued production of red pigment in young forms.

(5) The action of monochromatic light upon the pigment-formation of Hippolyte is entirely different from that of a monochromatic background in white light.

(6) In pure red light, yellow pigment develops. In some cases this leads to a green coloration: in others the colour remains yellow.

(7) In green light a carmine pigment is produced, and any red or yellow pigment existing in the experimental batch is either destroyed or disappears almost completely.

(8) On a red background in white light, Hippolyte becomes reddish-orange.

(9) On a green background in white light, Hippolyte becomes green, but the colour is not retained if the batch is transferred to an absorbing dark background.

(10) Continued exposure to daylight and a white background produces hypertrophy of the red pigment along the nerve-cord and a disappearance of the red and yellow pigment elsewhere.

(11) The production of sympathetic colouring in the shallower zones of the coast is explained as a background effect, in which the incident diffused light plays little part. The influence of background is predominant. The production of crimson colouring in deeper water is explained as due to diffused green light.

(12) There is no evidence that the pigments of the food (algæ) are the sources of the pigments of Hippolyte.

TABLE 1.—Wrasse, CRENILABRUS MELOPS. Experiment I: Light reflected from Various Backgrounds. Plymouth, 1907.

The specimens of this wrasse were of two sizes, averaging 10 mm. and 17 mm. in extreme length. They were of the usual faintly barred type of coloration (fig. 2 and description on p. 544). The ground colour is a dull, semi-transparent green due to the blue pigment suffusing the fin-rays and to the blue endoskeleton (and partly also to the opalescent scales) being seen through a network of yellow chromatophores. The brown transverse bars are due to red and black chromatophores aggregated in greater numbers in these regions than elsewhere. These young wrasses were taken at low tide round Drake's Island in the Sound. They were fed with Amphipods and Nereis.

Date.—Experiment begun on August 9th.	White background.—White enamelled bell-jar, clear glass cover. Circulation from the tanks. 2 specimens put in.	Black background.—Blackened bell-jar and clear glass cover. Circulation from the tank. 2 specimens used.	Darkness. A slate tank with circulation.	Brown weed (Laminaria saccharina).—The wrasses were placed in a museum jar, and this was immersed in a bell-jar filled with weed. Both vessels were provided with a circulation and the weed changed twice a week. Light reflected.—Nearly all from red to green; extremely dim. 4 wrasses, all faintly barred.	Green weed (Ulva).—Arrangement similar to that for brown weed. Light reflected.—Red to green. 3 wrasses, all faintly barred.	Red weed (Nitophyllum and Dasya).—Arranged as for brown weed. Light reflected.—Red-orange, bright green, very dim. 4 faintly barred specimens.
August 14th	Pale transparent green	Brown	—	2 brown with tinge of green. 2 brownish-green. (4 were added on August 15th)	2 green. 1 brown-barred. (4 were added on August 15th)	All distinctly green. 1 small one with reddish-brown tail. Red weed added. (4 of usual faintly barred type added on 15th).
August 19th	3 faintly brown-banded specimens added (10–11 mm.)	All very dark-coloured; well banded with brown, greenish on ventral surface	—	Greenish-brown with well-marked bands	Only one left, bright green.	Green; tails reddish-brown.
August 20th	—	—	—	Brownish with tinge of green. Bands at first faint, but showed up by flushing at once on exposure	3 pale-banded specimens added, 18, 21, and 24 mm. long	No change.

August 21st	Pale transparent green	All very dark and well banded	—	No change	No change	Distinctly browner.
August 22nd	Ditto. These were now transferred to another experiment (green weed)	Ditto	—	6 dark and well-banded. 2 rather paler (greenish) ground colour	1 green. 3 brown-banded	Showed brownish bands; red chromatophores temporarily expanded.
August 25th	—	Ditto	(8 brown specimens 20–30 mm. long put in August 26th)	2 with faint bands on green body-colour. 2 greenish	1 green. 3 brown	Greenish, with brown bands.
August 27th	—	Ditto	Very transparent blue. Extreme contraction of chromatophores. (Temporary expansion on August 28th)	No change	1 green. 1 greenish brown. 2 brown-barred	Ditto.
August 30th	—	Dead, transparent blue with fair bars	Transparent bluish. Two with tinge of brown	Put on black background to ensure expansion. Well-barred with brown on greenish ground. More red pigment than in experiment with red or green weed	Put on black background. 2 greenish-yellow. Microscopic examination showed good yellow network. 2 yellow with brown bands	Put on black background. 2 faintly brown-barred on green-ground, yellow green with faint bands. Yellow pigment fairly well developed and expanded. Red pigment, slight or fair.
Inferences	The usual effect of white background	The usual effect of black background. Darkest of all the experimental fish	Extreme contraction of chromatophores, comparable to nocturnal colour-phase of Hippolyte	Brown weed seems to act like black background	Result not unlike that of red background. The red-orange rays apparently the effective ones	This unexpectedly pale result seems to show that the dominantly red rays induce formation of yellow pigment.

TABLE II.—Experiment II. CRENILABRUS MELOPS. Light transmitted through Weed.

Date.—August 23rd, 1908.	A. Green weed transmitting orange to green and a little red light.—Two rectangular vessels fitted one inside the other, with a space of 1 cm. between the two. This space was filled with Ulva (3-4 fronds). The inner contained the fish and was without weed. 3 specimens, 8–10 mm., greenish; 2 specimens, 8–10 mm., brownish.	B. Brown weed transmitting orange and a trace of red and green, light of low intensity.—Similar receptacles with Laminaria.	C. Red weed transmitting the same as B, but in addition a broad band of red light. Similar receptacles with Dasya and Nitophyllum.—2 specimens, 17 mm., greenish; 2 specimens, 17 mm., faint barred; 1 specimen, 10 mm., brown barred.
August 26th	2 green, 3 brown-barred. (1 small green examined: — pale blue pigment diffused round the gut. Yellow pigment well expanded)	5 green specimens	2 greenish; 3 faint-barred.
August 27th	1 green, 3 pale grey-brown barred	All green	—
August 31st	All brown, considerable amount of red pigment in 3	Ditto	3 green (2 with tinge of brown).
Inference	Conversion of green colour to brown by development of red pigment. The effect of orange-green light favours this change	The amount of light was probably insufficient to do more than act as dimness, which produces a greenish coloration	Under the influence of red light the complementary colour is retained or developed.

TABLE III.—Coloured Light Experiment. HIPPOLYTE VARIANS, 1908.

Date.—August 8th.	White light.—An 800 c.c. clear glass beaker standing on dull greenish brick in tank. Chorda filum and adhering fine brown weed for food. 8 pale-lined specimens 7–10 mm. long. Light: direct and diffused sunlight.	Red light.—2 large glass dishes with a 2 cm. layer of erythrosin between. Tank-water circulation in inner vessel. Ceramium for food. A little Enteromorpha added on the 20th. 10 pale-lined specimens 6–8 mm. long. Light.—Diffused sunlight.	Red light.—August 15th.—Two large beakers separated by 400 c.c. erythrosin 1·5 cm. thick. Ceramium for food. Light.—Direct or diffused sunlight. Standing in tank 17–17·5° C.	Green light.—August 15th.—Two beakers separated by a 60 per cent. solution (1·5 cm. thick) of $CuCl_2$ with trace of pot. chromate. Light.—Direct and diffused. Standing in tank.	Darkness.—A slate tank containing museum jar, through which water circulated. Ceramium for food. Covered with black cloth.
August 17th	—	5 with red and yellow chromatophores, 2 with red and some yellow, 1 red only. After a short exposure to black background in dim light to obtain maximum expansion there were recorded, 1 large surface orange chromatophores, 1 brownish-lined form, 1 faint red-lined form	—	—	All red lined. (Another lot of 20 started on August 18th.)
August 19th	5 red-lined forms, 1 pale pink	—	6 typical red lined, 2 pink, 3 yellowish red lived forms	All very red-lined, 4 full red, 5 good red, 1 fair red. General effect.—More intense red than those in red light	—
August 20th	—	6 reddish-lined forms, 1 yellowish-green, yellow pigment much developed and expanded	—	—	Very transparent. 2 good red-lined, 2 faint red-lined. Dead on August 28th.
August 29th	3 left. All full brown-lined forms. Very large red and yellow chromatophores on carapace. Deep chromatophores with same pigments well-developed	4 green (2 good green, 1 fair green, 1 green-lined form). 1 yellowish (much yellow and some red). Yellow.—Well expanded and plentiful, much increased. Red.—Contracted and with blue halos	6 brown-lined forms similar to white-light result. Much red and yellow in superficial and deep chromatophores.	1 bright carmine. Chromatophores all of a pure crimson pigment, well-developed both deep and superficial. Eye stalks a very vivid crimson. Yellow pigment absent. Blue halos well marked around the red pigment	(From August 18th.) 20 good or fair reddish-brown lined forms. 1 or 2 colourless.
Sept. 4th	—	2 left, both greenish	—	—	—

TABLE III (continued).—HIPPOLYTE VARIANS, 1909.

In all these vessels light entered from below as well as from above (see p. 555). Ceramium (red weed) for food.

Date.—June 8th, 1909.	White light.—A 1200 c.c. beaker. Air circulation. Fine brown weed. About 25 specimens of the type shown on Pl. 23, fig. 4.	Red light. A 2 mm. layer of lithium carmine around inner beaker. Air-circulation	Red light. Lithium carmine.	Green light.—Two beakers separated by a 1 cm. layer of $CuCl_2$ + trace of pot. bichromate.	Darkness.—Started June 3rd. Museum jar covered with bolting silk and enclosed in slate tank. Constant circulation of water. Ceramium (red weed) for food. About 25 started.
June 10th	—	—	—	—	All well-marked red-lined forms.
June 15th	—	—	—	—	Very pale, transparent, faint red-lined forms.
June 17th	12 pale red-lined forms. Much reflecting yellow substance. Unaltered	12 brownish with yellow lines, 2 pinkish, 1 colourless	Started with specimens which had become red-lined on red weeds in white light (June 2nd—17th)	8 red-lined forms. Red fully expanded	12 left; all fair or faint red-lined forms.
July 10th	—	1 greenish, 4 yellows. Much reticulated yellow pigment. Much expanded vermilion pigment	1 brown-lined, 1 brownish yellow, 1 red-lined, 1 pink. Yellow pigment forming a network. Vermilion coloured chromatophores well developed both on the surface of the body and below it	3 bright crimson. Only traces of the superficial chromatophores. Dense arborescent masses of carmine pigment round the gut and nerve-cord	Only 1 left owing to bolting silk giving way. Deep brown-lined form. Few surface chromatophores. Vermilion and yellow in bushy masses round gut and nerve-cord.

TABLE IV (1908–9).—Summary of Influence of Light on the Development of Pigments in HIPPOLYTE VARIANS.

Starting point for these experiments: Small specimens (4½–5½ mm. long), transparent colourless (on black background), faintly red-lined (on white background). Yellow and red chromatophores present, but not in sufficient quantity to give rise to a definite colour (Pl. 23, fig. 4).

Light.	Background.	Weed (for food).	Length of experiment in weeks.	Resulting colour.	Characters of pigmentation.
None	(Darkness)	Red and brown	1½	Reddish-brown	Both yellow and red increased, especially in the deeper layers. Surface pigments disappear.
			2	Red-lined	
			6	Brown-lined	
White	Avoided by uniform illumination	Ditto	1½–3	That of weed	Surface and deep pigments well developed.
	White	Ditto	1	Almost colourless	Surface pigments absent.
			2	Deep crimson below	Deep crimson on gut and nerve-cord.
	Green weed	Green	2 days	Green in 66 per cent.	Evenly distributed.
	Red weed	Red	2 days	Red in 80 per cent.	Deep pigments better developed.
Red	Glass resting on slate	Red and then green	2	Reddish	Red disappeared, yellow developed, blue developed.
			4	Green and yellowish green	
	Avoided by uniform illumination	Red	4½	Yellow and greenish	Red present, yellow developed, blue in one.
Green	Glass resting on ivory-glazed brick	Red	3	Crimson	Carmine and blue only.
	Avoided by uniform illumination	Ditto	4½	Ditto	Carmine. Surface chromatophores have almost disappeared.

TABLE V.—Summary of Results showing the Colouring obtained by subjecting Young, almost Colourless, HIPPOLYTE VARIANS to Diffused Transmitted Monochromatic Light. See figs. 5–7.

Period in weeks.	Red light.		Green light.	
	1908.	1909.	1908.	1909.
1	Reddish	Brownish-yellow.	Red	Red.
2	Reddish and brown	—	Carmine (pure)	—
3	Green and yellow	Brownish-red	—	Carmine.
4	Greenish	Yellow and greenish	—	—
Food	Ceramium and (latterly) a little fine green weed	Ceramium	Ceramium	Ceramium
Final pigmentation of chromatophores. (Figs. 8–9)	Much yellow. Trace of red (vermilion). Much blue or green	Much yellow. Some red (vermilion). Blue in one (greenish) specimen	Carmine abundant. Blue (fair). No yellow. No vermilion	Carmine. Trace yellow.

TABLE VI.—Showing Effect of Background on the Development of Pigments in young HIPPOLYTE VARIANS.

	1908. White porcelain vessels with an air-circulation. Fine red weed used for food. One vessel covered with a sheet of green glass and two layers of Baker's green gelatine giving pure green light.		1909. Large museum jars painted with several coats of flatting, a clear space being left in the front. The red flatting reflected red light only, the green flatting reflected green light and a trace of blue. Fine red weed was used for food. Water-circulation employed. 25 specimens.	
Time in weeks.	White light. White background.	Green light. White background.	White light. Green background.	White light. Red background.
1	All remained very faint red-lined forms.	20. Pale transparent faint red-lined forms; transferred to white light	Colourless (7), reddish (4), greenish (4)	Colourless (2), red (4), greenish (4).
2	Superficial chromatophores had disappeared. Deep carmine ones clustered round the gut and nerve-cord (see Pl. 23, fig. 11). Specimens appeared transparent with a narrow crimson line down the centre	—	Colourless or faint greenish (8)	Orange (7).
6	—	—	4 left; all faint green, but reverting to red-lined forms on exposure to dark background	Bright reddish-orange (2).
Inferences	Remarkably protective development of crimson pigment in bright white light	On reflecting backgrounds, green light inhibits formation of pigments when employed for a short time.	Green light suffused with bright white light has no distinctive effect. It merely acts like dim white light	Red light suffused with white light has a definite effect, encouraging the development of red and yellow pigments.

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EXPLANATION OF PLATE 23,

Illustrating Professor Gamble's paper on "The Relation between Light and Pigment-formation in Crenilabrus and Hippolyte."

Fig. 1.—Young Crenilabrus melops (× 5) in the dark-banded phase induced by exposure to dark backgrounds.

Fig. 2.—The green phase in the same fish induced by exposure to red light transmitted by red weed, and also by exposure to backgrounds of red weed for one week.

Fig. 3.—The reddish brown banded phase assumed by exposure for a week to light transmitted through green weed. The red colour is a shade too pronounced in the figure.

Fig. 4.—Young Hippolyte varians in the almost colourless condition in which it is taken among weeds when 4–5 mm. long. (× 24.) These colourless Hippolyte formed the starting-point for the experiments recorded in this paper.

Fig. 5.—The brilliant carmine colouring induced in Hippolyte by exposure to pure green light for three to four weeks. (× 22.) Food-plant, Ceramium.

Fig. 6.—The green colouring induced in Hippolyte exposed to red light for four weeks. Food-plant, fine green weed.

Fig. 7.—The yellow colouring induced in some Hippolyte exposed to red light for four weeks. Food-plant, Ceramium.

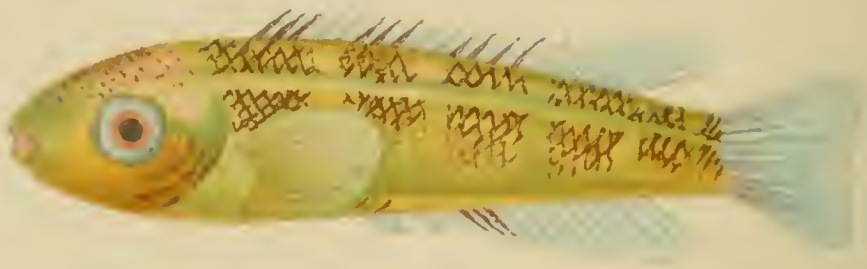
Fig. 8.—Chromatophores from fig. 7, highly magnified. (× 390.)

Fig. 9.—Chromatophores from fig. 6, highly magnified. (× 390.)

Fig. 10.—Chromatophores from fig. 5.

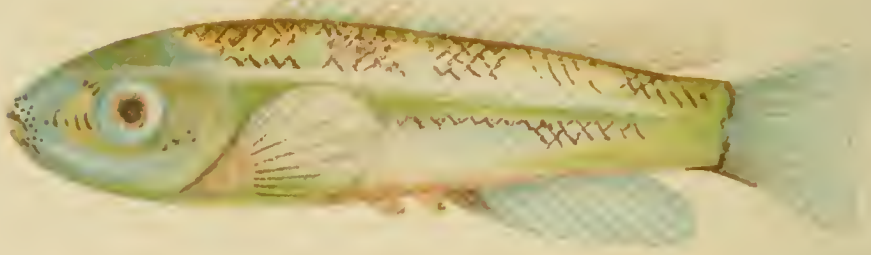
Fig. 11.—Chromatophores from Hippolyte exposed to white reflected light for one month.

Over brown weed.



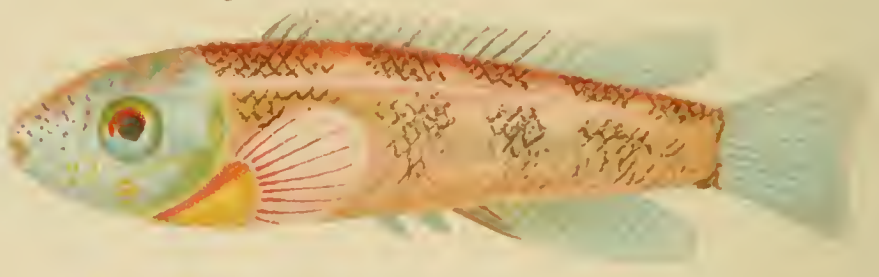
1.

Under red weed.



2

Under green weed.



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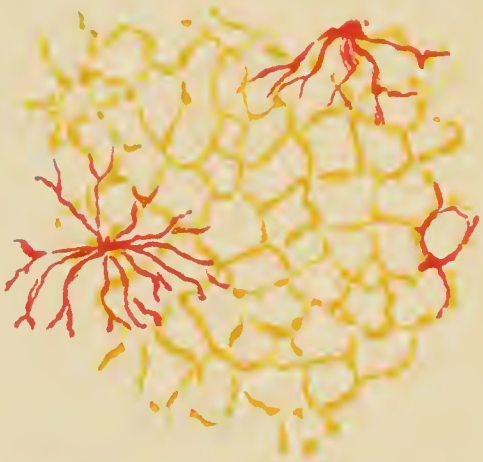

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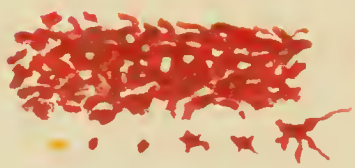
9.



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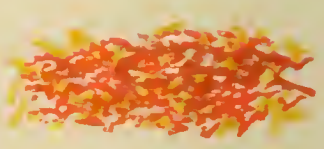


11.

Red light 7.

Red light 6.

Green light 5.

Original Stock 4.

Huth Lith: London

Is the Trophoblast of Hypoblastic Origin as Assheton will have it?

By

A. A. W. Hubrecht.

With 7 Text-figures.

In the 'Quarterly Journal of Microscopical Science' there has lately appeared (vol. 54, part 2) an article by my friend Assheton, in which he points out certain objections which he feels inclined to raise against some of the views that were developed by me in a contribution to the fifty-third volume of this Journal, entitled "Early Ontogenetic Phenomena in Mammals."

Although I regret that he has not seen his way to comply with the invitation which I addressed to my fellow-embryologists in October, 1901 (it was published on p. 5 of my article on "Tarsius" in the 'Verh. Kon. Akad. v. Wetenschappen te Amsterdam,' vol. viii, No. 6, 1902), and which was intended to minimise printed disputes, where personal inspection of the preparations might bring about consensus of opinion, still, I accept his challenge (loc. cit., p. 221), and will now "discuss more fully the difficulties which have arisen in the minds of some who are unable to accept (my) theoretical conceptions." In doing so I wish to remind my readers that I am not going to treat all the objections raised by Assheton one by one. Many of them will remain sub lite until new facts have been discovered, settling the point in dispute either one way or the other. I will on this occasion

restrict myself to a point of very fundamental importance on which Assheton's and my own views are diametrically opposed to each other, ever since 1898. If the new facts which I bring forward in this paper should be convincing enough to change the minds of those who feel inclined—following Assheton's example—to look upon the trophoblast as hypoblastic, I have no doubt that my proposal to exclude from the phylogeny of Eutherian mammals any ancestor who deposited megalecithal eggs, like the Sauropsids and the Ornithodelphia, will find a more easy acceptance on their part.

Assheton's reasons for considering the trophoblast as an essentially entodermal fœtal envelope were first developed in 1898, in his article on "The Segmentation of the Ovum of the Sheep" ('Quart. Journ. Micr. Sci.,' vol. 41). Plate 18 of that article presents us with a series of diagrams most delicately shaded in red and blue, which were meant to explain the mutual relations of trophoblast, epiblast, and hypoblast in ten different genera of mammals, and to compare them with the Sauropsidan arrangement.

These diagrams have not found favour with later authors on this subject, and have been taken no notice of in Hertwig's extensive 'Entwickelungsgeschichte,' in three volumes. At that time I refrained from entering into any polemical discussion, considering that later observations would show the untenability of Assheton's ingenious but unsatisfactory generalisation. In writing his latest article Assheton has, however, allowed himself to come too strongly under the influence of his own hypothesis of twelve years' standing. I see no necessity for entering upon any detailed discussion concerning the numerous and different arguments which have led other embryologists as well as myself to reject that hypothesis of Assheton's now that new facts have come to light concerning the very earliest segmentation stages of Galeopithecus. This very archaic genus may be looked upon as a derelict representative of a group that in earlier geological epochs gave rise to the modern bats. There are

certain points of agreement between its early development and that of Pteropus, whilst Leche's anatomical work ('Kgl. Svenska Vet. Akad. Handl.,' Bd. xxi, 1886) upon Galeopithecus points in the same direction. Of this genus I have now in my possession several series of sections made through segmentation phases, some of which I have here figured.

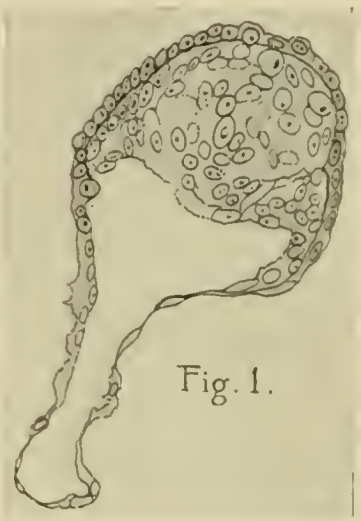
These sections leave no doubt that the trophoblast of Galeopithecus originates by delamination at as early an age as the two- and four-cell segmentation stage, and render it utterly futile to try and explain the Galeopithecus trophoblast as "due to an overflow of the yolk or hypoblast cells over the epiblastic rudiment" (Assheton, l.c., p. 228).

If we look more closely at the three stages of Galeopithecus here figured and begin with the one that is the furthest developed (Text-fig. 1), we find full coincidence with a similar stage described by Assheton for the sheep (l.c., 1898, Pl. 16, figs. 14, 15), by Keibel for the stag ('Arch. f. Anat. and Phys. Anat. Abt.,' 1902, p. 292), by Weysse ('Proc. Amer. Acad.,' vol. xxx, p. 283) for the pig, by van Beneden for the rabbit and bat ('Archives de Biologie,' vol. i), by myself for the hedgehog, for the shrew ('Quart. Journ. Micr. Sci.,' vol. 30, Pl. 17; vol. 31, Pls. 36, 37), for Tupaja, for Tarsius ('Verh. Akad. Wetensch. Amsterdam,' vol. iv, 1895, Pls. 1, 2; vol. viii, 1902, Pls. 1, 2), and for Nycticebus ('Keibel's Normentafeln,' 1907), as well as by other embryologists for various other mammals. This is the common starting-point in which there is a trophoblast and an embryonic knob with a cavity below it, and in which a hypoblast is not as yet distinctly developed, although just beginning to make its first appearance. It should be borne in mind that this very stage is thus characteristic for genera of mammals so diverse as those mentioned above. The way in which Assheton attempts to prove from yet earlier stages of the sheep that the outer trophoblastic layer is in reality a derivate of the hypoblast appears to me to be so pre-eminently artificial (c.f. l.c. his figures 9–14) and the argumentation so weak, that I must ascribe to a similar

incredulity on van Beneden's part that this latter author in the important article which appeared one year later than Assheton's ('Anat. Anzeiger,' 1899, p. 305), does not take the slightest notice of the English author's view that the trophoblast (van Beneden's "couche enveloppate") should be looked upon as an entodermal derivate.

If we now return to Text-fig. 1 of this paper and inquire how this stage in the ontogeny of Galeopithecus has been reached,

Text-fig. 1.



Section of a blastocyst of Galeopithecus with embryonic knob and enveloping trophoblast, just before the establishment of the continuous hypoblast.

we see that it has been preceded by the stages of which Text-figs. 2 and 3 are the representatives.

In Text-fig. 2 the centre of the different sections is occupied by comparatively large nuclei, evidently belonging to a central group of cells—the mother cells of the embryonic knob. Outside this embryonic knob and forming the peripheral layer in these sections is protoplasm in which distinct cell-boundaries are not visible, but in which a certain number of nuclei (smaller than those of the embryonic knob) clearly indicate that in the live blastocyst a peripheral cell-layer was differentiated in addition to the embryonic knob.

Going back yet one stage earlier, in which the two first cleavage-cells are just on the point of splitting up into four (as is distinctly indicated by the karyokinetic figures of fig. 3 *C*), we notice, besides the two cleavage-cells, three polar bodies of comparative large size, as they are known for mammals in general. Moreover, at the periphery of the two cleavage-cells we find separate nuclei, indicating the very first origin by an early delamination process of the cells which in Text-figs. 2 and 3 constitute the continuous layer of trophoblast.

TEXT-FIG. 2.

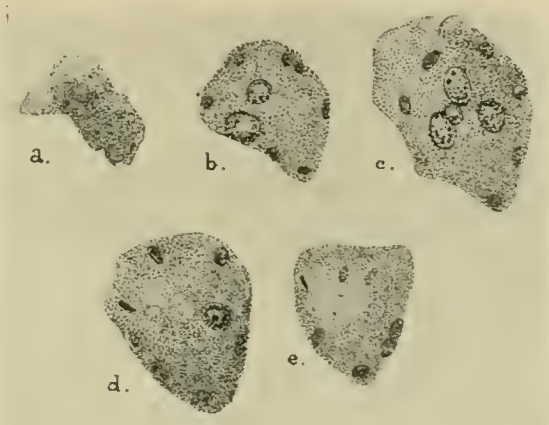


Fig. 2.

Galeopithecus. A series of five sections through a stage of cleavage preceding the blastocyst of Text-fig. 1. Embryonic knob with bigger nuclei contrasting with the smaller peripheral trophoblast-nuclei. No central space as yet developed.

In the mammalian genera hitherto examined with respect to the origin of the trophoblastic layer (Tarsius, Tupaja, rabbit, sheep, pig, stag, dog, mouse, guinea-pig, etc.), the trophoblast undoubtedly makes its appearance at a somewhat later stage of cleavage, or rather the distinction in the morula stage between the mother-cells of the embryonic knob and those of the trophoblast is not so soon evident as it is in Galeopithecus. However, the karyokinetic processes by which in this latter genus the trophoblastic nuclei separate from the segmentation nucleus (which in its turn owes its

origin to the union of the male and female pronucleus) at so early a moment are not revealed by my preparations, and we cannot for the present come to any sound conclusion as to which of the two modes of formation of the trophoblast is the more archaic one.

Recognising that the definite answer to this question can only be given when a number of new observations will be at our disposal, I may still be allowed to call attention to the fact that in Galeopithecus the spot where the polar bodies are

Text fig. 3.

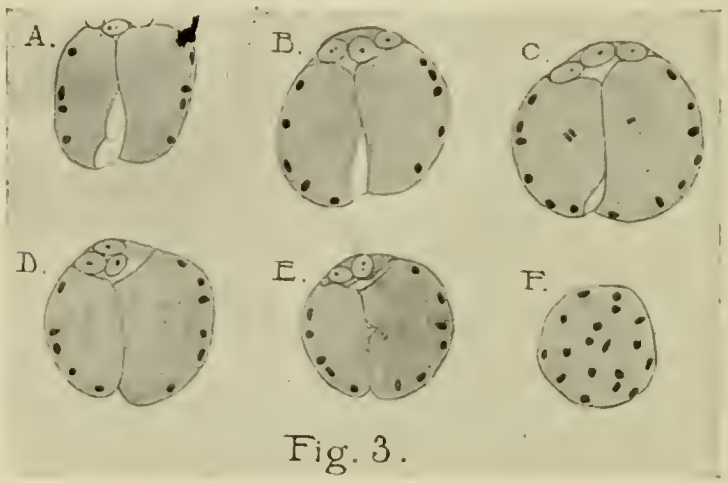


Fig. 3.

Galeopithecus. A series of six sections through a cleavage stage just preceding the formation of the second pair of cleavage-cells. In *C* karyokinetic figures indicate this. In *B—E* the polar bodies are visible. Apparent trophoblast nuclei are situated peripherally.

applied against the egg (see Text-fig. 3 *B—E*) remains without trophoblast nuclei somewhat longer than other parts of the egg's surface. The question presents itself—supposing the process is more primitive in Galeopithecus—whether this particularity might have led (in such mammalian genera that should be considered as phylogenetically younger) to the arrangement which has induced van Beneden, Duval, and Assheton (in his later publications) to consider the cleavage-process of those mammals as revealing epibolic characteristics. In case this question will later have to be answered in the

affirmative, the so-called blastopore which van Beneden (1875) described in the rabbit's morula-stage might correspond to the spot referred to in the three figures (Text-fig. 3 *B*—*D*), where the polar bodies lie.

Having thus shown that Assheton's hypothesis of the hypoblastic nature of the trophoblast is irreconcilable with the phenomena in Galeopithecus, I emphatically repeat my conclusion that we are not justified in accepting it for any other vertebrate. He himself will admit that, such being the case, the comparison of the trophoblast of mammals with the "deckschicht" of fishes comes to the foreground with increased validity.

I have already stated above that it is not my intention in this paper to follow Assheton's criticism step by step. A more extensive article on the ontogeny of Galeopithecus will appear in the course of this year. I shall there find occasion to reply more fully to other parts of Assheton's criticism. There is, however, one point on which I feel bound to apologise, viz. that I have not allowed enough space for the recognition of the fact that my kephalo- and notogenesis had already been partly forestalled in several of Assheton's papers, and had by him been termed proto- and denterogenesis. I ought to have particularly mentioned these names in my paper of 1908. Still, I must maintain my terminology now that Assheton himself states (l. c., p. 240) that his and my names "signify a different interpretation," and now that he maintains that mine "does not represent the actual facts." As matters stand I feel that the important issue which is at the base of the whole question of gastrulation in vertebrates (very fully treated in Keibel's contribution to vol. x of the 'Ergebnisse der Anatomie und Entwickelungsgeschichte,' but since then looked upon in a somewhat different light after his and my own short papers in the 'Quart. Journ. Micr. Sci.' [vol. 49] and in the 'Anat. Anzeiger' [vol. xxvi] had appeared) renders any polemics about the nomenclature that should be adhered to untimely. Very numerous investigations

are yet necessary, and will undoubtedly soon be undertaken, before we dispose of the comparative material which is necessary for settling this important point in Vertebrate ontogeny, and for finally deciding which nomenclature ought to be adhered to. I gladly leave the latter decision to others, but would not let this paper see the light without recognising that until lately I have not sufficiently been aware that Assheton already in 1894 expressed opinions to which Keibel and myself have come along other roads, and which, though far from identical, still overlap each other in many respects.

Appendix.

While this paper was in the press, attempts were made by

Text-fig. 4.

Fig. 4

Part of a section through the blastocyst of Manis. The ectoderm (*ec.*) and endoderm (*en.*) of the embryonic knob are transversely cut. The trophoblast cells appear darker in this figure.

me to ascertain whether other genera of mammals might perhaps exist which furnish evidence concerning the early phases of the trophoblast that might further corroborate the facts such as they are presented by Galeopithecus. I was all the more anxious to obtain information concerning the earliest stages of the scaled ant-eater (Manis), as, by a regrettable lapsus calami, which disfigures both the English and the German version of my "early ontogenetic phenomena in mammals, etc.," a gastrula stage of Manis is erroneously attributed to Galeopithecus.

It is fig. 18 on Pl. C, in vol. 53 of the 'Quart. Journ. of Micr. Sci.,' and fig. 46 in the German publication. I here

reproduce the misnamed figure of this early Manis, and have since had the good fortune of obtaining sections of yet earlier cleavage stages of the same animal.

Sections of early blastocysts of two specimens of Manis are

TEXT-FIGS. 5A AND B.

Two consecutive sections of very early blastocysts of Manis, which show what is presumably the earliest trophoblastic covering of the mother-cells of the embryonic knob.

reproduced here in Text-figs. 5 *a*, *b*, and 6. The stage of Text-fig. 5 is presumably a two-cell, the other (as far as I can follow it up in the consecutive sections of the series) a four-cell cleavage stage (purposely but incorrectly not counting the

TEXT-FIG. 6.

Another section through another blastocyst of the same genus in the same stage.

trophoblast cells as such). In both the differentiation between the mother cells of the embryonic knob on the one hand, and the already so much more numerous trophoblast cells, leads to the inevitable conclusion that the phenomenon of the separation of the larval trophoblast from the remaining

embryonic cells takes place at quite as early a moment as we have above described it for Galeopithecus, and that also in Manis it is perfectly excluded to look upon the trophoblast cells as hypoblastic. And so the early Manis may be joined to the early Galeopithecus as fatal to Assheton's interpretation of the trophoblast. I cannot yet say with certainty, but I have reason to believe that also in the very young hedgehog similar peculiarities occur.

At the same time it is very suggestive that the quaint and aberrant mode in which the trophoblast cells of Galeopithecus and Manis arise offers so many points of mutual resemblance between these two genera, and differ not inconsiderably from what we find in Primates, Rodents and Carnivores.

Later investigations will have to decide whether the phenomenon, as it presents itself in Galeopithecus and Manis, is one of precocious segregation.

The Origin and Formation of Fibrous Tissue Produced as a Reaction to Injury in Pecten Maximus, as a type of the Lamellibranchiata.

By

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Beit Memorial Research Fellow; and

W. De Morgan, F.Z.S.

With Plate 24.

Contents.

Introduction.

The experiments described in this paper were performed on Pecten maximus at the Laboratory of the Marine Biological Association at Plymouth.

The object of our work was to investigate the histology of the reaction of the tissues to the presence of a foreign body, and to determine the origin and method of formation of the fibrous tissue formed around it.

As one type of foreign body we chose sterile agar jelly, which has little or no irritative or toxic action on the tissues, and is not removed by phagocytosis. As another type we chose masses of gill-tissue and of the tissue of the digestive gland, taken from an animal of the same species. Neither of these could be injected under aseptic conditions, and both were capable of removal by phagocytosis. Considerable irritation was set up by the implantation of these tissues, especially in the case of the digestive gland. This produced marked degeneration of the neighbouring tissues, possibly owing to the liberation of ferments and consequent digestive action.

Pecten maximus was selected for these experiments on account of the large size of its adductor muscle, which presents a homogeneous mass of tissue particularly suitable as a site for implantation of foreign bodies. Before making this choice, experiments were tried on several other animals, but it was found that in most cases the technical difficulties encountered in endeavouring to make implantations into small masses of tissue, and in determining the exact relation of the underlying organs to the superficial anatomy, were too great to render these animals suitable subjects for experiment.

Such experiments were tried on Carcinus mænas, Pagurus bernhardus, and others of the smaller species of crabs, on Palæmon serratus, Ligea oceanica, Aphrodite aculeata, Patella vulgata, Aplysia punctata, Archidoris tuberculata, and many Lamellibranchs, but none offered such promise of success as Pecten maximus.

Methods.

Pecten maximus can be readily obtained in the Salcombe Estuary. It was found necessary to allow these animals to become acclimatised to living in the laboratory tanks before proceeding to the experimental work. When first placed in the tanks the mortality was heavy, often amounting to 30

per cent. in the first three days, but after the lapse of about a week the survivors appeared to be fully acclimatised to the changed conditions, and often remained healthy for some months.

Experiments on animals whose health was doubtful were of no value, both because the shock consequent on the injection of the foreign body frequently caused death, and also because the reaction of the tissues was not normal in unhealthy specimens. When a Pecten is healthy it lies with the valves of the shell slightly apart, the tentacles are expanded, and it responds rapidly to any stimulus by closing the shell; when held up in the air, the water which drains away is clear and contains no slime. An unhealthy specimen lies with the valves of the shell wide open, there is little or no response to stimuli, and the valves only close under pressure. The tentacles are retracted, and the gonads, gills, and tissues generally, look flabby and unhealthy. The water which flows out between the valves is slimy and viscid, and this is generally the first sign of deterioration.

All instruments used in the experiments were carefully sterilised in boiling water.

The transplanting needle resembles a large hypodermic needle about 1 mm. in diameter and 6 cm. long. Into the hollow needle a somewhat longer stylet fits closely and works like a piston. Any material taken up in the point of the needle is sucked in by drawing the stylet back, and again ejected by pushing it forward.

For injecting into the muscle, a solution of agar in sea-water, coloured by a little hæmatein, was used. The agar jelly was liquefied by heating in boiling water, and was drawn up into the transplantation needle. On cooling it forms a cylinder, of the diameter of the needle, which is easily introduced into the muscle.

The adductor muscle of Pecten maximus is so large that there is no difficulty in selecting a spot at which to bore the shell. The apex of an equilateral triangle, having for its base the line of junction of the posterior auricula with the

right valve, marks roughly on the surface a point at which the shell may be bored without damage to any organ. But as the animal gapes when removed from its tank, it is easy to slip a cork between the valves and select a spot by inspection.

The holes were drilled in the convex or right valve by an ordinary dentist's drill, the head of which was prevented from penetrating too deep by a lapping of thread.

The spot selected for drilling was sterilised with a saturated solution of corrosive sublimate, washed off with a solution of hydrogen peroxide (30 vols.) or distilled water, care being taken not to allow any of the sublimate to run between the valves. The transplanting needle was then introduced to the required depth, slightly withdrawn, and its charge projected into the channel. The hole was then thoroughly dried, and stopped with sealing-wax. If the drying is thorough the wax will adhere after the animal has been returned to the tank. It would, of course, have been possible to implant directly into the muscle through the opening of the valves, but the risks of sepsis would have been greater.

When required for examination, the shell was opened by cutting the adductor muscle at its attachment to the right or convex valve, and a portion of the muscle containing the implanted material removed. This was fixed by three or four hours' immersion in Gilson's fluid, then thoroughly washed, passed through the alcohols, cleaned in xylol, and embedded in paraffin wax. It was then cut into serial sections eight μ thick.

Delafield's hæmatoxylin, followed by Van Gieson's stain, or Benda's iron mordant and hæmatoxylin were used for staining.

Description of the Tissues of Pecten maximus involved in the Experiments, and the Normal Process of the "Clotting" of the Blood.

The adductor muscle of Pecten maximus consists of two portions, bound together by the same sheath of connec-

tive tissue, but differing in structure. The larger, semi-transparent and whitish, consists of striated fibres. The fibres of the smaller, which is opaque and dead white, and lies against the posterior surface of the larger mass, are non-striated. It was into the larger mass that all material in our experiments was introduced.

There is a large blood supply to the muscle from the adductor artery (Dakin, 2), and it contains numerous lacunar spaces. Scattered through it are numerous strands of connective tissue. These contain fibroblasts with deep staining nuclei and long fibrillar processes.

The digestive gland has a tubular structure and completely surrounds the stomach, into which its ducts open. The ducts break up into numerous alveoli, which ramify and ultimately form cæca. The ducts are lined with ciliated epithelium, and the alveoli with secreting cells. These secreting cells are said to degenerate and become filled with a granular pigment, and are ultimately shed into the lumen of the ducts (Dakin, 2). Thus in their younger stages they appear to have a secretory, and in their later stages an excretory function. In addition to these glandular cells, fibrous connective tissue and unstriated muscle-fibre are present. The ducts contain particles of food material, algæ, diatoms, and bacteria, and consequently as a rule septic conditions prevail in the experiments.

The blood of Pecten maximus is a slightly cloudy, colourless fluid. It does not coagulate, but when shaken a number of small, white, flocculer masses appear, which soon fall to the bottom of the tube, leaving the supernatant fluid clear and transparent. These masses consist of blood-corpuscles agglutinated to form plasmodia.

The corpuscles, although varying in size, appear to be only of one kind. They are amœboid bodies, which when expanded protrude a number of slender pseudopodia. When contracted, they are ovoid or spherical. There is a single compact nucleus, staining readily with methylene-blue. The cytoplasm is finely granular, and stains with eosin, but there are

no large eosinophile granules. According to Cuénot (1), they originate in a "glande lymphatique" situated at the base of the gills.

One of us (Drew, 4) has shown in the case of Cardium norvegicum that when the corpuscles come in contact with a rough foreign body, or with injured tissue, they possess the power of agglutinating and forming a compact plasmodial mass. In this way bleeding from a small wound is stopped. When the edges of a wound are covered with this mass of agglutinated corpuscles, protoplasmic strands are formed across the wound, connecting the plasmodia; these strands thicken and contract and so approximate the edges of the wound. So far as our observations go, there is no reason to suppose that the blood of Pecten maximus differs in any of these particulars from that of Cardium norvegicum.

That Lamellibranch blood-corpuscles are capable of a phagocytic action towards degenerated cells has been shown by De Bruyne (3) in the case of Mytilus edulis, Ostrea edulis, Unio pictorum, and Anodonta cygnæa. Sir Ray Lankester (5 and 6) has shown that certain corpuscles of Ostrea edulis have a phagocytic action on diatoms and minute green algæ, and it has been shown by Drew (4) that the corpuscles of Cardium norvegicum have a phagocytic action on bacteria, and are attracted towards extracts of dead tissues.

The Formation of Fibrous Tissue in the Site of the Implantation of a Mass of Gill-Tissue.

As bacteria are normally present on the gill-filaments, the conditions when gill-tissue is implanted differ totally from those obtaining when sterile agar is used.

The implantation soon produces an intense inflammatory reaction on the part of the animal. The blood-spaces in the immediate neighbourhood of the implanted tissue become distended and crowded with corpuscles, which escape from the lacunar spaces and migrate towards the source of irrita-

tion, travelling in all directions between the muscle-fibres. On reaching the gill-tissue the corpuscles come to rest, and form a dense, agglutinated, plasmodial mass, completely surrounding and shutting off the gill-tissue from the neighbouring muscle (fig. 1). They soon appear as if they had undergone some degree of pressure and the nuclei are slightly flattened, probably owing to the contraction of the plasmodial mass as it tightens round the implanted gill-tissue (Drew, 4). In time the corpuscles show signs of degeneration; the nuclei become irregular in outline, and the chromatin is represented by numerous granules staining darkly with hæmatoxylin. The degenerated mass of corpuscles is then invaded by fresh blood-cells, and is more or less completely removed, apparently partly by a process of phagocytosis and partly by autolysis.

While this is going on, the cells of the gill-filaments have degenerated, their outlines are ill-defined, and the nuclei no longer discernible; the bacteria present multiply considerably.

The degenerated gill-tissue is then invaded by blood-corpuscles which have penetrated through the surrounding mass of agglutinated cells, and in most cases the bacteria and epithelial débris are removed by phagocytosis, leaving only the chitinous supporting-rods of the gills.

In the course of this process many of the invading cells also are destroyed, and appear in their turn to be removed by other phagocytes. In time the whole space originally occupied by the gill-tissue becomes filled with a loosely packed mass of blood-cells, among which the chitinous supporting bars are the only relics of the original implanted mass. In many of our experiments bacteria multiplied so rapidly that the phagocytes were unable to cope with them. Consequently the bacteria invaded the neighbouring tissues, entered the blood-spaces, and rapidly caused death.

In preparations from obviously unhealthy animals, it was commonly found that the bacteria had penetrated beyond the protecting mass of agglutinated cells and had invaded the

muscular tissue, which showed signs of degeneration in its somewhat swollen fibres and faint striation.

When a blood-space had been entered, bacteria were often seen ingested by the blood-corpuscles, but in later stages it was obvious that the number of bacteria was so out of proportion to the number of corpuscles that they could not all be removed by phagocytosis, and were of necessity distributed all over the body in the blood-stream.

During these processes the fibroblasts in the walls of the blood-spaces, and in the intermuscular connective tissue in the neighbourhood of the implanted mass, undergo rapid division. This rapid division, resulting from the reaction of the tissues to the irritation caused by implantation, appears to be always amitotic. Mitotic division was only observed in much later stages, when the source of irritation had been removed by phagocytosis, and the rate of division of the fibroblasts was much slower.

Before amitotic division the fibroblasts lose their spindle shape and become oval; a split then appears at one end, and progresses in the plane of the long axis of the nucleus until two daughter nuclei are formed, attached to each other at one extremity, and inclined at an acute angle to one another. These gradually straighten out until they form an hour-glass-shaped mass of nuclear material. Finally the two nuclei are separated at the constriction and become almost circular in shape.

As a result of this active multiplication of the fibroblasts, the strands of connective tissue bounding the blood-spaces and forming the intermuscular connective tissue become crowded with nuclei. The bodies of the fibroblast cells become very indistinct, and little beyond rows of elongated nuclei is discernible. As the multiplication becomes more rapid the typical spindle shape of the nuclei is lost, and they become first oval and finally circular.

There appears to be a constant migration of these cells, with round and oval nuclei, towards the site of implantation. They have very little cytoplasm, and from this, and their

smaller size, are easily distinguished from the blood-corpuscles (figs. 2 and 3). These fibroblasts largely follow the course of the strands of fibrous tissue bounding the blood-spaces, and they appear to travel along in the spaces, being most plentiful near the walls. At the same time, when they multiply very rapidly, many migrate in all directions between the muscular fibres towards the implanted tissue, and are not confined to travelling only in the proximity of pre-existing connective-tissue strands.

On reaching the degenerating layer of agglutinated corpuscles surrounding the implanted tissue, they arrange themselves in rows, and their nuclei elongate in such a direction that their long axes form arcs of a circle surrounding the implanted tissue. Some fibroblasts penetrate among the degenerating cells of the gill-tissue, which are being removed by phagocytes, and in this position start the formation of fibrous tissue.

The surrounding layer of fibroblasts gradually thickens, and presents a somewhat stratified appearance. At first this layer contains a number of blood-corpuscles, but these eventually are removed, probably by autolysis, leaving only the fibroblasts, which can now be seen to be connected with each other by numerous fine processes of the cytoplasm, the whole presenting a somewhat reticulated appearance. In time this tissue becomes more compact, and the reticulation vanishes. It would appear that this has been caused by the contraction of the processes of the fibroblasts, with consequent approximation of the cells. Finally, the nuclei become long and spindle-shaped, the amount of cytoplasm slightly increases, and a layer resembling normal fibrous tissue results.

In our experiments the great variation in the rapidity with which the various changes described took place was very noticeable. The health of the animal after the experiment seems an important factor in accounting for this, for the slow rate of fibrous tissue formation in unhealthy, as compared with healthy animals, was very marked.

Unfortunately none of the animals into which gill-tissue

was implanted lived long enough for all the elements of the gill-tissue to be completely replaced by fibrous tissue, but in healthy specimens most of the signs of inflammation had vanished, and the implanted tissue was surrounded by a wall of apparently healthy fibrous tissue, in four or five days.

Formation of Fibrous Tissue around the site of implanted Digestive Gland Cells.

After the implantation of portions of the digestive gland, a marked degeneration of the muscular fibres in its neighbourhood is noticeable. They swell slightly, all trace of striation is soon lost, and they stain less intensely. The area of degeneration gradually extends, and the muscular fibres in the immediate neighbourhood of the gland tissue are slowly dissolved. This action is presumably due to the presence of ferments in the digestive gland, which digest and render soluble all tissues in the immediate neighbourhood. At the same time the cells of the gland itself degenerate and appear to undergo auto-digestion, so that eventually only the brown pigment-granules originally contained within the secreting cells remain. Under these conditions bacteria do not seem to multiply, though they must have access to the cæca of the digestive gland, as these are in direct communication with the alimentary canal. In none of our sections have we been able to find bacteria, though it is quite common to find the siliceous skeletons of diatoms in the cæca. It seems, therefore, probable that the presence of digestive ferments inhibits the multiplication of bacteria.

As a result of the implantation of this tissue a condition of intense inflammation is set up, and all the blood-spaces in the neighbourhood become distended with blood-corpuscles. There appears to be an endeavour on the part of the organism to shut off all the implanted gland, together with the area of muscular tissue which has undergone degeneration, from the general blood-stream. This is effected by the formation of a layer of agglutinated blood-corpuscles around

the whole of the affected area (fig. 4). It was very noticeable in our preparations that the degenerated area was always larger in specimens that had been implanted with the digestive gland for some time (up to six days), than in those implanted for shorter periods, and thus it would seem that the range of action of the digestive ferments gradually increases. The degenerated area was always found surrounded by a layer of agglutinated corpuscles, though in different specimens this layer varied considerably in thickness. It would seem that while the degenerative process is spreading the layers of corpuscles must be continually dissolved, and others formed a little further back by the spread of the digestive ferments. During this process the fibroblasts undergo division as in the case of the gill-tissue, but while the inflammation is much more acute, the multiplication of fibroblasts is not so rapid, and they are not nearly so noticeable a feature in the sections. In the form of rounded cells, with oval or spherical nuclei, they migrate in small numbers towards the layer of agglutinated blood-corpuscles. Here they share the fate of the corpuscles, being dissolved by the digestive ferments, and accordingly there is no formation of fibrous tissue.

We were never able to keep the animals alive for more than six days. At the end of this time all that remained of the digestive gland was the brown pigment-granules and a little epithelial débris. This was surrounded by a space from which most of the muscular tissue had been dissolved, and this again by a relatively large area of degenerated muscle-fibres. Finally, the whole was surrounded by a layer of agglutinated blood-corpuscles, into which a few fibroblasts were making their way.

These experiments show that the protective layer of corpuscles must very completely shut off the space it encloses from the neighbouring tissue. If this were not the case the digestive ferments, once they had gained access to the blood, would rapidly become disseminated over the whole body. Instead of this, we have distinct evidence that there is a slow and

steady invasion of the tissues by the ferments, and that the area of their action is always contained within a protective layer of agglutinated blood-corpuscles. It seems probable that the digestive gland, when implanted, contains little or no free enzyme, and quickly becomes surrounded by the protective layer of corpuscles, and that later the enzymes are slowly evolved from the zymogens contained within the cell. The vitality of these cells has been impaired by removal from their normal connections and by implantation into the muscle tissue, and accordingly they are dissolved by the enzymes they have themselves evolved.

The Reaction of the Tissues to Implanted Agar Jelly.

Sterile agar jelly has no irritative action on the muscle, and so differs from the tissues previously described.

Agar jelly may be regarded as a physiologically inert substance, and as in these experiments it was made from sea-water in which the Pecten were living, it was approximately of the same salinity as their blood (Dakin, 2), and so was of the same osmotic concentration. Further, the cylindrical rods of agar are remarkably smooth, and if unbroken present no rough surface, except possibly at the extremities.

One of us (Drew, 4) has shown that in the case of Cardium norvegicum, the agglutination of the blood-corpuscles (*in vitro*) is much influenced by the nature of the substance on which they impinge, and that it occurs very much more readily when they come in contact with a rough surface from which a large number of small points may be imagined to project, than when they impinge on a smooth, polished body. It seems probable that similar conditions obtain in the case of the blood of Pecten maximus.

In accordance with these properties of the agar jelly, it was found that absolutely no inflammation resulted from its implantation in the muscle. No layer of agglutinated corpuscles was formed round it, and there was no sign of the collection of unusual numbers of the corpuscles in the

vicinity, nor of any distension of the blood-spaces. The fact that the rod of jelly was always implanted as far as possible parallel to the long axes of the muscle-fibres, and that they were usually rather separated from each other, than cut by the insertion of the transplanting needle, probably contributed towards this result.

After a period of about seven to eight days there were signs of division of the fibroblasts in the neighbourhood of the implanted mass, and a slow migration of the new-formed cells towards the agar took place. By about the tenth day these cells had arranged themselves so as to form a thin and delicate ensheathing layer. The process presents marked differences from that which occurs after the implantation of a substance which causes an inflammatory reaction, with the consequent development of a protecting layer of agglutinated corpuscles. The division of the fibroblasts, instead of being rapid and amitotic, is comparatively slow, and frequently, though not always, mitotic. The nuclei of the young fibroblasts retain their elongated shape, and though the nuclei of the dividing cells lose their typical spindle-like appearance and become oval, they do not become round, as in the case of rapid division after inflammation. The layer of fibrous tissue formed is thinner and less compact, the proportion of cytoplasm to nucleoplasm is greater, and the nuclei assume their typical spindle shape more rapidly. The process seems to be complete by the tenth day, and the appearance is almost identical with that shown in fig. 5, which represents the condition after seventeen days.

In some of our experiments the sealing-wax with which the drill holes were closed became detached in the tank. The holes were re-sealed as soon as this was noticed, but the animals seldom survived long. On sectioning, an area of inflammation was usually found surrounding the agar, and rapid division of the fibroblasts in the vicinity was in progress. In specimens that survived longer a complete sheath of fibrous tissue had formed round the agar, and the condition resembled that resulting from implantation of gill-

tissue. It seems that in these cases bacteria must have entered through the drill-hole, and, travelling between the agar and muscle, have caused an inflammatory reaction. In one other case, in which the hole had not come unsealed, inflammation and formation of fibrous tissue occurred, but as this only took place once out of twenty-six implantations made with sterile agar, it is probable either that the sealing-wax plug leaked at the edges or that bacteria found their way in when the agar was introduced.

Summary of Results.

Our experiments show that the implantation of a tissue, such as that forming the gills, accompanied by the bacteria which adhere to it, produces an intense inflammatory reaction. This is characterised by the active migration of blood-corpuscles, which form a plasmodial mass around the implanted tissue, shutting it off from the general circulation. This protective layer is gradually removed by phagocytosis and autolysis, and at the same time the gill-tissue is invaded and removed by phagocytes. While this is going on, rapid amitotic division of the fibroblasts in the neighbourhood occurs; they lose the typical spindle-shape of the nuclei, and the new-formed cells consist of rounded or oval nuclei, with a scarcely perceptible amount of cytoplasm. These rounded cells migrate towards the implanted tissue, and arrange themselves in layers around it, the nuclei become elongated, and the proportion of cytoplasm increases. Finally, a layer of typical "scar" fibrous tissue is formed, enclosing the chitinous skeletons of the gill-bars.

In the case of the implantation of digestive gland tissue a similar protective layer of agglutinated corpuscles is formed, but this is continually dissolved up and reformed, as the sphere of action of the enzymes in the cells of the digestive gland extends. All the muscle-fibres within this protective layer soon lose their striation, swell, and are partially dissolved, presumably by the digestive enzymes. The fact that

there is a progressive extension of this digestive action shows that the layer of agglutinated corpuscles performs its protective function very completely, as otherwise the enzymes would escape into the general circulation. Simultaneously the fibroblasts in the vicinity multiply and migrate, as in the case of implanted gill-tissue, but the multiplication does not seem to be so rapid. No permanent layer of fibrous tissue is formed, as the migrated fibroblasts are dissolved in the course of the extension of the sphere of action of the digestive ferments.

In the case of the implantation of sterile agar jelly, made with sea-water, no inflammation results, and for some time there is no sign of any reaction of the tissues if absolute asepsis has been ensured. After seven or eight days there is a slow and often mitotic division of the neighbouring fibroblasts; they migrate and rearrange themselves to form a thin layer of fibrous tissue around the agar.

It is noteworthy that though the tissues and the blood, especially in its manner of forming a "clot," present marked differences from those in Vertebrates, yet the formation of fibrous tissue, as a reaction to injury, does not differ in any essentials from the process which takes place in the higher types.

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DESCRIPTION OF PLATE 24,

Illustrating the paper by Messrs. G. H. Drew and W. de Morgan on "The Origin and Formation of Fibrous Tissue produced as a Reaction to Injury in Pecten maximus, as a type of the Lamellibranchiata."

Reference Letters.

ag. Agar. *agg. lyr.* Agglutinated layer of blood-corpuscles. *b.c.* Blood-corpuscles. *deg. gill.* Degenerated gill-tissue. *deg. msl.* Degenerated muscle. *dig. gl.* Digestive gland-tissue. *div. fbl.* Dividing fibroblasts. *fbl. lyr.* Fibroblast layer. *mig. fbl.* Migrating fibroblasts. *msl. fbr.* Muscle-fibres.

[N.B.—In the figures the bundles of muscle-fibres are shown as a whole: the individual fibrils and their striations are not differentiated. The size of the muscle-bundles differs considerably in different parts of the adductor muscle.]

Fig. 1.—× 400. Gill-tissue which has been implanted for sixteen hours. A layer of agglutinated corpuscles divides the degenerated gill-tissue on the left from the muscular tissue on the right. Corpuscles are making their way between the muscle-fibres to join the agglutinated layer.

Fig. 2.—× 300. A later stage of fig. 1, taken seventy-two hours after implantation. A definite layer of fibrous tissue has been formed round the gill-tissue, which is completely degenerated and invaded by phagocytes. The fibroblasts are dividing and migrating towards the lesion.

Fig. 3.—× 700. A more highly magnified portion of one of the blood-spaces drawn from the same section as fig. 2. The fibroblasts are undergoing amitotic division, and migrating towards the gill-tissue, where they arrange themselves to form a layer of fibrous tissue.

Fig. 4.—× 450. Digestive gland-cells (on the left) which have been implanted for ninety-six hours. External to them is a region of degenerated and partially dissolved muscle-fibres, which is divided from the normal muscle by a thin layer of agglutinated corpuscles. These are also rapidly degenerating, but are reinforced by the continued arrival of fresh corpuscles. The cellular structure of the alveoli of the digestive gland has been lost, leaving little beyond traces of the original cell walls and the brown pigment-granules.

Fig. 5.—× 450. Agar jelly (to the left) which has been implanted for seventeen days. It is divided from the muscle-tissue by a delicate layer of fibroblasts.


deg. gill.
msl. fbr.
Agg. lyr.
b.c.
1.
msl. fbr.
deg. gill.
b.c.
mig. fbl.
2.
fbl. lyr.
Ag.
5.
fbl. lyr.
mig. fbl.
msl. fbr.
msl. fbr.
b.c.
3.
div. fbl.
mig. fbl.
fbl. lyr.
big. gl.
msl. fbr.
b.c.
mig. fbl.

The Division of the Collar-Cells of Clathrina coriacea (Montagu): A Contribution to the Theory of the Centrosome and Blepharoplast.

By

Muriel Robertson, M.A., and E. A. Minchin, M.A.

With Plates 25 and 26.

Introductory.

At the present time there is a great deal of confusion in the use of the words "blepharoplast" and "centrosome." Two distinct questions arise with regard to the significance of these bodies; the first is the question of the homology of blepharoplasts and centrosomes; the second is that of the nature of the centrosome, and more particularly whether or not it is to be regarded as equivalent primarily to a nucleus.

With regard to the first of these questions, it is now generally admitted that blepharoplasts and centrosomes are essentially bodies of the same nature, for reasons that will presently be considered at greater length. The difference between a centrosome and a blepharoplast, on this view, is entirely a matter of divergence of function. A centrosome may be briefly characterised, in a general way, as a body which exerts or governs kinetic functions in relation to the division of the nucleus; a blepharoplast may be defined as a centrosome which governs the movements of motile organs, such as flagella, which arise from it and are in direct or indirect connection with it.

With regard to the second of these questions, namely, the nature of the centrosome, two opposite views are current,

which may be summarised as follows: (1) The centrosome is to be regarded as primarily a body of achromatic[1] nature, elaborated and evolved, in all probability, in the nucleus or in connection with it, but not itself equivalent to a nucleus; (2) the centrosome is regarded as the equivalent of a nucleus, and as representing primarily a nucleus which has become modified and specialised both in function and structure. These two theories may be termed conveniently the achromatic and the nuclear theory of the centrosome respectively. According to the second of these views, which has recently been revived and advocated by Hartmann and Prowazek (6), every cell is to be regarded as primarily and essentially binucleate; the two nuclei, at first, doubtless, equivalent and similar in all respects, became modified in two directions respectively, the one becoming specialised for trophic, the other for kinetic functions, with corresponding differentiation of structure. In the metazoan cell, according to this theory, the nucleus represents the original trophic nucleus deprived of all kinetic structures, while the centrosome represents the kinetic nucleus deprived of all "vegetative" functions and of its chromatic apparatus. On this interpretation of the centrosome, the minute granules which are the centre of kinetic functions are termed "centrioles," in order to distinguish them from the centrosomes as a whole. In fact, from the point of view of the nuclear theory of the centrosome, the centriole requires to be defined in exactly the same way as the centrosome itself on the achromatic theory.

The confusion produced by these two theories of the centrosome reaches its height in the nomenclature of the different parts of the body of a trypanosome. In these organisms, and in allied genera of flagellates, there are three distincts parts of the nuclear apparatus to be reckoned with. First, there

[1] Meaning by the term "achromatic" something which is not composed of chromatin, not necessarily something which is not coloured by stains. All is not chromatin that stains, even with a so-called nuclear stain. In our opinion a great deal of error and misconception has arisen by identifying as "chromatin" all bodies in the cell that are coloured black, for instance, by the iron-hæmatoxylin method.

is a chromatic body, which may be denoted temporarily by the symbol *N*, situated usually in or near the middle of the cell-body, and in no special connection with the flagellar apparatus. Secondly, there is a second chromatic body, which may be denoted by the symbol *n*, distinctly connected with the flagellum or flagella, when they are present, and apparently kinetic in function. In the genera Trypanosoma, Herpetomonas, Leishmania, and Crithidia, *N* is always much larger than *n*, but in Trypanoplasma the reverse may be the case. Finally, the flagella arise, probably in all cases, from basal granules, which are often very minute and exhibit staining reactions quite different from either *N* or *n*.

According to the nuclear theory of the centrosome advocated by Hartmann and Prowazek, these three parts of the trypanosome body are to be interpreted and named as follows: *N* is the trophic nucleus, while *n* represents the second nucleus of kinetic function, in other words, the centrosome, which, since it controls the activities of the flagellar apparatus, is to be termed a blepharoplast. The basal granule is a mere thickening of the proximal end of the flagellum, of no special significance, or at most representing a centriole. Thus a trypanosome would represent the ideal binucleate cell of Hartmann and Prowazek in a very primitive state.

An interpretation of the trypanosome body, quite different to that of Hartmann and Prowazek, has been advocated by one of us (**12**), which may be briefly stated as follows: *N* is a trophic nucleus, which contains its own centrosome or division-centre in itself; *n* is a distinct kinetic nucleus, a specialisation of the nuclear apparatus for a particular function; it has nothing to do with a true centrosome, though it may, like the trophic nucleus, contain a body of this kind, nor is it to be regarded as a blepharoplast, a body which is represented by the basal granule of the flagellum.[1]

[1] It is not our purpose here to summarise the various views that have been put forward with regard to the morphological interpretation of the trypanosome-body, but only to select two which show in sharp

In consequence of these divergent theories and interpretations, a great confusion in nomenclature has arisen, especially with repard to *n*, which is always termed the blepharoplast in German works, the centrosome in French works, and in this country is sometimes named the micronucleus, but more usually the kinetonucleus.

In Flagellata other than the trypanosomes and their allies there is usually only one structural element other than the principal nucleus (*N*) to be reckoned with in the nuclear apparatus, namely, a deeply staining grain or set of grains, from which the flagellum or flagella take origin, and to which the name "blepharoplast," or the synonymous term "diplosome,"[1] is commonly applied. The question at once arises, How is the arrangement seen in a trypanosome to be compared to that of other flagellates, and to which element in the nuclear complex of a trypanosome should the blepharoplast of an ordinary simple flagellate be compared? Does it represent the basal granule (true blepharoplast, on our view) or the kineto-nucleus (*n*)? In our opinion, the bodies in question are true blepharoplasts, comparable to the basal granules of the flagella of trypanosomes, and the kinetonucleus or German blepharoplast of the trypanosomes and their allies is a nuclear body peculiar to them, and not found in ordinary flagellates. To this extent, at least, we are in agreement with the idea expressed by Hartmann, who has placed the trypanosomes and forms regarded as

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contrast opposed views with regard to the nature of the blepharoplast and the proper application of this word. Thus Laveran and Mesnil in their well-known work on trypanosomes use the term "centrosome" for *n*; so also Moore and Breinl, who contrast the extra-nuclear centrosome (*n*) with the intra-nuclear centrosome (karyosome of *N*).

[1] "The term "diplosome," meaning literally and etymologically a double body, is commonly applied, by an abuse of language, to the single grain from which a flagellum arises. It should, of course, be used only for those cases where twin granules give origin to two or more flagella, that is to say it should not be regarded as synonymous with blepharoplast or basal granule, but as implying a condition in which such bodies are doubled.

allied to them in a separate order of Flagellata termed the Binucleata. (The question as to whether or not the Hæmosporidia should be included in the Binucleata is one which, in the present memoir, we do not wish to raise or discuss.) A trypanosome is, in our opinion, a binucleate organism, possessing a trophic nucleus (*N*), a kinetic nucleus (*n*), and a blepharoplast (basal granule).

In order to settle these disputed points, more knowledge is required regarding nuclear and other structures connected with the locomotor apparatus in other organisms, and in the hope of throwing some light on these questions we have studied the division of the collar-cells of a calcareous sponge, of which preserved material was in the possession of one of us. A collar-cell, although occurring as tissue-element of a Metazoan organism, is essentially a flagellate organism, comparable in every way with an individual of the Choano-flagellata. It has recently been pointed out by one of us (**13**) that there are two types of collar-cells in calcareous sponges. In one type, characteristic of the family Clathrinidæ, amongst Ascons, the nucleus lies at the base of the cell, far removed from the origin of the flagellum, which arises from a distinct basal granule or blepharoplast situated at the apex of the cell. In the other type, characteristic of the Leucosoleniidæ amongst Ascons, and of the Heterocœla generally, the flagellum arises directly from the pear-shaped nucleus, which is usually situated in the upper part of the cell, close to the point at which the flagellum emerges from the body of the cell. These two differences in arrangement are also paralleled amongst free-living Flagellates, for instance amongst the two genera of Mastigamœbæ described by Goldschmidt (**4**), and there can be no doubt that the conditions are perfectly comparable in the two cases—that is to say, that when the flagellum arises from a basal granule distinct from the nucleus, the basal granules are homologous structures. As the result of our investigations we have obtained, as will be apparent in the sequel, evidence of a most convincing kind as to the identical nature of centrosomes and

blepharoplasts; but before proceeding to the detailed account of our observations it will be useful to give a brief resumé of previous work on this subject. For this we have relied chiefly on the excellent summaries given by Wilson (20) and Erhard (3).

The most convincing and abundant evidence of the identical nature of blepharoplasts and centrosomes has come from the study of spermatogenesis in animals and plants. These researches have been summarised by Wilson and Erhard, and it will be sufficient here to refer specially to the memoirs of Henneguy (7) on the spermatogenesis of Bombyx mori, etc., and of Belajeff (1) on that of Gymnogramme and Marsilia spp. Henneguy found, as we have done, the blepharoplast (in this case a diplosome in the true sense of the word) acting as a centrosome in the mitosis while still preserving its function as a blepharoplast. Similarly Belajeff found that the body which acted as a centrosome in the mitosis became subsequently the blepharoplast.

In the case of tissue-cells other than spermatocytes in Metazoa, the relation of flagella and cilia to bodies of centrosomic nature has been studied by Joseph (9A), in whose memoir will be found very full references to the work of others. Joseph's researches have led him to support very definitely the theory of Lenhossek and Henneguy, according to which the basal corpuscles of the cilia arise from the centrosome; and in his conclusions he states (l.c., p. 71): "Viele, vielleicht alle eingeisseligen Zellen sind Centralgeisselzellen, d. h. ihr Geisselfaden steht in Verbindung mit dem Centrosom." Erhard (3) has reviewed the whole question in the light of renewed investigations, and comes to the following conclusions: "Das Diplosom in Flimmerzellen als Teilungsorgan wirkt, also ein echtes Centrosom darstellt. . . . Die ausserordentliche Seltenheit von Mitosen in Flimmerzellen darauf schliessen lässt, dass die Diplosomen in allgemeinen eine andre Rolle als die der Teilung auszufüllen haben. . . . Zwischen Basalkörpern und Centrosomen kein erlei Beziehungen bestehen . . . die Basalkörpern an der

Teilung der Metazoenflimmerzellen keinerlei aktiven Anteil nehmen, so kann fur diese Zellen die Henneguy-Lenhossekschе Theorie nicht mehr aufrechterhalten werden." Thus while maintaining the centrosomic nature of the diplosome, Erhard denies it for the basal granules of the cilia in ciliated cells.

As regards the basal granules of the flagella in Protozoa, evidence bearing on their nature is scanty to a disappointing degree. The majority of investigators appear to ignore these bodies. Schaudinn (17) found in Paramœba the "Nebenkörper" acting as a centrosome in the mitosis; the flagella of the swarm-spore appear to arise quite independently of the Nebenkörper, a body which, from Schaudinn's investigations, gives the impression of being rather of the nature of a kinetonucleus than of a centrosome (pace Hartmann and Prowazek), and which very probably contains its own centrosome (or centriole), which acts also as the centrosome of the principal nucleus in the mitosis. Prowazek (15) points out that the flagellum of Flagellata may arise within the nucleus ("Kernendogener Ursprung") or outside it; in the latter case the flagellum may terminate in a "diplosome," which again may be quite free from the nucleus (as in the collar-cells studied by us) or may be connected with the nucleus by a "rhizoplast." In the nuclear division of Entosiphon, Prowazek (16) found a "basalkörperartige Verdickung" at the origin of each flagellum, and from this body a rhizoplast passing back to the nucleus. At the division of the nucleus a "Centronukleolusspindel" is formed. The basal granules do not appear to influence the division of the nucleus in any way; they divide, and two new flagella grow out from each pair.

In his famous investigations on the trypanosome of the little owl, Schaudinn (18) gives the following account of the origin of the flagellar apparatus. The nucleus of the oökinete contains a karyosome in which a "central grain" is surrounded by eight chromosomes. By heteropolar division the single nucleus divides into a larger nucleus, the trophic nucleus, and a smaller, the kinetonucleus ("blepharoplast"). The kineto-

nucleus is "a complete nucleus with centrosome and eight chromosomes, not merely a centrosome, karyosome, nucleolus, or a simple ectoplasmic thickening." (The contrast drawn between a nucleus and a centrosome in this sentence is instructive.) The kinetonucleus then divides by another heteropolar mitosis and gives rise to a third nucleus, the smallest of the three; this third nucleus forms a nuclear spindle composed of eight mantle-fibres and a "central spindle" or centrodesmose connecting the two centrosomes situated at the two poles of the spindle. The central spindle becomes the flagellum and the eight mantle-fibres the eight myonemes. By growth and elongation of the flagellum and myonemes, one centrosome is carried out at the tip of the flagellum, while the other remains as its basal granule. From these statements of Schaudinn, it may at least be said without expressing any opinion as to the accuracy of the details in the development described by him that he regarded the basal granule of the flagellum as a centrosome, and that he distinguished clearly between a centrosome and a nucleus, and in particular between the kinetonucleus and the centrosomic body from which the flagellum arises, although he used, in our opinion quite wrongly, the term "blepharoplast" to denote the kinetonucleus, instead of applying it to the basal granule of the flagellum. This mistake, as we consider it, in Schaudinn's terminology is the more remarkable, since he seems to have understood so clearly the true centrosomic nature of the basal granule of the flagellum, and to have realised its existence independent of the kinetic nucleus.

The most important contribution to the question of the blepharoplast in the Protozoa is the memoir of Jahn (8) on the swarm-spores of one of the Mycetozoa, Stemonitis flaccida. He finds that at division the centrosomes at the poles of the nuclear spindle give rise to the daughter-flagella while still actually engaged in their centrosomic functions; a state of things entirely parallel to that which we have found in the collar-cells we have studied.

Hamburger (5) found in Dunaliella the paired flagella

arising from a basal granule which is connected with the nucleus. At division the basal granules divide and each gives off two flagella; though they do not appear to control the division of the nucleus in any way, nevertheless each basal granule is connected with the dividing nucleus by two streaks, giving an appearance very similar to that figured by us on Plate 25, figs. 4 and 5. (Jahn also figures a very similar condition.) Dobell (2), in his investigations on Trichomonas, etc., appears to support a view similar to our own. Lastly, Yamamoto (21), who has studied the locomotor apparatus of various organisms by methods which seem to us unduly violent and severe, describes the flagellum of a trypanosome as arising from a basal granule ("proximal centriole"); his statements, in matters of fact, simply confirm those of Schaudinn.[1]

Observations on the Division of the Collar-cells.[2]

The material on which this work was done consists of a number of specimens of Clathrina coriacea preserved by one of us at Roscoff, and embedded in paraffin at the time.

[1] Yamamoto states that he has obtained preparations of trypanosomes (species not stated) showing myoneme fibrillæ, of which he states I deny the existence. This is a glaring misstatement on his part, seeing that I have described and figured the myonemes of Trypanosoma percæ and T. granulosum in full detail (vide 'Proc. Zool. Soc. Lond.,' 1909, pl. v, figs. 84, 96, 97).—E. A. M.

[2] I greatly regret that in my account of the Sponges in Lankester's 'Treatise on Zoology' (Part II, 1900, p. 56) I gave an entirely erroneous account of the division of the collar-cells of Clathrina coriacea, stating that after division of the nucleus the cell divides transversely to its long axis, and then the basal portion forms a new collar and flagellum. I have re-examined the figures and preparations on which these statements were founded, and see that I was misled by sections passing obliquely through the epithelium, so that the top part of a dividing cell, with the nucleus at the apex, appeared superposed on the base of an ordinary cell, with its nucleus in the usual position. The account given in the present memoir will show clearly the error of my former statements.—E. A. M.

Most of the sponges were preserved in osmic acid followed by picrocarmine, a good method for showing clearly the cytoplasmic structures, especially the collar and flagellum, but not suitable for demonstrating the finer details of the nuclear apparatus. Some of the material, however, had been preserved in Hermann's fluid, and it is on this that we base the results set forth in this memoir. Sections cut from sponges preserved in this way were stained with various stains, more particularly by Heidenhain's iron-hæmatoxylin method, and counter-stained with eosin or Lichtgrün, the latter being found to be of great assistance in making out the details of the collar and flagellum, since these parts are tinged by it.

(1) The Resting Collar-cell.—In Clathrina coriacea, as in all sponges of the family Clathrinidæ, the nucleus lies invariably, in the ordinary "vegetative" or resting condition, at the base of the columnar collar-cell, that is to say, at the end which is furthest from the collar and flagellum. At the apex of the cell, in the centre of the area enclosed by the base of the collar, lies a minute granule—the blepharoplast—from which the flagellum takes origin. These structures, no less than the general form of the collar-cell and its position in the epithelium, of which it forms a part, give a definite orientation to the cell; any direction parallel to an imaginary axis continuing that of the flagellum and passing through the blepharoplast and nucleus may be termed vertical, while any plane at right angles to the vertical axis may be termed horizontal.

The form of the collar-cell and the dimensions of their different parts vary considerably with the condition of the sponge, whether expanded or contracted, and may be different also in different parts of the same sponge. In specimens in which the pores are fully open, and in which all appearances indicate that the collar-cells are in full functional activity, the bodies of the cells are fairly broad, and about 12–13 μ in height by 5–6 μ in breadth; the collar reaches a length of 10–11 μ, and the flagellum some 25–27 μ. When, on the other hand, the pores are closed up and the sponge is partially con-

tracted, the collar-cells become taller and narrower and the collar much shorter. In each cell the basal three fourths of the body is broader and more or less cylindrical in shape; this part of the cell is in contact with the neighbouring cells, and constitutes the main body of the cell. We have not found processes connecting the bodies of the cells with one another. It has been shown by Minchin and Reid (**14**) that when the collar-cells are carefully brushed away and the wall of the sponge is stained with picro-nigrosin, a delicate blue-stained network is visible in surface view, representing a honeycomb-like structure, the spaces in which were originally occupied by the bodies of the collar-cells. Hence in life the bodies of the collar-cells are probably not in actual contact, but are separated by a delicate extension of the gelatinous ground-substance of the body-wall of the sponge. If, as would seem probable on theoretical grounds, the bodies of the collar-cells are connected across this intervening substance by protoplasmic fibrils, such connections have escaped our notice, possibly on account of their being of extreme tenuity and requiring, perhaps, other methods of technique, in order to demonstrate their existence, than those employed by us for the study of the mitosis. It is well known that in other sponges the collar-cells may be connected by protoplasmic processes, as, for instance, in Hexactinellids, where such processes are extremely obvious, forming the so-called membrana reticularis.

The cylindrical basal portion of the cell ends in a distinct rim or flange, and from this level arises a narrower portion, which may be termed the "neck," and which is quite free from any contact with neighbouring cells. The summit of the neck is rounded off, forming a convex lens-like area enclosed by the base of the collar, and giving off centrally the flagellum. The so-called collar has more the form of a cuff or sleeve when fully expanded. It is distinctly thicker and more rigid in its basal portion, becoming very delicate at its distal end, which is usually more or less shrunk and distorted in preparations. The uppermost limit of the collar is

often very difficult to make out. It is best preserved in the osmic-picrocarmine preparations; after Hermann's fluid it appears collapsed and shrunk or frayed out. A short way above its origin the collar usually shows a distinct thickening, visible as a horizontal hoop-like structure, especially when the collar is a little contracted; when it is expanded to its fullest extent the hoop is difficult to make out as a horizontal line, but its presence is marked by the fact that all the part of the collar below it stands out stiff and firm, and is not creased and folded like the part above. It is evident from the appearances seen both in the resting and the dividing cell that the collar for about 2 μ from its origin is thickened and strengthened as compared with its distal portion.

The nucleus of the collar-cell is about 5 μ in diameter and more or less spherical in form, sometimes slightly flattened in the vertical direction. The most conspicuous element in its structural composition is a large grain, which stains deeply with iron-hæmatoxylin, and appears to be of the nature of a karyosome. This structure is always present, and sometimes double (figs. 1 *c*, 3, 7). The karysome is sometimes lodged in a clear space (fig. 36, *d*, *e*, *f*); its position in the nucleus varies. The remainder of the nuclear contents appear granular, but in thin sections of the nucleus a fine network can be made out (figs, 36, *e*, *f*, *g*), in the nodes of which the granules of chromatin are lodged. These granules vary very much in different nuclei in the same preparation, being sometimes so fine as to be scarcely visible individually, while in other nuclei they are coarse and irregular in size and shape (figs. 30 and 36, *a*, *b*, *c*). All transitions can be found between the finely and the coarsely granular condition, but the two extremes form two well-marked types, which may be characterised as the light and the dark type respectively. It is worthy of note that nuclei of cells about to divide are always of the light type, as will be pointed out in the next section.

The above description of the nuclei refers to preparations stained with iron-hæmatoxylin. In material preserved and

stained by the osmic-picrocarmine method the nuclear structure is not shown at all as a rule, but the nucleus simply stains evenly pink. Sometimes the karyosome can just be made out, sometimes not. A peculiar feature of the preparations is that the red stain often does not extend up to the nuclear membrane; the stained portion forms a mass lying in the centre of the nucleus, and between this stained mass and the nuclear membrane a clear space remains, which can often be seen to be traversed by delicate radiating lines, as if fine filaments started from the membrane to support the central stained mass. Comparison with nuclei stained with iron-hæmatoxylin shows in many of the latter a distinct alveolar border to the linin-framework; sometimes the alveolar border is relatively very broad (fig. 36 *e*), and shows the radiating partitions of the alveoli very distinctly. It would appear as if the action of the osmic-picrocarmine method was to cause a shrinkage within the alveolar border, with the result that this inner portion of the nuclear framework contracts and appears as a homogeneous mass, which contains all the chromatin and stains deeply, leaving the alveolar border unstained. It should be noted that by no means all the nuclei of the collar-cells show the clear border within the membrane; many of them stain evenly up to the membrane, and this is always so in those cells which are about to divide.

The blepharoplast and flagellum stain black with iron-hæmatoxylin, but by the osmic-picrocarmine method they are not stained.

The cytoplasm of the collar-cells is finely granular and usually very vacuolated. The neck is free from vacuoles as a rule, but in many cases a round vacuole-like structure, which differs in appearance from the other vacuoles, can be seen in the neck region. The ordinary vacuoles in the body of the cell are clear and appear as empty spaces, doubtless representing drops of fluid in the living condition, but in the direct line between the nucleus and blepharoplast there is generally to be seen a vacuole, which has finely granular

contents and sometimes a minute central granule (fig. 30, cell on the extreme right). This body is sometimes nearer the blepharoplast, sometimes nearer the nucleus, but usually it lies at a level midway between the neck and the main body of the cell or in the neck itself; its significance is doubtful.

In addition to the vacuoles, the cytoplasm almost always contains one or more coarse refringent granules of irregular, angular form and yellowish-brown colour. They are lodged in any part of the cell and are often present in the vicinity of the blepharoplast. They probably represent excretion-grains. After the iron-hæmatoxylin stain they become darker, but still retain their characteristic yellowish-brown tint, and can be easily distinguished from chromatin grains. No other enclosures, as a rule, are to be found in the collar-cells, but occasionally they contain large rounded bodies (figs. 31–35 and 50, 51), which stain deeply with iron-hæmatoxylin and appear to be of the nature of organisms, though whether they represent parasites or food ingested by the cells is difficult to say. In some parts of the sponge they are found more commonly than in others, and in one case (fig. 34) no nucleus could be made out in the cell; it may, however, have been cut off in the section.

(2) Preparations for Division.—Before the nucleus begins to show any of the changes in its minute structure which initiate mitosis certain events take place in the cell, namely, the migration of the nucleus bodily from the base to the summit of the cell, the disappearance of the flagellum, and the division of the blepharoplast. As a general rule these three events take place in the order named, but not invariably, so that a number of different combinations arise in different cases.

The migration of the nucleus is always the first sign that a collar-cell is about to divide, and this peculiarity is a great aid to the study of the division, since in a section of the sponge which shows the collar-cells cut vertically those that are dividing or preparing to divide arrest the attention at once, even with a comparatively low power of the microscope,

owing to the fact that the nucleus is no longer in its usual position at the base of the cell, but has either migrated to the apex or has been preserved in the act of doing so, and is found in some position between the base and the apex (figs. 1–5, etc.). Such cells are also characterised by being much broader and stouter than the ordinary resting cells, but they do not increase in height to an appreciable extent.

By this process of migration the nucleus comes to lie immediately under the blepharoplast, and at this stage a curious appearance has been observed in two instances (figs. 4 and 5); the nucleus is seen to be flattened on the side nearest to the blepharoplast, and from the blepharoplast itself two streaks appear to radiate to the two ends of the flattened side of the nucleus. Careful examination of each of these preparations gives the impression that these two streaks are in reality the optical section of a cone-shaped mass of protoplasmic substance, the base of which rests on the flattened side of the nucleus, and which is, perhaps, the cause of the flattening. A comparison with the resting cell suggests that this conical mass is derived from the peculiar vacuole with granular contents, which was described in the last section as situated in the direct line between nucleus and blepharoplast, and that by the upward migration of the nucleus the vacuole in question is pushed up until it is caught, so to speak, between nucleus and blepharoplast, when, coming under the influence of the forces of attraction or repulsion exerted by the blepharoplast, it assumes the conical form seen. If this is a correct interpretation of the phenomena, the vacuole should, perhaps, be regarded as an archoplasmic vesicle, such as has been described in other cases, and which supplies some part of the material of the achromatic spindle in the mitosis. In fig. 4 it is seen that the flagellum is still present, though short, while in fig. 5 the flagellum has entirely disappeared and the blepharoplast has divided.

The disappearance of the flagellum and the division of the blepharoplast are two events which take place independently so far as their relative sequence in time is concerned, that is

to say, the flagellum may disappear completely before the blepharoplast divides or may persist until after this has taken place. In either case the two daughter-blepharoplasts migrate inwards and place themselves on opposite sides of the nucleus in order to become, as will be seen, the two centrosomes in the mitosis. If the flagellum persists during this process of events it remains attached to one of the two blepharoplasts (figs. 6 and 10), and becomes drawn into the body of the cell, as seen in figs. 7–9; in each of these three specimens the flagellum, though greatly shortened, is still persistent, and can be seen passing into the body of the collar-cell and terminating in one of the two blepharoplasts, while the other blepharoplast can be seen on the other side of the nucleus quite independent of the flagellum. On the other hand, figs. 5, 11, and 12 show the two blepharoplasts very close together at the apex of the cell and apparently very recently separated from one another, with no trace of a flagellum.

The exact method in which the flagellum disappears is difficult to determine simply by comparison of different stages in sections; it could only be made out satisfactorily by watching the process in the living cell. In collar-cells in which the upward migration of the nucleus is taking place, the flagellum almost always appears much shorter than in the surrounding cells, an appearance too constant in occurrence to be explained simply as due to artificial curtailment of the flagellum in the process of section-cutting, especially when the collar is intact and the flagellum does not project beyond it (figs. 9 and 10). But a remarkable feature of this stage is the frequent occurrence of a protoplasmic projection, like a small pseudopodium, from the apex of the cell round the base of the flagellum (figs. 7, 9, 39, 40); this process persists for a time after the flagellum has completely disappeared (figs. 14, 41). The appearances suggest that the cell throws out a pseudopodial process, by the help of which the flagellum is retracted and absorbed at its base;

in all cases the protoplasmic process in question is very short in proportion to the length of the original flagellum.

The division of the blepharoplast takes place with formation of a distinct centrodesmose connecting the two daughter-blepharoplasts (figs. 6, 7, 13).

During these changes the collar remains practically unaltered, except that it begins to show more or less clearly the appearance of shrinkage and degeneration characteristic of the succeeding stages of the division.

(3) The Mitosis.—The general course of the mitosis in the collar-cell is similar to that known to occur in the cells of other Metazoa generally, and described for sponges by Maas (**10**, **11**) and Jörgensen (**9**). It is unnecessary, therefore, to do more than describe its most characteristic features.

As already stated in a previous section, the nucleus of a collar-cell about to divide, but before any changes preparatory to division have begun in the chromatin contents, is of a pale type—that is to say, the granules of chromatin distributed over the general framework are very fine and scattered evenly, so as to give the nucleus an almost homogeneous appearance relieved only by the karyosome, stained a deep black, after iron-hæmatoxylin, in contrast with the pale grey tint of the remainder of the nucleus (figs. 1, 10, 11). The nucleus at this stage is also distinctly larger than the average nucleus of a resting cell.

The first changes to be observed in the chromatin contents of the nucleus are that they stain darker and become more blotchy and uneven in appearance, apparently as the result of the minute granules of chromatin being clumped together to form coarse grains or masses. Figs. 6 and 7 show early stages in this process; the masses of chromatin still stain faintly, appearing to be loose in texture and ill-defined in outline, and the karyosome stands out sharply. In later stages (figs. 8, 12) the chromatin masses become more definite in outline and somewhat smaller, and the deep stain they take gives the impression that they are more closely knit and of denser texture; the karyosome, however, is still distinct.

Finally, the chromatin masses become very definite and stain very deeply, and no distinct karyosome can be made out; this body seems to break up and to contribute by doing so to the general store of chromatin. At first the chromatin masses, or chromosomes, as they may now be termed, appear to be connected together by delicate filamentous junctions (fig. 9); this stage corresponds apparently to the spireme stage. Next, the connections between the chromosome disappear, and they are seen lying separately from one another as irregular rounded masses, showing more or less distinctly indications of division, each into two (fig. 15). In spite of much searching we have not been able to find any stages other than those described, and, in particular, nothing more nearly resembling an ordinary spireme stage than the specimen shown in fig. 9.

These changes in the interior of the nucleus also go on quite independently of the changes in the flagellum and blepharoplast described in the previous section. Thus the flagellum may have vanished, and the two daughter-blepharoplasts may have taken up their definitive position when the nuclear contents are at the beginning of their changes (fig. 14); or the nucleus may be comparatively far advanced when the blepharoplast has only just divided (fig. 12), or before the flagellum is absorbed (figs. 8, 9). Finally, however, a stage is reached when the nucleus has resolved itself into a mass of separate chromosomes, and the two blepharoplasts, or, as they may now be termed, the centrosomes, are placed on opposite sides of it, indicating the two poles of the future nuclear spindle (fig. 15); when this stage is reached the nuclear membrane is absorbed and cannot be discerned.

The formation of the nuclear spindle is seen in the two stages drawn in figs. 16 and 17. After the absorption of the nuclear membrane the chromosomes arrange themselves to form an equatorial plate, to which delicate rays can be seen to pass from the centrosomes, forming the characteristic achromatic spindle. The two centrosomes appear to be pushed further apart by the formation of the spindle, so that they

come to lie at the extreme surface of the cell. The spindle is lodged in that portion of the cell which we have termed the neck in a previous section, and the centrosomes are situated about midway between the origin of the still persistent collar and the flange. The chromosomes appear massed together, and are difficult to distinguish individually when the equatorial plate is seen in side view (figs. 17, 18), but can be seen better in cells cut parallel to the plane of the equatorial plate (fig. 22). The number of chromosomes appears to be about sixteen.

At this period, while the equatorial plate is still simple and undivided, an important event takes place. From the centrosomes at the two poles of the spindle the two daughter-flagella grow out, appearing as two minute hair-like projections from the surface of the cell (figs. 18–21). This stage is a very common one, and it is, in fact, rare to find a collar-cell with a mitotic spindle without the two daughter-flagella projecting from the two centrosomes; this indicates that the first formation of the flagella must be an extremely rapid one. Sometimes only one daughter-flagellum is to be seen, but in such cases the cell is usually slightly oblique, and the missing flagellum has probably been cut off by the knife in cutting the section. The two new flagella are formed entirely outside the original collar, which is still persistent. The condition of the collar is best studied in osmic-picrocarmine preparations (figs. 42–45), in which it is seen that the formation of the nuclear spindle causes the cell to become much broader, with the result that the base of the collar is greatly stretched. The thicker portion of the collar, below the hoop, retains its form more or less, but the portion above the hoop tends to collapse and fall together.

From the stage with the single equatorial plate the diaster-stage arises in the usual way (figs. 23, 24). It is remarkable that we have succeeded in finding but few specimens of the diaster-stage, and, unfortunately, most of those have been cut obliquely or horizontally, and hence do not show well the relation of this stage to the cell as a whole. Figs. 23 and 24

show the two best diaster-stages we have found. Fig. 23 shows the spindle well, but the cell is cut almost horizontally, and the collar and one daughter-flagellum are sliced off; in fig. 24 the cell is cut more vertically, and shows the collar, but the plane of the spindle lies obliquely, and only one centrosome and daughter-flagellum can be made out. The scarcity of the diaster-stage indicates that it is passed over very rapidly, and this conclusion receives further support from the fact that in the subsequent stages, when the daughter-nuclei are being reconstituted, the daughter-flagella are scarcely longer than they were in the stage with the undivided equatorial plate.

After the diaster-stage, and with the reconstitution of the daughter-nuclei, the cell-body begins to divide (figs. 25–28A). Between the two daughter-nuclei there are seen for a time streaks, the remains of the achromatic spindle, stretching across from one to the other (figs. 25–27); these streaks persist until the division of the cell-body is far advanced. The details of the reconstitution of the nuclei are difficult to make out clearly; the chromosomes appear to fuse together into a compact mass in which their individuality is masked, if not lost. The division of the cell is effected by means of a constriction in the vertical plane, producing a cleavage which is much more marked at the upper than at the lower end of the cell. The cleavage goes right through the old collar, and leads to its destruction and disappearance; it appears to break down into a granular mass which disintegrates and vanishes.

When division of the cell-body is complete the new collars of the daughter-cells grow out round the short but growing flagella. At their first origin the new flagella projected in an oblique direction from the dividing cell, as figs. 18–28 show clearly; they took origin from that portion of the surface of the parent-cell which lies between the flange and the base of the collar. When the division is nearly complete (figs. 28A and 47), the point of origin of the flagella becomes slightly shifted so as to be placed at the uppermost level of

the cell, with the result that the young flagella come to point vertically upwards. After complete division the form of the two daughter-collar-cells undergoes a change, becoming elongated in the vertical direction, so that the cell as a whole acquires a slender columnar form, with a shallow collar surrounding the short flagellum at the upper end (figs. 29, 30, 48). A curious feature of these stages, both those in which cleavage of the cell is taking place (figs. 25–28A) and those in which division is recently completed (figs. 29, 30, 48), is that they are found in the sections at a higher level than the rest of the epithelium, as shown in figs. 30 and 48; the bases of the young cell are on a level with the flanges of the ordinary resting collar-cells. This peculiarity is very marked when the recently divided cells have assumed the columnar form; they project so much above the general level of the collared epithelium that they become very conspicuous objects in the sections of the sponge, and are consequently very easy to find. Later they appear to push their way down amongst the other epithelial cells, and so find their normal level (fig. 49).

The nuclei of the young collar-cells, at first compact masses, soon become looser in texture; the karyosome reappears and nucleus acquires the structure of the ordinary resting nuclei, from which it differs only in its smaller size. In osmic-picrocarmine preparations the young nuclei show the marginal clear zone very distinctly (figs. 48, 49). Immediately after division the nucleus is at the apex of the collar-cell (figs. 29, 30, 48), but it now begins to migrate towards the base of the cell (fig. 49), and so resumes the position characteristic of the resting cell. The collar and flagellum grow to their full length, and the latter arises from a basal granule or blepharoplast which, as is clear from the development that has been described and depicted, is one of the two centrosomes of the nuclear spindle in the mitosis, derived from the division of the resting cell.

Summary and Conclusions.

The course of events that take place in the division of the collar-cells may be summarised briefly as follows, omitting the details of the mitosis, since they present no special peculiarities.

The nucleus of the collar-cell migrates from the base to the apex of the cell, and so comes to lie immediately under the blepharoplast. The flagellum then disappears and the blepharoplast divides. The two daughter-blepharoplasts travel to opposite sides of the nucleus and take on the function of centrosomes. The nucleus breaks up into chromosomes, its membrane disappears, and a mitotic spindle is formed in the ordinary way, with the two centrosomes at its poles. The two new flagella then at once begin to grow out from the two centrosomes, outside the original collar, before the equatorial plate is divided. The mitosis is completed, and as the cell-body divides the original collar breaks down and disappears. The centrosomes become the blepharoplasts of the two daughter-cells, the flagella continue to grow out from them, the new collars grow up round the new flagella, the reconstituted daughter-nuclei migrate back again to the bases of the cells, and the two daughter-cells resume the structure and appearance of the ordinary resting collar-cells. Thus it is seen that the blepharoplast-centrosome is a permanent cell-organ, which multiplies with the cell; but that the collar and flagellum are formed afresh at each cell-division, quite independently of the collar and flagellum of the parent cell.

In this process of division the feature to which we wish to draw special attention is the fact that the bodies which have the function of blepharoplasts in the resting-cell have that of centrosomes in the dividing cell. In fact, it is seen that during a certain stage in the division, the stage, namely, of the nuclear spindle, when the daughter-flagella are growing out from the centrosomes at the poles of the spindle, one and

the same body functions at one and the same time as a blepharoplast and a centrosome, thus furnishing a decisive proof of the identical nature of these bodies, at least in the class of cells that we have been studying.

We are therefore in entire agreement with those authors who regard blepharoplasts as bodies of centrosomic nature. It is very obvious in the case which we have studied that the terms "blepharoplast" and "centrosome" denote merely two different functional activities of the same body. It may well be that in other cases division of labour may lead to structural differentiation, and that two distinct and independent classes of bodies occur, centrosomes controlling nuclear division and blepharoplasts giving rise to locomotor cell-organs. But in all cases alike we regard centrosomes and blepharoplasts as organs similar in nature and identical in phyletic origin.

It only remains to discuss how far the results we have obtained throw light on the state of things in other cases, and more particularly with regard to the vexed question of the true blepharoplast in trypanosomes, that is to say, whether the name "blepharoplast" should be given to the kinetonucleus, or to the basal granule of the flagellum in these organisms. With regard to this point, it may be stated at once that there is nothing whatever in the structure or behaviour of the centrosome-blepharoplast of the collar-cells to justify a comparison between it and the kinetonucleus of a trypanosome, or, indeed, a nucleus of any kind. We are fully in agreement with those who, following Schaudinn, regard the kinetonucleus of trypanosomes as a body of the nature of a nucleus, and it is precisely on this ground that we regard it as a body of a different nature from a true blepharoplast, such as that which is seen in the collar-cells, and which cannot, in our opinion, be identified as a nucleus by any stretch of the imagination. On the other hand, the body, which in a trypanosome corresponds in every way to the true blepharoplast, is the basal granule or centriole of the flagellum.

Our position, therefore, with regard to the nuclear apparatus

of a trypanosome is that the basal granule of the flagellum represents the true blepharoplast, a body of the nature of a centrosome, and that the kinetonucleus or German blepharoplast is an accessory nucleus which is not represented in the economy of a collar-cell or in flagellated organisms generally, but which is a special feature of the genus Trypanosoma and its allies, especially the genera Trypanoplasma, Herpetomonas, Leishmania, and Crithidia, a nucleus which doubtless possesses its own centrosome or centriole. With regard to the function of the kinetonucleus, its close association with the blepharoplast and the flagellar apparatus has generally been held sufficient to justify the assumption that it possesses a kinetic function, that is to say, that it is a nucleus specially concerned with the regulation of the function of locomotion. We require, however, more knowledge with regard to the relations of the kinetonucleus to the life-cycle as a whole, and more particularly to the phenomena of sex and sexual conjugation in these flagellates before this point can be decided. We may refer in this connection to the interesting experiments of Werbitzki (19), who was able to obtain trypanosomes without a kinetonucleus (termed by him "blepharoblast"), and found that such individuals showed no difference, as regards their movements, from the trypanosomes of normal structure. This result seems to us to indicate that the flagellar apparatus of a trypanosome is not so dependent on the kinetonucleus as is generally supposed, and also to be strongly in favour of our view that the basal granule of the flagellum, and not the kinetonucleus, represents the true blepharoplast. Werbitzki seems, in fact, to have reduced his trypanosomes artificially to the more primitive condition found in other flagellates and also in collar-cells, a condition in which the organism possesses a nucleus and a true blepharoplast, but no kinetonucleus.

It may be objected to our conclusions that they are based only on analogy, and that a collar-cell is too far removed from a trypanosome in phylogeny and affinities to permit of

conclusions being drawn with regard to the homologies of the flagellar apparatus of trypanosomes. It is, of course, possible that the conclusions drawn from the one do not strictly apply to the other, and it is certainly very desirable that these points should be studied in flagellates generally, and in forms allied to trypanosomes particularly, more than has been done at present. On the other hand a collar-cell, although it forms part of the epithelium of a sponge, is as much a flagellate organism in all points of structure and function as any free-living flagellate; and the study of cytology tends rather to demonstrate the essentially uniform nature of permanent cell-structures throughout the whole range of living organisms, whether animal or vegetable.

Lister Institute, Chelsea, S.W.
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EXPLANATION OF PLATES 25 AND 26.

Illustrating Miss Muriel Robertson and Mr. E. A. Minchin's paper on "The Division of the Collar-cells of Clathrina coriacea (Montagn): A Contribution to the Theory of the Centrosome and Blepharoplast."

[All the figures are drawn from sections of material fixed with Hermann's fluid and stained by Heidenhain's iron-hæmatoxylin method; the outlines were traced with the camera lucida at a magnification of 2000 linear, with the exception of figs. 36 and 37, which are magnified 3000 linear.]

PLATE 25.

Fig. 1.—Six collar-cells in their natural arrangement; five of them are in the resting state; the sixth (*d*) shows the nucleus in its migration towards the blepharoplast, and has a very short flagellum.

Fig. 2.—Early stage in the upward migration of the nucleus; the blepharoplast in the act of division, with shortened flagellum.

Fig. 3.—Similar stage to the last, the blepharoplast distinctly divided, the nucleus with two karyosomes.

Figs. 4 and 5.—Stages showing the nucleus in close proximity to the blepharoplast and distinctly flattened on the side nearest the blepharoplast, from which two streaks are seen to come down to the two ends of the flattened border of the nucleus; these two streaks appear to be the optical section of a cone-shaped figure. In fig. 4 the flagellum is seen to be still present, but shortened; in fig. 5 no flagellum is seen and the blepharoplast is divided.

Fig. 6.—Stage showing the divided blepharoplasts connected by a centrodesmose; the flagellum is still present and of fair length.

Figs. 7, 8, and 9.—Stages showing complete division of the blepharoplast with persistent flagellum in each case; the two daughter-blepharoplasts in each dividing cell have travelled inwards and placed themselves at opposite sides of the nucleus, drawing in with them the root of the flagellum, and the free portion of the flagellum has its base surrounded by an upgrowth from the apex of the cell. In fig. 7 the adjacent resting cell is drawn for comparison; in the dividing cell a centrodesmose is seen between the two blepharoplasts, and there are also indications of a streak running down from one of the blepharoplasts to a granule in the body of the cell, but this streak appears to be due merely to the arrangement of vacuoles in the cytoplasm, and not of the nature of a

centredosmose. These three figures also show three different conditions of the nucleus preparatory to mitosis. In fig. 7 the karyosome is very distinct, while the remainder of the chromatin is pale, but beginning to aggregate into larger masses. In fig. 8 the karyosome is also distinct, but the rest of the chromatin is darker and the coarse granulation is more distinct. Fig. 9 shows the stage which appears to correspond to the spireme-stage; the chromatin is in darkly staining masses (chromosomes), connected by fainter lines, and no karyosome can be made out. All three cells are from the same slide.

Fig. 10.—Blepharoplast divided, remnant of flagellum still present; nucleus not showing any preparation for mitosis. Cell cut somewhat obliquely.

Fig. 11.—Blepharoplast divided, flagellum entirely absent; nucleus as in last.

Fig. 12.—Blepharoplast and flagellum as in last; nucleus showing beginning chromosome-formation, but karyosome still distinct.

Fig. 13.—Cell cut obliquely, showing two blepharoplasts connected by a centrodesmose.

Fig. 14.—Cell showing the flagellum completely withdrawn, and represented only by a little upgrowth from the body of the cell; the two blepharoplasts (centrosomes) have placed themselves on opposite sides of the nucleus, which is still in a very early stage of preparation for mitosis, with distinct karyosome and pale chromatin.

Fig. 15.—Similar stage, but with the chromatin of the nucleus completely broken up into chromosomes. No karyosome is to be made out. One centrosome is seen on the right at the side of the nucleus, the other on the left, rather low down and almost under the nucleus.

Figs. 16, 17.—Stages showing the formation of the nuclear spindle. In fig. 16 the chromosomes are still irregular in arrangement, while in fig. 17 they are arranged to form a definite equatorial plate. No flagella have as yet grown out from the centrosomes.

Figs. 18–21.—Stages with the nuclear spindle and with daughter-flagella growing out from the centrosomes (blepharoplasts). In fig. 18 the spindle lies slightly obliquely, and only one daughter-flagellum is seen. In fig. 21 the cell is cut obliquely.

Fig. 22.—Nuclear spindle cut in the plane of the equatorial plate, which is seen from one of its flat surfaces.

Figs. 23, 24.—Diaster-stages. Fig. 23 shows a cell cut obliquely, and only one of the daughter-flagella is seen. In fig. 24 the nuclear spindle lies obliquely, and only the left-hand centrosome and daughter-flagellum can be seen.

Fig. 25.—Late diaster-stage, with beginning reconstitution of the daughter-nuclei. Slightly oblique; only one daughter-flagellum to be seen.

Figs. 26–28, 28A.—Stages in the division of the cell-body, with reconstitution of the daughter-nuclei. In all figures, except 28A, the remains of the original collar can be seen clearly. In figs. 26 and 27 the remains of the achromatic spindle can be seen between the two daughter-nuclei. In fig. 28A the division is practically complete.

Figs. 29, 30.—Pairs of young, recently divided collar-cells. In fig. 30 some of the adjacent cells are drawn to show the way in which the cells at this stage are raised up above the surrounding cells.

Figs. 31–35.—Collar-cells showing enclosures of various kinds, some of them perhaps of parasitic nature. In the cell shown in fig. 34 the nucleus seems to have disappeared, but may have been cut off.

Fig. 36.—Nuclei of resting collar-cells, magnified 3000 linear. *a*, *b*, *c*, dark nuclei; *d*, a light nucleus; *e*, *f*, *g*, thin sections of nuclei showing the reticular structure; in *g* the karyosome does not come into the section.

Fig. 37.—Transverse sections of collar-cells in the region of the collar. *a* passes through the base of the collar, and *b* just above this level; both show the blepharoplast centrally. In *c* the collar is cut transversely with the flagellum in the centre.

PLATE 26.

[All the figures are drawn from sections of material fixed with osmic acid and stained with picro-carmine; magnification throughout 2000 linear.]

Fig. 38.—Two collar-cells, one of the normal resting type (on the left), the other with the nucleus migrating towards the apex of the cell preparatory to division.

Figs. 39, 40.—Collar-cells showing the nucleus at the apex of the cell, and the flagellum in process of retraction by means of a pseudopodium-like process from the cell.

Fig. 41.—On the left a normal resting cell; on the right a cell with the nucleus at the apex and the flagellum completely retracted, but represented by the still persistent pseudopodium-like process seen in the two preceding figures.

Figs. 42, 43.—Stages with the daughter-flagella growing out from the poles of the nuclear spindle, and with the collar beginning to collapse. The achromatic elements, namely, spindle and centrosomes, are not stained and are not visible in the preparation, but the equatorial plate

is seen. In fig. 43 the collar contains a foreign body, as in the right-hand cell in fig. 48.

Figs. 44–46.—Diaster-stages with daughter-flagella. In fig. 44 a resting cell is drawn for comparison; in fig. 46 the cell is cut obliquely and does not show the collar.

Figs. 46A, 47.—Stages in the division of the cell-body. In fig. 46A the collar is still seen; in fig. 47 it has disappeared.

Fig. 48.—Two young, recently divided collar-cells, drawn with three ordinary resting collar-cells to show the manner in which the young cells project above the level of the epithelium. The collar-cell on the extreme right shows a foreign body lodged in the lumen of the collar.

Fig. 49.—Four collar-cells, of which the two middle ones are evidently a pair of sister-cells, the product of recent division, showing the nuclei in the act of migrating down to the base of the cell.

Figs. 50, 51.—Two collar-cells showing bodies (parasites?) in the cytoplasm.

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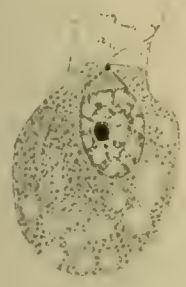

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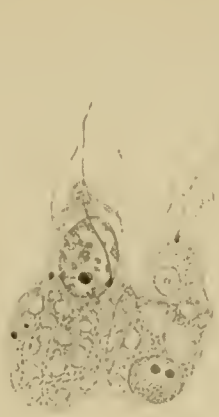

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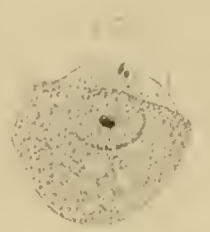

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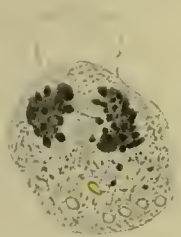

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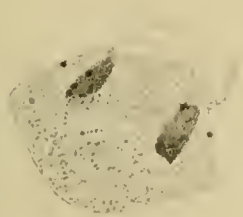

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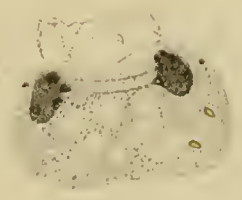

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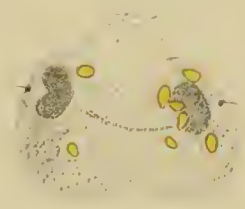

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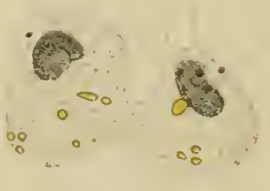

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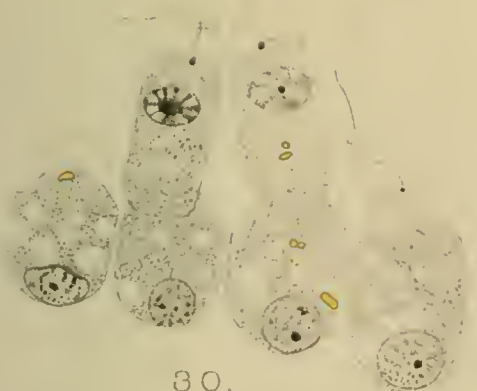

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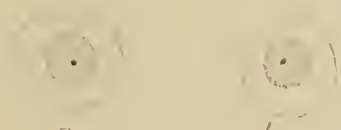

a. *b*. *c*

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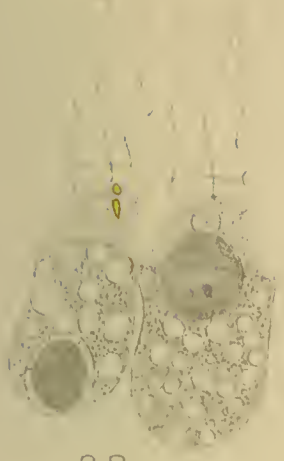
Huth, Lith^r London

OLLAR-CELLS.



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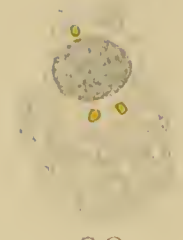

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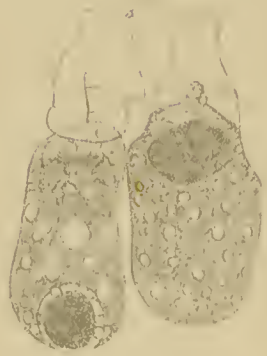

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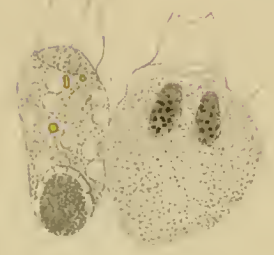

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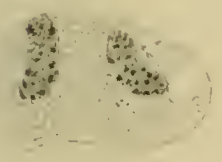

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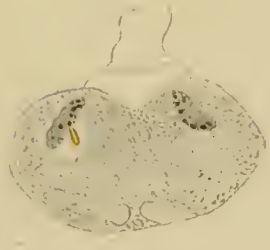

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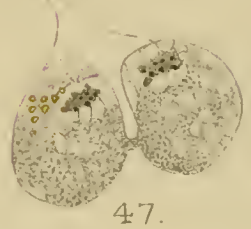

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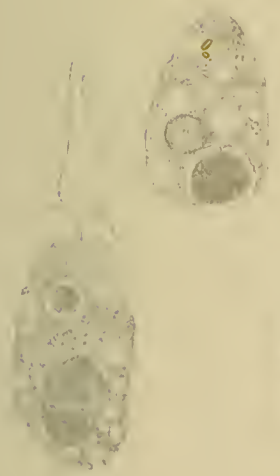

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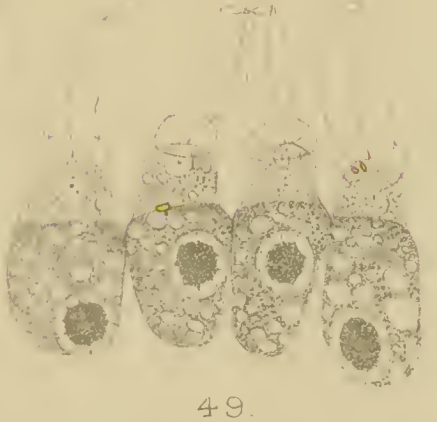

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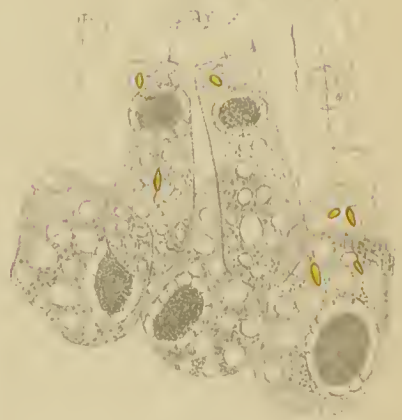

48.

DIVISION OF COLLAR-CELLS.

Studies on Avian Hæmoprotozoa.

I. On certain Parasites of the Chaffinch (Fringilla cœlebs) and the Redpoll (Linota rufescens).[1]

By

H. M. Woodcock, D.Sc.(Lond.),

Assistant to the University Professor of Protozoology.

With Plates 27—31.

Contents.

1. Introductory.

My reason for taking up the study of Avian Hæmoprotozoa has been the desire to obtain, if possible, some definite enlightenment on the important question of their life-cycle. The far-reaching conclusions bearing upon this subject, to

[1] This research was carried out as Mackinnon Student of the Royal Society during the year 1907–1908. The publication of the results has been delayed for several months owing to a long stay at Rovigno in the endeavour to supplement this work by the study of the actual parasites described by Schaudinn in Athene noctua.

which the celebrated protozoologist, the late Fritz Schaudinn, was led as the result of his well-known researches (**27**) on certain parasites of the little owl (Athene noctua), have been largely discredited by many subsequent workers in this field. This is chiefly due to the suggestion, first put forward by the American workers, Novy and McNeal, that there is nothing in Schaudinn's description to show that the author took sufficient precaution against the liability of confusing the life-histories of what were really separate and independent parasites. Novy and McNeal, in their endeavour to confirm Schaudinn's views, investigated the trypanosomes of various birds (**14**), and also made a study of the flagellates occurring naturally in mosquitoes (**15**). As a result of their work they have maintained that Schaudinn was entirely wrong in regard to all his main conclusions. They consider, on the contrary, that the trypanosomes of birds are quite distinct from intra-cellular parasites (such as Halteridium), and further, that they do not undergo any part of their life-cycle in an insectan host, the flagellates occurring in the latter having no connection with the trypanosomes.

I chose avian forms on which to work for the following reasons: In the first place, a considerable amount of research has now been done on various trypanosomes parasitic in other vertebrates, e.g., fishes and mammals, which will be referred to in due course. Secondly, it is from a study of avian forms, if any, that one may reasonably expect to learn how far Schaudinn's views and statements were justified. As a matter of fact, at the present time the trypanosomes of birds are those about which the least is positively known, for Novy and McNeal's work, while it has undoubtedly re-opened the entire question, does not, on the other hand, contribute much to its definite settlement. In my opinion, many of the conclusions reached by these authors are equally open to criticism. They themselves have certainly not brought forward adequate or sufficient evidence to justify the negative views adopted by them.

Hosts Selected to Work upon.—It was my intention

to study first the parasites of the "little owl" itself. In spite of all my efforts, however, I could not obtain a supply of these birds here at home, so that I was obliged to turn my attention to other birds. Recent observations have shown that many kinds of birds harbour trypanosomes, and it is probable that their infection with these parasites is fairly widespread in nature (cf., for instance, the numerous American species which Novy and McNeal found to be infected). The only worker, to my knowledge, who has published any notes relating to the occurrence of avian trypanosomes here in England is Petrie (**21**), who observed the parasites in the blackbird, swallow, house-martin, song-thrush, chaffinch, and yellow-hammer; he failed to find them in the crow, sparrow, starling, or jackdaw.

Had it been my object to find trypanosomes in as many different birds as possible and to content myself with noting their presence, it would have sufficed to shoot various kinds of wild birds and examine them at once. This habit of describing and naming trypanosomes from one or two casual observations is unfortunately far too prevalent; it is one which adds little or nothing to our knowledge of the really essential points on which light is needed. For the purposes of my investigation I felt it was best to restrict myself to birds which could be obtained without much difficulty, and which were hardy and would live well in captivity. Hence, with a few exceptions at the commencement of the work, when I was endeavouring to "lay a course" as it were, I have used small native cage-birds, obtained from various dealers. Mentioning the exceptions first of all, in order to give a complete list, I began with some Java sparrows (Padda oryzivora), from which host a trypanosome, T. paddæ, has been described by Thiroux. But after spending some time fruitlessly in attempts to find this parasite, which was not present, and my limited supply of these birds giving out, I relinquished the search. In spite of great efforts to trap common birds, the only result was a

blackbird caught for me at Elstree, which died two days after receiving it. Neither in this, nor in another blarkbird, purchased, were any trypanosomes found. A barn-owl (Strix flammea), which was kindly given me by Dr. Dean, also proved negative.[1] I may add here that in one of the Paddas and in one blackbird Halteridia occurred, but sparingly; I thought it best, however, not to take up this aspect of the question at first, but to continue my search for hæmoflagellates and concentrate my attention on them in the first place, turning to the Hæmosporidia later, as should appear desirable.

The small birds, of which I have examined most, are closely allied members of the finch family (Fringillidæ, sub-fam. Fringillinæ), namely, greenfinches (Chloris chloris), chaffinches (Fringilla cœlebs), redpolls (Linota [Acanthis] rufescens), and linnets (L. [A.] cannabina). Trypanosomes were found only in the chaffinches and redpolls, so that for the greater part of the time I have occupied myself entirely with these. Unfortunately during the spring these birds also were very scarce and difficult to procure, and I was unable to replenish or augment my stock when I particularly wished to do so.

The occurrence of the parasites in these two hosts cannot be considered as at all rare. Out of twenty-two chaffinches examined, five were found to be naturally infected, sixteen birds were certainly uninfected, and one was doubtful. Neglecting this last,[2] the percentage works out at about 24. As regards the redpoles, trypanosomes occurred in three out of fifteen; eleven were uninfected, and one, again, was uncertain. This gives an approximate percentage of 21·5, which is not very different from that in the case of the chaffinches. As far as they go these proportions are reliable, because they are exhaustive—that is to say, the

[1] In the case of blackbirds this was not conclusive as to the absence of the parasites, for no cultures were made (cf. below, p. 658).

[2] Also in the case of the first chaffinch and redpoll no cultures were taken, as I had no tubes ready at the time.

negative side also can be relied upon, for reasons which are given below ; in this respect they differ from most previous tables and estimates of trypanosome-infections of birds. The figures suffice to show that, so far as occurrence is concerned, the birds with which I have worked do not bear out the dismal statistics given by many of the researchers (e. g. Ziemann, the Sergents, Dutton and Todd, etc.)

Intra-cellular Parasites in the Chaffinch. — In several of the chaffinches I noticed, when looking for trypanosomes, the presence of Halteridia ; except in one case, which I shall describe shortly, these were only scanty in number. I have also observed, in three cases, an interesting leucocytic parasite, which is quite different in appearance from the celebrated Leucocytozoon ziemanni of owls.

What is undoubtedly a similar parasite has been observed independently by Dr. Stevenson, of University College, in smears of the blood of a greenfinch, which he has kindly shown me for comparison.

2. Methods of Work ; Attempts at Transmission by Mosquitoes ; Technique.

Fresh blood was always taken, in the living bird, from a fairly large marginal vein of the wing, prominent where it crosses the arm on the inner side, immediately below the elbow-joint. A fine-pointed surgical needle of the triangular-bladed kind was used. It is essential that the point be sharp. Unless a clean prick is obtained, the blood does not exclude freely in a good drop, but suffuses beneath the skin, raising a swelling from which blood cannot be got satisfactorily. As a rule bleeding stops quickly. Should it give any trouble, a swab of cotton-wool, dipped in lysol, is applied to the wound and the wing closed up over it and held to the side of the body for a few minutes. The vein soon recovers from this little operation, and can be used again, if desired, in a couple of days or so.

Culture-tubes.—The use of culture-tubes has been of the

greatest service to me. I have developed and extended Novy and McNeal's method, making use of it not only on the dead bird, but also—what is much more difficult—on the living bird. In taking drops of blood for culture-tubes, the great desideratum is to get the region of the arm above-mentioned sterile if possible. The part is very well washed and gently rubbed first of all with cotton-wool soaked in lysol, particular attention being paid to the skin near the base of the feathers. The lysol must then be washed away with distilled water, which has been well boiled. Lastly, the water is absorbed as well as possible with more cotton-wool, which has been boiled along with the water, and from which the hot water is quickly pressed out. This is preferable to using loose wool and serves to take up most of the water, the warmth also helping in drying the part. It is most important to have the arm as dry as possible before pricking the vein, otherwise the blood spreads and runs over the surface. As it exudes, the blood is taken up by a sterilised Pasteur pipette, the drawn-out tube of which is long enough to pass into the expression-water of the culture-tube.

It is, of course, a much easier matter to get sterile inoculations from the bone-marrow, heart, etc., if the ordinary precautions are adopted.

If a culture-tube can be successfully inoculated with four or five drops of blood, I have found that in a few days (usually five to seven, sometimes fewer) one can generally say with confidence whether the bird was infected, according as the tube develops trypanosomes or not. Unfortunately, even with the greatest care, the inoculated tubes are sometimes badly contaminated before that time has elapsed. In such circumstances I never rely upon a negative indication, though I may add that now and again a positive result has been obtained where the medium had become contaminated. When I have been unable to get any cultures to develop in two or three sterile tubes taken from a bird, subsequent examination and culture of the bone-marrow after death have also proved negative. Hence I have regarded the

above as a reliable test of the presence of the trypanosomes in the living bird.

Culture Media.—The parasite from the chaffinch and redpoll lives and multiplies readily in a blood-agar medium, prepared either after Novy and McNeal's recipe, or according to Mathis' modification. At first I followed the American authors (see **14**, p. 265), but added only an equal volume of defibrinated rabbit's blood to the sterilised meat-agar, as I found this to be quite sufficient. Tubes so prepared always have an ample quantity of expression-liquid, in which the parasites thrive at any temperature from 20° to 25° C. A temperature of 28° to 30° C. was found to be too high, if it was desired to keep the tube for any length of time, as the trypanosomes soon die off, owing to their too rapid multiplication and exhaustion of the nutrient material. At the lower temperature the tube is all right for about twelve or fourteen days, and some of the trypanosomes will remain alive longer if a little salt-citrate solution is added to replenish the medium. If it is desired to keep the culture going for some time, however, it is necessary to make a sub-culture, after ten or twelve days, by transferring a drop of the medium containing the parasites to a fresh tube. By this means I have kept a continuous series of cultural forms, both from the chaffinch and from the redpoll, thriving and multiplying for six and a half weeks, the one having been transferred (sub-cultured) four times, the other, I think, only thrice. Had it not been for the accident of the temperature of the incubator rising to nearly 30° C. for two or three days, whereby the trypanosomes were all killed off, the cultures could apparently have been kept for as long as I wished.

The great drawback to this method is that, where, as in my case, a large number of the tubes are used, too much time and labour are involved in obtaining sufficient rabbit's blood. Mathis' modification (**10**), which I have now followed for some time, avoids this difficulty. In this method, ox-blood, which can be readily got from a slaughter-house, is used instead. A quantity is allowed to fall direct into a sterilised

receptacle, and at once defibrinated. As before, equal volumes of blood and agar are mixed. The tubes, when prepared, must be sterilised by the fractional method at a temperature of about 100° C. (under rather than over), for an hour or so on two successive days. This is necessary to ensure sterility.

Owing to this process, however, tubes prepared thus are often deficient in expression-liquid; to remedy this 1 or 2 c.c. of boiling salt-citrate solution (·75 per cent. salt + 1 per cent. sodium-citrate), are added to each tube, which is then left for a day or two before being inoculated; the liquid absorbs nutrient material from the solidified part. The trypanosomes will not live in salt-citrate solutions alone. I have tried various combinations of salt, sodium-citrate, and (or) citric acid, similar to those used in cultivating the Leishman-Donovan bodies, but with no success. For the practical purpose of ascertaining whether a bird is infected or not I have found these tubes to be, as a rule, as serviceable as the others; but I do not think they suit the parasites quite so well. The culture does not start quite as easily, and multiplication is often somewhat slow at first. It is at least four or five days before the trypanosomes can be found at all readily in a small drop taken for examination, whereas in the case of the other tubes three or four days usually suffice. Again, after a week or nine days the parasites tend to become very granular and altered, and large agglomeration-clusters form sooner. In short, the trypanosomes do not live "healthily" so long in this kind of culture as in the other.

I may point out, with regard to the macroscopic appearance of infected tubes, that in the case of the parasites with which I have been working, there is normally nothing indicative of their presence to be seen. A culture (if free from bacteria) looks just like an uninoculated tube. Even when the parasites are very abundant, the expression-liquid remains clear and unaltered in colour. Not once have I found the parasites on the solid part of the medium. They never form visible colonies or masses there. The only

instances where anything unusual is to be noticed are in old, used-up tubes, in which the liquid is full of clumps of agglomerated parasites, and many are degenerating and dying. These masses tend to settle to the bottom of the liquid, and may be apparent as a small quantity of whitish-yellow scum.

Inoculation of Birds with Trypanosomes.—I endeavoured to produce an infection with trypanosomes in birds which I had found to be uninfected. So far, the only means at my disposal of doing this has been by inoculating; and most, certainly, of my attempts in this direction failed. In all about twenty-five inoculations were performed, and only in three cases was any positive result afterwards observed, which might be due to the inoculation. Many of the failures resulted from attempts to inoculate other (uninfected) birds with the trypanosome of the chaffinch and redpoll. Thus, a couple of linnets, one of them inoculated twice, proved negative. Also a barn-owl was tried with no more success. I was rather surprised, however, to find that a canary, which I thought would be very likely to prove susceptible, refused to become infected. It was inoculated three times, twice from cultures, and once from fresh (infective) blood, mixed with a little salt-citrate solution.

A few words in connection with the modus operandi. To begin with, I inoculated the birds intra-pleurally, as recommended by Novy and McNeal, but I lost two or three redpolls straightway as a result of the operation. It was very cold weather at the time, and this may have conduced to their collapse. Since then, I have always found it much more satisfactory to do the birds intra-peritoneally or intra-muscularly (in the pectoral muscles). None of the birds so inoculated suffered any ill-effects, even though, occasionally, they were done in both ways at once. The "dose" was generally four or five drops (from one eighth to one sixth of a cubic centimetre) of the liquid in the tube. This contained, of course, numbers of parasites.

With regard to the three cases in which the trypanosomes were observed subsequently, I may point out that I had made

sure, by means of good cultures, that all three birds had no trypanosomes in the blood prior to the inoculation, and therefore I considered them to be free from those parasites.[1] Hence these are in all probability instances of successful inoculation. One case was that of a chaffinch inoculated with the parasites from a redpoll; another was that of a redpoll inoculated with a culture from a chaffinch. With regard to the third case, that of a chaffinch inoculated with a culture from another chaffinch, I have been very uncertain, owing in part to the different course the infection took, whether the appearance of the trypanosomes in this instance was really due to the inoculation, or was connected with the presence in this bird of Halteridium. I now think this was also a case of successful inoculation, for reasons which are discussed below (see p. 678).

Attempts to Transmit the Parasites by Mosquitoes.—It was a great disappointment to me that all my efforts to get mosquitoes infected with the trypanosomes from the birds have been fruitless. Both from Schaudinn's description of the infection of Culex with the trypanosomes from the "little owl," as well as on account of the known rôle of this insect as alternate host of the Proteosoma (Hæmoproteus) of birds, I thought it most likely that mosquitoes would prove to be the transmissive agents of the parasites—at any rate, the trypanosomes—of the chaffinch and redpoll.

Unfortunately I was baffled in the very initial stage of all the experiments. I was never able to get the mosquitoes to bite the birds. I have tried at different seasons of the year, late spring, summer, and early autumn, and at periods when the temperature has been quite high for this country. Most of my attempts were made with females which were bred out from larvæ. None of them, however, showed the slightest inclination to bite. Nor would they feed on a guinea-pig, with which I tried them occasionally. They would only take

[1] I have worked throughout on the assumption that if trypanosomes are present, they will occur, if sparingly, in the general circulation.

such things as sugar-water, banana-juice, or mashed date. And if they were not provided with something of this kind they soon died off.

I also obtained several batches of "wild" mosquitoes (females), thinking these might at any rate bite. Indeed, Prof. Minchin, who sent me some from Norfolk, said they were biting the horses in the open fields at the time. But here again I had no better luck. In fact, the Culex seemed to starve instead of feeding on the bird. I have kept batches under observation without food,[1] and seen their bodies gradually become attenuated, until, although placed for a couple of nights consecutively with a bird, and without other food, by the fourth or fifth day (since they last took food) many of them would be dead. The mosquitoes were nearly always placed with the bird in the late afternoon, and left with it all night. Care was taken, of course, that they should be perfectly able to get to it and feed if they wished. Now and again, also, I held a tube containing a few hungry-looking insects to the bird's body for a little time, displacing the feathers so as to expose the skin; and similarly with the guinea-pig. I tried keeping the mosquitoes in a biological incubator at a temperature of about 25° C. (77°–78° F.), for a day or two before using them, but this did not make any difference. Even small pieces of organs containing blood from freshly killed rats remained untouched so far as I could see. In short, all my efforts to induce Culex to take blood were unavailing.

What is the probable explanation of this unwillingness experienced of the insects to bite? Such a total failure in this respect was quite unexpected. Taking into consideration the results in this connection—fortunately more successful—since gained at Rovigno, I think that there is probably more than one reason for the above negative results. In the first place, the question of temperature and moisture in the air is very important. I found this to be the case at Rovigno.

[1] But not without water, a small dishful of which was always kept in the cage.

Until the beginning of June I had the same difficulty there. As soon, however, as the regular summer weather set in—a moist, sweltering warmth—there was no difficulty in getting the Culex to bite (once, at any rate). It must be remembered that all the research done on Culex hitherto, in this connection, from which it is known both to transmit certain hæmatozoa and to harbour flagellates (which in many cases are most probably hæmoflagellates), has been done in countries where a much higher average summer temperature is experienced than in England. And I do not think that I succeeded in getting sufficiently favourable environmental conditions in my laboratory attempts in London.

There is another probably equally essential point, of which I was not aware at the time of my (the above) experiments. According to Mr. E. H. Ross, in a report on the prevention of fever on the Suez Canal (Cairo : National Printing Department, 1909),[1] the mosquitoes (females) apparently desire to suck blood only after having been fertilised. As it happened, in my early work I kept the bred-out females separate from the males, of which I took no account, thinking they were not required (as, of course, they do not take blood). Hence those females used were certainly not fertilised. As regards the caught "wild" ones, however, it is just as likely that they were fertilised as not, so that some of these ought to have bitten, had other conditions been suitable.[2]

Another Possible Insectan Host.—Owing to my lack of success in this essential preliminary, I was left in the dark as to whether Culex was the alternate host of the Hæmatozoa of the chaffinch or not. I may point out in passing that a study of the cultural forms of the Trypanosome which I have

[1] See 'Nature,' vol. lxxix, 1909.

[2] In working at Rovigno, where I was able to breed out the Culex in greater abundance, I left the two sexes together, for the sake of convenience in dealing with the insects. In this case many females were fertilised, for I frequently noticed the little "egg-rafts" floating on the dishes of water in the cage. Probably those females which sucked blood had been fertilised.

obtained, and their comparison with various flagellates described in blood-sucking Invertebrates (cf. below), leaves no doubt whatever in my mind that these bird-trypanosomes have some alternate (doubtless insectan) host. But it is quite possible that, in the present instance, some other insect than Culex performs this rôle. I endeavoured to ascertain what other biting insect was likely to be concerned. Mr. Austen, of the British Museum, very kindly informed me of a small hippoboscid fly, of the genus Ornithomyia, which is an ectoparasite of various birds, especially to be found on nestlings.[1] Up to the present, however, I have been unable to obtain a supply of these insects.

It seems to me not at all unlikely that it is in this direction one must look for the alternate host. If this be the case, it is very probable that infection usually occurs while the birds are quite young, and before they leave the nest.

Early in the autumn I obtained a young redpoll, infected with trypanosomes, which could not have been more than two months old, if that, when bought; and as most of these little cage-birds are caught, I am told, as soon as they can look after themselves and before they finally leave the nest, this may very well be a case in point.[2] Unfortunately, owing to the hampering restrictions of wild birds' protection acts, etc., I could not get hold of any nests containing fledgelings for examination. Towards the end of the close season a bird-seller did procure a chaffinch nest for me, from which the young birds only flew away as he approached. This was well searched for insects, but contained none. I may add that I have never noticed any insects (fleas, lice, etc.) on my birds

[1] The Sergents have recently found (30) that a hippoboscid fly belonging to the genus Lynchia is most probably concerned in the transmission of the Halteridium of the pigeon. Lynchia, however, is not met with in Britain.

[2] An interesting observation noted by Danilewsky of trypanosomes being present in a young roller-bird only a week old also supports this view. The only alternative would be that of hereditary infection, which is extremely doubtful.

when examining or inoculating them; they always seemed to be free from anything of this kind.

Technique.—All my permanent preparations are in the form of smears made on slides. As a rule, the thinner the smear the better the result. In the case of very stout trypanosomes it happens occasionally that they are rather flattened out if the smear is too finely drawn; but in thick smears the parasites are often not well stained by the Romanowsky method, being too blue in appearance. As regards smears of the cultural forms, I experienced some difficulty at first, on account of the expression-liquid (the medium containing the parasites), of which the drop to be smeared consisted. This was quite clear in the fresh condition, but formed a sort of coagulum after fixation, which stained very readily. Hence the trypanosomes appeared to lie in a layer of substance, stained reddish, which was often somewhat dense immediately around them. This coagulated layer was much more noticeable in smears made from the first kind of tubes than it was when I used the second kind, to which salt-citrate solution was added. The only means of obviating the trouble was to make the film as thin as possible and to take care that no stain was deposited on the slide.

Fixation.—Most of my preparations have been fixed with osmic acid vapour; the few smears not so fixed were of little value as regards the trypanosomes. I make use of a 4 per cent. solution of osmic acid, placed in the bottom of a stain-tube, to which two or three drops of acetic acid are added. The slide to be fixed is placed in the tube as quickly as possible after the film has been drawn. A fairly deep or thick glass ring in the liquid at the bottom of the tube prevents the slide itself from getting wet. Slides are left in contact with the vapour from twenty seconds to half a minute, the shorter time particularly in the case of a smear from a culture. After fixing, the slide is placed in absolute alcohol for fifteen to thirty minutes, according to convenience. If the smear is to be stained by the Romanowsky method, it is not advisable to leave the slide in absolute alcohol for much

longer than half an hour; I have always found a longer period to be detrimental to the staining. I found this method of fixation to be the best for giving a correct idea of the size and general appearance and morphology of the parasites, whether trypanosomes or intra-cellular forms; and, for the sake of uniformity, all my figures are of individuals so fixed, so that one may be compared at once with another, without any ulterior considerations having to be taken into account.

Staining.—Nearly all my preparations are stained by some variety of the Romanowsky method. I have made use of two stains (or stain mixtures): one of them is the ordinary Giemsa solution, the other is a combination which I have found particularly good for cultural forms. The Giemsa solution was always used in the customary proportion of one drop of the stain to 1 c.c. of water. The length of time for which slides were allowed to stain varied in different cases. The period required to give the best results varies considerably at times, even when the smears have been fixed, so far as can be told, in exactly the same manner. For one thing, the temperature made considerable difference. I used the stain at the laboratory temperature, and whereas in the winter and spring forms in the blood required to be stained for twelve to eighteen hours to be successful, in the summer they would be excellently stained in three or four hours.

Cultural forms stain much quicker than the parasites in the blood, and need only about fifteen to twenty minutes in the stain; but the Giemsa solution was found to be not nearly so suitable for smears of cultural forms as the other method which I adopted; by this latter method the parasites themselves are more sharply stained, while the coagulated layer, which is often unpleasantly prominent as a reddish ground-substance, after Giesma, hardly stains at all.

In my particular method three solutions are made use of, as follows:

(1) A 1 per cent. solution of azure I, in equal parts of glycerine and methyl-alcohol.

(2) A 1 per cent. aqueous solution of methylene-blue (Höchst—an essential point), to which 5 per cent. of pure sodium-carbonate is added. This solution is kept warm at a temperature of 40° to 45° C. for a couple of days or so, when it is made up, after which it is ready for use.

(3) A 2 per cent. solution of eosin (also Höchst).

In using the stain, I have found that a mixture made up in the following proportions gives very good results[1]: four drops of each of the three solutions are added to 10 c.c. of distilled water. The different liquids are poured from small drop-bottles of equal size, the drop-bottles being the same as are generally used for Giesma. (The drops themselves of the different liquids are not, it may be noted, of the same size.)

By this method cultural forms are excellently stained in six to eight minutes; and if any stain is deposited in the ground-substance it comes away readily with orange-tannin afterwards. In fact, on a good smear of cultural forms thus stained, it is often scarcely apparent macroscopically that there is anything at all on the slide. For staining trypanosomes in the blood, only forty to fifty minutes is required.

In all cases, whichever method of staining was used, the slide was well rinsed with tap-water after staining, and then a few drops of orange-tannin were poured on the slide for half a minute or so, to remove the excess of stain. If, after further washing with water, the parasites still appeared to be over-stained, either more orange-tannin or else acetone was added. The latter must be used extremely cautiously and quickly rinsed off, for though at first it only extracts the blue, it soon begins to take out the red from the flagellum. Eventually the slide was washed with distilled water and allowed to dry.

I have since regretted that, owing to the great scarcity

[1] These proportions can be varied, of course, as is found most suitable, in other cases. I may mention that I experimented some time using either (1) or (2) alone in combination with (3), in various proportions, but I never obtained anything like the good results that I did after using both (1) and (2) together.

of the trypanosomes in the blood, I was not able to make use of the iron-hæmatoxylin method of staining. For there is one distinct drawback to the Romanowsky method and its variations. While it may be regarded as giving, after fixation with osmic, a perfectly reliable presentation of the form and general structure of the body, it is now quite clear from the most recent research (see, for example, Minchin [**12**] and Minchin and Woodcock [**13**]) that the nuclear structure and details cannot be interpreted correctly by the aid of stains of this kind alone. This is owing to the invariable tendency of Romanowsky stains to deposit the red colour in excess around certain organellæ, especially small granules, which are thus overloaded with stain and artificially enlarged to many times their real size, often with the result that other cytological features are quite obscured.

Nevertheless, this characteristic behaviour of the Romanowsky stains being now proved and recognised, due allowance can be made therefor, and hence one is not likely to be seriously misled in the case of a study such as is here described, which deals chiefly with the comparative morphology and behaviour of different types of form. Further, it may be pointed out that results obtained by the use of the same methods throughout may be compared with confidence.

3. The Parasites in Relation to Their Hosts.

Numerical Scantiness of the Trypanosomes.—As a rule, the trypanosomes are extremely scarce in the peripheral circulation of an infected host. This fact renders it often an excessively slow and wearisome process to get hold of the parasites at all in a living bird, and hampers any work upon them more than can be imagined until such research has been attempted. Unfortunately, there is all but unanimous agreement among observers upon this point,[1]

[1] The only exception of which I am aware is indicated by a statement of Vassal (**36**) in describing a trypanosome from an Annam pheasant.

which it would be tedious to cite in detail (cf. the remarks by the Sergents [29], Novy and McNeal [14], Laveran [6], Dutton and Todd [4], and others). I will only add that Petrie, in the note already referred to, states that he could not find the trypanosomes in the blood of any of the infected birds, but only saw them in the bone-marrow. With respect to this numerical scarcity, birds are certainly the most trying of all vertebrate hosts. There can be no doubt that, owing to this factor, an erroneous idea has often been obtained of the prevalence of trypanosome infections among birds. This has been well shown by Novy and McNeal, whose adoption of the culture method is of very great value in this connection. It will sufficiently illustrate this to give the statement of these authors that, in the case of forty-three various birds where microscopic examination had failed to reveal trypanosomes, nineteen, or 44 per cent., were proved by means of cultures to have been infected.

To give now my particular experiences. Out of five naturally infected chaffinches only in one were trypanosomes ever seen in freshly drawn peripheral blood; in this case, I once saw an individual in a cover-slip preparation. The same bird was examined at intervals during three months subsequently, but I never saw any living parasites again. That they were still present in the general circulation, however rare, was proved nevertheless on three occasions by means of cultures. Once, determined to find this elusive parasite if possible, I took a few drops of blood and made several smears, which were fixed and stained. In six good-sized films, which were minutely and thoroughly searched, representing a labour of several days, only one trypanosome was seen! It is important to note that these observations were made during the early spring, from January to April. In the case of the trypanosome parasitic in the redpoll I was

This writer was in the happy position of being able to say that the parasites were not infrequent in the peripheral circulation. An individual could be found in every two or three fields (of an oil-immersion lens).

not able to see it in the peripheral blood at all during the first five months of the year, although in two cases I knew by means of cultures that the birds were infected. During the early autumn, however, I was able to find it in smears from a very young bird, which had probably not been long infected. The number of parasites on a fair-sized film varied from six to ten in September, but only from four to eight in films made in October.

Principal Habitat.—In general, the trypanosomes are most numerous in the bone-marrow; this is certainly their principal habitat. Two or three parasites can usually be found in a fresh cover-slip preparation from one of the long bones of an infected bird. But even here, at times, considerable search is necessary,[1] since the parasites are apt to be hidden by clumps of leucocytes, erythroblasts, etc. However, there is generally no difficulty in finding the trypanosomes in a carefully made smear of a small, teased-up fragment of bone-marrow. Thus, when the chaffinch above alluded to was killed, some of the smears from the bone-marrow contained twenty trypanosomes or more.

Artificial Infection.—Only in a couple of instances up to the present have I had the pleasure of finding trypanosomes at all plentiful in the peripheral circulation. One of these cases, at any rate, was certainly the result of successful inoculation. This was a chaffinch which was infected with a culture of the form from the redpoll. Examined previously, no parasites had been found in this bird. On December 19th it was inoculated intra-peritoneally with a fifteen-day culture. On December 21st, twelve days later, examination of the blood showed at least five trypanosomes in two fresh cover-slip preparations, which were not exhaustively searched; and permanent smears made at the same time proved to contain quite a considerable number of parasites—twenty to twenty-five or more on a good-sized

[1] Certainly in one instance, where I failed to find any parasites in a careful search of the bone-marrow, the trypanosomes subsequently appeared in a culture taken from this organ.

film. On New Year's Day also, two parasites were found in a living preparation without much difficulty. When next examined, however, on January 10th, only one trypanosome was seen in two cover-slip preparations, which were thoroughly searched; this indicated a marked diminution in numbers. And in one permanent smear taken at the same time I could not find a trypanosome at all. This bird was not looked at again until the beginning of February, when no trypanosomes were seen in a living preparation. Nevertheless, the parasites were still present, for a tube inoculated subsequently developed a culture; evidently the parasites had by this time diminished in number to their customary scantiness. Unfortunately, this chaffinch accidentally escaped soon afterwards, flying away through an open window.

A Strong "Mixed" Infection.—I have left to the last a consideration of my most interesting case. On March 20th I inoculated a chaffinch with a seven-day culture of the chaffinch form. Three good (i.e. sterile) tubes had been inoculated from this bird previously, and had not developed any parasites. Hence I was practically certain that there were no trypanosomes present in this bird. Examination of the blood at intervals from March 26th until April 3rd, that is, until fourteen days had elapsed since inoculation, proved negative, no cover-slip preparations showing any parasites, so that I was very doubtful whether the inoculation had been successful. About three weeks afterwards the bird was again examined with a like result, but to make the matter certain, a tube (the first)[1] was then taken. To my surprise this developed a culture, the presence of the trypanosomes being thus proved, although I had never seen them in the fresh blood. I propose to leave aside, for the present, the question of whence these trypanosomes had come.

[1] I had not made a culture on the occasions of the earlier examinations, thinking that if the inoculation had been successful the parasites would have been readily observed in the circulation, as in the other instance described.

This bird was then left alone for some weeks,[1] until with the approach of summer I decided to look at it again and see if the oncoming season appeared to make any difference in the number or condition of the parasites. Examining a cover-slip preparation on the afternoon of June 16th I was surprised to see numerous microgametocytes of Halteridium. The stimulus of cooling was causing many of them to rupture the red blood-corpuscles, and rapidly form and liberate the active male gametes. I had never seen any Halteridia in the preparations or smears made previously from this chaffinch; if this parasite was present then it must have been extremely scarce in the peripheral circulation.

I was so occupied with watching this process of the liberation of the gametes and in endeavouring to see actual conjugation stages (unfortunately without success) that I did not search these fresh preparations for trypanosomes. In permanent smears made at the same time, however, trypanosomes occur, but they are not numerous (half a dozen or so on a slide).

Having this abundant Halteridium-material, and knowing the bird to be infected with trypanosomes also, I determined to examine it in the night-time to see if I could obtain any phases connecting these two types of parasite. Blood taken at 1.30 a.m. on June 18th showed the same condition as regards the Halteridia, and, in addition, Trypanosomes were easily found, three and four respectively being seen in two cover-slip preparations without any difficulty; and there were probably several more in each. The trypanosomes seen were manifestly much larger than the Halteridia, and I saw no indications of a rapid transformation of the Halteridia into trypanosomes, or vice-versa; indeed, the only Halteridia observed free in these living preparations were the adult gametocytes, male or female, behaving in the

[1] The bird was not made use of during this period because I had now given up making permanent preparations when a living drop failed to show the parasites. I had learnt that the probability was so much against my finding any trypanosomes in a reasonable time.

usual manner. Many smears of the blood were made, some at once, others after waiting a moment or two, and with or without the addition of a drop of salt-citrate solution.

The bird was again examined on the afternoon of June 22nd, when one trypanosome was seen in two cover slip preparations after some searching. Another night examination was made about 1 a.m. on June 30th. Compared with the previous night examination there appeared to be as many trypanosomes present, but the mature Halteridia did not seem to be quite so numerous as before. After a similar procedure I at length killed the chaffinch (about 2.30 a.m.) in order to obtain smears from the internal organs—heart, liver, spleen, bone-marrow, kidneys, etc. Most unfortunately, I omitted to make any preparations from the lungs—an oversight which I have since greatly regretted. I need only mention here that the trypanosomes were afterwards found to be comparatively few in number in preparations from the bone-marrow, while in smears from the liver, etc., they are very scarce. As regards the peripheral circulation, the parasites are certainly more numerous in these night-slides than they are in those taken (from the same situation) in the daytime (afternoon). Hence there would seem to be, to some extent, a wandering of the trypanosomes from the internal organs (probably chiefly from the bone-marrow, which is their principal "internal" habitat) into the peripheral circulation during the night-time.

Halteridium in Relation to the Corpuscles.—As already indicated, the Halteridial infection of this bird was a very strong one, and the parasites were very numerous at this time; in fact, in some smears, for instance, from the liver, they are almost abundant. The Halteridia are of all sizes, from minute forms up to fully grown adults. Nearly all the parasites are intra-cellular. Until recently the only cases in which I observed any forms free from the corpuscle[1]

[1] Of course, ripe sexual individuals, which have become rounded off and liberated themselves from the corpuscles, are not included in this statement; neither are distorted or irregular individuals, which have

—in spite of much searching—were four or five instances in which a special kind of individual, with peculiar features, was found free in the plasma. Having been led, however, as a result of my observations at Rovigno, to again examine very carefully certain of my preparations made at night, I have now found here and there a few individuals of small or intermediate size, and apparently of normal appearance, free in the blood. It is noteworthy that these free individuals have been seen only in smears from the peripheral blood, and not, for instance, in preparations from the liver, where the parasites are most numerous. Hence I do not think that the first impression I formed, namely, that the Halteridia do not leave the blood-corpuscle in the course of their growth, can be sustained.

Occurrence of the Leucocytozoon. — The new leucocytozoon which I have observed occurred in three chaffinches. In two it was very scanty, only one or two isolated individuals having been noticed, and they were small. In one bird, however, which happened to be that which was successfully inoculated with Trypanosomes from the redpoll (see above, p. 659), the Leucocytozoon is not at all infrequent. The parasites are nothing like so numerous as the Halteridia are in the case just described, but there are certainly as many or more Leucocytozoa than there are trypanosomes on any smear. On one film more than twenty-five have been marked, and the slide has not been exhaustively searched for all the minute forms. Unfortunately, I did not detect this parasite in living, cover-slip preparations. For one thing, I was examining the chaffinch in which it occurred for Trypanosomes, which can be readily seen; further, as this species does not produce the characteristic spindle-like appearance of the host-cell, as in the case of nearly all other Leucocytozoa so far described, there was nothing about the parasites to catch

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obviously been accidentally set free from a ruptured corpuscle in making the preparation, such as are occasionally met with.

the eye. If I passed over one in my search I doubtless took it merely for a large leucocyte.

4. Description of Trypanosoma Fringillinarum, n. sp.

(a) As Found in the Birds.

The trypanosomes from the chaffinch (Fringilla cœlebs) and the redpoll (Linota rufescens) most probably belong to one and the same species. The trypanosome once noted, but not described, by Ziemann, in 1898, was most likely this form; and the same applies doubtless to Petrie's observations (21) in 1905. The occurrence of trypanosomes in the redpoll has not been known hitherto; this bird is a new avian host for the parasites. I regard the trypanosome from these two birds as a distinct and new species, for which I propose the name T. fringillinarum.

I discuss below the question of the specificity of different trypanosomes, with reference particularly to avian forms. I will merely give here the chief reasons which lead me to consider all the different types met with in the chaffinch and redpoll as belonging to one species. In the first place the ordinary, or definitive form of the parasite, the type, that is, which affords in the existing state of our knowledge the chief basis of morphological comparison in a systematic study of different Trypanosomes, appears to be essentially the same, as regards form and structure, both in the chaffinch and in the redpoll (cf. for instance figs. 4 and 31 of individuals from a naturally infected chaffinch with figs. 3 and 32 respectively of parasites from a naturally infected redpoll). Again, the forms which appeared in the blood of a chaffinch as the result of inoculation with a culture of the redpoll-parasite are also of a similar type (cf. figs. 1 and 28).[1] Secondly, although considerable polymorphism is shown, transition forms occur, which are intermediate between the more

[1] The fact that the inoculation of the parasites from the redpoll into the chaffinch was successful itself points to the specific identity of the two forms.

extreme types noticed and serve to connect them. Lastly, it may be added that the various cultural forms to which the parasite from the chaffinch gives rise are quite similar to, and cannot be distinguished from, those developed from the trypanosome of the redpoll.

The ordinary or definitive type of T. fringillinarum is elongated and slender in appearance (figs. 1–4, 27, and 28) The aflagellar end is long and finely tapering, at times being, indeed, extremely attenuated (fig. 27).[1] The free flagellum is usually comparatively short. The trypanosome possesses a well-developed undulating membrane, which has three or four folds or pleats, broad and deep. The average dimensions of a full-sized "adult" individual are as follows:

Total length, including flagellum	41 to 45 μ
Greatest width, including undulating membrane	$4\frac{1}{2}$ to 5 μ
Greatest width of undulating membrane	$1\frac{3}{4}$ μ
Length of tapering aflagellar portion of body, i. e. the distance from kinetonucleus to extremity	5 to 7 μ
Length of free flagellum	5 to 7 μ

The trophonucleus (nucleus) is situated near the middle of the body, often slightly in the aflagellar half. It lies generally somewhat nearer to the undulating membrane than to the opposite side. The nucleus is more frequently ovoid in shape, but it may be approximately round (figs. 1 and 29); in the former case it may measure as much as 3 μ by 2 μ, and in the latter case it may have a diameter of $2\frac{1}{2}$ μ; but these dimensions are not always attained.

The kinetonucleus appears as a relatively large body,

[1] This aflagellar prolongation is very delicate and liable to be broken off and lost in the preparation of the specimen; hence, now and again a parasite is seen which appears to have no "snout" at all, and where the body appears to be terminated by the kineto-nucleus; this is certainly an artificial condition, for it is characteristic of the fully grown ordinary individuals to have this long attenuated process at the aflagellar end.

ovoid or rather oblong, which occupies the entire width of the parasite at the point where it is situated. Its apparent size is about $1\frac{1}{4}$ to $1\frac{1}{2}$ μ by 1 μ. It is nearly always intensely stained after Romanowsky stains, and shows no structural details.

The flagellum, at its proximal end, nearly always stops short of the kinetonucleus; only very exceptionally does it appear to come into contact with the latter organella. In this connection it may be emphasised that my specimens are all from films properly fixed with osmic-acid vapour—none from air-dried smears. Moreover, at the point where the flagellum terminates, a definite granule, staining rather more deeply, can sometimes be made out quite clearly (figs. 4, 28). Unfortunately in many cases the root portion of the flagellum, which is probably intra-cytoplasmic, is not well stained, and in these the granule cannot be made out.

The cytoplasm stains pale blue, and is of fairly uniform structure, appearing in some instances finely alveolar. Occasionally a few small vacuoles or spaces are to be seen in the cytoplasm, but I have not observed anything that could be regarded as a definite, regularly occurring organella of that kind. In some of these forms the cytoplasm is free from granules; in others, however, granules which stain bright red, and are of varying size, occur in greater or less number (figs. 1, 3, and 27). These granules are most probably of a chromatoid nature, derived from the nucleus.

The structure of the undulating membrane shows an interesting feature. Running longitudinally in the broad folds or pleats, usually about the middle, is a prominent line, which stains blue—not red, like the flagellar border (figs. 1, 2, 4, 28–32). With a good light it is not difficult to make out that the part of the fold nearer to the body appears slightly denser than that on the outer side of this line, and stains faintly but distinctly blue, whereas the outer part is practically colourless. The explanation of this structure is that it represents a delicate intrusion of the

endoplasm, running part of the way into the pleat of fold, between the two (otherwise) closely apposed ectoplasmic layers which constitute the membrane. The longitudinal line about the middle of the fold is the edge or limit of this inner endoplasmic layer. Laveran, in his account of T. avium (6), calls attention to a "rib" or longitudinal striation in the membrane. This striation corresponds, in all probability, to the limit of an endoplasmic intrusion similar to that just described.

Apart from the undulating membrane, I have never seen indications of an ectoplasmic layer. The trypanosomes I have studied show no sign of a well-developed, red-staining "periplast," such as has been described by several workers in the case of T. lewisi, for example. As a matter of fact, I should not expect to see any such appearance here, since the ectoplasmic part of the folds of the membrane is itself generally quite colourless, as already mentioned, and at most shows in one or two instances the faintest possible tinge of pink colour, which would be quite lost against the stronger blue of the body. Nevertheless, there is no reason to doubt that the parasites have a delicate ectoplasmic sheath, investing the body generally.

I will leave until later the consideration of the minute structure of the trophonucleus.

The above type of the parasite is the form which I have found in the blood of the host—at any rate, in the chaffinches—during the winter and early spring months, when the numerical factor is low, the infection being, as it were, persistent, but in a quiescent and somewhat scanty condition.

Young individuals, not yet full-grown, which belong to this ordinary definitive type, can be readily recognised. They are, of course, somewhat smaller, but their form and general appearance agrees in most respects with that of the adult parasites. The chief point of difference is that the "snout" is usually not so elongated and drawn-out; it is more conical, but still sharply pointed (figs. 31–33). This aflagellar part of the body attains the extreme degree of

attenuation only in the fully grown forms. An intermediate condition is seen in figs. 29 and 30. It will be noticed that there is often considerable variation in the size of the nuclei in these young or intermediate-sized individuals (cf. figs. 29–33), even where the parasites appear very similar in size and form. This feature is met with also in other series of forms to be described (see below, p. 672). I do not think much stress need be laid on apparent differences in size of these organellæ in comparing parasites otherwise similar.

Unfortunately, as already mentioned, I could not obtain any stained specimens of the parasite in the blood of the redpoll during that period, owing to its scarcity, although I had obtained cultures on two or three occasions. It was early autumn before I could obtain series of permanent preparations showing the trypanosomes in this bird; and in these smears, parasites which belong to the type above described are relatively scarce and outnumbered by another type. I have not found in this host at this period any ordinary forms which have attained quite the dimensions of the fully grown individuals occurring in the chaffinch in the early part of the year. The individuals observed, however, correspond closely to the slightly smaller forms of the parasite, which have been described above (cf., for instance, figs. 33 with fig. 4, and, again, fig. 32 with fig. 31). Hence I have little doubt that they represent that phase of the same species, bearing in mind also the other considerations stated already. It is probable that if I could have obtained examples of the trypanosome in the blood of the redpoll in the early part of the year I should have found "adult" definitive forms similar to those in the chaffinch.

The predominating form of the trypanosome in the blood of the redpoll in the autumn (September, and again in October), is a very large parasite. Some of the individuals of this new type are, in all respects, the largest trypanosomes I have observed in the birds, being not only as long as the longest ordinary individuals, but also much stouter. The individual drawn in fig. 37, for example, measures 48 μ in total length

and $6\frac{1}{2}$ μ in total breadth, while that in fig. 35 is 44 μ by $6\frac{3}{4}$ μ. Even the rather smaller forms of this kind (figs. 34, 36, and 38) are distinctly wider than the full-grown definitive parasites, their breadth varying from $5\frac{1}{2}$ to 6 μ. Hence, in general appearance these trypanosomes differ considerably from those of the first type.

The aflagellar end is prolonged for some distance (6 to 8 μ) beyond the kinetonucleus; it may be fairly wide and somewhat blunt (fig. 36), or slender and tapering (fig. 37), but it is never so finely drawn-out and attenuated as in the case of the definitive individuals. The free flagellum is usually short, only about 4 to $4\frac{3}{4}$ μ long. The undulating membrane is well developed, but the folds or pleats are not usually so sharply separated from each other as in the case of the other forms.

The cytoplasm of these massive forms stains blue, deeply and intensely.[1] In structure it is quite different from that of parasites belonging to the other type. As a whole it is much coarser in texture and more granular. In the majority of cases it does not appear to be of uniform character throughout the body (figs. 34 to 36). In the aflagellar third or so of the body it is loose and spongy, with large granules more or less uniformly distributed; but in the other two thirds or so, i.e. in the region from the trophonucleus to near the flagellar end, it is more compact, and the granules tend to be closely arranged in longitudinal rows, of which there are usually five or six. Thus the cytoplasm in this part of the body appears made up of narrow dark bands (composed of more prominent granules, packed together), with between them paler bands or zones of more finely granular (and hence less deeply staining) cytoplasm. The extent to which this serial arrangement of the larger granules is developed varies in different individuals. In some they extend through two

[1] There is no question of this difference being due merely to accidental variations in the staining; individuals representing the two types of form have been found on the same smear, and within a short distance of one another.

thirds or more of the length of the body, while in others they occupy only the middle portion (fig. 35). Now and again these bands appear very narrow, but in no case can they be considered as lines or striations; I do not think they have any connection with, or themselves indicate, actual myonemes. Dutton and Todd (4) have described what is probably a similar cytoplasmic differentiation in Trypanosoma mega and T. karyozeukton. They distinguish the loose, spongy aflagellar region as "spongioplasm," and the region of the longitudinal bands as "hyaloplasm." The chief difference in their cases is that the dark bands are very broad and very compact, showing less obviously their granular structure, while the alternating, less granular zones are very narrow and pale, and appear as clear stripes.

I have never seen any indications of division in any parasites belonging to either of the above types.

The next series of forms of Trypanosoma fringillinarum to be described consists, on the whole, of small parasites, some of which are extremely small. These forms have been found in two cases. The first instance of their occurrence noted was in the bone-marrow of a naturally infected chaffinch, which was killed about the middle of March. This bird had the usual scanty number of ordinary definitive trypanosomes in the general circulation, and these are also present in the bone-marrow, along with the parasites of small type. The other case was in the chaffinch which was found to have a mixed infection of Halteridia as well as trypanosomes towards the end of June (cf. p. 660). In this bird the trypanosomes were comparatively numerous in the blood; but no individuals of the ordinary large type have been found in any of the preparations, whether from the blood or organs. As I shall frequently have to distinguish between these two cases, it will be convenient, and will, I hope, render the description clearer, to refer to them as case A (the former, earlier case), and case B (the second, later case), respectively.

I will begin the account of this small type of form by

describing the parasites which occur in the later case (B). The smallest individuals have been found in the bone-marrow. The trypanosomes are distinctly less frequent in the bone-marrow than they are in the general circulation, and the individuals which do occur in this situation are nearly all small or minute in size. One of the smallest forms seen is drawn in fig. 40. Its total length is 15 μ, that of the free flagellum alone being 4 μ; hence the length of the body itself is 11 μ. The width is a trifle under $2\frac{1}{2}$ μ. It is only necessary to compare this parasite with some of those above described to realise the great difference in size which may be shown by different individuals of the same species of avian trypanosome. Another very small individual (fig. 5) has a total length of 18 μ, partly accounted for by the rather longer flagellum of 8 μ, and its greatest breadth is 3 μ.

On the other hand, the largest individuals belonging to this series of forms which I have observed are seen in figs. 44 and 45. The parasites are of only medium size; they do not really come in the category of large forms. The trypanosome of fig. 45 has a length of $33\frac{1}{2}$ μ, its flagellum alone is $8\frac{1}{2}$ μ, and the greatest breadth is $5\frac{1}{2}$ μ. The dimensions of the other individual are rather less. Between these two extremes of this type parasites of all intermediate sizes occur—forming, indeed, a regular gradation. This is illustrated by figs. 6, 42, and 43. The trypanosome in fig. 6, for instance, is 23 μ in total length, of which the flagellum is $6\frac{1}{2}$ μ, and has a width, including the undulating membrane, of $3\frac{3}{4}$ μ; again, the individual of fig. 43 is 27 μ long, the flagellum alone 6 μ, and the breadth $4\frac{1}{2}$ μ.

As will be noticed, there is a general similarity in form between all these parasites. The body is fusiform or spindle-shaped, and fairly wide in proportion to its length; it is quite distinct in appearance from the body of a definitive individual. The aflagellar end is drawn out and pointed, but it is not so elongated and attenuated as in the case of the definitive parasites described above. In the smallest indi-

viduals the undulating membrane is narrow and inconspicuous (figs. 40 and 5), but with the increase in size of the body it becomes wider and more prominent. The kinetonucleus may be relatively large, more particularly in the small individuals; in the parasite of fig. 5 it appears to be four-lobed, as if it were composed of four small masses. The free flagellum is fairly long, varying from $6\frac{1}{2}$ to $9\frac{1}{2}\,\mu$. A modification of this type occurs, but it is very uncommon in this series; certain parasites are relatively very wide, and have the aflagellar end very short and abruptly conical, which gives the trypanosome a stumpy appearance (fig. 41). The dimensions of this individual are: Total length, $18\frac{1}{2}\,\mu$; of the flagellum alone, about $3\frac{1}{2}\,\mu$; while the width is as much as $5\frac{1}{2}\,\mu$.

Comparing now the small forms present in the earlier case (case A), the parasites are quite numerous in the bone-marrow, and to this situation they appear restricted. They are of varying size, but I have not found individuals quite so minute as the smallest of those above mentioned. Parasites which are fairly small, nevertheless, are shown in figs. 46 and 47. The former is $25\,\mu$ in length and $3\frac{1}{4}\,\mu$ wide, the flagellum alone being as much as $9\frac{1}{2}\,\mu$; these two trypanosomes correspond fairly closely with that of fig. 6 from the other series, the chief difference being the longer flagellum. Here, again, it will be seen that there is considerable difference in the size of the kinetonucleus in the parasites compared. But on the same slide as the parasite of fig. 46, actually only two or three fields away, is another individual almost identical except that its kinetonucleus is nearly twice as large. Compare also figs. 44 and 45, and again, figs. 52 and 53.

Rather larger forms are seen in figs. 49–51. Most of the parasites in this earlier case, however, are comparable rather with the wide, stumpy form alluded to above, than with the fusiform individuals. Typical examples are seen in figs. 52–54. The parasite in fig. 54 has a total length of $27\,\mu$, the flagellum being $9\,\mu$, and its breadth is $5\frac{3}{4}$ to $6\,\mu$; the corresponding dimensions of the trypanosome in fig. 53 are

29 μ, 10 μ, and 6½ μ respectively. The flagellum of these trypanosomes is usually comparatively long (from 9 to 11 μ), being often longer than in the largest individuals of the fusiform kind. The kinetonucleus is always very near the aflagellar end, which is short and conical. The trophonucleus varies in shape; it may be more or less round, but it is often considerably elongated in a direction transverse to the longer axis (figs. 52–54).

It is noteworthy that in this earlier case no forms have been observed which correspond to the larger fusiform trypanosomes of the other series (case B). The parasites, which are no longer very small—which are becoming intermediate in size—such as the individual drawn in fig. 50, are obviously approaching in character the wide, stumpy forms, and differ appreciably from the intermediate-sized individuals of the fusiform variety in the features already indicated, namely, the broader body, the longer flagellum, and the abruptly terminating aflagellar part (cf. with figs. 43, 44, from the other case).

Many of the individuals in the above-described series of "small" parasites, including both fusiform and stumpy ones, show a cytological peculiarity which is at first somewhat puzzling. This feature is a row or chain of granules, which take up the red stain strongly, and which are very closely apposed to each other, giving the idea of a thick, beaded line (figs. 42–44, 47–50, and 54). This chain runs approximately parallel to the flagellar border of the undulating membrane, often following its curves closely, and it is frequently more deeply staining and prominent than the flagellar border itself. It begins near the origin of the flagellum, and always ceases with the limit of the body, at the opposite end, i.e. it never becomes free, as anything corresponding to a free flagellum. At first sight this line might be regarded as representing a new flagellum, formed either de novo or by a splitting of the old one, the parasites showing this appearance being therefore in the act of commencing division. After studying several of these individuals,

it is clear, I think, that this structure has really nothing to do with a flagellum. The line is usually most prominent in parasites which show numerous red-staining (probably chromatoid) granules in the cytoplasm; and, in suitable instances, it can be seen quite well that it is situated at the edge of the endoplasmic intrusion in the membrane (figs. 42, 47, 49). Further, when present, it can usually be traced right along the course of the membrane from end to end.

If we had to deal here with a case of division or formation of a new flagellum, individuals showing either an earlier or later phase in the process might be expected to occur, for this appearance is not at all infrequent; but I have not found any such. Again, in most cases, there is not the least indication of nuclear division. Lastly, in one of the exceptionally few instances where any indications of division are present, in addition to the kinetonucleus having divided into two, the true flagellum can be seen to be itself double for a short distance near its proximal end, probably as a result of splitting (fig. 54).[1] The granular chain is also present, and, as before, quite separate from the flagellum. Hence there is no reason for regarding this structure as in any way connected with a flagellum, much as it simulates one at times.

The small stumpy trypanosome in fig. 41 shows what is probably an early stage in the development of this line. Here there is a row of red-staining granules, quite separate, and not closely apposed to constitute a chain, which run parallel to the flagellar border, doubtless at the limit of the endoplasm. The granules are apparently quite similar to others which are seen in the general cytoplasm. I have no idea what is the explanation of this aggregation of chromatoid granules into a compact chain, lying in the position described. I have never seen it either in the ordinary definitive trypanosomes or in parasites of the other large type. I may add that I have observed the same feature in the case of a trypanosome from a blackbird (Turdus merula), at

[1] Cf. also the micro-photograph reproduced in fig. D.

Rovigno, the parasites which showed it being also of the same type of form.

There is still another variety of form to be mentioned, which occurs in case A (in the bone-marrow). This is a fairly small trypanosome (figs. 55 and 56), which is very narrow in proportion to its length. The aflagellar end is comparatively long and finely drawn out, and may approach the attenuated condition. The flagellum is fairly short, and the undulating membrane has well-developed folds. The dimensions of the individual in fig. 55 are: total length, 27 μ, breadth (including membrane), 3 μ, and length of flagellum $6\frac{1}{2}$ μ. The kinetonucleus is relatively large. These parasites strongly resemble in appearance young ordinary or definitive trypanosomes.

With regard to the multiplication of these small forms the only evidence I have been able to obtain is very slight.

I have observed three or four individuals (and not more) of the wide stumpy kind from case A, in which the kinetonucleus is in two parts (figs. 48 and 54); and in one solitary instance, just alluded to, the flagellum is partially doubled. In no case have I seen two trophonuclei. The condition in fig. 48 is the nearest approach to trophonuclear division that I have observed; this may represent commencing division because other organellæ of this parasite are dividing. The flagellum has not yet begun to divide, but as a prelude thereto, the centrosomic granule at its proximal end ("blepharoplast") is clearly double. So far as the fusiform series (of the other case) is concerned, I have observed absolutely no signs of division at any phase.

General Remarks.—The significance and relation to each other of all these manifold forms of the trypanosome is a somewhat difficult question. Where transitional forms or division phases occur they afford, of course, considerable help. Beginning with the small forms, the stumpy parasites of case A, in which indications of division can be found, probably give rise, as a result of that process, to small individuals like those in figs. 46 and 47, which grow into

somewhat larger individuals of the fusiform type (figs. 49 and 51). The stumpy trypanosomes themselves are best regarded, I think, merely as division-forms of young to medium-sized individuals of fusiform type. Hence, in this case, it may be said that the fusiform parasites present are of small to medium size and tend to multiply, by passing into the stumpy division form, rather than grow, at any rate at this period, into large trypanosomes. Next, with regard to the very thin, slender forms (e.g. figs. 55 and 56): when first seen they appeared in such sharp contrast to the prevailing stout type of parasite that I was somewhat disposed to think they represented male forms. As above mentioned, however, I am now more inclined to look upon them as young definitive parasites, which would grow into medium-sized ones, such as those in figs. 4 and 31, and so to full-grown adults, as in figs. 2, 28 (all from this series).

Turning again to case B (the later case), we find no ordinary forms present. Fusiform individuals of medium size are not uncommon, and between these and very small forms parasites of all intermediate sizes occur. There are very few stumpy forms, and none of those found show any actual signs of division.[1] Hence, the main condition here is undoubtedly a series of steadily growing fusiform individuals.

There remain two or three interesting questions in connection with the different type or phase of the infection occurring at different periods, in regard to which I can only put forward those surmises which seem to me the most probable. In the first place, comparing the condition found in a chaffinch (case B), in the summer, with that obtaining in a redpoll in the early autumn, where the parasites are mostly of the large massive type (e.g. figs. 34–36), I think it is most likely that the fusiform parasites of the former case (such as those of figs. 44, 45), would grow ultimately into individuals corresponding to those of the latter. The body-form is essentially similar in the two cases. The size of the

[1] It is possible, however, that the two or three small stumpy individuals seen in this case may be about to divide.

parasites found in the autumn is of course greater, but the difference is not relatively more than that between the larger and the smaller fusiform individuals in the summer. A difference which might appear of more importance is that in the character of the cytoplasm in the two cases. This can probably be explained, however, by supposing that the cytological features shown by the large massive individuals in the redpoll have become more developed and consequently more prominent, as a result of the increase in size. And, on the other hand, there is no evidence whatever that the fusiform parasites will pass directly into the characteristic ordinary type.

Assuming, then, this connection between these two sets of forms, how are we to explain the condition met with in the winter and early spring, when the only type of individual in the blood is the ordinary definitive form? The answer to this depends largely, I think, on what significance is to be assigned to the large massive forms just referred to. Are they to be considered as sexual individuals —of the female type? This is, of course, possible, but more than that cannot be said. And if this is the case, I certainly do not know which are the individuals of male sex; there do not appear to be any forms present at the same time which could be so regarded. On the other hand, I think it is at least quite as probable that the massive individuals have grown to this size prior to multiplication; they may later undergo some process of multiple fission or segmentation, occurring in one of the internal organs, and so give rise to the small forms. This supposition would fit in very well with the condition found, for instance, in case A (in the spring), where, as we have seen, small parasites are numerous in the bone-marrow, along with the ordinary forms, the latter being probably to some extent replenished from them. And here there are no signs of the large massive individuals. At all events, in view of Chagas' recent important work (2), showing that a new human trypanosome, Schizotrypanum cruzi, has a method of multiplication by multiple fission or

schizogony, I think it is not at all unlikely that naturally occurring trypanosomes—about whose life-cycle in the Vertebrate host very little is yet really known—may show some such schizogonic process more commonly than has hitherto been supposed.[1] In default of such a process in the present case, I have no idea how the small forms are developed, since they certainly do not appear to be derived from the adult ordinary individuals.

Another question is, What becomes of the ordinary, definitive forms of the trypanosome? As I have obtained many successful cultures from birds where this was the only type present in the blood, the natural inference would be that this form can be transmitted to the insectan host; but the same applies equally, it must be noted, to the fusiform parasites of case B, since I obtained cultures from them also. And I cannot be certain that both these types would develop naturally in the insect. Some of the ordinary forms, later on in the season, may pass into the large, massive type; this is not at all unlikely, if the latter is really a multiplicative form. The individual drawn, for instance, in fig. 39 may perhaps represent an intermediate stage in such a transition. Another possibility, of course, is that this definitive type disappears altogether in the summer, its place being taken by the fusiform type; the condition of the infection would then correspond with that of case B. I do not think this is likely. Case B most probably represented a recent infection (see below); in such the condition may quite likely differ from that found in an old established infection. Moreover, in the earlier case A (about the middle of March), parasites of the ordinary type are quite numerous, and do not look like disappearing; and further, in the autumn, in the redpoll, this type is also present.

It remains for me to say a few words with regard to the origin

[1] A most interesting piece of evidence bearing upon this point is supplied by Minchin (**12**), who mentions and figures the occurrence of a large individual of T. percæ, which is apparently in an encysted condition. Such a form might very well be about to undergo schizogony.

of the infection in this later case B, the chaffinch in which there was also an abundant halteridial infection. As I have stated in my note (**38**) on this interesting Halteridium, I was at the time inclined to think that the very small trypanosomes might have been developed directly from the Halteridia. Paying attention, for the moment, only to the trypanosome side of the question, in addition to the fact that in this case we have certainly to do, not with division, but with growth and increase in size from the minute forms up to comparatively large ones, there were other reasons which led me to take this view. This chaffinch, originally free from trypanosomes, was inoculated with cultural forms, but the subsequent course of events was very different from that in the case of the other successful inoculation described. In the latter case the parasites soon became comparatively numerous in the blood, whereas in the former they were not found at all at first, and only after some weeks were they shown to be actually present, by tubing (for further details, cf. p. 660). When at length they did become sufficiently numerous to be found without difficulty in stained preparations, they proved to be, as we have seen, quite different in form from the ordinary individuals developed in the other case. Hence, taking all things into consideration, I considered that the trypanosome infection was probably not due to the inoculation (which, in several cases, it must be remembered, did fail), but to the presence of Halteridium.

I admit now that I have changed my opinion about this case since writing my former note. In spite of the many features which seemed either to point strongly to this view, or at least to favour it, I think after all the trypanosome infection was not really connected with the Halteridial one, but was due to the inoculation (for further discussion of this subject, see under Halteridium). There remains the question, Why was the course of the infection so different in the two cases? Of course, in the one case where the parasites developed quickly, the inoculation was made with cultural forms which had come from a redpoll, while in the other they came from a

chaffinch; but I do not think this sufficiently explains the difference, because everything points to the species being the same in both birds. Since I have been able to study my cultural forms, I have come to the conclusion that the progress of the infection may have been so different, on account of a difference in the condition of the two cultures. The chaffinch-culture, from which resulted, we must suppose, the slowly developing infection, was one of six days' age, and certainly contained the characteristic trypaniform individuals to be subsequently described (cf. below, p. 690); for permanent preparations were made at the same time which showed this type. On the other hand, the redpoll culture used in the other (earlier) case was a fairly old original one of fifteen days; preparations were not made from this culture actually on the day when it was used for inoculating the bird, but in smears taken a couple of days before, none of these forms had been seen; the culture appeared quite healthy, and consisted almost entirely of the usual trypanomonad forms, to which, presumably, the infection must be ascribed.

It is an interesting question in which of these cases the course of the infection, so very different in the two, more nearly resembles that occurring naturally, i. e. by the inoculation of the right developmental forms from the insect. As will be seen on reference to one or two papers discussed below (p. 709), the remarkable trypaniform type alluded to is thought to be probably the true propagative form, which produces the infection of the vertebrate host. If this is so, it would seem to follow that the later case (case B), where the infection developed slowly, agrees most with the course of events in a natural infection.

(B) The Trypanosomes as Found in Cultures.

Before beginning an account of the cultural forms, one or two introductory remarks are necessary. When I commenced to make use of the cultural method, I did so solely because, from Novy and McNeal's work (14), it was evident

that it is of very great service in ascertaining whether a bird is infected with trypanosomes or not. I think now that I must have been unusually fortunate in my first experiences of the culture method.[1] I had no difficulty in getting the parasites to develop in my cultures, and, moreover, in a perfectly healthy manner. I soon had no trouble in distinguishing between what could be regarded as normal types, of regular occurrence, and what were abnormal, irregular forms. Hence, I admit that I modified my former attitude towards this method, and came to the conclusion that the cultural forms were probably, for themselves, well worth studying. I claim some excuse for my earlier opinion, since at that time this method had only begun to be adopted for trypanosomes, and in the early descriptions of cultural forms most of the figures depict what can only be described as altered appearances, which certainly belong to the category of abnormal phases. As a result of my own work, the view I now hold, and which I have expressed in my article in Lankester's 'Protozoa' (**39**), is that the cultural forms of trypanosomes may afford indications of value as to the developmental phases of the parasites occurring in the invertebrate host.

As I have already indicated, the chief cultural forms developed from the trypanosomes in the redpoll are quite similar to, and practically indistinguishable from, those to which the parasite from the chaffinch gives rise. I have had, however, a much greater number of successful cultures from the latter bird than from the former; hence I have found a greater variety of intermediate phases in my cultures from the chaffinch, and have had the good fortune, moreover, to observe one or two particular phases which I have not seen in cultures from the redpoll. This is doubtless due, however, merely to lack of sufficient material in the

[1] I may mention incidentally that I have since had a full measure of the trials and troubles which may attend the cultural method, for at Rovigno, in connection with the trypanosomes of the little owl, I had no success at all with it.

latter case, and I have no reason whatever to think that one set of cultural forms shows any intrinsic differences from the other, which would imply that the trypanosomes from the chaffinch and the redpoll, respectively, are distinct parasites.

The predominating type of the trypanosome in the cultures is a well-defined and characteristic form, which may be termed the trypanomonad form of the parasite, deriving this convenient general designation from one of the various alternative (synonymous) names (viz. Trypanomonas) given by Danilewsky to certain parasites described by him. This type is elongated and slender, the width usually varying but slightly in the middle of the body, and diminishing more or less gradually towards the aflagellar end. The essential diagnostic characters are: (1) The two nuclei are always close together, and situated either about the middle of the body, or else distinctly in the aflagellar half; and (2) the flagellum is attached for some distance to the side of the body, forming a distinct undulating membrane. The membrane may be at times fairly prominent, and possess a wavy edge, indicating a slight development of pleats or folds. The kinetonucleus is never near either end of the body. It is important to note that the flagellar end of the body is drawn out with the flagellum, as it were, and ultimately thins away, leaving the flagellum free. This condition is of very general occurrence, of course, among trypanosomes (as seen in the blood), and is the natural consequence of the presence of an undulating membrane. In respect of all the above features, therefore, the trypanomonad type differs essentially from a herpetomonad form.

Typical examples of the trypanomonad form, showing parasites of medium to large size, are seen in figs. 7, 8, 71–75, and figs. 13, 77–79, from preparations of cultures from the chaffinch and redpoll respectively. To give an idea of the size of these forms, three principal measurements may be taken: (A) length of body alone, (B) greatest width of body, and (C) length of free flagellum. These dimensions, in the case of some typical individuals, are as follows (in μ); fig. 72—(A)

21, (B) 3, (C) 9; fig. 73—(A) 25, (B) $2\frac{1}{2}$, (C) 10; fig. 7—(A) 25, (B) $2\frac{1}{2}$, (C) 29; fig. 75—(A) 26, (B) $3\frac{1}{4}$ (opposite nucleus); (C) 11; and again, fig. 79—(A) 21, (B) $2\frac{1}{2}$, (C) 15; fig. 77—(A) 23, (B) 3, (C) 19; fig. 88—(A) 26, (B) $3\frac{1}{2}$ (opposite nucleus), (C) 14. The measurements are given in a slightly different manner from that adopted in the case of the parasites when in the bird. In the cultural forms the length of the body by itself affords a better means of comparing the size of different individuals than the length of the body plus that of the flagellum. This is because of the great and apparently indiscriminate variation in the length of the flagellum, which cannot be said to bear any relation to that of the length of the body. This is well seen by contrasting figs. 80 and 81, from a redpoll culture, with figs. 84 and 83, respectively, from a chaffinch culture. This diversity is chiefly due to the manner of division, as will be explained shortly.

Smaller forms, very similar in appearance to some of the larger ones indicated, are seen in figs. 85 and 86; the former is 17 by $1\frac{3}{4}\,\mu$ and its flagellum $7\frac{1}{2}\,\mu$. The smallest parasites observed, however, belong to, or result from, a slightly modified variety of the above type. This is somewhat different in appearance (figs. 8, 97), but it really represents only another facies, as it were, of the same trypanomonad type, from which it is derived by the gradual drawing back of the nuclei well into the aflagellar half of the body, and by a somewhat modified manner of division which is then found (concurrently).

As the process of multiplication plays an important part in the development of these various forms, it may be as well to give a general morphological description of it here before proceeding farther. The mode of division by which the long, slender trypanomonad forms are produced is that of equal or subequal fission of the body. Sometimes the two daughter-flagella are practically equal (figs. 11, 96), but in the majority of cases one of the flagella is distinctly longer than the other (figs. 91–95). In all the instances I have noticed, the division of the cytoplasm begins at the flagellar

end. It generally happens that, as the split extends, the parasites tend to separate from one another, turning outwards, away from each other as it were (figs. 92, 93); eventually the two daughter-individuals come to lie in one line (which may be more or less curved), with the flagella,waving freely at opposite ends, the parasites only remaining connected by what is actually the still undivided aflagellar end (figs. 94, 95). The fact, therefore, that we may find either equal or sub-equal cytoplasmic division in which the daughter-flagella differ considerably in length, explains the great variation in this respect which is met with among the ordinary trypanomonad individuals.

In many cases the division of the two nuclear bodies does not take place in a direction quite transverse to the long axis of the body, but in an oblique direction, one pair of daughter-nuclei lying somewhat nearer to the aflagellar end than the other pair. In this manner are produced forms such as are seen in figs. 8, 97, and 100. These individuals in which the nuclei have progressed into the aflagellar part of the body have the undulating membrane very well developed; it may be said that the trypanomonad condition is here accentuated. In such forms of the parasite the mode of division is also distinct, being markedly unequal in character (figs. 98–100). The two resulting individuals are not of the same type (cf. figs. 12, 103, and 107). One, the larger parasite, is of the same type as the parent individual, and possesses from the first a conspicuous membrane, but the other, the smaller daughter-individual, is at first pear-shaped and stumpy, and has only a short, inconspicuous membrane. This mode of division presents a general resemblance, it will be noted, to one of the types of division characteristic of T. lewisi. Indeed, in the present case, the process might also be regarded as a "budding-off" of a daughter-individual from the parent. I have never observed, however, more than one bud formed, i.e. the process appears always to retain its character of binary fission and never to be of the multiple type. When set free, the smaller

daughter-individual elongates a little and becomes spindle-shaped instead of pyriform; the membrane also becomes more conspicuous. I have not seen any transitional phases between these fusiform individuals and the type represented by the parent form, and have therefore no indications as to whether they (the former) grow or otherwise pass into the accentuated trypanomonad type again. By successive multiplication according to this manner the size of the parasites become considerably reduced. In fig. 102 is seen a very small couple of the kind described. Examples of free parasites, of different sizes, representing accentuated trypanomonad daughter-individuals are given in figs. 105 and 108–110, 108 being from a redpoll culture, the others from a chaffinch one. The smallest form (fig. 110) is $10\frac{1}{2}\,\mu$ long, its flagellum is $13\,\mu$, and its breadth is $2\frac{1}{4}\,\mu$. The small fusiform parasite of fig. 111, representing a pyriform daughter-individual, is $9\,\mu$ long, its flagellum is $7\frac{1}{2}\,\mu$, and its width $2\frac{1}{2}\,\mu$.

The great majority of the parasites in thriving cultures belong to the above-described types. After a fresh culture-tube has been inoculated (from a bird) about five days, by which time the trypanosomes have generally multiplied sufficiently to ensure that there will be a fair number of parasites on a permanent smear—in other words, that an individual can be found without much searching—practically all the parasites present conform to the trypanomonad type. And up to the end of a week or so this type persists with great constancy, notwithstanding the rapid multiplication. The only variations that are numerically important are those already indicated, in the direction, that is, of an accentuated trypanomonad type and of a fusiform one. Further, if a sub-culture of these normal forms is made (preferably not later than the seventh or eighth day) the development of similar forms continues steadily in the sub-culture. Thus the parasites drawn in figs. 74, 109, are on a preparation from a second sub-culture, and the total interval that had elapsed since the blood was originally taken from the bird was twenty-six days, or over three weeks.

Certain other phases or developmental forms of the trypanosomes, however, have been encountered in cultures which were in a normal healthy condition, but these have been, as a rule, scanty in number, contrasting markedly with the abundance of the prevailing types. In cultures of six or seven days' age or more a small percentage of the individuals—and usually only a very small percentage—show a tendency to lose the fusiform or more active type of form, and to develop a pear-shaped or rounded, more passive type of form. In most of my culture-series (including sub-cultures), these pyriform or ovoid forms are very infrequent and have to be carefully searched for, even on slides where the ordinary parasites are most abundant. The individuals of this character are generally of medium, or less than medium size, but occasionally are large and massive. Pear-shaped forms are seen in figs. 112–114, that of fig. 113 being from a redpoll culture, the others from different chaffinch ones. The dimensions of these parasites (flagellum excluded), are, for example, 8 μ by 5 μ (fig. 113), and $6\frac{1}{2}$ μ by $3\frac{3}{4}$ μ (fig. 114). Medium-sized ovoid forms are 8 μ by 6 μ (figs. 116 and 117). The large ovoid individual of fig. 118 is 13 μ by 7 μ and has a very long flagellum of 24 μ; the small corresponding form (fig. 115), is 6 μ by 4 μ. Although I have distinguished these parasites as more "passive" forms, it is difficult to know whether to regard them as being about to enter on a "resting-phase," for in all cases where I have observed them in what were normal, healthy cultures, these individuals possessed a flagellum. I may mention here that the only instance where I have found rounded-off parasites which lacked a flagellum was in a culture (original), nineteen days old, which was full of atypical, altered forms (cf. below, p. 696).

This type of parasite, whether pyriform or ovoid, to rounded, is almost certainly to be derived from forms in which the alteration in nuclear position has occurred, and in which the modified method of multiplication, by unequal fission, has made its appearance. Pear-shaped individuals, such as

those of figs. 112 to 114, are probably simply the smaller daughter-individuals which have retained the pyriform shape, instead of taking on the fusiform, more active one. On the other hand, most of the ovoid or rounded forms, especially where they are of medium to large size, would seem to arise from the accentuated trypanomonad type of daughter-individual. Transitional phases can be found, showing different degrees in the retraction of the drawn-out flagellar end and the concurrent reduction or disappearance of the undulating membrane. Thus, both the large and the small ovoid individual (figs. 115 and 118) have still a delicate but distinct continuation of the body along the proximal part of the flagellum, which doubtless corresponds, for the most part, to undulating membrane. And in others of these rounded forms, indications of the original membrane are still afforded by the attachment of the flagellum to the side of the body for some distance, the flagellum curving with it—at times partly curling round it, as it were—before becoming free (fig. 119).

In general these rounded forms of the parasite do not, apparently, undergo division. In most instances where I have observed these forms, they are, as I have mentioned, of small or only medium size, and these never show indications of division. One of my culture-series, however, for some reason or other for which I was unable to account, but which was probably due to some variation in the condition of the culture medium, behaved differently from the usual manner. In this culture a pronounced tendency in the development of the parasites was the production of large, massive forms, which are sometimes ovoid or rounded in shape. Examples are seen in figs. 120–123. The parasites in my preparations of this series (taken when the culture was seven days old) are certainly not degenerate or abnormal; this is clearly shown by a comparison of their structure with that of distinctly atypical or degenerate forms (c f. below, p. 693). There is none of the irregular multiplication of organellæ, nor of the alteration in the cytoplasmic constituents which is apparent in the latter. I consider that the unusually large proportion

of broad or ovoid massive forms in this series was probably due to a greater growth activity than was usually met among the cultural parasites. And just in this case, it is interesting to note, I have found not infrequently various stages of division in ovoid or rounded individuals (cf. figs. 121, 123–125). Making allowance for slight differences due to the more massive form, the process appears to follow, in the main, the unequal method of fission. In all these forms, whether dividing or single, it may be as well to state, the flagellum was present; none of them showed any signs of absorbing or otherwise losing this organella.

The next type of cultural form of the parasites which I have to describe is quite distinct from the preceding ones, being markedly trypaniform. By the term trypaniform is understood the condition characteristic of a trypanosome, where the kinetonucleus lies much nearer to the aflagellar end of the body than does the trophonucleus, and where, consequently, the flagellum is attached by an undulating membrane along the greater part of the length of the body.

In my cultures I have found trypaniform phases, differing slightly in character, at two different periods of the development. As regards one case, I came across this type of the parasites rather accidentally as it were, in the following manner. I inoculated culture-tubes from the chaffinch which had a strong halteridial infection, in addition to small forms of Trypanosoma fringillinarum, in the peripheral circulation. These culture-tubes were examined much earlier than it was my custom to do, namely after forty hours had elapsed. This was not on account of the trypanosomes, as I knew from former experiences that at this early period they would probably not have multiplied sufficiently for me to be able to find an individual on a smear without prolonged searching; it was because I wished to see what development, —if any—was undergone by the halteridia in the culture.[1] In examining a good living drop to see if I could find any halteridial oökinetes, I noticed one or two trypanosomes which

[1] See below, p. 727.

were very active, travelling much more rapidly than was customary in the case of these cultural forms. In the course of looking for halteridia on a permanent smear (made at the same time), I happened very fortunately to come across a trypanosome, and this was so different from the usual trypanomonad type that I subsequently examined my preparations of this series thoroughly to ascertain whether this was the prevailing type. Unfortunately the trypanosomes are very scarce, only three or four on a large film. It is noteworthy, however, that all the parasites seen as a result of systematic searching are in the same trypaniform phase, and show only slight individual variations.

The type is extremely thin and slender, the parasite having a distinctly vermiform appearance (figs. 10, 126, and 127). The body is from 21 to 25 μ in length, excluding the flagellum, and its greatest breadth only from $1\frac{1}{4}$ to $1\frac{1}{2}$ μ. The aflagellar region is very long and finely tapering. The kinetonucleus is far removed from the trophonucleus, and generally lies about midway between the latter and the aflagellar extremity. Its actual distance from this end varies from 6 to 9 μ, depending upon the degree of attenuation. The undulating membrane is in most cases very narrow, and practically distinguishable only by its flagellar border. In some individuals the flagellar border originates, not in close proximity to the kinetonucleus, as is usually the case, but from a point some little distance beyond, i.e. on the aflagellar side of the kinetonucleus (figs. 10, 127). A distinct granule (blepharoplast or basal granule) can often be made out at its commencement. The length of the free flagellum is from 8 to 11 μ. The trophonucleus, instead of being the usual shape, namely, oval or rounded, is considerably elongated in the long axis of the body, this being in relation, in all probability, with the narrow form.

The other instance of the occurrence of parasites of a trypaniform type in my cultures was in a series from a six-day (original) culture of the chaffinch-form, taken when the trypanosomes, of the ordinary, definitive type, were very

scanty in the blood. The parasites are numerous, nearly all being, of course, in one or the other variety of the trypanomonad phase. Exceptionally, however, individuals occur which show the trypaniform condition; for example, on a smear containing between two and three hundred parasites there are four or five such, three of which are drawn in figs. 129–131. I have not found any which correspond exactly to the individuals of this type just described. The parasite in fig. 129 approximates fairly closely to those of figs. 126 and 127, but it is distinctly shorter and relatively not quite so slender. The two other individuals, on the other hand, while altogether much larger, are still very slender in proportion to their length; and in these the aflagellar part is very prolonged and vermiform. While agreeing in general form and character with the parasite, for instance, of fig. 10, they represent, it would seem, an older, later condition. The individual of fig. 130 has attained, probably, the fullest development of this type, at least as far as the culture is concerned; it constitutes, I consider, a most important phase.

The length of the body alone is 36 μ, and its greatest width 2 μ; the distance of the kinetonucleus from the aflagellar extremity is $11\frac{1}{2}$ μ. The free flagellum is only 8 μ long. The trophonucleus of this individual presents a remarkable appearance (fig. 130). The chromatin is arranged in a series of short transverse bars, forming a longitudinal row—hence the description "ladder-like." I have found a quite similar condition in two other examples of this type; but in the other large vermiform individual I have figured (fig. 131) the chromatin is not arranged in such a definite ladder-like manner, but appears to form a fairly regular double row of grains.

None of the trypaniform parasites which I have found—in either case—showed any indications of division.

The types above described include all the cultural forms of the trypanosome observed, which I have no hesitation in regarding as perfectly normal and regular. As I shall mention more particularly later, they are closely paralleled

by flagellate forms known to occur in various blood-sucking invertebrate hosts.

I may now contrast with them certain other cultural forms found, most of which I have equally little hesitation in considering as abnormal or atypical forms, developed by the parasites as a result of unfavourable conditions in the medium. These forms are found in old, original cultures of, say, twelve days or more, in which multiplication has gone on to a very great extent. It must be borne in mind that such a medium no longer corresponds at all to any condition met with in an insectan host. In an insect, the digestion of the imbibed blood—the medium of the parasites—and its absorption are completed in the course of a few days at most; by this time the parasites remaining in the digestive tract have passed into the resting, attached phase. In an old culture, on the other hand, the fluid medium is still present, presumably containing a certain amount of nutriment of a kind, but now considerably altered in character by the addition of waste products of the metabolism of the parasites, which have doubtless a deleterious action on the trypanosomes. In subcultures made at sufficiently short intervals, these abnormal forms are usually not found at all. In this case it is as if the transferred parasites remained continuously in a pure medium, which may be looked upon as a substitute for the medium in the stomach of the insect—at any rate during the early period of digestion.

A most interesting feature of the morphology of these forms is that very few of them show the trypanomonad phase; nearly all the parasites have passed into a more or less herpetomonad-like condition. The earliest indication of an alteration in the character of a culture is afforded by the appearance of such forms. They are to be met with in cultures of ten or twelve days and onwards. At first, of course, these individuals are very few in number.

Examples of this "pseudo-herpetomonad" condition, as I propose to term it, are seen in figs. 140–146; figs. 140, 145, and 146 are from a chaffinch culture of twelve days; fig. 141

is from a redpoll culture of nine days, and figs. 142–144 from one of nineteen days. The body is fusiform to long and slender in shape. The two nuclei are situated distinctly in the flagellar half of the body; they lie usually fairly close together. The appearance of the flagellar end of the body and its relation to the flagellum is in general intermediate between that found in the trypanomonad type and that in a typical herpetomonad form. The flagellum itself is only connected with the body for a comparatively short distance, and is usually not obviously attached along one side of the body to any extent (figs. 140, 141, 143, and 144); hence there are no indications of an undulating membrane. This proximal portion of the flagellum is, in the majority of cases, chiefly intra-cytoplasmic, constituting simply a rhizoplast, and corresponding to the rhizoplastic part of the flagellum in the trypanomonad forms (before it passes to the surface to become the border of the membrane). On the other hand, the flagellar end of the body, while sometimes fairly sharp and acute, approximating to the condition in an ordinary herpetomonad (cf. figs. 140, 141, and 147), may taper more or less gradually (figs. 142, 144, and 145); hence, in these cases, where it is drawn out a little with the flagellum, the latter may be regarded as "attached" for a short—or very short, distance. For this reason, and because the two nuclei are closer together than is customary in a herpetomonad, this condition is preferably distinguished as pseudo-herpetomonad. The difference will be readily understood when it is remembered that all these individuals are derived from trypanomonad forms by the more or less complete loss of the undulating membrane and its attached flagellar border; hence, of course, parasites showing all manner of intermediate stages in the process are to be met with.

In the early formed individuals of this pseudo-herpetomonad variety there is nothing about them to indicate that they are actually abnormal or unhealthy. As I shall discuss subsequently, however, I think it is very probable that the occurrence itself of this unusual condition is the consequence

merely of the unusual environment; I am very doubtful whether it can be regarded as representing a normal phase of the life-cycle. In any case, however, as the age of a culture increases, and these forms multiply and predominate —the trypanomonad phase as quickly declining—numerous irregular forms of the parasites are met with, which are manifestly unhealthy. As might be expected, the form and size of these individuals varies considerably (cf. figs. 145–154, taken either from a twelve-day chaffinch culture or from a nineteen-day one from a redpoll). Some of them are long and narrow, others pear-shaped, while others are large and massive, ovoid, or of ill-defined shape.

The abnormal condition of these forms is particularly indicated by certain cytological characters, which I have never observed in normal individuals. A common feature is the occurrence of a peculiar altered appearance in the neighbourhood of the rhizoplastic part of the flagellum. Sometimes there is a cluster of red-staining granules in this region of the cytoplasm (figs. 145, 146). In the more massive forms there is usually a greater or less amount of a diffuse, indefinite substance, which also stains red. This substance is often more or less streaky in form, one or more streaks commencing in the neighbourhood of the rhizoplast and running backwards in the cytoplasm for a short distance (figs. 150, 151, and 153). In a few individuals the streaky condition is combined with the occurrence of the granules (fig. 152). I am unable, unfortunately, to offer any certain explanation of this interesting character, owing to the fact that I have only had material stained with Giemsa in which to observe it; very likely the appearance is different after other methods of staining. So far as the granules are concerned, they do not differ in their staining reactions from the ordinary chromatoid granules which are often found in normal trypanomonad types; the latter, however, are scattered more or less generally throughout the body, whereas the particular granules under consideration are always concentrated near the rhizoplast. Hence, it is not certain that the granules

have the same significance in the two cases. With regard to the curious streaky substance, its position in relation to the basal part of the flagellum certainly suggests some association with this organella; it seems to me not at all unlikely that its presence is connected with the disappearance of the trypanomonad character, and, indeed, a comparison of figs. 119, 149, and 150 prompts the query whether it may not possibly represent the remains of a flagellar border which has been actually absorbed by the parasite in the case of some of these massive forms.

Another cytological character often apparent in fairly old cultures is vacuolisation. One or two small vacuoles in the cytoplasm may be seen occasionally in individuals of quite regular form; but, on the whole, in my cultures parasites belonging to the definite types recognised above are free from vacuoles. The occurrence of a few small vacuoles in an individual doubtless signifies nothing very abnormal; when, however, the cytoplasm either appears practically full of vacuoles, or else contains one or two huge ones (fig. 154), this ought most probably to be considered as an unhealthy sign.

Very marked indication of a disturbance in the mutual balance of the various cell-constituents is frequently seen in an irregular distribution of the nuclear organellæ. Parasites with two trophonuclei and a single kinetonucleus are not uncommon (fig. 156). These are not to be interpreted as individuals which are in an early stage of division, the process having been begun by the trophonucleus. On the contrary, they are the result of a division in which the nuclei have been unequally apportioned between the two daughter-parasites. This is clearly shown by fig. 157, where the cytoplasm is splitting in such a manner that one daughter-individual has both the trophonuclei and the other only a kinetonucleus. The remarkable feature is that these forms without a trophonucleus can live alone, at any rate for a certain length of time, for I have observed four or five examples in the course of examining my slides of this series (fig. 155). I have never found an active, flagellated form with a trophonucleus

but without a kinetonucleus. In some of the large massive parasites numerous nuclei and flagella are present (figs. 162 and 163), the number of the different organellæ not by any means corresponding. Successive multiplication of the latter has taken place without concurrent division of the cytoplasm; later, the cytoplasm would probably split into three or four portions, and it might very well happen as a result that one of the individuals thus formed would be happy in the possession of three trophonuclei (fig. 158).

Another interesting irregularity in division is met with rarely. This consists in the unequal splitting, longitudinally, of the cytoplasm of certain large individuals, a thin form, with (fig. 159), or possibly without (fig. 161), a flagellum being cut off from the side of the parent. An important point is that these forms have no definite nucleus of either kind—i.e. they are apparently without both tropho- and kinetonucleus. In fig. 161 the individual—if such that portion of the cytoplasm can be termed—about to be cut off has a clump of granules, but that in fig. 159 has nothing at all. I have not observed a narrow form of this kind actually free; in fig. 160, however, an active pear-shaped individual is drawn which also has no definite nucleus, but which possesses many red-staining granules. I have no doubt whatever that these forms are purely "freaks," the result of a degenerative mode of division, and die off quickly after being set free. There is a general resemblance, it will be noted, between this production of enucleate forms, in my cultures, and the formation of sickle-like (so-called "spirillar") forms in cultures of Leishmania donovani, described by Leishman and Statham (8). It is highly probable that, in that case, too, the process is due to an abnormal condition of the Leishmania parasites (which, of course, ultimately degenerate and die off in cultures), and that such forms have nothing to do with any natural developmental phase in the insectan host.

Reference has been made already to the occurrence of rounded forms lacking a flagellum. These have been seen only in an old culture of nineteen days, in which they are not

infrequent. A few are medium in size (fig. 135), but most of them are small (figs. 137, 138, and 139). It is quite obvious from their appearance that these forms of the parasite, in the culture at any rate, are not merely "resting," persistent phases, but are degenerating and dying. And it is interesting to note that the process of degeneration takes place by a gradual disappearance of the nuclear elements. These no longer stand out, sharply stained, in the cell. They lose their distinctive affinity for the stain and become less and less distinguishable from the general substance of the body; at the same time they tend to diminish in size, as if they were being dissipated in the cytoplasm. The last stage of the parasite is an indefinite body, which stains a dull or faint red. Hence, so far as the cultural forms are concerned, all the evidence I have goes to show that the loss of the flagellum means approaching degeneration and death (contrast, for example, the parasite of fig. 136 and that of fig. 138, which are on the same slide and within a few fields of each other).

The above description includes all the different types and the chief varieties of form which I have observed among the trypanosomes in cultures.

Agglomeration.—I have, next, a few observations to make upon the characteristic feature known as agglomeration. I have seen many instances of this occurrence in my cultures. I have never found it in early original cultures (i.e., of less than six or seven days), nor in subcultures. Agglomerated clusters are only met with when the parasites have become abundant in the medium. The clumps are of all sizes, from small ones composed of a few individuals (a dozen or less) up to large masses containing hundreds of parasites. Now and again, in these large aggregations, the parasites are clustered round more than one centre, i.e. in these cases there is an approach to the condition of secondary agglomeration, distinguished by Laveran and Mesnil from primary (single) clusters. In all the clusters seen the parasites have their flagella directed towards the centre of the rosette.

On more than one occasion I have noticed the commencing formation of a clump in a cover-slip preparation of living parasites, where every field contained numerous individuals. Here and there are small numbers of parasites, which have become entangled by their flagella, the distal portions of which appear to be inextricably intertwined.

Once started, the increase in size of cluster may take place in two ways: (1) by the addition of fresh individuals from the surrounding medium, which are continually being attracted; and (2) by the multiplication of forms already present. The increase is undoubtedly due much more to the former method than to the latter; during the early stage, at any rate, it is probably almost entirely due to the accession of more individuals. In short, these clusters are formed mainly by agglomeration. As a matter of fact, dividing forms are comparatively rare in all the clusters I have examined (cf. figs. E–G, Pl. 5). I once left a cover-slip preparation containing a great many free, active parasites for two or three hours; when I returned to it I found several large clumps which had not been there before. It was impossible that these rosettes could have arisen otherwise than by agglomeration; they all had their flagella centrally directed and resembled the cluster of fig. G, except for the fact that some were even larger.

An early stage in the formation of a cluster is seen in the micro-photograph reproduced in fig. E. The individuals composing it differ appreciably in form and size; some of them, at the periphery, had apparently only recently been attracted, and were not yet firmly attached. Only two individuals are undergoing division. The beginning of a secondary agglomeration is instructive. Parasites continue to be attracted to the clump, but owing to the number already present the newcomers are unable to penetrate in between them and become firmly attached. Hence they tend to form a subsidiary cluster for themselves (figs. E and F). The large agglomeration-cluster of fig. G is apparently made up of individuals attached around three centres, two of which, the

older two (in the upper right-hand part of the figure), are partially confluent.

It is important to note that agglomerations are formed of individuals which are of a quite normal type. Nearly all the parasites of the clusters figured, for example, are definitely trypanomonad in character, either fairly long and fusiform, or belonging to the pyriform variety of individual. Agglomerations of less typical forms, pseudo-herpetomonad in character, also occur, but I have not met with them to any extent, even in old cultures.

Novy and McNeal, in their account of cultures of avian trypanosomes (14), make a great point of distinguishing between multiplication rosettes and true agglomeration clusters. They regard all rosettes in which the parasites are joined by their flagella, corresponding, that is, to those I have just described, as arising by successive multiplication from a single individual, which starts the culture. Only those cases, on the other hand, where the parasites are united by their aflagellar ends, are considered to be true agglomeration clusters. Until I myself came to work with cultures, I had no idea but that the view of these authors was correct, and that these two opposite kinds of clusters resulted from quite different processes. Studying Novy and McNeal's description and figures in the light of my own work, I feel sure that these authors have given an entirely wrong interpretation of the clusters, which they regard as multiplication rosettes. Novy and McNeal consider that the whole process starts from a single cell, which is more or less rounded off, and has no flagellum. This gives rise, by division, to a few cells, which now possess flagella; by further multiplication, a typical rosette of spindle-like forms is produced.

Novy and McNeal's figures on Plates 8 and 9, which are from excellent micro-photographs, are most instructive, and are, in my opinion, convincing evidence that the view these authors put forward is incorrect. Most of the figures represent simply clusters, large or small, of different forms

of the parasite, certain of which appear distinctly unhealthy. The authors state that all the figures on the plates to which I am now referring (as well as others) are of parasites from a culture in the seventh generation, grown for seven days, by which I understand them to mean a sixth subculture, itself of seven days' age. This long-continued cultivation doubtless accounts both for the varieties of form present, as well as for the number of clusters. Their fig. 2, Pl. 9, supposed to represent an early stage in rosette-formation, shows a large indefinite-shaped parasite, in which irregular multiplication of the nuclei is going on. There is no indication of the development of any flagella, and I have no hesitation in regarding this individual as an abnormal, degenerating form. That it would ever give rise to a rosette of active, flagellate parasites is most improbable. Again, fig. 3, Pl. 9, represents an agglomeration cluster of four or five somewhat similar forms, three or four of which, however, are not quite so degenerate, as they still possess flagella; but the same irregular multiplication of the nuclei is shown.

Phases such as these have, I venture to say, no connection whatever with the rosettes of more typical parasites figured on Pl. 8. Fig. 2, here, is a small cluster of a dozen pyriform individuals, each with a single, centrally directed flagellum. Not one of the individuals shows the least sign of division. Similarly in fig. 1, Pl. 8, there is a cluster of about eighteen parasites. Hence, in neither of these rosettes is there any evidence that they are going to give rise to one of many more individuals, such as that of fig. 4, Pl. 8, by multiplication. And, from my own experience, I know that such rosettes can be formed very quickly indeed. In this cluster of fig. 4 there are several individuals at the periphery, which are manifestly only loosely attached, and whose flagella cannot be connected with the central core (cf. my own figures). There can be no doubt that these are the individuals which have been most recently attracted to the cluster.

A point in favour of this view of Novy and McNeal's would be furnished by evidence which went to show that two

typical daughter-parasites often remain entangled by their flagella after division. Now, as I have stated, the flagellar ends of the two individuals resulting from division (i.e. longitudinal fission) always become widely separated, and I have never seen any instance of such an occurrence. Even in the rare cases where multiple (quadruple) longitudinal fission is proceeding, the flagella are all distinctly free from one another, and when the cytoplasmic division was completed, the daughter-individuals would doubtless separate. Moreover, from Novy and McNeal's figures, it is obvious that the dividing forms in their cultures behaved in a similar way (cf. figs. 1, 2, and 5, Pl. 7).

Hence, to conclude, I regard Novy and McNeal's rosettes, in which the parasites are attached by their flagella, equally with those in my own cultures, as true agglomeration clusters, originating, and in the main increasing, by the coming together of independent individuals. There can be no doubt, it may be pointed out, that agglomeration of trypanosomes by the flagellar end does occur in the invertebrate host; the process has been described, for instance, in the case of T. lewisi, when in a louse, by Prowazek (22), and when in a flea, by Swingle (33).

On the other hand, there is no reason to doubt that in certain types or phases of the parasite agglomeration in cultures may take place by the aflagellar end; this is stated by Novy and McNeal to occur in the case of their "spirochætes." I have never had cultures which showed a sufficient number of parasites belonging to this type for agglomeration to occur, and so am unable to say more upon this point. It is interesting to note, however, that agglomeration of trypanosomes in the blood of the vertebrate hosts takes place by the aflagellar (kinetonuclear) end, and these "spirochætes" are also definitely trypaniform; in contra-distinction to these, parasites of the trypanomonad type form rosettes which have their flagellar ends attached.

Summary and General Remarks on the Cultural Forms.

From my observations on the cultural forms of T. fringillinarum a few interesting and important data have been obtained, relative to the course of the development of the parasites on passing into the culture-medium. The earliest type of form which I have found is a slender, trypaniform phase. This is soon replaced by the characteristic trypanomonad phase, into which most of the trypaniform individuals pass. This trypanomonad phase is the predominating cultural form, and it is persistent, apparently, so long as the condition of the medium remains healthy. During this period, however, in a culture of six days' age, trypaniform individuals have also been seen, though they were extremely few in number. Further, rare instances of another form have been found, which is distinguished by its vermiform appearance, and by the remarkable ladder-like character of its trophonucleus. This phase is doubtless simply a further development of the ordinary trypaniform type. Whether these later trypaniform individuals represent forms of this character which have been persistent from the commencement of the infection, or whether they indicate a second development of this phase from the trypanomonad type, I have not sufficient evidence to decide. I am rather inclined to think, however, that the latter may be the case; for one or two individuals have been found which might correspond to transition-forms in such a passage (fig. 128).

Since the above research was carried out, I have been studying, in conjunction with Prof. Minchin, the parasites of Athene noctua, and I have observed the early developmental phases of a trypanosome (most probably T. noctuæ) from this bird, in the stomach of the mosquito (Culex pipiens). We hope to publish in due course a full account of this work, but I wish to refer here to one or two general facts. In the first place, to answer any possible criticisms, it may be stated

expressly that the flagellates which I am about to mention were derived, beyond all question, from the little owl.

The parasites occur both in the trypanomonad and in the trypaniform phase. Some of the latter individuals resemble the vermiform type of figs. 10 and 137 closely, the only difference being that the attenuation may be even more pronounced. In fig. 132 is drawn such an example, which shows the extraordinary slenderness of the body. Hence, so far as I am able as yet to compare the two cases, this elongated trypaniform type develops to a much more marked extent in natural conditions than was the case in my cultures; in the latter, for some reason or other, it was soon almost entirely superseded by the trypanomonad type.

The occurrence of anything approaching a herpetomonad phase has only been seen in cultures of a certain age, in which there is every reason to believe the condition of the medium must be becoming abnormal and unhealthy for the parasites. Even then, it is only very seldom that an individual is found which corresponds at all closely to a true herpetomonad (fig. 147); most of the parasites assume what I have called a "pseudo-herpetomonad" condition, which is readily distinguishable from that of an ordinary herpetomonad. With regard to the occurrence of rounded-off "resting" phases, forms of this kind without a flagellum were seen also only in old cultures, full of altered forms, and the individuals which were in this condition were manifestly degenerating and dying. Hence, from such individuals no conclusions can be drawn respecting the occurrence of rounded, aflagellar phases as a normal part of the life-cycle in the insectan host. Such a phase may occur or it may not.

What may be regarded as highly probable, however, is the occurrence in natural circumstances of forms which correspond to the small fusiform or pyriform individuals of the culture (cf. fig. 111) in an attached condition, i.e. with the flagellum more or less shortened or retracted, and serving as fixative organella. The predilection that such forms have for forming groups or clusters in the cultures (cf. fig. G, Pl. 31, and

also Novy and McNeal's figures of so-called multiplication-rosettes on Pl. 8) is probably to be regarded, indeed, as indicating the tendency of these forms to become attached, when in the natural insectan-medium. In the culture-medium, however, there is nothing for them to attach themselves to, excepting these commencing clusters of their fellow-individuals. Hence, the probable explanation—in great measure, at any rate—of the clumps or clusters which have their flagella centrally directed, is that they represent the attached phase in the insect. This is of well-known occurrence, both among trypanosomes (cf. Prowazek [l.c.], figs. 53 and 54), and among insectan flagellates (cf. especially Patton [**16**, Pl. 9, fig. 22], where a number of Crithidia sp., in Gerris are clustered around a food-particle, and again, Swingle [**32**], who states that a rosette of Crithidia in the sheep-ked, Melophagus, may be formed around a free epithelial cell). In the case of parasites in cultures, when one, two, or three individuals have become entangled by their flagella, the interlocked ends furnish doubtless the "nucleus" for the attachment of many other parasites, with the result that a large cluster is soon formed.

An important point brought out decisively by my cultures is that this avian trypanosome does not proceed to form rounded-off, resting phases immediately on passing from the vertebrate host into the cold medium. And further, I may mention, there is not the least indication of any such behaviour in the case of the trypanosome of the little owl when it passes into the stomach of the mosquito.

Up to the present only one or two accounts of cultural forms of trypanosomes have been published which describe and make any attempt to distinguish between the different types of form and phases developed at different periods in the culture. Of these, the most important for purposes of comparison with my own results is the paper of Novy and McNeal, to which reference has been made. In this connection it must be emphasised that most of the authors' figures of cultural forms (and apparently their descriptions also) are

based upon the parasites present after cultivation has been continued for some time, i. e. in sub-cultures of the sixth or seventh generation, when the culture was fully developed and "enormously rich in flagellates." In such cultures of a trypanosome, regarded by the authors as T. avium, the great majority of the trypanomonad forms were found in clusters, some of which were large enough to be visible macroscopically as patches in the medium. The interesting point is that parasites in the form of "spirochætes" were of common occurrence, sometimes abundant; "spirochæte," it may be as well to state, is the term applied by Novy and McNeal —somewhat misleadingly—to individuals of the slender, trypaniform type, similar to those seen in my figs. 10, 126, and 127.[1] These trypaniform individuals were mostly free, very active, and some were undergoing division.

Hence the condition found by Novy and McNeal obviously represents a much later period in the development of the culture than any I have described above, and I cannot find any account of the early course of the development, i.e. during the first five or six days or so. The authors do not say at what intervals of time their sub-cultures were made, but it is evident, from the number of the "generation" given, that the trypanosomes must have been cultivated for at least some weeks. In the case of T. fringillinarum, I was unable to obtain any development in my cultures corresponding to that found by Novy and McNeal in T. avium. If I did not sub-culture frequently enough the parasites become abnormal and degenerative, so that a preparation would show nothing but altered, pseudo-herpetomonad forms and so forth, and when I sub-cultured frequently the trypanosomes retained, for the most part, the trypanomonad phase. I never continued subculturing for so many generations as Novy and McNeal did; it is only since I have come to study carefully my preparations and to compare

[1] Although in one or two cases these parasites show indications of an extended nucleus, in no case is a definite ladder-like appearance figured or described.

my results with those obtained by Novy and McNeal that I realise some additional knowledge might have been gained by continuing to cultivate longer. In one case I subcultured four times at fairly slow intervals; this was done chiefly with a view to seeing how long I could keep a culture of the trypanosomes alive (cf. above, p. 647). Unfortunately, being kept away for a few days by ill-health, I missed an opportunity of examining this fourth subculture at a time when the parasites would have been very numerous; and before my return an unfortunate accident had terminated their career. Possibly this subculture might have shown more trypaniform individuals.

Novy and McNeal go to the length of founding two new species of Trypanosoma upon the different behaviour and appearance of certain of their cultural forms. In fact they distinguish several types or varieties chiefly or entirely upon a basis which is most inadequate and misleading, namely, on a comparison of the multiplication-rosettes (really the agglomeration-clusters) and the free "swarming" parasites in the different cases. I only wish to point out here that, in the case of both their new species, viz. T. laverani and T. mesnili, the free-swarming forms which they compare with the slender, trypaniform type of the other species dealt with (T. avium) and contrast with the rosette-forms, are in reality not trypaniform ("spirochætes") at all, but are ordinary trypanomonad forms, which do not differ essentially from those constituting the rosettes. This is perfectly obvious from a comparison of their figures on Pls. 5–7.

The matter amounts simply to this: In the case of these two species, the authors have not got a development of the trypaniform type at all. Many of Novy and McNeal's figures of these forms, especially of T. mesnili on Pl. 6, are of individuals which show pronounced vacuolisation, and which, in my opinion, appear distinctly unhealthy; also the cluster of individuals of T. laverani, reproduced in fig. 3, Pl. 7, I regard as partly composed of abnormal forms. In short, from a comparison of the figures given of T. laverani and

T. mesnili with most of those of T. avium, I am strongly inclined to say that the cultural development of the former parasites was not proceeding so successfully—at any rate, when the preparations concerned were made—as that of the last-named species.

Slight differences in the constitution of the medium may certainly influence the rapidity of growth of these cultural forms, as I have stated above, and probably also, to a certain extent, the manner of their development. Further, it is quite likely that different species of trypanosomes, when cultivated in the same medium, may also differ in their rate of growth and in the development of the different types of form. Hence, I think we may agree with Novy and McNeal, although on quite different grounds, that the parasites which they name T. laverani and mesnili are at any rate different from the other (T. avium). Moreover, it may be reasonably inferred that under slightly different conditions—in one way or another—of the medium, these forms would also develop a trypaniform phase. For it will be seen from the subsequent context of this paper that there is every reason to suppose such a phase is of regular occurrence at some period in the development of a trypanosome outside the vertebrate host. As a matter of fact, T. laverani itself appears to be very closely allied to the trypanosome with which I have been working.

The only other paper dealing with cultural forms, to which I need refer is a note by Thomson (35), on the cultivation of trypanosome (probably T. danilewskyi), from the goldfish, which gives instructive indications of the course of development of that parasite in cultures. It is most interesting to find that there is a general resemblance between the course of events in the case of that piscine form, as outlined by Thomson, and in the avian parasites discussed above. Thomson does not describe any developmental forms occurring earlier than the seventh day. By this time the parasites are in a phase corresponding to my accentuated trypanomonad type; and division by a quite similar method of unequal

fission is taking place, a small fusiform or pyriform individual being cut off from the large, more or less club-shaped parent-form. Several of Thomson's figures are, indeed, almost identical with some of my figures. Another important point is that distinctly trypaniform individuals were present, and such forms were found to be more frequent later on, for instance in a culture of the forty-second day.

As Thomson says, it is probable that earlier phases in this development might have been found before the seventh day. It is interesting to note that Thomson figures an unaltered trypanosome (as it left the blood of the fish) in the culture of seven days. Thomson's view is that the large, club-shaped trypanomonad individuals are derived directly from such trypanosomes by an alteration of the body-form, most of the protoplasm becoming concentrated in the aflagellar part of the parasite, which thus becomes greatly swollen in appearance. According to Thomson, there is no prior multiplication of the parasite in an ordinary trypanomonad condition. Hence in this case a type of form very similar to that which I have found in my cultures (cf. figs. 97, 98) is attained by a quite different process; in the culture of the avian parasites, the trypanosome-phase is quickly lost and active multiplication in the ordinary trypanomonad phase goes on.

It is evident from this that the development of the piscine type in cultures proceeds much slower than that of the Avian form, and this bears out, in an interesting manner, the facts so far known relative to the development of the two types in the true invertebrate hosts (leech and insect) respectively.

The Significance of these Cultural Forms of Trypanosomes in Relation to the Question of an Alternate Invertebrate Host.

When we come to compare the chief types of form described above as occurring in cultures of trypanosomes from different vertebrates with the flagellates described by various authors from blood-sucking invertebrates, which they have considered

as being phases in the life-cycle of some vertebrate trypanosome, we find at once a fundamental resemblance, while in one or two particular cases there is a strikingly close similarity in detail. It would occupy too much space to follow out this comparison at length. I must content myself with a reference to various papers, and with a few indications as to the chief points of agreement.

It may be noted, as a preliminary, that I follow Patton's definition of, and distinction between, a herpetomonad form and a crithidial or trypanomonad form; the terms "crithidial" and "trypanomonad" are practically interchangeable, but I prefer to use the latter, at all events when referring to this phase in connection with a vertebrate trypanosome.[1] Further, it is necessary to emphasise the fact that the characterisation of these two types is based upon their structure when in the active, extended, flagellate condition; in other words, the diagnostic form of the parasites is only seen when they are in this condition. Rounded, resting phases, whether possessing a flagellum or lacking one, cannot be regarded by themselves as representing either a herpetomonad or trypanomonad phase, simply because, when the parasites are in this condition, the features used for distinguishing between the two types are not present. It is certainly due to Patton that we are at last able to realise that there are these two perfectly definite types, a herpetomonad and a crithidial or trypanomonad one, and to distinguish clearly between them. Until Patton separated the two types upon the above basis, the greatest confusion often prevailed as to whether a given parasite belonged to one or the other; and it must be admitted this confusion was chiefly due to the unsuitable diagnostic characters used by Léger in his earliest descriptions of these forms.

The memoirs in question are those by Miss Robertson (**23**, **24**, and **25**), Minchin (**11**), Prowazek (**22**), Stuhlmann (**31**), and Roubaud (**26**). In all the parasites described, namely, T.

[1] There have been, hitherto, two quite different meanings attached to the term "crithidial" (cf. also below).

raiæ and T. vittatæ (Miss Robertson), T. grayi (Minchin), T. lewisi (Prowazek), T. brucii (Stuhlmann), and T. gambiense, cazalboui, and congolense (Roubaud), a trypanomonad phase occurs, and is usually prominent. In all of them a definite trypaniform phase (i. e. one in which the kinetonucleus is some distance on the aflagellar side of the trophonucleus), is also met with. And in two cases, namely, T. brucii in Glossina fusca (Stuhlmann), and T. raiæ in Pontobdella (Miss Robertson), the occurrence of a greatly elongated trypaniform type with an extended, ladder-like nucleus is described. These are the only cases of which I know where this characteristic type of form has been seen in an invertebrate; and it is highly significant, I think, that a similar form occurs, beyond all question, as a developmental phase of more than one avian trypanosome. Unfortunately I am not yet able to add anything to our knowledge of the purpose or meaning of this interesting form, which has been variously considered as possibly a male form, and—more likely—as a propagative individual infecting a vertebrate host.

The same close agreement holds good also for another important point, namely, the absence—apparently the entire absence—of anything corresponding to a true herpetomonad phase in these parasites when in the Invertebrate host. Out of a total of some hundreds of figures in the above memoirs, there is not one which shows a typical herpetomonad individual, such as, for instance, Herpetomonas muscæ-domesticæ, lygæi, jaculum, etc., or Leishmania. There are only one or two figures, e. g. in one of Miss Robertson's accounts (**24**, figs. 12, 21, and 22), which could be regarded as in any way approaching a herpetomonad condition; and it is precisely in such a case, moreover, that the essential proviso noted above must be borne in mind. The individuals figured are manifestly intermediate stages in the development from a rounded resting-phase to an active flagellate type of form. Further, they are all dividing, and one of the daughter-individuals (fig. 21, right-hand side) is already acquiring the

trypanomonad condition. Hence these cannot be regarded as representing in themselves determinative phases, but are rather only transitory stages in the development of a trypanomonad (or it may be a trypaniform) type, such as is exemplified in most of Miss Robertson's figures of active, flagellate individuals. On the other hand, what is far more important is that none of the numerous elongated "monadine" forms figured by Roubaud (26) show any indication of herpetomonad affinity. Last, but not least, the so-called herpetomonad forms of T. grayi—the extremely slender ones, which proceed to encystment—have nothing whatever to do with the herpetomonad type, as indeed Patton has already pointed out, but are unmistakably of the trypanomonad type. This mistake arose, of course, simply by following Léger's mode of distinguishing between the two types chiefly by means of the body-form.

There can be no doubt, I think, that this briefly outlined comparison enhances the probability that the various accounts to which I have alluded do actually relate to phases of the life-cycle in an invertebrate host of the different vertebrate trypanosomes which they purport to do; in my own opinion, and in that, I venture to say, of most other people, the matter is certain.

I should like to offer a few further remarks upon the still disputed question of a vertebrate trypanosome in its alternate host versus a natural flagellate of the invertebrate. In the first place, two classes of invertebrates are principally concerned, namely leeches and insects. The former I intend to leave altogether out of account, as up to the present not the slightest evidence has been brought forward of the occurrence of any flagellate parasites in this class of hosts, which are not developmental forms of some vertebrate trypanosome. In the case of insects the subject is much more complicated; since in many non-blood-sucking insects flagellates occur which can be only parasites of the one host.

As a result of the above comparative observations, one general proposition can be stated, I believe, which ought to

prove of considerable help in this connection. It is this: Parasites exhibiting a trypaniform condition in a blood-sucking insect must be considered as belonging to the life-cycle of a vertebrate trypanosome, until the contrary is definitely established; and the onus probandi lies with those who maintain the opposite view.

Another conclusion which appears indicated is that, in general, such parasites do not pass into a true herpetomonad condition; in other words, they have not a definite herpetomonad phase in the life-cycle. Bearing in mind that many, at any rate, of the vertebrate trypanosomes which have an insect as their alternate host are almost certainly to be derived from a herpetomonadine form, which was originally a parasite solely of the insect, it will be understood, of course, that in certain circumstances the parasites may revert, as it were, to a psendo-herpetomanad condition, or even to a herpetomonad one, as I have found in the case of my avian trypanosomes in cultures. But with this qualification, all the observations so far recorded point to the above conclusion.

As a matter of fact, the occurrence of typical herpetomonad forms in blood-sucking insects has not been described in nearly as many cases as would appear, at first sight, to be the case. In many of the papers that I have seen which profess to describe such forms, a study of the figures shows that the authors have been dealing really with trypanomonad (crithidial) forms; these are merely further instances of the confusion formerly existing in regard to the diagnosis of these two types. Thus the Herpetomonas algeriense described by the Sergents (**28**) from Culex pipiens does not appear to have anything in common with a true Herpetomonas; from the figures given it must be regarded as a trypanomonad form.[1]

[1] Instances, on the other hand, of what are apparently true herpetomonad forms occurring in mosquitoes and restricted to this host are given by Patton ('Brit. Med. Journ.,' 1907, ii, p. 78) and also by the Sergents (l.c.); but there is not likely to be any difficulty in distinguishing such parasites from phases of a vertebrate trypanosome. I may

Again, Novy, McNeal, and Torrey, in their paper on the flagellates of mosquitoes (15), distinguish two parasites, namely, Crithidia fasciculata and Trypanosoma (Herpetomonas) culicis. These authors also followed Léger's unfortunate definition of a Crithidia, restricting the name to small oval or pyriform parasites with a truncated flagellar end and a short flagellum. The whole objection to this definition lies in the fact that such forms are merely resting or attached phases (in natural conditions) of either crithidial (trypanomonad) or herpetomonad forms. However, in the case of their Crithidia, the figures given show that, in a more elongated condition, it conforms on the whole to the trypanomonad type. Similarly, their other parasite, Trypanosoma (Herpetomonas) culicis, also has a well-marked trypanomonad phase, as, indeed, is implied by the generic position which the authors assign to it; apparently it is placed in the sub-genus Herpetomonas because of its monadine form. I may observe here that these papers by the American authors have been most difficult for me to comprehend, because the indications afforded or suggested by their plates often appear to be opposed to the account given in the text. I have only really grasped the significance of their first paper on avian parasites and their cultural forms since working on my own birds and cultures; and I am sure, from the interesting plates of mosquito-parasites in the authors' second paper, that a further study of the phases and forms which they figure is essential to a correct understanding of their significance. Hence I do not propose to criticise them further at present.

This much, however, must be said in regard to all these cases of the occurrence of trypanomonad forms in mosquitoes. It is at least quite as likely that the flagellates observed were phases of vertebrate trypanosomes—say of avian forms—as that they were purely insectan parasites. I have referred

say here that in the development of T. noctuæ in Culex pipiens I have not come across the slightest indication of a herpeto monad phase.

above to the undoubted indications I have obtained that a trypanosome of the little owl undergoes developmental phases in Culex pipiens. There is, therefore, no reason whatever to doubt any longer that some, at all events, of the flagellate phases described by Schaudinn in mosquitoes which had fed on infected birds were also actually phases of Trypanosoma noctuæ. Moreover, in regard to Crithidia fasciculata itself, the type-species of that unfortunate genus, no one has yet shown that it is solely an insectan parasite. In first describing it, Léger very wisely admitted the possibility that it was only a phase of a vertebrate trypanosome, and this still remains the most logical assumption with regard to it.

Similarly with regard to crithidial forms in other blood-sucking insects, e.g. C. tabani, Patton (18), C. melophagia, Swingle (32), etc., by far the most likely and reasonable view is that these parasites are merely the trypanomonad forms of a trypanosome.[1] One or two cases have been described, however, of the occurrence of crithidial forms in what are alleged to be non-sanguivorous insects, e.g. C. gerridis from Gerris fossarum, Patton (16); such parasites may apparently be regarded as true Crithidia, by which we may understand flagellates that have developed a trypanomonad condition, but which are restricted to an invertebrate host.

Two or three parasites have recently been described, and, moreover, from non-biting insects, which have been regarded as "trypanosomes." They are Trypanosoma drosophilæ, Chatton and Alilaire (3), and two peculiar herpetomonad forms termed Leptomonas mirabilis, from Pycnosoma putorum and L. mesnili, from species of Luculius, which

[1] As regards C. melophagia, I have quite recently obtained evidence which makes this almost certain. After prolonged examination of the blood of a sheep on which were "keds" infected with this parasite, I had the good fortune to find a typical, active trypanosome. This is the first occasion, so far as I know, of a (natural) trypanosome having been found in this domestic animal. There can be little or no doubt that the "Crithidia melophagia" is simply a developmental phase of this sheep-trypanosome in its alternate, insectan host.

have been described by Roubaud (26). In the case of the first-named, the individuals figured certainly appear to be in a definite trypaniform condition, possessing a distinct, though narrow, undulating membrane. The two other parasites are very remarkable, in that typical herpetomonad forms appear to have also a "trypanosome" phase in their life-cycle, and all intermediate conditions between these two extremes are figured. So far as I can judge from the figures given, however, the so-called "trypanosome" phases do not represent a true trypaniform condition in the sense in which it has been understood in the above pages. To begin with, the flagellar end of the body is not drawn out at all, but the flagellum emerges straightway from it. The kinetonucleus is, indeed, near the aflagellar end of the body; but in all cases the course of the flagellum, from the point where it comes into contact with the cytoplasm up to the kinetonucleus, is shown running through the middle of the cytoplasm; it is never drawn lying at the side, still less as showing any undulations. I think this is an important point, and one which tells very much against the presence of an undulating membrane in these Leptomonas. For in the great majority of preparations of trypaniform parasites, however attenuated they may be, and however narrow the membrane, the attached flagellum lies nevertheless at one side (cf. my figs. 10, 126–132, and also Minchin's figures of T. grayi). I think, therefore, that in these peculiar phases a considerable part of the flagellum is intra-cytoplasmic, forming, as it were, a long rhizoplast, consequent on the passage of the kinetonucleus to the opposite end of the body. These forms appear to be quite distinct both from ordinary herpetomonad parasites and from the true trypaniform type. "T" drosophilæ, on the other hand, appears to exemplify the trypaniform condition.

The above summary represents, in my opinion, the present position of this difficult problem of the flagellates occurring in blood-sucking invertebrate hosts. My view on the subject

is the same as that I have maintained in my article on the Hæmoflagellates in Lankester's Protozoa (**39**), as will be seen by anyone who cares to compare that account with the above pages. As a matter of fact, there is now no doubt whatever that one of Schaudinn's far-reaching conclusions was correct, namely, that vertebrate trypanosomes undergo a definite part of their developmental cycle in an invertebrate host, and that true cyclical infection occurs by means of the latter; for conclusive experimental proof has been recently brought forward by Kleine, Bruce and others, Minchin and Thomson. To indicate the work of these authors, however, would be going outside the scope of this paper; moreover, in this discussion, I have preferred to limit myself to the above comparative observations, since most of them provided material on which I relied for support in my article (l.c.).

Patton has of late occupied himself in reiterating his view that in all those instances considered above, as well as in every other case where an author has purported to describe phases of a trypanosome in an invertebrate, the parasites in question were merely natural flagellates of the invertebrate, which had no connection with a vertebrate host. Patton's view is that of scarcely anyone else; even Novy and McNeal have not gone quite so far in this wrong direction. I do not intend to argue the matter with Patton; a perusal of his recent papers suggests that he is unable to appreciate argument. In his latest review (**20**), Patton has adversely criticised, in somewhat forcible terms, my article in Lankester's treatise, chiefly because I have maintained the opposite view to himself. I do not think it necessary to reply at length to Patton's remarks; it is obvious that Patton is hopelessly biassed, and in one or two places I consider he oversteps the boundary of legitimate criticism. I venture to say, however, in justice to my editor as well as to myself, that if a student of tropical medicine and protozoology follows Patton's judgments on our knowledge relating to the hæmoflagellates and their allies, as set forth in his "critical" review, he will obtain a distinctly erroneous and misleading

impression of the group, and one which is further from the truth than the views expressed in my article.

(c) Notes on Nuclear Cytology and Division.

My material, having been all stained by the Romanowsky method, has not proved very suitable for a study of the minute structure of the nucleus (trophonucleus). Nevertheless, in the light of the interpretation which Minchin and Woodcock (**13**) have shown is to be placed upon the "Giemsa-picture" of the nucleus of a trypanosome, I am able to say that, in the case of many, at any rate, of the parasites observed, the type of nuclear structure certainly agrees with that described in that paper. Unfortunately, in the parasites figured from the blood of the bird, the nucleus often shows the usual granular appearance; now and then, however, the definite clear region can be seen, corresponding to the central, plastinoid part of the karyosome, which contains a deeply staining granule in the middle—the intranuclear centrosome (figs. 30, 34, and 51). For some reason or other cultural forms show this appearance, which is to be regarded as the typical one, much more frequently, indeed quite regularly (figs. 7, 8, 72, etc.). The trophonucleus of the individual in fig. 3 is in an interesting condition; it is more faintly stained than usual, the nuclear sap apparently containing little or no chromatin (cf. the numerous chromatoid granules scattered in the surrounding cytoplasm). Whether the deeply-stained central body represents in this case a small karyosome or a greatly enlarged central granule, it is impossible to say. Other instances of an unusual appearance of the trophonucleus are seen in the parasites of figs. 38 and 39; here there appear to be a certain number of separate chromatic masses, of varying size. This condition perhaps represents a fragmentation of the single large karyosome usually present.

The blepharoplast, or basal granule, at the proximal end of the flagellum is sometimes visible in the parasites from the

blood (figs. 4, 28); but frequently the proximal, rhizoplastic portion of the flagellum is not well stained, and then the blepharoplast cannot be made out. In preparations of cultural forms it is generally conspicuous, and now and again very prominent (figs. 10, 71, 81, etc.).

As regards the details of commencing division, the trypanosomes in the blood have provided me, as already stated, with hardly any indications at all. On the other hand, I have obtained a nice series of stages among the cultural parasites. The first act in the process is apparently the division of the blepharoplast at the base of the flagellum (fig. 120). This is followed by the splitting of the flagellum for some distance, which may be fairly short or fairly long (figs. 100, 104, 121, and 123); the splitting never extends, however, throughout the whole of the attached part of the flagellum. In the case of this avian parasite, the splitting-off of a portion of the old flagellum to form the foundation of the new daughter one appears to be of general occurrence. I have observed nothing which would indicate that the daughter-flagellum is formed as an entirely independent outgrowth from the second blepharoplast. Fig. 89 shows a flagellum caught in the act of dividing, the proximal portion being drawn out transversely, as a broad band, prior to splitting. In figs. 88, 100, 104, and 123, the newly formed part is still connected at its tip with the old flagellum; and in fig. 121 the new portion, in this instance only short, has just separated. Of course, once the rudiment, as it were, is cut off, its further growth is quite independent.

The division of the nuclei may begin while the splitting of the flagellum is proceeding (figs. 104, 123), or it may be delayed until the latter process is completed (figs. 90, 121); there is apparently considerable variation in this respect. The first stage in the division of the trophonucleus is most probably the division of the intra-nuclear centrosome, which acts as a division-centre; this is clearly shown in fig. 88. All that can be said from Giemsa-stained preparations as to the rest of the process is that the nuclear substance becomes

extended in a direction more or less transverse to the long axis of the body, this being doubtless brought about by the separation of the daughter-centrosomes (cf. fig. 99); the two centrosomes remain connected by a fibril, which at a later stage may become considerably drawn out (figs. 124, 125). The nuclear material becomes aggregated around these two division centres; as the latter continue to separate, it is pulled out more or less into the form of a dumbbell and finally constricted into two halves, the daughter trophonuclei. With regard to the division of the kinetonucleus, the process, so far as can be judged from the phases seen in figs. 101 and 104, appears to be similar to that occurring in the division of the other nucleus. A distinct thread or band connects the separating halves; this probably indicates a fibril, corresponding to the other, which may also have its terminations in two intra-nuclear division-centres. If this is really the case, not only the trophonucleus, but also the kinetonucleus, possesses an intra-nuclear centrosome.

(D) Comparison of Trypanosoma fringillinarum with other Avian species.

The reasons which have led me to consider all the manifold forms of the trypanosome met with as belonging to one and the same species have been given at the commencement of the description of the parasites, and also alluded to elsewhere in the account, so that I need not recapitulate them here. This illustration of the very great polymorphism which may be shown by one species is most instructive. If, for instance, only two types of form, at opposite extremes as regards size, had been observed, it might readily have been supposed that two different trypanosomes were concerned. And there can be no doubt that many observers, not only of avian parasites but also of others of cold-blooded vertebrates, who have based their descriptions on casual observations of the parasites, have fallen into such an error. So long as the mammalian forms, and among these chiefly the lethal ones, with their comparatively modest variations in form and size, remained

those with which research was principally occupied, the possibility of such striking polymorphism was insufficiently recognised. It is evident, I think, that the safer plan for workers on these naturally occurring trypanosomes will be to regard all the forms met with in any one host as belonging to one species until they have satisfied themselves that this is not the case.[1]

On the other hand, for the purpose of distinguishing different species of trypanosomes, I certainly continue to think that what may be called the biological consideration is, in the present state of our knowledge, the most reliable and useful guide. By this I mean that the less closely related, zoologically, two hosts are, the greater the probability that their trypanosomes are distinct species. As a general indication it may be said that the same parasite may, in certain cases, be parasitic in different species of host, or even in closely allied genera,[2] but where the hosts in question belong to different families, or still more, to different orders, it may be safely assumed, as a working rule, that their trypanosomes are distinct species. The best practical test for this criterion is, of course, the production or non-production of cross-infection.

In making use of resemblances or differences in morphology in comparing two trypanosomes, I think the ordinary adult form of the parasite furnishes the best indications. Take the case of T. lewisi, for example; neither the young daughter-individuals resulting from multiple fission, nor the large, stout, multiplicative individual itself is regarded as the definitive form, the form of every-day occurrence, as it were. Now I think we can carry this comparison very usefully to other cases. Small, fusiform, or stumpy individuals are more

[1] I consider, for instance, that Wenyon (37) has done wisely in including the quite different types of form found, on the one hand, in the guinea-fowl (Numida) and, on the other hand, in a lizard (Mabuia), under one species in each case, viz. T. numidæ and T. mabuiæ.

[2] In this connection attention must be paid to the question of distribution.

likely to be young forms; these may, perhaps, themselves undergo division, as in T. lewisi, and, moreover, in many cases, owing to a slow rate of growth and increase in size, these small forms may give the impression of being distinct parasites. On the other hand, very large, massive forms are likely in many cases to be essentially multiplicative individuals. Of course the possibility must not be overlooked that, in some cases, large, stout forms may be sexual (female) individuals, but up to the present evidence pointing to the occurrence of sharply differentiated sexual forms is only forthcoming in a few instances. At any rate, so far as T. fringillinarum is concerned, I think there is a general parallel with T. lewisi in regard to the different types.

In the case of many of the avian species so far described, the account has been based in all probability upon the ordinary adult type, e. g. T. avium, as emended by Laveran, T. paddæ, Thironx, etc. But in other cases, where only stumpy forms have been described, such as T. hannæ, another T. sp. from Senegambian birds,[1] and T. laverani, these probably do not represent the definitive type. Passing on now to compare T. fringillinarum with certain other trypanosomes, we may begin with the type-species, T. avium. This name was originally given by Danilewsky, who followed his own methods of nomenclature, to trypanosomes found both in owls (sp. indet.) and in a roller-bird (Coracias garrula). Laveran (6) has rightly restricted this specific name to a parasite from an owl (Syrnium aluco), which he considers to be the same form as that observed by Danilewsky; the other trypanosome, from the roller-bird, is in all probability a different species. T. fringillinarum, while showing a general similarity in size and form with T. avium, as described by Laveran, differs in two respects,

[1] This parasite, described by Dutton and Todd (4), occurred in a bird (Estrelda) in which the very different form T. johnstoni was found. It is not at all improbable, I think, that T. johnstoni is the ordinary form, and the broad, stumpy parasite a multiplicative form, of one and the same species.

namely, in the length of the free flagellum, which is much shorter, and in the appearance of the aflagellar end, which is more elongated and attenuated. In addition, the hosts are, of course, quite different in the two cases.

Novy and McNeal have included in the species T. avium a number of parasites they have found in various North American birds. They distinguish two chief forms, viz. large and small parasites, each of which shows considerable variations in size. How Novy and McNeal have been able to ascertain any details with regard to form and size, if they had not better preparations to study than those from which their excellent photos have been taken, it is impossible to say. From their photos of the parasites in the birds, it is obvious that the trypanosomes were wretchedly fixed and stained; in scarcely any can the length of the flagellum or the true nature of the aflagellar end be made out. Hence, any real morphological comparison is out of the question. In any case, on the grounds of occurrence and distribution, it is very improbable that any of the parasites represented the true T. avium. This has been recognised by Lühe (9), who has placed all these forms identified by the Americans as T. avium in a new species, T. confusum—a very apt name. I do not for a moment suppose, however, that all the forms described belong to one species. Novy and McNeal rely partly on the cultural characteristics shown, which they say were similar in all these cases. All their photos of cultural forms of this group of trypanosomes are taken from preparations of a single culture, from one bird only. I should prefer to see figures of cultural forms from the other birds first of all.

The trypanosome which Novy and McNeal distinguish as T. laverani, from an American goldfinch, Astragalinus tristis, is most probably closely related to T. fringillinarum, although I am hardly inclined to think the two forms are identical. The authors only figure a solitary example from the blood, which, from the size given, and from what can be made out from the photo, agrees very well with the small, fusiform individual of T. fringillinarum. There is a

general agreement also, both in regard to appearance and size, between the trypanomonad forms in cultures. The reason which weighs most with me in keeping the two parasites distinct is the different hosts and their different distribution. Unfortunately Novy and McNeal do not describe, as I consider, the definitive type of the parasite, and so I am unable to compare it with that of T. fringillinarum. Other reasons are that T. laverani is said to have a very sparse and slow growth in cultures, and the cultural forms themselves show very generally a peculiar rod-like structure near the aflagellar end of the body. I have certainly never seen this feature in any of the cultural forms of T. fringillinarum.

5. Note on Halteridium fringillæ (Labbé).

I have already published a short paper (38) relating to the chief features of interest which I have observed in connection with this parasite; and I do not propose to repeat in detail the description there given. I wish, rather, to add here a few general remarks and comments.

I am now able to publish many of the actual drawings from which the text-figures in my previous note were made; and these—especially the coloured figures—bring out certain distinctive points very clearly. It is particularly in such a case as this, I may say, that the value of the different tints and depths of colour, produced by the Romanowsky (Giemsa) stain, is apparent. Firstly, in regard to the dimorphism of the nuclear constituents (cf. especially figs. 14, 15, and 17). The smaller nuclear body, representing the kinetonuclear element, is seen to be quite distinct in its staining reactions from the larger body, the ordinary nucleus. These two nuclear portions correspond closely in appearance (leaving out of account the marked difference between them as regards size) to the trophonucleus and kinetonucleus of a trypanomonad parasite, where these two organellæ are close together or in contact.

Again, with respect to the so-called "indifferent" individuals, which are very scanty in number, compared with the female or male forms, figs. 15, 17, and 64, show the characteristically clear cytoplasm, not at all granular, and staining very faintly, of these individuals—readily distinguishable from the granular, deeply staining cytoplasm of female forms.[1] Further, in most of the parasites of this kind which I have found, the kinetonuclear element is relatively large, and may approximate in size to the other nucleus (cf. fig. 64). What exactly is to be understood by the term "indifferent" as applied to these forms, and what their significance is, it is difficult to know. If they are neither male nor female they are not gametocytes; that much is obvious. At the time when I wrote my earlier note on this Halteridium, I was strongly inclined to think that these neutral individuals passed, in certain conditions or circumstances, directly into small trypanosomes. Unfortunately I have not been able to obtain any more evidence in support of this view, either from a renewed study of my own preparations of the chaffinch parasite, nor—which is even more important—from the study undertaken of Halteridium noctuæ, so far as this has yet progressed. Hence the meaning of these "indifferent" individuals, which certainly appear to be quite distinct from the forms of male or female character, has still to be ascertained. I have never found indications of division in them, any more than in the other types.

In fig. 16 is drawn one of the two or three instances I have observed of the remarkable form of individual occurring free in the blood-plasma, which shows a conspicuous line running down the greater part of the body, near one side. This line stains distinctly red, like a flagellum; it appears to start in close proximity to the nuclear masses, and ends in a definite granule. The pigment-grains in this parasite are all aggregated together near one end of the body—that farther away from the nuclei. I regarded the halteridia in this phase

[1] Of course there is no possibility of confusing these forms with male gametocytes, which have a large, diffuse, pale-staining nucleus.

as being about to pass actually into little active trypanosomes, in a manner similar to that described by Schaudinn, that is to say, by getting rid of a portion of the cytoplasm containing the effete pigment-grains and by the development of a flagellum, the proximal, attached part of which constituted the flagellar border of an undulating membrane. In spite of much searching, I have not succeeded in finding any further stages in this developmental change. I cannot suggest any other satisfactory explanation of this peculiar structure, however, and I still continue to think it has some connection with a flagellar development, as will be seen in a subsequent paragraph.

The halteridial parasites of small or intermediate size, which I have now found to occur occasionally free from the corpuscles (cf. p. 663), seem to be quite ordinary in character and show nothing unusual. I have seen nothing at all in these to indicate that they undergo any transition to a trypaniform phase. The same observation applies equally, I am sorry to have to say, to Halteridium noctuæ, where, in one or two cases of very strong infection, I have found free individuals, of varying size, to be quite numerous.

As I pointed out in my note, the possession by an intracellular parasite of nuclear dimorphism, in the sense in which I have used this term, is very significant and important evidence in favour of a flagellate affinity or connection of the parasite exhibiting this feature. Indeed, on à priori grounds, the undeniable occurrence of this distinctive character in Halteridium is, even regarded by itself, a very weighty argument in support of Schaudinn's view of the ontogenetic connection of this intra-cellular form with a trypanosome. When, in addition, the other evidential points to which I alluded were taken into account, such as the occurrence, now and then, of individuals attempting (as I consider) to develop a flagellum, and the occurrence of very small trypanosomes at the same time, which were no larger than the full-grown Halteridia, the most reasonable conclusion did appear to be that the two forms of parasite were indeed connected.

I admit, nevertheless, that I am now doubtful of such an actual connection, especially since I have been working at Rovigno. I am more inclined to think that an intra-cellular parasite may exhibit nuclear dimorphism, in certain conditions or phases as a result of a close phylogenetic relationship with a parasitic flagellate (say a trypanosome), without necessarily being any longer ontogenetically connected with one. Put into other words, this is to say that a parasite, such as Halteridium, which shows this feature, is probably derived from a trypanosome which has become adapted entirely to a resting, intra-cellular condition, and has coincidently lost, more or less completely, the ability to develop an active trypaniform phase.

Berliner, in a recent paper entitled "Flagellaten-Studien" (1), has incidentally corroborated my account of the occurrence of nuclear dimorphism in Halteridium by describing it in the case of H. noctuæ, i.e. in the very parasite in which Schaudinn first maintained it was present. Berliner's figures are very striking and interesting. His preparations were stained with iron-hæmatoxylin, and another most important point brought out by this method of staining is the close correspondence between the structure of the (chief) nucleus in the Halteridium and that of the trophonucleus of a trypanosome. I need not dwell upon this point here, as Professor Minchin and myself have already referred to it in our paper (13), showing the essential difference which exists, on the other hand, between the nuclear structure of a hæmogregarine and of a trypanosome; and we shall have more to say about it in our own account of the parasites of Athene noctua.

This fact furnishes, however, strong additional evidence in support of the (modified) view of a close relationship between Halteridium and the hæmoflagellates, which I am inclined to prefer. On this view the gradual "Rückbildung" of the kinetonucleus—which is associated principally, of course, with the locomotor activities—can be readily understood, and is, indeed, to be expected. It accounts, further, for the com-

paratively small size of the kinetonuclear element, as well as for the fact that it is not always distinguishable as a separate organella, differentiated from the main nucleus. On the other hand, such a phylogenetic connection of Halteridium with a trypanosome would also render it quite possible that, in certain cases, such as the incidence of an unusual stimulus or under some other special circumstances, the parasites might attempt to pass into—to revert to, as it were—a trypaniform condition. Thus would be explained the peculiar form of individual I have above described, which appears to have developed a flagellar thread.

This view agrees in substance, it will be seen, with Hartmann's ideas (5) of the Hæmosporidia as a whole, which he has united with the hæmoflagellates in one group—the Binucleata—the common character being the possession of a binuclear condition, i.e. of nuclear dimorphism. So far as the hæmogregarines are concerned I do not think they show any evidence at all of this feature (cf. Minchin and Woodcock, l.c.), and therefore consider that these forms, at any rate, should be kept separate.[1] With regard to the malarial parasites (e.g. Plasmodium and Proteosoma), Hartmann considers that these show indications of nuclear dimorphism; apparently, however, the kinetonuclear element is in a more "rückgebildet" condition than is the case in Halteridium. Hartmann thinks, further, that these forms show other evidences of a hæmoflagellate ancestry, such as the presence of a delicate, narrow, undulating membrane, with flagellar border in the microgametes. This opinion was maintained also by Schaudinn in the case of the microgametes of Halteridium.

Not having personally studied the finer structure of the malarial parasites, I cannot say much about Hartmann's opinion. If the above view is correct, as I consider it to be,

[1] In a later paper on this subject, which I have seen just as my MS. is about to go to the press, Hartmann and Jollos ('Arch. Protistenk.,' xix, p. 81, 1910) have apparently come to the same conclusion, and remove the hæmogregarines from the Binucleata.

in the case of Halteridium, there is nothing inherently improbable in supposing that it holds good for the malarial parasites as well; this was, it will be remembered, Schaudinn's idea also. The first essential point, however, is to show that these parasites possess a nucleus (trophonucleus) of the true hæmoflagellate type (such as is shown by the trypanosomes and Halteridium), as revealed by a stain like iron-hæmatoxylin.

As regards the finer structural details of the microgametes of Halteridium, I have been unable to assure myself of the presence of an undulating membrane and flagellar border. I have examined both faintly stained and intensely stained individuals, which, for all I know to the contrary, were as fully developed and mature as if they had been taken from the stomach of the insect; I have studied them with the best objectives and with the best possible illumination. I think the photos reproduced give very accurate representations of these delicate and minute organisms; and neither my friend, Dr. Reid, who has most kindly taken these photos for me, nor I myself, can make out such a structure. It may be there or it may not; I must leave the point unsettled.

Certain of the microgametes in the photos show clearly the centrosomic granule at one end. The opposite end is finely tapering, and comparable to a cytoplasmic tail; as Schaudinn pointed out, it does not appear to be of flagellar nature. The end possessing the centrosomic granule is to regarded as the anterior end; it is by this end that the microgamete penetrates the female element, as can be distinctly seen in fig. J.

As I mentioned in a former section, I examined particularly cultures inoculated with blood containing these ripe gametes, with a view to finding stages in the development of the oökinetes. Somewhat to my surprise, I could find no indications of any developmental changes in the halteridia in the cultures. I saw no oökinete-like phases, and, indeed, only one or two halteridia which had become liberated from the corpuscles, and these appeared to be degenerating and dying.

6. Note on Leucocytozoon fringillinarum, n. sp.

Habitat.—There has been considerable discussion with regard to the exact nature of the host-cell in which these Avian leucocytozoa are parasitic, some authorities stating that it is a leucocyte, while others regard it as an erythroblast, or else an altered red cell. I have been able to assure myself that in the case of this species the host-cell is undoubtedly a uninucleate leucocyte, and not an immature red cell or erythroblast.[1] After once carefully comparing them there is little difficulty in distinguishing between these two types of cell. Examples of immature red cells are seen in figs. 22 and 57, and of uninfected uninucleate leucocytes of about the same size, or a little larger, in figs. 23 and 58. The nucleus of the leucocyte is relatively larger than that of the other type of cell, occupying, indeed, most of the body; moreover, it is nearly always eccentric in position, with the result that the cytoplasm lies chiefly on one side, whereas the nucleus of the erythroblast is central. The appearance of the two nuclei is also different. The latter contains many small chromatic masses; that of the leucocyte, on the other hand, appears to have a few large masses, which by the Romanowsky method of staining do not stand out so sharply from the general nuclear substance as in the other case. Further, the cytoplasm of the leucocyte is always distinctly paler than that of the other kind of cell.

From the immature red cell all transitional stages occur to the ordinary full-sized red blood-corpuscle; but I have seen no connection whatever between such cells and the others—the uninucleate leucocytes—which are entirely distinct. Moreover, in no case have I found the parasites occurring in the former type of cell, but always only in the leucocytes. Wenyon, in his account of L. numidæ (37), figures uninfected cells belonging to this type of immature red cell, above

[1] From the observations which I have so far been able to make upon L. ziemanni, in the little owl, I am strongly inclined to think that the same is true for this parasite also.

described. He also figures a young Leucocytozoon in a cell which obviously corresponds to the uninucleate leucocytes (cf. his fig. 4 with my figs. 24 and 60). But he does not figure the true type of host-cell (uninfected) at all; this, I gather, he considers to be an immature red cell, such as he figures. I have no hesitation in saying—what, indeed, is apparent from my figures—that the uninucleate leucocytes (fig. 23) are the host-cells, and not immature red cells or erythroblasts (fig. 22).

Effects on the Host-cell.—The young Leucocytozoon always penetrates the leucocyte on the side where there is most cytoplasm. It never becomes actually intranuclear, but it often has a curious position in relation to the nucleus during its early growing phases, appearing to be lodged in a deep depression or pit in the side of the nucleus (fig. 62). At times the parasite is almost entirely enclosed by the nucleus (fig. 19). This result is probably due partly to a tendency of the Leucocytozoon to push or sink further inwards, and partly to the growing out or extension of the nucleus, which undergoes a certain amount of hypertrophy, in the form of a wide crescentic or semi-circular mass, at the sides of the parasite. Coincidently, the nucleus undergoes an alteration in character, losing all indications of large, separate chromatic masses, and taking up the stain quite uniformly. As the parasite grows and expands, the free ends of the semi-circular nucleus are pushed outwards, and no longer enclose the Leucocytozoon. When the latter is full grown the nucleus of the containing host-cell is seen as a thick, curved mass at one side (figs. 20, 21, 25, and 26).

In my preparations all the leucocytozoa are intra-cellular. I have never observed more than one parasite in one host-cell.

My observations, as also those of Wenyon (l. c.), of young and intermediate-sized gametocytes, intra-cellular in habitat, and manifestly growing into the adult individuals in a similar situation, do not support in the least Schaudinn's view with regard to the origin of the adult gametocytes.

Schaudinn considered that these were simply the resting-phases of large, sexual trypanosomes, which had come into relation, in a peculiar manner, with the leucocytes, causing the host-cell to become greatly extended and altered in form. I agree with Wenyon that this view cannot be sustained.

Structure of Gametocytes.—In stained preparations the parasites occur in two well-marked and distinct forms, which represent without doubt male and female gametocytes, since they agree very well with these types in other leucocytozoa. The parasites occur in all sizes, from very young forms up to what are probably fully grown, mature individuals (figs. 19, 20, 24, and 25). Even in fairly young individuals the male or female character can be often recognised (figs. 19 and 24). The diameter of a rounded individual averages about $8\frac{1}{2}$ to 9 μ; the ovoid parasite of fig. 26 is 11 μ by $6\frac{1}{2}$ μ. Female forms appear to attain a slightly larger size than male forms.

Comparing a male gametocyte with an individual of female sex, the cytoplasm of the former stains much paler than that of the latter, and appears to be more homogeneous in structure. The cytoplasm of a female individual is distinctly granular. The nucleus of a male form is large and somewhat diffuse; it appears to contain a number of small chromatin granules (probably really chromatin "dust," which stain pinkish. The female nucleus is small, compact and dense; its chromatin grains stain darker and more intensely than in the other case. Both in the male, as well as in the female form, a definite small chromatic body is sometimes found outside, but close to the chief nucleus (figs. 20, 25, and 26); it has also been seen in small parasites (figs. 19 and 60). This small body corresponds to that associated with the nucleus of L. ziemanni, where it was first described by Schaudinn. As I hope to have something to say subsequently, in conjunction with Professor Minchin, upon the nuclear structure of the latter parasite, I will not discuss this point at present, especially as my material is limited and all stained by the Romanowsky method.

One feature about this new Leucocytozoon is of great interest and importance, the fact, namely, that in no instance observed is the cytoplasm of the host-cell extended in the form of a spindle at both sides. Even where the body of the parasite is oval in shape, and more comparable in form to the deeply stained portion of the body in other leucocytozoa, there is no sign of any extension of the protoplasm of the leucocyte. If in the case of other species, e. g. L. ziemanni, L. numidæ, this great drawing out of the ends of the host-cell is due merely to the parasitic influence of the Leucocytozoon, why does the parasite not produce the same effect here? I certainly think it is quite as probable that, in those cases where the spindle-like appearance is shown, there is some more material cause for this constant shape, and that there is really a prolongation of the body of the parasite,[1] in the nature, perhaps, of a faintly staining ectoplasmic layer, for some distance at the two sides, to which is chiefly due this extension of the cytoplasm of the host-cell. Upon this point, also, I shall be able to say more when I have studied the preparations of L. ziemanni. If this is the correct explanation, it is evident that the Leucocytozoon of the chaffinch has lost its ectoplasmic layer, at least so far as can be made out. This development would indicate a closer adaptation to the intracellular condition, which is also seen, perhaps, in the rounded form of the parasite, the other species known being much more fusiform.

I propose the name L. fringillinarum for this new species of Leucocytozoon from the chaffinch; the parasite found by Stevenson in the greenfinch probably also belongs to this same species, since, so far as I can ascertain from the preparation kindly given me by Stevenson, it also has the rounded form and does not cause the host-cell to become spindle-shaped.

Of the many species of Leucocytozoon now known, only two or three, so far as I am aware, have been described as having the rounded form, and with the host-cell lacking the

spindle-like prolongations. The descriptions of these forms are to be found in a series of notes by Mathis and Léger (**10A–10D**). I wish to point out that as regards one at any rate, and possibly more than one, of their parasites, the authors, in describing the gametocytes (and their host-cells) as rounded, appear to have been dealing simply with individuals which had begun the active process of rounding themselves off preparatory to rupturing the host-cell and becoming liberated as ripe gametocytes. Now, in preparations of the fusiform species (L. ziemanni and others), which show gametocytes caught in this act, it is generally impossible to recognise any longer the typical fusiform shape, the cytoplasm of the host-cell having been quickly disorganised.

In the case of Mathis and Léger's forms L. caulleryi (a rounded form) and L. sabrazesi (spindle-like), both from the same host, namely a fowl (Tonkin), I feel sure that the latter parasite is the typical intra-cellular form of the former. Thanks to the authors' kindness in sending some of their preparations of these parasites to the Lister Institute, I have been able to compare them. On a slide containing L. caulleryi all the individuals found are quite rounded-off, and, moreover, there is no sign of the host-cell in connection with them, i.e. the latter has been ruptured and disorganised, and the parasites are seen as ripe, free gametocytes. A slide containing L. sabrazesi, on the other hand, shows the parasites still within their host-cell, the latter having the usual spindle-like prolongations. Mathis and Léger themselves say, in their note on L. caulleryi (**10A**), that only exceptionally did they see the nucleus of the host-cell—evidence that the latter had been ruptured and disorganised. Hence I myself have no doubt, especially when the fact of these two parasites being found in the same host is considered, that L. sabrazesi is only a synonym for L. caulleryi, and that this species (L. caulleryi) belongs really to the fusiform group.

On the other hand, in the case of the species I have described, L. fringillinarum, there is no doubt that it is

quite distinct from the fusiform group, since in all stages—from young forms right up to large gametocytes—the parasite and its host-cell retain the rounded form. Apparently Mathis and Léger's form, L. marchouxi, from Turtur humilis (**10**c), also agrees with this type, for in this case the authors find the host-cell intact, the whole appearance of parasite and leucocyte being, so far as can be judged from the account, similar to that of L. fringillinarum.

The Lister Institute,
April, 1910.

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EXPLANATION OF PLATES 27—31,

Illustrating Dr. H. M. Woodcock's paper "I. On certain Parasites of the Chaffinch (Fringilla cœlebs) and the Redpoll (Linota rufescens)."

[All the drawings on Pls. 1—4 are drawn to a uniform magnification of 2000 diameters. For several of the coloured figures on Pl. 1 and for two or three of the drawings on each of the other plates I am indebted to Miss Rhodes, who has kindly done them for me.]

Plates 27 and 28. With the exception of figs. 7–13, 22, 23, 57, and 58, all the figures relate to the parasites as found in the birds.

PLATE 27.

Figs. 1–6.—Trypanosoma fringillinarum, n. sp.

Fig. 1.—Adult, ordinary individual from the blood of a chaffinch inoculated from a redpoll-culture.

Fig. 2.—Ditto, from the bone-marrow of a naturally infected chaffinch.

Figs. 3 and 4.—Slightly smaller forms; 3, from the blood of a redpoll; 4, from the bone-marrow of a chaffinch.

Figs. 5 and 6.—Small forms of the fusiform type (case B), from the bone-marrow of a chaffinch.

Figs. 7–13.—Cultural forms of the trypanosome; 7–12 from chaffinch cultures; 13 from a redpoll one.

Figs. 7–9, and 13.—Trypanomonad forms (6 and 7 days).

Fig. 10.—Early trypaniform type (40 hours).

Figs. 11 and 12.—Examples of equal and unequal binary fission.

Figs. 14–18.—Halteridium fringillæ (Labbé).

Fig. 14.—Female individual.

Figs. 15 and 17.—"Indifferent" individuals.

Fig. 16.—Special form, free in the blood-plasma, with chromatic

line. (Unfortunately the terminal granule has not come out in the plate.)

Fig. 18.—Very young form.

Figs. 19–21, 24–26, Leucocytozoon fringillinarum, n. sp.

Figs. 19 and 24.—Young gametocytes, female and male.

Figs. 20 and 26.—Large female gametocytes.

Figs. 21 and 25.—Large male gametocytes.

Fig. 22.—Immature red blood-corpuscle.

Fig. 23—Leucocyte (uninfected).

PLATE 28.

Figs. 27–56.—T. fringillinarum.

Figs. 27–33.—Ordinary definitive forms of the parasite of varying size; 27 from a chaffinch inoculated with redpoll culture, 28–31 from naturally infected chaffinch, 32 and 33 from naturally infected redpoll.

Figs. 34–38.—Large, massive forms, from a redpoll.

Fig. 39.—? Transitional form, intermediate between ordinary type and that last mentioned, from a redpoll.

Figs. 40–45.—Series of fusiform parasites from very small to a moderate size, from a chaffinch (Case B).

Figs. 46–54.—Small forms from a chaffinch (Case A), fusiform or broad and stumpy; 48 and 54 show indications of division. Many of the individuals in both these series show the granular chain or line.

Figs. 55 and 56.—Remarkably slender individuals (? young, definitive forms).

Figs. 57 and 58.—Immature red blood-cell or erythroblast and uninfected leucocyte, respectively.

Figs. 59–62.—Leucocytozoon fringillinarum.

Fig. 59.—Male individual.

Fig. 61.—Female individual.

Figs. 60 and 62.—Young forms, probably female individuals.

Figs. 63–70.—Halteridium fringillæ.

Figs. 63, 65, and 66.—Medium-sized to large female forms.

Fig. 64.—"Indifferent" individual.

Figs. 67–69.—Small or intermediate-sized individuals.

Fig. 70 *a* and *b*.—Male gametes.

PLATE 29.

Figs. 71–111.—Cultural forms of T. fringillinarum.

[All the figures are from original cultures of 6–8 days, except figs.

74 and 109, which are from a second sub-culture of 26 days, specially for comparison.]

[(C) indicates chaffinch culture; (R) redpoll-culture.]

Figs. 71–86.—The ordinary trypanomonad type, showing variations in size and in degree of development of the membrane.

Figs. 71–76, 83–86 (C); figs. 77–82 (R).

Figs. 87 and 88.—Individuals in which the kinetonucleus is a trifle on the aflagellar side of the trophonucleus; in fig. 88 division is just being inaugurated. Both (C).

Figs. 89–95.—Stages in equal binary fission. All (C) except fig. 83, which is (R).

Fig. 96.—Division-form of sub-equal character, giving rise to individuals of the accentuated trypanomonad kind.

Fig. 97.—Accentuated trypanomonad individual (C).

Figs. 98–104.—Various stages in the unequal division of the accentuated trypanomonad individuals. Figs. 100 and 103 are (R), the rest are (C).

Figs. 105–111.—Illustrative of the two kinds of individual which result from unequal fission. Figs. 105, 107 (upper half), 108–110, accentuated trypanomonad forms, often more or less club-shaped, with nuclei far back and well-developed membrane; Figs. 107 (lower half), 106 and 111 A and B, fusiform individuals, with only slightly developed membrane; note the comparatively short flagellum. Figs. 106 and 108 (R), rest (C).

PLATE 30.

Figs. 112–131, 133–163.—Cultural forms of T. fringillinarum (contd.)

Figs. 112–114.—Pear-shaped forms, probably derived from the smaller halves of unequal divisions, which have not become fusiform. Figs. 112 and 114 (C), 113 (R).

Figs. 115 and 118.—Small and large individuals of the accentuated trypanomonad kind, passing into the ovoid or rounded condition. Both (C).

Figs. 116, 117, and 119.—Medium-sized rounded forms. (All C).

Figs. 120–125.—Individuals from the (C) culture which showed a pronounced tendency to develop large massive forms. Many of them are undergoing division.

Figs. 126 and 127.—Early trypaniform individuals. (C) forty hours.

Fig. 128.—? Transition form from trypanomonad to trypaniform type. (C) 6 days.

Fig. 129.—Small trypaniform individual. (C) 6 days.

Figs. 130 and 131.—Greatly elongated trypaniform individuals. (C) 6 days.

Fig. 132.—Trypaniform phase of a trypanosome of Athene noctua from the stomach of Culex pipiens.

Figs. 133, 134, and 136.—Rounded forms still possessing a flagellum, but lacking any signs of an undulating membrane. In the two first a large vacuole is present. Fig. 133 (C), figs. 134 and 136 (R).

Figs. 135 and 137.—Rounded forms without a flagellum (R).

Figs. 138 and 139.—Small rounded forms in a dying condition; the two nuclei are gradually disappearing (R).

Figs. 140–146.—"Pseudo-herpetomonad" forms, illustrating various degrees in the loss of the membrane and attached part of the flagellum. Figs. 140, 145, and 146 (C), 141–144 (R).

Fig. 147.—Herpetomonad form (C).

Figs. 148 and 149.—Pear-shaped forms, with little or no attached part to the flagellum (R).

Figs. 150–163.—All these forms are from a (R) culture of 19 days.

Figs. 150–153.—Large, altered, unhealthy parasites, with a development of granular substance in the region of the base of the flagellum.

Fig. 154.—Parasite showing two large vacuoles.

Fig. 155.—Individual with a kinetonucleus, but no trophonucleus.

Fig. 156.—Individual with one kinetonucleus and two trophonuclei.

Fig. 157.—Dividing parasite, showing how the unequal distribution of the nuclei, as found in the two last forms, is brought about.

Fig. 158.—Parasite with three trophonuclei for one kinetonucleus.

Fig. 159.—Showing the splitting off of an individual with a flagellum, but with no nuclear substance at all.

Fig. 160.—A free, active individual, with no definite nucleus of either kind, but with scattered granules.

Fig. 161.—Showing the splitting-off of a portion of the cytoplasm containing only a few granules.

Figs. 162 and 163.—Forms showing irregular multiplication of the different organellæ.

PLATE 31.

[The micro-photographs on this plate were all taken for me by my friend Dr. D. J. Reid, to whom I wish here to express my deep sense of his kindness and to offer my sincere thanks. It is as well to point out, perhaps, that the more deeply stained parts have come out, in most cases, relatively far too dark.

The magnifications are as follows (approximately): Figs. A–D 1630, fig. E 620, fig. F 500, fig. G 550, figs. H and J 1630, fig. K 1840, fig. L. 1220.]

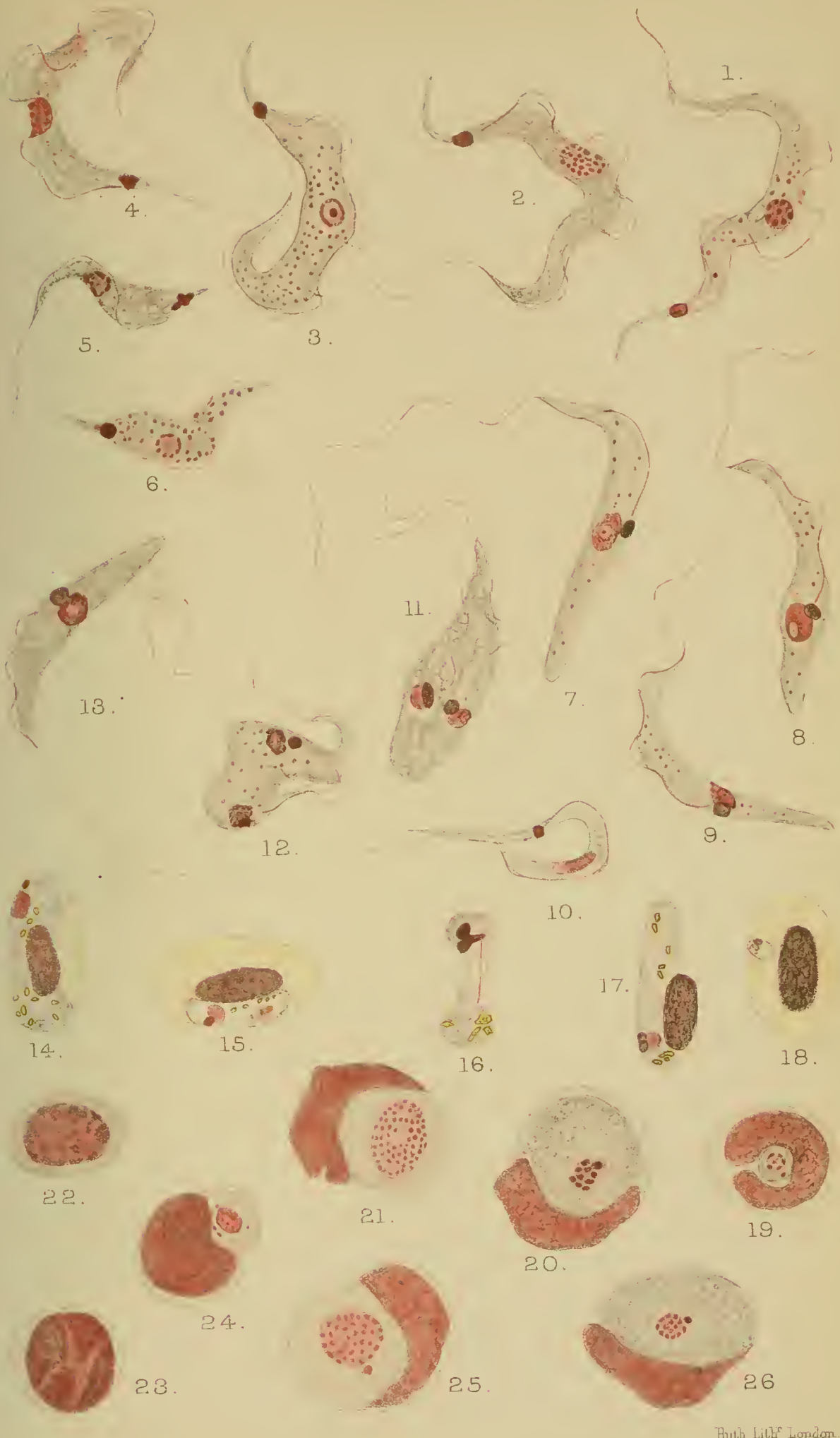
Figs. A–D.—Trypanosoma fringillinarum, as found in the birds. For description of these figures see under figs. 2, 3, 28, and 54, which are of the same individuals respectively. [In the reproduction the whole length of the delicate aflagellar prolongation, which is visible in the actual photos, cannot be made out. Unfortunately there are two small pieces of débris lying on the parasite of fig. B, which are, of course, reproduced. One lies about one third of the distance from the kinetonucleus to the trophonucleus; the other on the fold of the membrane opposite to the nucleus. In the drawn figure (fig. 3) these particles are omitted.]

Figs. E–G.—Agglomeration clusters of various sizes of T. fringillinarum in cultures. [The parasites of the first two clusters are not so nicely stained, unfortunately, as those of the third, but they show the manner of formation of the cluster.]

Fig. H.—Halteridium fringillæ; female individual showing nuclear dimorphism (the same is drawn in fig. 14).

Fig. J.—Fertilisation of a macrogamete by a microgamete. Note that the latter is penetrating by the end which has the centrosomic granule.

Figs. K and L.—Microgametes.



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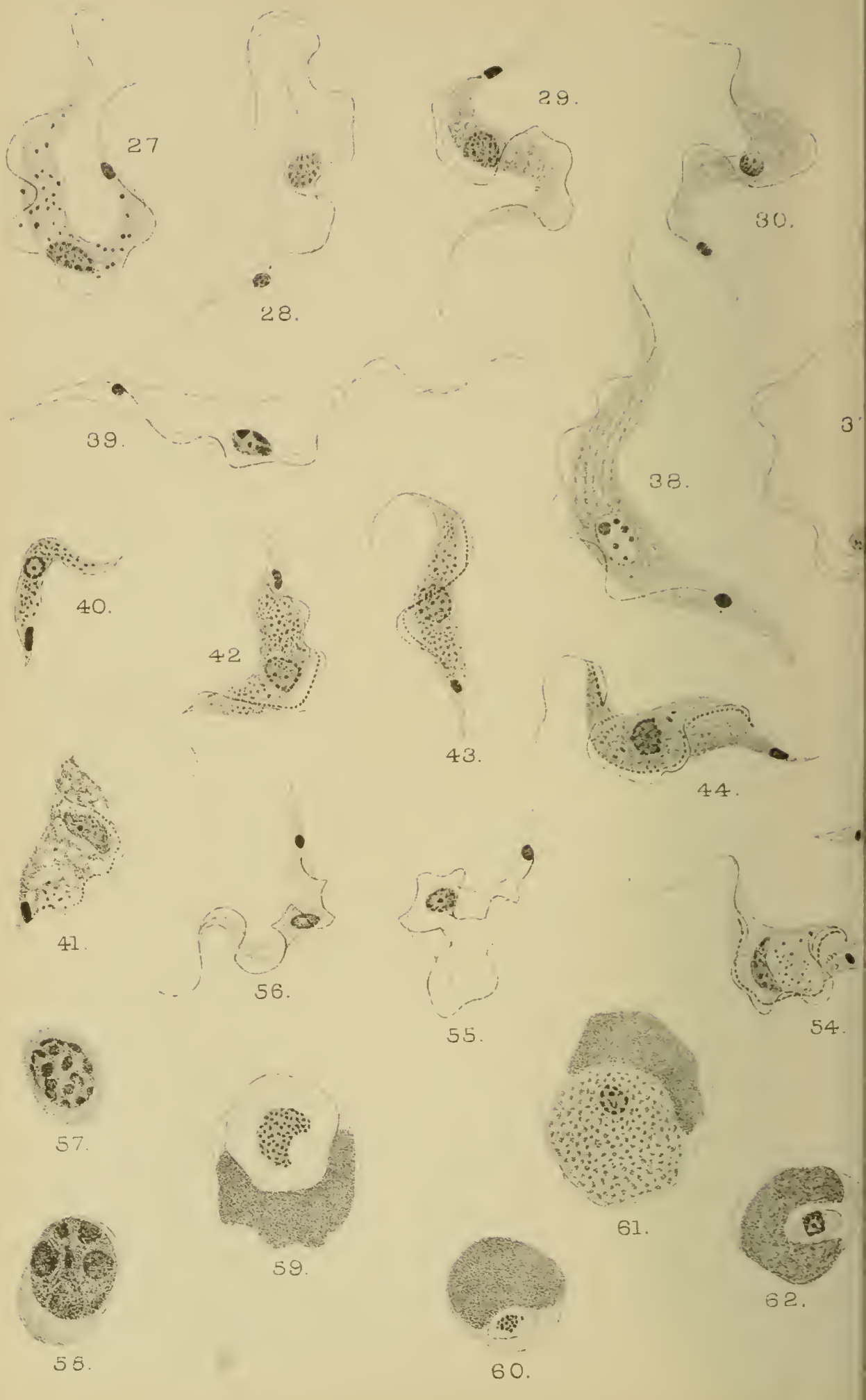
AVIAN HÆMOPROTOZOA.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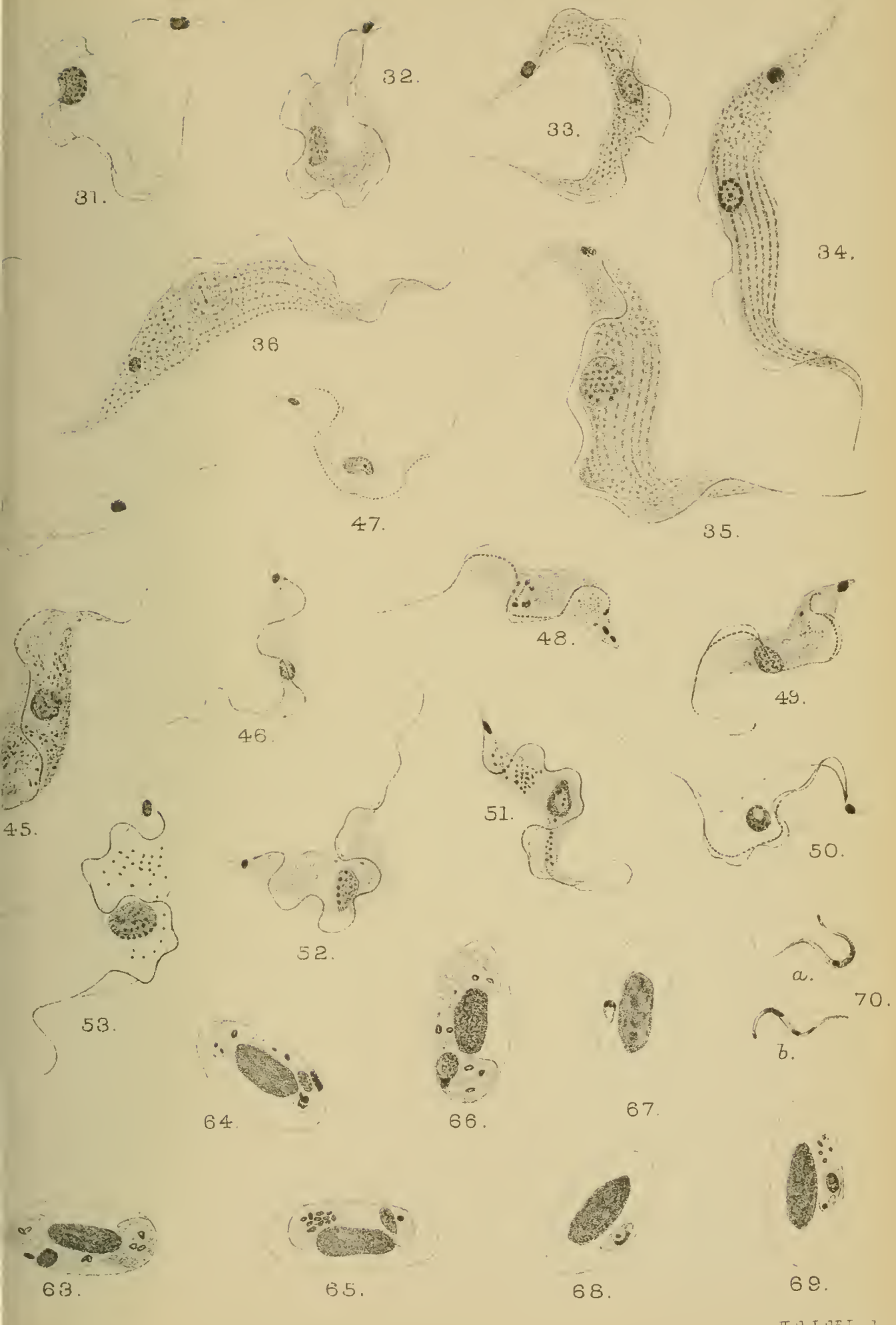

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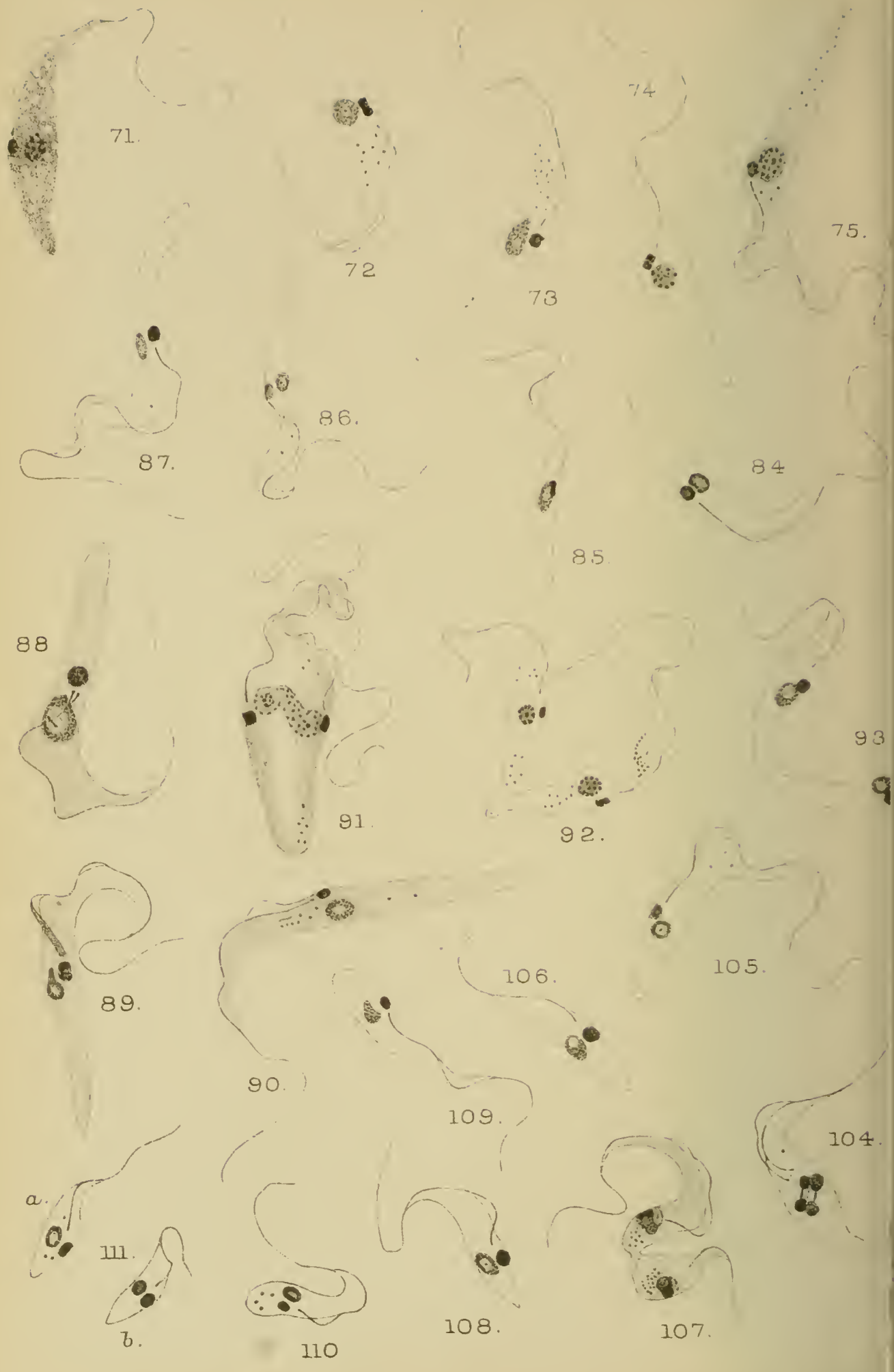
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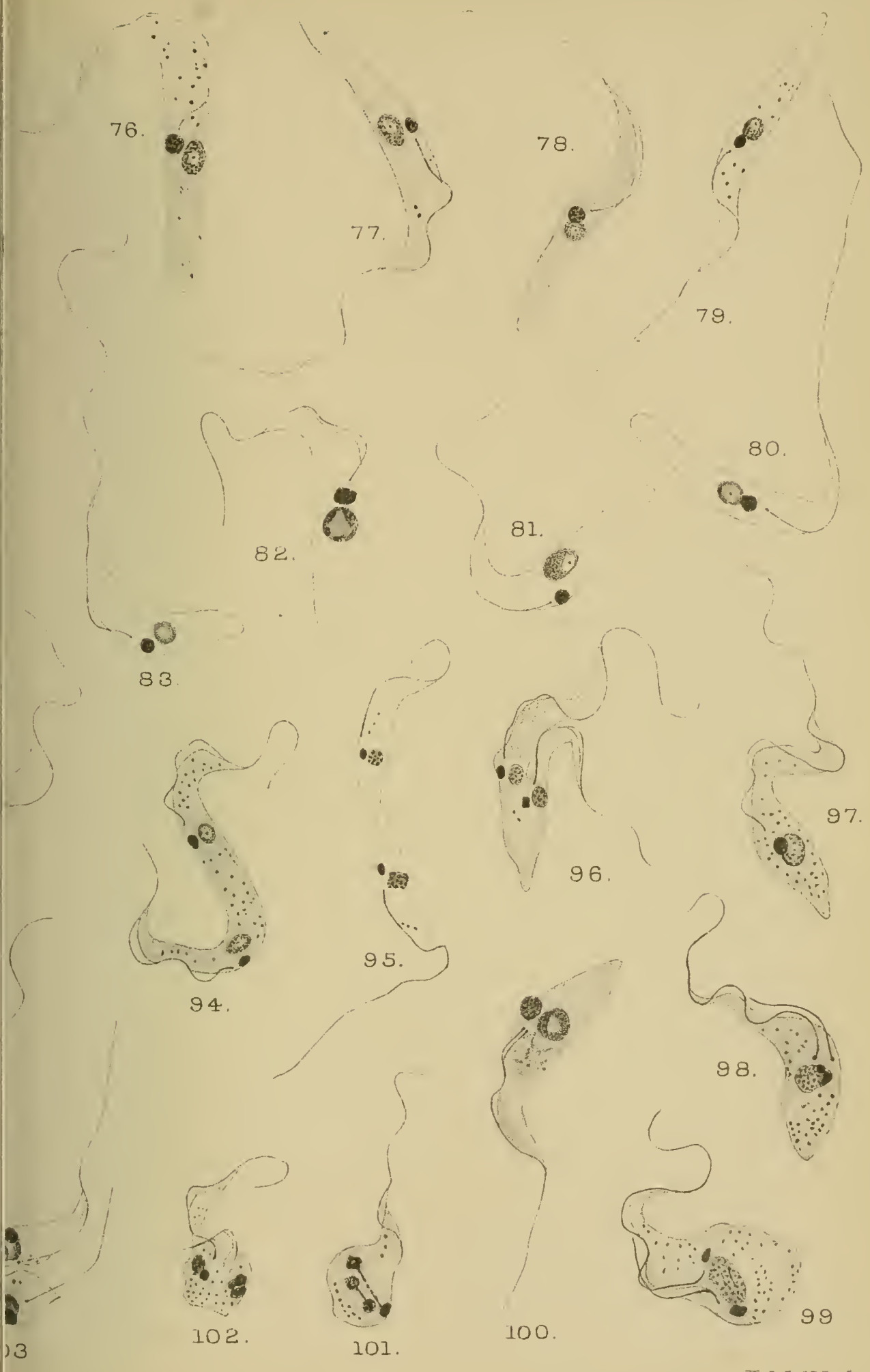

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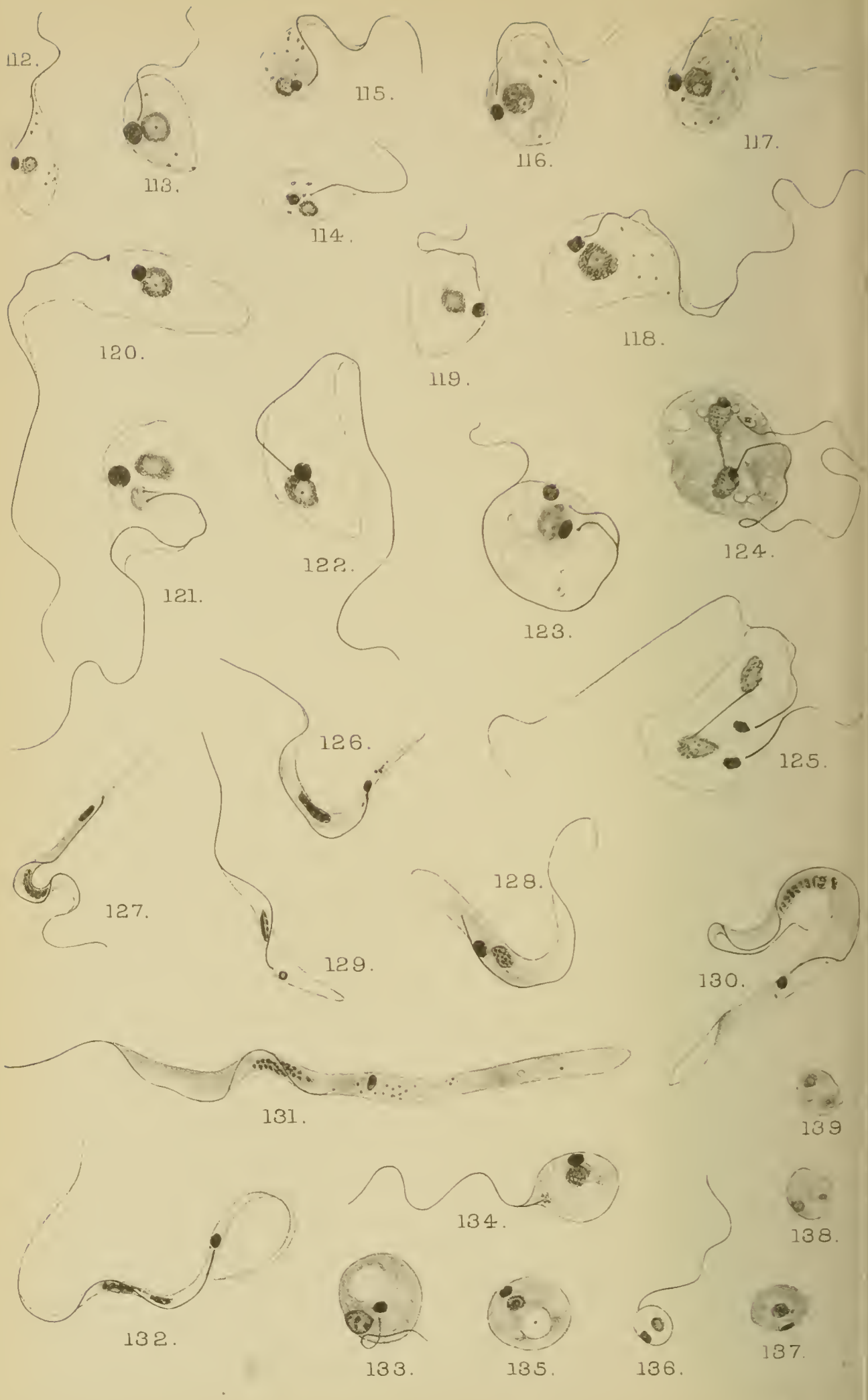
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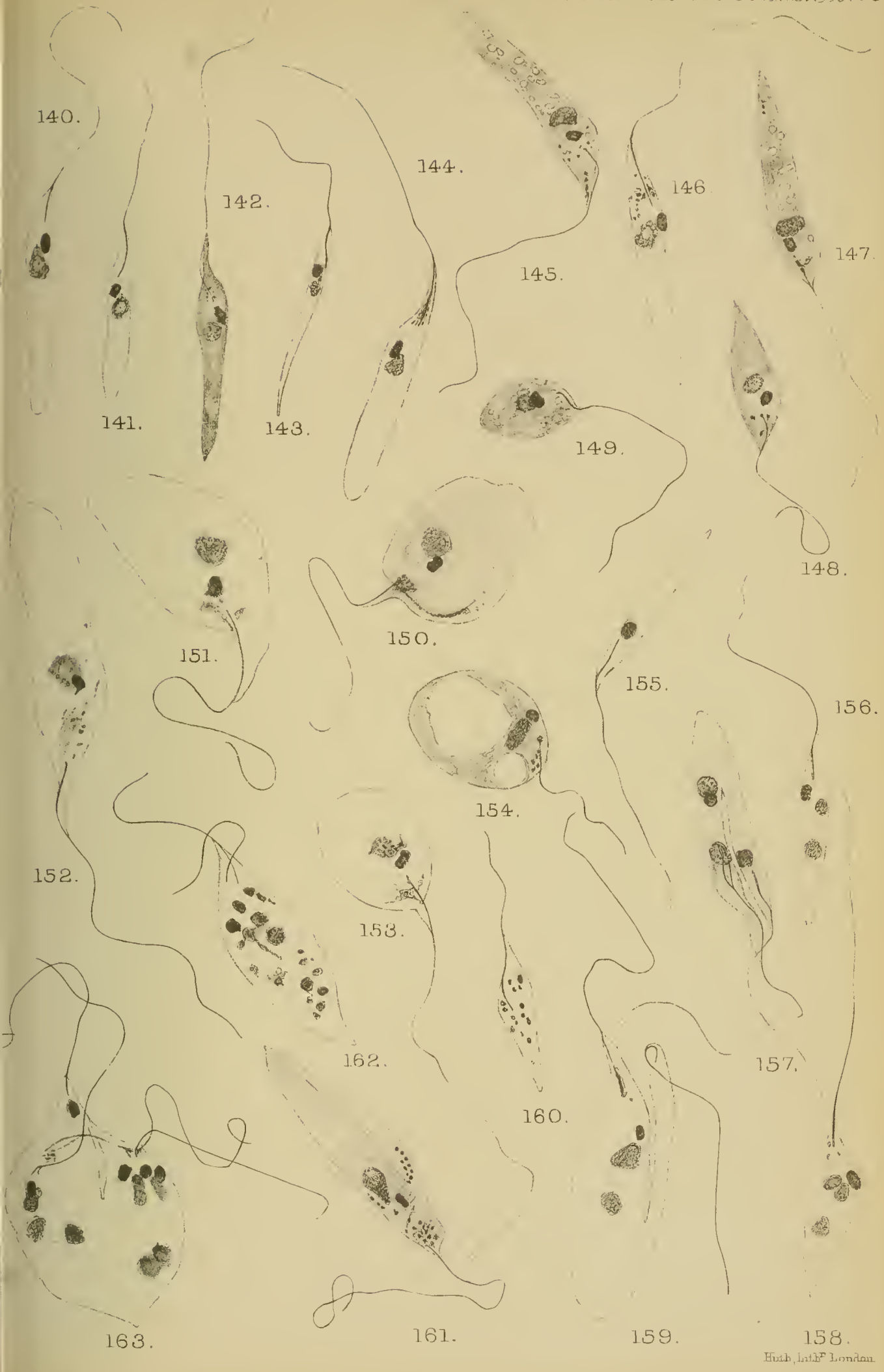

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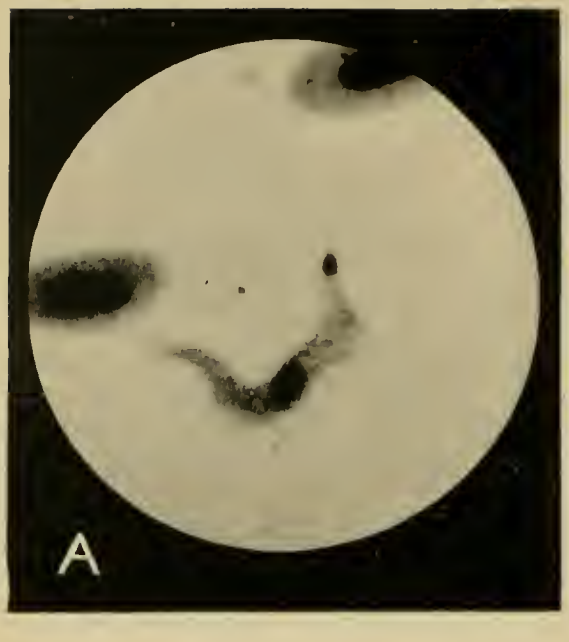
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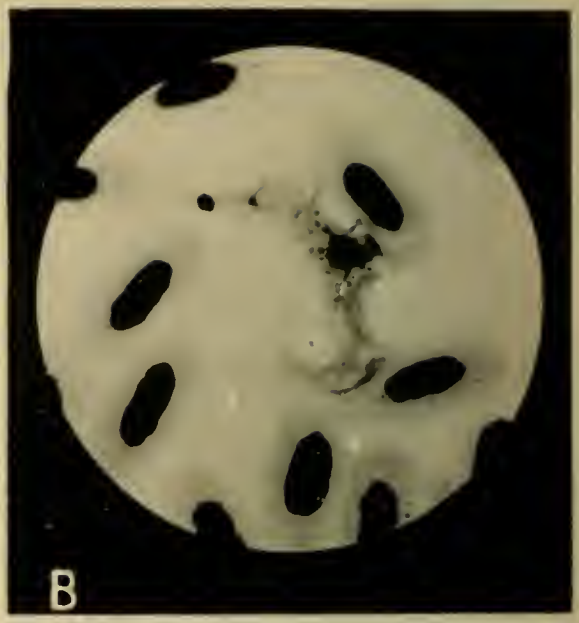
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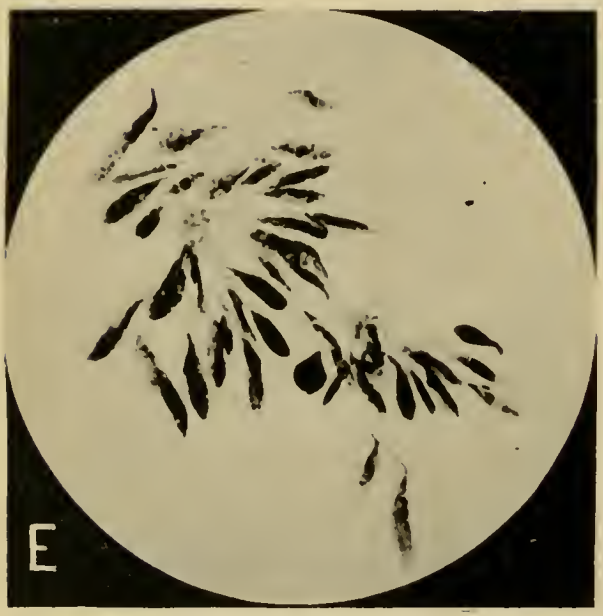
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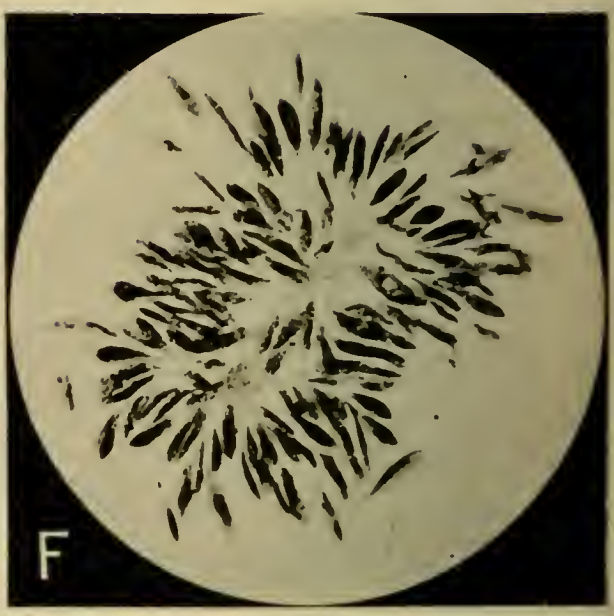
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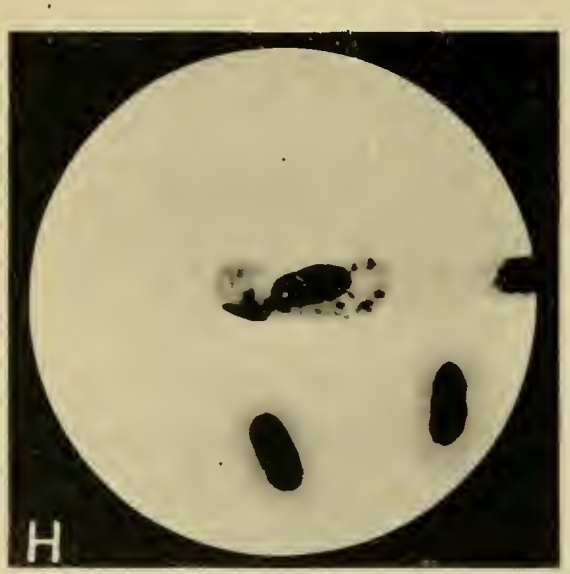
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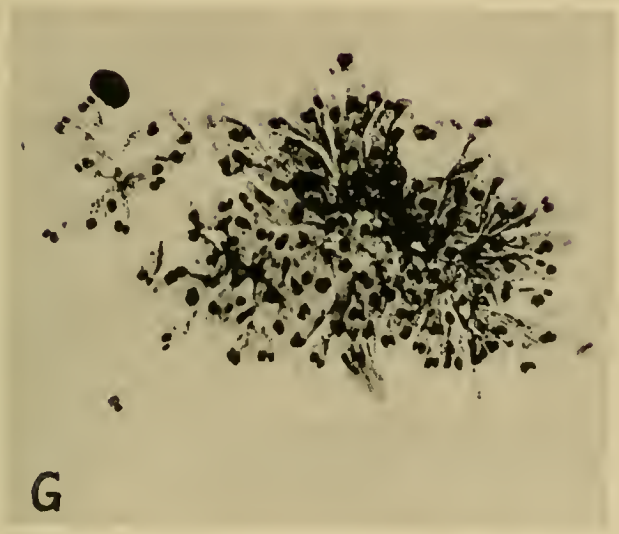

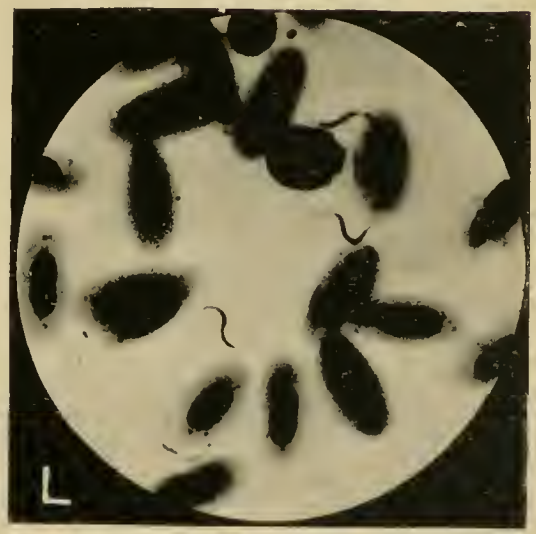

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MOPROTOZOA.

Studies on Ceylon Hæmatozoa.

No. II.—Notes on the Life-Cycle of Hæmogregarina nicoriæ, Cast. and Willey.

By

Muriel Robertson, M.A.

With Plates 32–41 and 1 Text-figure.

Contents.

In 1904 Drs. Castellani and Willey (**3**) described a hæmogregarine from the blood of the common lake-tortoise of Ceylon, Nicoria trijuga. They named the parasite Hæmogregarina nicoriæ after its host. Shortly afterwards these authors gave a somewhat fuller account of their observations in the 'Quarterly Journal of Microscopical Science' (**4**).

While in Ceylon in 1907–08 I was able, largely through the kindness of Dr. Willey, to collect the material described in the following pages. My observations agree in the main with those of the earlier observers already cited. I have, however, been able to supplement their results and to give

an account of some of the processes which take place in the intermediate host, the leech Ozobranchus shipleyi.

I. Occurrence of the Parasite.

Nicoria trijuga occurs in very large numbers all over Ceylon. It generally frequents ponds, lakes, and rivers, but specimens are sometimes found living a semi-terrestrial existence in places removed from water. The tortoises which have adopted the drier habitat occasionally show ticks, but I have never found them infected with the hæmogregarine. I did not, however, examine a sufficiently large number of individuals to be able to draw the conclusion that the dry-dwelling tortoises are never infected.

The Nicorias from the usual aquatic habitat are very often infected with the hæmogregarine. It does not seem to produce any pathogenic effects even when present in large numbers. No other blood-parasites were ever observed in association with the hæmogregarine. The intestinal parasites were not investigated, but it may be noted in passing that a Bodo-like flagellate was found on two occasions in the gall-bladder.

The only ectoparasites present were ticks on dry-land tortoises and leeches on the water-dwelling tortoises. The leeches belonged to a species of Ozobranchus; only once was an isolated Glossiphonia found upon a Nicoria. I found that tortoises from all parts of Ceylon showed the hæmogregarine. I never, however, investigated individuals from more than an elevation of 1500 feet. Generally speaking I found the up-country reptiles were free from blood-parasites.

II. Brief Summary of the Life-history.

For the sake of clearness it is, I think, advisable to give a brief account of the life-history of the form under discussion, in so far as it has been made out, before treating the various points in detail.

The hæmogregarine in the blood of the tortoise shows the usual two types, a bean-shaped and a recurved type. Certain of the bean-shaped individuals, namely, the large forms with a nucleus in which the chromatin is rather loosely arranged, give rise by a process of schizogony in the lung to a great number (about seventy) of large merozoites. Another type of schizogony is found in the circulating blood-corpuscles, and arises also from bean-shaped individuals. This results in the formation of a small number (six to eight) of merozoites of quite small dimensions. It appears that the form which gives rise to this second type of merozoite is itself derived from the schizogony in the lung. It is probable that the small merozoites give rise to the gametocytes. The reason for this assumption is given in another part of the paper. When the hæmogregarines are taken into the crop of the leech, Ozobranchus shipleyi, together with the blood of the tortoise, certain of the hæmogregarines pass into the intestine, and are there found as motile vermicules. They penetrate into the intestinal wall, where the differentiation of the hitherto indistinguishable gametes takes place, culminating in a process suggesting anisogamous conjugation. The zygote breaks up to form eight sporozoites, which pass through the intestinal wall into the blood-spaces. The hæmogregarine is probably passed into the blood of the Nicoria through the contamination of the wound by the leech while feeding.

III. Phases of the Hæmogregarine in the Blood of the Tortoise.

In the living state the hæmogregarine may easily be distinguished as a clear sausage-shaped inclusion in the red blood-corpuscles. The protoplasm is slightly more granular at one end than the other, and the nucleus can be seen as a sharply defined clear area. The parasites do not show any sign of movement when they are observed upon a sealed slide, but free vermicules are very occasionally found in

serous blood that has been allowed to stand exposed in the air. The addition of salt-solution to the blood sometimes causes the hæmogregarines to quit the corpuscle, but never in large numbers. Altogether, it may be said that H. nicoriæ shows far less tendency to become motile in the blood than the majority of the species of hæmogregarines.

The greater part of the blood-films made were preserved by the drying method and stained with Giemsa; a few were, however, fixed while still wet in sublimate-acetic, and treated by wet methods throughout. These wet films were stained with iron hæmatoxylin, and it has been clearly shown that wet fixation followed by hæmatoxylin, hæmalum, or other suitable stain, gives far truer pictures than those obtained by the Giemsa method. All the detail of structure, etc., described were worked out on the wet films.

Parts of the various organs, such as the spleen, liver, and lungs, were also preserved (in Flemming, corrosive-acetic, and Bles's fluid) and sections made. Bles's fluid was found to give an exceedingly good fixation of the blood-corpuscles and of the parasites they contained, especially in the tissue from the lung. When stained with hæmalum a very clear and precise picture was obtained, and the results derived from a study of the films could thus be corroborated and criticised by means of the section material.

In the stained films it can be seen that the parasite is surrounded by a delicate sheath or capsule. The nature of this capsule shows the greatest possible variation in different members of the genus Hæmogregarina. In some species it is a thick refractile envelope, which opens to let out the enclosed parasite when the motile phase is adopted. Even when the capsule is more delicate it is often capable of persisting for a time after the hæmogregarine has escaped. This has been observed by many workers; Castellani and Willey (4) have shown it in H. mirabilis, Dobell, in a form from Boa constrictor (6); I have myself seen the same thing in H. triedrus. In H. nicoriæ the capsule is rather

difficult to demonstrate; iron-hæmatoxylin films or those counter-stained with eosin are the best for this purpose. The capsule never persists after the parasite has escaped, in fact it seems that in this case the envelope may be said to disintegrate rather than to be shed in the usual way. The capsule is to be seen quite clearly in the live state in individuals from the crop of the leech, especially at the time when the blood-corpuscle has been already digested away, but before the parasite has passed down to the intestine, where it becomes motile.

The protoplasm is delicately alveolar, and is sometimes slightly granular; chromatoid particles outside the nucleus are very rare, and this form does not show the curious eosinophile inclusions found, for instance, in H. vittatæ (11). The nucleus consists, as a general rule, of a number of isolated chromatin granules arranged, often rather symmetrically, round a small central body (see figs. 1–3). The peripheral grains of chromatin may be connected by strands with the central granule. This central granule cannot be called a karyosome in anything approaching the same sense in which this word is applied in protozoan literature generally. In the nucleus of this hæmogregarine it is only the position that marks off the central body from the peripheral chromatin granules; it is in no way distinguished from them in size or staining reaction, and in those cases where the chromatin granules are less regularly arranged (fig. 9A) it is quite impossible to pick it out with certainty. Nevertheless, it appears to me to be of a different nature from the other nuclear elements, in so far that, in the very primitive nuclear division, it seems to form a kind of centrodesmose. Not infrequently the peripheral chromatin granules are joined to one another, a chromatin ring being thus formed all round (see figs. 2, 5, and 8). It must not be supposed that this chromatic ring is truly a nuclear membrane; it takes the chromatin stains deeply, and assumes a bright red colour with Twort's stain. It is thus in sharp contrast to the green membrane found by this method round the nucleus in, for instance, some trypano-

somes and certain amœbæ. I am inclined to think that it is simply formed by the running together of the grains of chromatin. Forms are sometimes found in the blood of the tortoise which show the chromatin arranged in an inner and outer ring; this type is shown in fig. 5. Finally, forms are also seen in which the chromatin is in the shape of a large number of irregularly disposed granules, which may at times give the appearance of a kind of reticulum (figs. 6, 17, and 18). In H. nicoriæ, as in almost all the species known, there are, in addition to the young forms, two types in the blood of the vertebrate host, the one a bean-shaped organism with an approximately central nucleus, the other a long recurved creature with its nucleus situated in the broader limb near the bend (see figs. 1, 3, 8, 9, 17 and 18). The bean-shaped form is always present in far greater numbers than the fully developed vermiform individuals, but specimens are very common where the more slender recurved limb is only about half as long as the broad limb (figs. 2 and 9A). This is one of the points in which the wet fixation method is so much superior to the dried films. In the latter the great majority of these specimens, where the recurved limb is shorter than the broad one, appear simply to be bean-shaped, the drying having artificially obscured the recurved limb. There are no very marked or constant nuclear differences in these types; it may, however, be observed that generally speaking the larger bean-shaped forms show the more scattered arrangement of the chromatin. The smaller bean-shaped individuals and the half recurved creatures have usually the more symmetrical circular type of nucleus, while the large vermiform specimens have a slightly elongated nucleus, with a tendency for the chromatin masses to run together at their edge. A glance at the figures will make these points clear.

Two main theories as to the significance of the bean-shaped and vermiform (fully recurved) creatures have been put forward: (1) That the bean-shaped individuals are macrogametes or macrogametocytes, and the recurved ones microgametes or microgametocytes. These two different types or

their immediate derivatives are by this view expected to conjugate in the intermediate host and give rise to the sexual cycle. (2) The second view considers that the bean-shaped creatures are responsible for the endogenous cycle within the vertebrate, while the recurved vermiform type carries on the life-history in the intermediate host (**15**). It appears that in H. nicoriæ, at all events, the schizonts (in both types of schizogony) are bean-shaped when they enter upon the process of schizogony (see figs. 10 and 19). I am inclined to think, however, that too much importance has been attached to the difference in shape between the recurved and bean-shaped individuals. The recurving is an appearance caused by the growth in length of the parasite inside its capsule, and there seems to be evidence (fig. 8A) which goes to show that the recurved part is capable of being reabsorbed as the parasite increases in width. It is therefore not improbable that certain of the schizonts are really derived from the vermiform individuals.

Although doubly and trebly infected corpuscles are to be seen, I have never come across any trace of binary fission nor of any process that could reasonably be interpreted as conjugation within the corpuscle. Hahn (**8**) has recently described this process, but I have not been able to corroborate his results.

Schizogony.—Two quite different types of schizogony occur in the vertebrate host. The one takes place in the lung, each schizont giving rise to a very large number (about seventy) of large merozoites. The other takes place in the circulating blood-corpuscle, each schizont producing six to eight quite small merozoites.

Schizogony in the Lung.—The first stage is shown in fig. 19, and is from a section of the lung; it represents a bean-shaped hæmogregarine, rather larger in size than those found in the blood-stream. There is a delicate envelope round the creature, the protoplasm is rather granular, and there is a single nucleus with the chromatin arranged in small irregular grains. The hæmogregarine is not contained

in a blood-corpuscle, but is apparently lying free in a capillary of the lung. The schizont now increases immensely in size, and the nucleus multiplies by successive divisions. The mitosis is of a very simple type; the amount of chromatin seems to augment by division of the granules, the nucleus becomes slightly elongated, the central body divides, and the strand of staining material which connects them appears to play the rôle of a simple spindle. The chromatin granules now become loosely grouped about each new central body, and the connecting strand disappears. From the scarcity of division-figures one is inclined to think that this primitive mitosis must take place very rapidly (figs. 16, 21).

During these processes of growth and nuclear multiplication the shape of the body is maintained, and there results a very large bean-shaped or sausage-shaped organism surrounded by a membrane. It is circular in section (figs. 21–23), and contains a large number of nuclei; I have counted about seventy, but the number appears to vary. A point of some interest is that very little, if any, diminution takes place in the size of the nuclei; it will be observed, also, in the figures that they are evenly distributed through the cell-body and not arranged at the periphery.

The protoplasm finally segregates round the nuclei, and there are formed a corresponding number of merozoites, which still lie within the envelope. They are presently set free as sausage-shaped hæmogregarines of 6 to 7·5 μ in length, that is to say, only little below the average size (8 to 10 μ)[1] of the hæmogregarines seen in the blood. They have usually rather regular nuclei of the rounded or slightly elongated type.

Schizogony in the Blood-corpuscle (figs. 10–16).—In the blood of practically all the infected tortoises examined multinucleate hæmogregarines were found in greater or less numbers. These forms may show any number of nuclei up to eight; generally, however, they do not show more than six. From a study of the early binucleate phases it is clear that these specimens arise from bean-shaped hæmogregarines (figs.

10 and 11).[1] The parasite remains inside the blood-corpuscle (fig. 11 is a case where the creature has been liberated mechanically in the making of the film), and does not undergo any increase in size. Finally, the protoplasm segregates round the small, slightly elongated nuclei, and a corresponding number of little falciform merozoites are formed inside the original envelope (figs. 14 and 15). This stage is rather difficult to find and must be of short duration, as it is somewhat rare in comparison with the number of multinucleate creatures to be found in the blood. In the cases I have found the number of merozoites is six, but I should expect that eight may sometimes be formed, as rare stages with more than six nuclei are to be seen (see fig. 13). Schizogony stages of this type occur in blood from any part of the tortoise. The merozoites, which are much smaller ($4\,\mu$) than those formed in the lung, finally escape and penetrate into another blood-corpuscle, where they proceed to grow. It is unfortunately almost impossible to trace the subsequent career of these young forms with any satisfying measure of certainty. There are, practically speaking, no distinctive features to lay hold of, and once they have increased in size there is nothing to distinguish them from other forms. The impression I have gained in my attempts to follow their development is that they grow into a compact bean-shaped creature of no great size (see figs. 4 and 7). The nucleus is inclined to stain deeply, and is composed of separate granules, which may be arranged irregularly or in a circle—the latter is on the whole the more common. Beyond this point I have not been able to trace these forms; I was always working with natural infections, which appeared to be of a chronic

[1] The question arises as to whether these bean-shaped forms which give rise to the schizogony in the peripheral blood are derived from the vermiform type. The evidence to be drawn from the infections of H. nicoriæ which I examined is very inconclusive. In H. vittatæ, a form parasitic in the tortoise Emyda vittata, however, the recurved type appears only relatively late in the infection, and I am therefore inclined to think it is associated with the later periods of schizogony and possibly with the process as it occurs in the peripheral blood.

type and generally of long standing. It is obvious that only by following the successive stages of the infection in a previously clean tortoise can points like this be really conclusively determined.

Interpretation of the Two Types of Schizogony.—There are three views which might be put forward in explanation of the facts: (1) That the schizogony in the lung with the large merozoites gives rise to the female gametes, and the schizogony in the blood-corpuscles to male gametes. This view is, I think, inadmissible, as it is very unlikely that the small male gametes should be produced in such small numbers, namely six to eight to one parent individual, while the female gametes are produced in large numbers—about seventy to one parent individual.

(2) The second, and I think more probable, explanation is that the schizogony in the lung is the endogenous asexual multiplication, and that certain of the merozoites thus formed proceed in turn to form gametocytes by the schizogony in the blood-stream.

(3) A third quite plausible explanation is that the schizogony in the lung is brought about by the newly injected parasite—that is to say, it is the first activity of the hæmogregarine upon arriving in the vertebrate host. Miller's (9) account of Hepatozoon perniciosum, Chagas' (5) work on Schizotrypanum, and Aragao's (1) on Hæmoproteus columbæ furnish parallels for such an interpretation. On this view the schizogony in the blood-corpuscle would be the later, and, so to speak, chronic process of multiplication, which would at some period culminate in gamete formation.

I think the evidence is strongest in support of the second view (2) put forward, namely that the schizogony in the lung is the asexual multiplication, and that in the blood gamete-formation. A somewhat important point against view (3) is the fact that the schizont in the lung does not appear to penetrate a lung-cell, which one would expect it to do did it arrive in the lung as a free vermicule (sporozoite). Moreover the possession of an envelope in so early a stage as that shown

in fig. 19 strongly suggests that it has had an endo-corpuscular existence. I have never seen any sign of the parasite reaching the lung by being engulfed by leucocytes, and I am therefore inclined to think that the schizonts in the lung must have come from the blood-corpuscles.

IV. Stages in the Leech.

Before giving an account of the stages of Hæmogregarina nicoriæ observed in the leech, it will, I think, be well to describe the more important features of the leech itself.

The form in question belongs to the Rhynchobdellid genus Ozobranchus. Mr. W. A. Harding, to whom the leech was sent for identification, found that it belonged to a new species, and called it Ozobranchus shipleyi. It is a small aquatic form carrying a row of feathery gills on each side of its body. The creature rarely reaches more than about one third of an inch in length even when fully extended. Generally speaking, it is found attached to the tortoise at the back of the neck, round the sockets of the limbs, and more rarely upon the ventral side near the throat. The leeches have a tendency to assemble together in groups—a habit they preserve even when kept in a glass dish. The gills of the Ozobranchus are kept in constant motion, and the animal dies if left out of water for any length of time. I was not very successful in getting the leeches to live for long in captivity, nor was I able to discover exactly what was amiss in the conditions to which they were exposed. Possibly the smaller quantity of water rose to too high a temperature. Leeches are usually very hardy and live well in captivity. I had no difficulty in keeping Pœcilobdella alive in Ceylon for months. I have often observed, however, that newly fed specimens are much less resistant than fasting individuals, and this seems true of a number of different species of leech. Almost all the Ozobranchus I got were either in the act of feeding or newly fed, and therefore in the least favourable condition. This leech seems to show a much closer adaptation to its host than generally

obtains among the group. Thus it was never found upon Emyda (the milk tortoise) living in the same lake with the Nicoria, nor upon the siluroid fish Saccobranchus, nor upon the water-snakes which shared the same habitat. Even in an area so restricted as a well, these leeches were only found to infest the Nicoria. Moreover, Ozobranchus lays its eggs upon the carapace of the tortoise; they are of a dark brown colour, closely resembling that of the tortoise, and are so firmly cemented on that it requires a knife or some fairly sharp instrument to detach them. It appears that the leeches move readily enough from one tortoise to another, but it is difficult to make out exactly how they are adapted to the terrestrial night-wandering of their host. The Nicoria spends all the day sleeping in the water and comes to land to prowl around at night, so most likely the leeches feed during the day and drop off at night. Generally speaking I got more leeches from nicoria caught in the evening, but there were, however, some exceptions to this; presumably these were cases where the tortoise had spent the night either in the water or in a damp place. Ozobranchus is capable of executing rather feeble swimming movements, and, in addition, can creep around upon its suckers in the usual way. The time taken to digest a meal seems to vary from about three to seven days, according to the size of the leech.

In Ozobranchus shipleyi the proboscis leads into the crop, which is a wide, very extensible sac dividing into two large lobes at its lower end. The intestine opens from the crop at the point where the division takes place. The upper end of the intestine, which is rather wide, shows four long diverticula on each side (see fig. in text, p. 753). This wide part of the intestine terminates in a kind of chamber which opens by a narrow communication into a simple coiled tube, which leads to the exterior at the anus. For some reason the most infected part of the gut wall is almost always this chamber at the end of the wide intestine. The accompanying diagram, which was made from reconstructions of sections by the glass-plate method, shows the relations of the various parts of the ali-

mentary tract. The cells lining the intestine are very large and richly ciliated; their protoplasm has a strong affinity for all nuclear stains, including the red element in Twort's stain. The nuclei are very large and reticulate, often showing several karyosomes.

The stages of the parasite in the leech had to be studied for the most part upon section material; sublimate acetic and Flemming's fluid were the fixatives used. The leeches were usually placed between two slides, so as to prevent undue

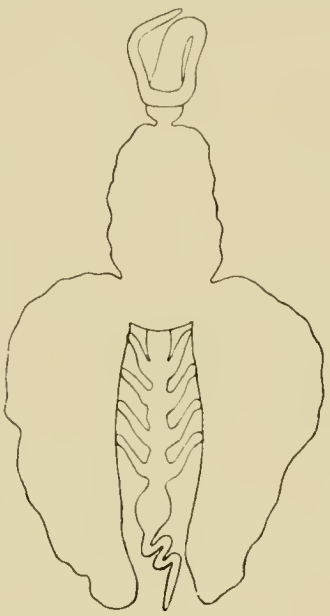

Diagram of the alimentary tract of Ozobranchus shipleyi.

retraction. As regards staining, Delafield's hæmatoxylin, Twort's stain, thionin, methyl-blue eosin, and Mayer's hæmalum were all used with good effect, hæmalum and Delafield being the most generally useful. Heidenhain's iron-hæmatoxylin was quite impossible, as it darkened the whole intestinal region so intensely that, long before that region was sufficiently colourised, the remainder of the section was completely bleached.

I am indebted to Mr. Peter Jamieson for the skill with which he has cut the many sections required.

I may mention in passing that the intestine of the leech is,

as a general rule, extraordinarily free from bacteria, schizomycetes, etc., and although a total of about 150 leeches were examined, I never found them to contain any flagellates, or, indeed, any protozoan parasites other than the hæmogregarine. A large number of observations upon live material from the leeches were made in the hope that the sequence of the processes might be followed by direct observation, but this proved to be impossible, as the development occurs in the tissues of the leech.

The blood upon being taken up by the leech is stored in the large crop, where the blood-corpuscles undergo a gradual degeneration. The blood passes in small quantities into the intestine, where it is digested and absorbed. Blood-corpuscles are never found, nor even their nuclei, in a recognisable state in the intestine, and this holds good even in the case of a newly fed leech. A large number of live observations were made, but no motile hæmogregarines were ever found in the crop. This particular hæmogregarine appears to be digested out of the corpuscle (figs. 25 and 26), and only to become motile when it passes into the intestine. I am persuaded that this cannot be universal amongst hæmogregarines; so many species react almost instantly to the mere shedding of the blood that I expect in other cases the parasites will be found to become motile at once upon being taken into the intermediate host. Motile hæmogregarines are to be found in the intestine at intervals all through the digestion, but except in cases where the blood is very rich in parasites, there are never a very large number present at one time. The hæmogregarine never makes any attempt to attack the wall of the crop.

A number of hæmogregarines seem to degenerate in the crop (fig. 27), but degeneration stages are only rarely found in the intestine; it seems to fare with these, as with the blood-corpuscles, that they disintegrate before reaching the intestine. So far as my observation goes, neither the large bean-shaped forms nor the completely recurved individuals are to be recognised in the intestine. The individuals which

are met with in this situation have a round or oval nucleus with the chromatin grains fairly regularly arranged (figs. 28–34), and seem, as far as morphological features are concerned, to be the motile phases of such types as are shown in figs. 1–3 and 9A from the blood of the Nicoria, and figs. 21 and 26 from the crop. The protoplasm of the hæmogregarines have very little affinity for most stains, and this is particularly true of the stages in the leech.

The motile creatures carry out movements of flexion and also of contraction and extension; in addition to this they can glide by means of very shallow undulations passing down the body. This constricting motion, as in analysis it really is, is most strikingly seen in H. leschenaultii (a hæmogregarine from Hemidactylus leschenaultii), but the difference is purely one of degree.

The faculty of contracting and extending the whole body shown by the motile forms of H. nicoriæ is a disturbing factor when an attempt is being made to divide the parasites into different categories. After much searching, I have come to the conclusion that the only distinction between the parasites while still in the lumen is one of size, and I consider this to have practically no value when one remembers the capacity of the creature for stretching, and the great difficulty in getting a correct idea of bulk in an animal of this type. The drawings have been made from sections, and here one has the additional danger of not always getting the animal in a perfectly horizontal position.

It was noticed not infrequently in the live specimens from the intestine that two equal individuals ranged themselves side by side, but complete fusion was never observed. In the sections this association in couples was again found (see fig. 35), and the individuals showed no differentiation. Here, also, stages indicating complete fusion were not seen; only the two cases figured (figs. 36 and 37) were observed, and as both these are cut obliquely they are not particularly convincing. I therefore think that if appearances such as those shown in figs. 35–37 relate to conjugation at all, they

are only instances of (perhaps precocious) association. After a time (figs. 38–41) the hæmogregarines penetrate the intestinal wall, where appearances quite different from those just described suggest conjugation of a type closely resembling that found by Siedlecki (**14**) in Adelea ovata, and by Perez (**10**) in Adelea mesnili. Fig. 44 gives a picture of an early stage; the macrogameta has become differentiated as a large rounded organism with a nucleus in which the chromatin is beginning to form a rather diffuse mass instead of being arranged in the definite granules seen in the motile phase. The nucleus of the microgametocyte is very compact, and stains deeply, the protoplasm has not fused with that of the microgamete, nor does it appear to do so subsequently.

From appearances such as fig. 45, the microgametocyte nucleus seems to divide into three or four, of which two or three, as the case may be, remain outside and degenerate; they sometimes persist for a long time, and are to be seen forming a dense mass of chromatin on the edge of the sporocyst (see figs. 49, 51, 52). The division of the nucleus of the microgametocyte into four is probably the more normal condition, the cases where three are formed being most likely due to the suppression of one of the divisions. One of the four microgamete nuclei thus formed appears to pass into the protoplasm of the macrogamete; unfortunately quite clear pictures of the fusion of the gamete nuclei and the first division of the zygote nucleus were not found. Fig. 47 B shows a condition suggesting the latter stage, but in view of certain reactions on the part of the host-cell to be noted later, I do not feel perfect confidence in this interpretation.

It is quite impossible to pass over these appearances without noting their very probable significance as conjugation and their close resemblance to the fertilisation of Adelea; at the same time I am fully aware of important gaps in the series. Great caution is required in interpreting these appearances, as degenerating hæmogregarines are occasionally found in the gut wall. Moreover, the host-cell seems sometimes in

strong infections to react to the presence of the parasite by the formation of internal masses resembling the mucoid globules described by Leger and Duboscq (8 A).

Formation of Sporozoites.

The further development of the parasite culminates in the formation of eight sporozoites. A membrane is secreted round the protoplasm, forming a kind of cyst-wall, but it appears to be thin and not very resistant. Fig. 46 shows an early stage where there are only two nuclei present. Subsequent divisions occur, and appearances such as fig. 48 are produced, where the larger nucleus at one end of the creature is preparing for division. Finally (see figs. 49–53), the protoplasm segregates round the nuclei, and there are produced eight individuals; these when fully developed show considerable resemblance to the free motile forms found in the lumen of the intestine, and are of much the same size. The sporozoites are set free in the wall and pass out into the blood-spaces (see fig. 47 c, 54–56), where they can be distinguished from the corpuscles of the leech by their shape and characteristic nuclear appearance.

There is a well-marked correlation between the processes of digestion in the leech and the condition of the parasite. In a recently fed leech the free motile forms are numerous in the intestine but no multiplicative stages are to be seen in the wall. Later on the hæmogregarines have penetrated the wall, but only the earlier stages are present. Still later ripe cysts with fully formed sporozoites are found in considerable numbers in good infections. Quite late towards the end of digestion, when the crop is empty, the sporozoites have for the most part escaped into the blood-spaces, and the intestinal wall is once more almost free from parasites.

I have not been able to carry my investigations beyond this point, and cannot say by what means the hæmogregarines are passed back into the blood of the tortoise. In spite of much searching I have never found motile stages of the para-

site in the proboscis, nor do they appear in this region in the sections.

V. General Remarks and Conclusions.

When the foregoing account was all but complete, I received Dr. Reichenow's (12) interesting preliminary note on H. stepanovi. The results I have obtained coincide in all essential points with those of Reichenow, and the evidence he has obtained upon the question of conjugation is much more conclusive than that brought forward by myself, as he has figured the first two divisions of the zygote-nucleus. The type of conjugation is clearly the same in the two cases. The only point of divergence in the two life-cycles is the schizogony in the vertebrate host; in H. stepanovi this takes place in the bone-marrow and always occurs inside the blood-corpuscle, the number of merozoites not exceeding twenty-four. This difference is the main justification for preserving the species name of H. nicoriæ.

There is scarcely a single point in the development of H. stepanovi as described by Siegel (13) which is in agreement with the results obtained by Reichenow, or with what I have myself observed in H. nicoriæ. I have never seen the formation of the minute microgametes, nor the sporulating stages in the blood-spaces of the leech, nor the worm-like sporozoites which he describes. It would appear that this worker must have been dealing with conditions differing widely from those presented by the leeches I examined.

It will be observed that the life-cycle of H. nicoriæ differs in one or two points from that of Hepatozoon perniciosum, the hæmogregarine of the rat, described very completely by Miller. The most important divergence occurs in connection with conjugation and the formation of sporoblasts, which in turn produce sporozoites. The sporozoites never become motile in the mite, and the parasite returns to the rat by way of the alimentary tract when the rat eats the mite.

The life-cycle of H. nicoriæ at once recalls the processes observed in Coccidia, but there are two points of difference which are, I think, important as diagnostic characters. Firstly, at no stage does H. nicoriæ show in its nucleus the karyosome so characteristic of the coccidia; secondly, the sporozoites are not enclosed in a resistant cyst, and become motile within a relatively short time after they are formed without the stimulus of transference to another host-individual. In all the coccidia hitherto described the sporozoites remain dormant, until by one means or another they pass to the exterior, and are taken up by another individual of suitable species where the sporozoites are set free. As regards the question as to whether the stages in the leech might not belong to an independent parasite, and have no connection with H. nicoriæ, the following points may be urged: The close correspondence between the stage of digestion and the development of the parasite, the strong morphological resemblance between such stages as those figured in figs. 1, 2, 3, 9A, 25, 26, 28–34, 38–41, 51, 54–56, derived respectively from the blood of the tortoise and different parts of the leech, and the apparent absence of the parasite in leeches taken from uninfected tortoises. Lastly, on the hypothesis that the stages in the leech are independent of those in the tortoise, the only other group in which the forms from the leech could be placed is that of the Coccidia. The points of divergence noted in the preceding paragraph are, I think, sufficiently important to distinguish them from any form belonging to that group. The point is, of course, one which could be determined experimentally when suitable material is available.

LISTER INSTITUTE,
April, 1910.

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EXPLANATION OF PLATES 32—41,

Illustrating Miss Muriel Robertson's paper on "Studies on Ceylon Hæmatozoa."

[The figures are all drawn with the Abbé camera at a uniform magnification of 2400 diameters.]

Figs. 1–24 represent stages from the vertebrate host Nicoria trijuga. Figs. 1–15 (with the exception of fig. 9) are stages from the blood treated by the wet method throughout and stained with Heidenhain's iron-hæmatoxylin. Figs. 16–24 are from sections of the lung stained with Mayer's hæmalum.

Fig. 1.—Bean-shaped hæmogregarine with circular type of nucleus.

Fig. 2.—Half-recurved specimen with circular nucleus; the chromatin is symmetrically arranged and the central granule is visible.

Fig. 3.—Bean-shaped specimen, the stain further extracted.

Fig. 4.—Small form derived from schizogony in the blood.

Fig. 5.—Bean-shaped specimen showing the chromatin in the nucleus arranged in two rings, one within the other. This creature has been set free mechanically in the making of the film.

Fig. 6.—Bean-shaped specimen with reticulate nucleus.

Fig. 7.—Small compact specimen.

Figs. 8 and 9.—Fully recurved (vermiform) specimens. Fig. 9 is from a dried film stained with Giemsa.

Fig. 8A.—Recurved specimen where the recurved limb is being reabsorbed.

Fig. 9A.—Half-recurved individual, rather broad, and with a large nucleus containing irregularly arranged chromatin.

Figs. 10–13.—Early stages of schizogony in the blood-stream.

Figs. 14 and 15.—Final stages of schizogony in the blood-stream. Fig. 15 has been decolourised to a greater extent than fig. 14; both show six merozoites.

Fig. 16.—Early stage of above type of schizogony from section of the lung; one of the nuclei is undergoing division.

Figs. 17 and 18.—Bean-shaped specimens from section of lung.

Fig. 19.—Earliest stage of schizogony in the lung.

Fig. 20.—Slightly later stage of schizogony; the schizont has increased in size and the nucleus has divided.

Fig. 21.—Still later stage; some of the nuclei appear to be preparing for division.

Fig. 22.—Multinucleate schizont cut across in section.

Fig. 23.—Late stage of schizogony in lung, the protoplasm beginning to segregate round the nuclei.

Fig. 24.—Fully formed merozoites; only a very few of the total number formed are shown in the section.

Figs. 25—56 represent stages in the leech Ozobranchus shipleyi Harding.

Figs. 25 and 26.—Non-motile stages from the crop.

Fig. 27.—Degenerating stage from the crop.

Figs. 28–32.—Free motile stages in the lumen of the intestine of the leech.

Figs. 33–37.—Association in the lumen of the intestine.

Figs. 38–41.—Early stages in the cells of the intestinal wall.

Fig. 42.—Precocious differentiation of microgamete.

Fig. 43.—Early stage of macrogamete.

Figs. 44 and 45.—Stages suggesting conjugation. In fig. 44 the microgametocyte is lying closely applied to the macrogamete. In fig. 45 the microgametocyte appears to be giving rise to the microgamete nuclei, one of which will fuse with the nucleus of the macrogamete.

Fig. 46.—Early stage of sporocyst showing two nuclei.

Fig. 47.—(A) Stage apparently representing a zygote; (B) stage showing what appears to be the first division of the zygote nucleus; (C) free sporozoites in the wall of the intestine.

Fig. 48.—Stage of above showing five nuclei, of which one is preparing to divide.

Fig. 49.—Sporocyst with eight nuclei: the protoplasm has not yet divided up; the rejected microgamete nuclei are still visible.

Figs. 50–53.—Sporocysts showing sporozoites.

Figs. 54 and 55.—Motile sporozoites escaping through the cells of the intestinal wall.

Fig. 56.—Sporozoite in blood-space of the leech.

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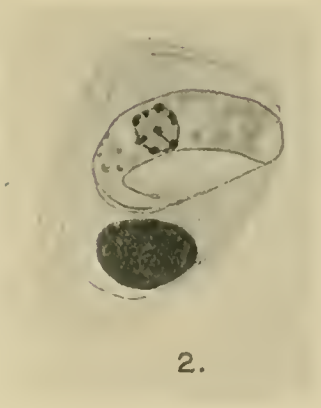

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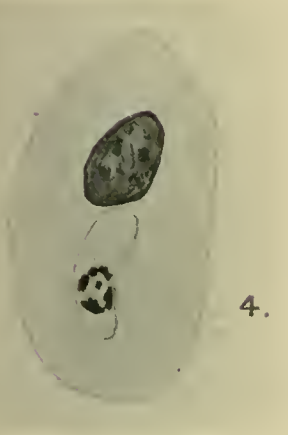

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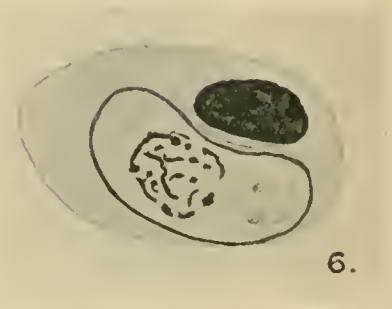

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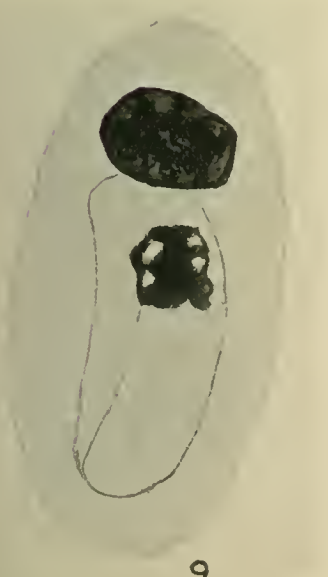

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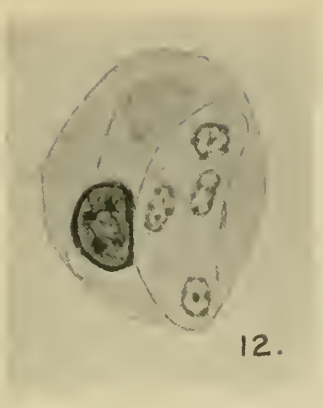

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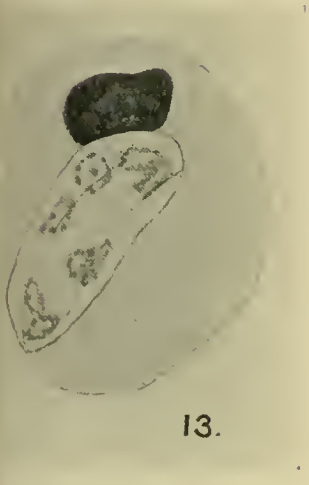

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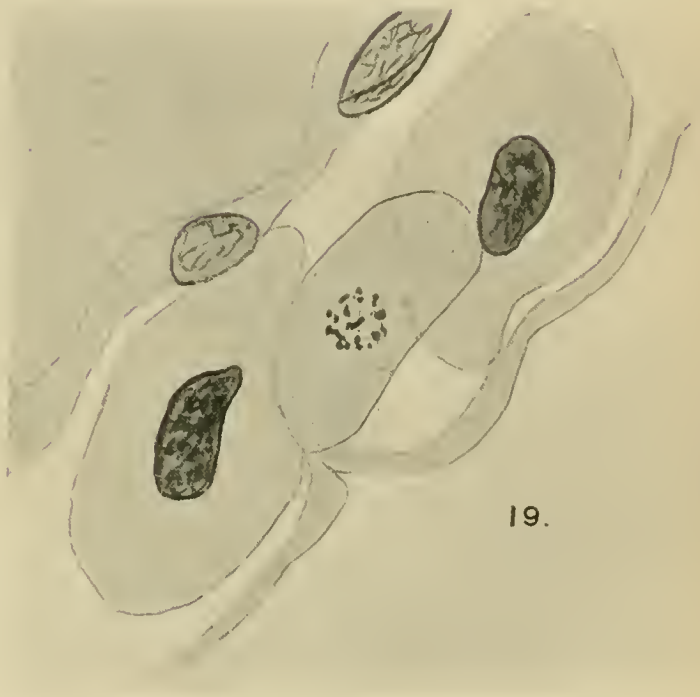

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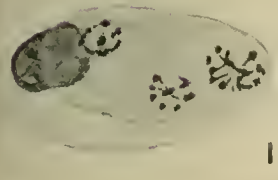

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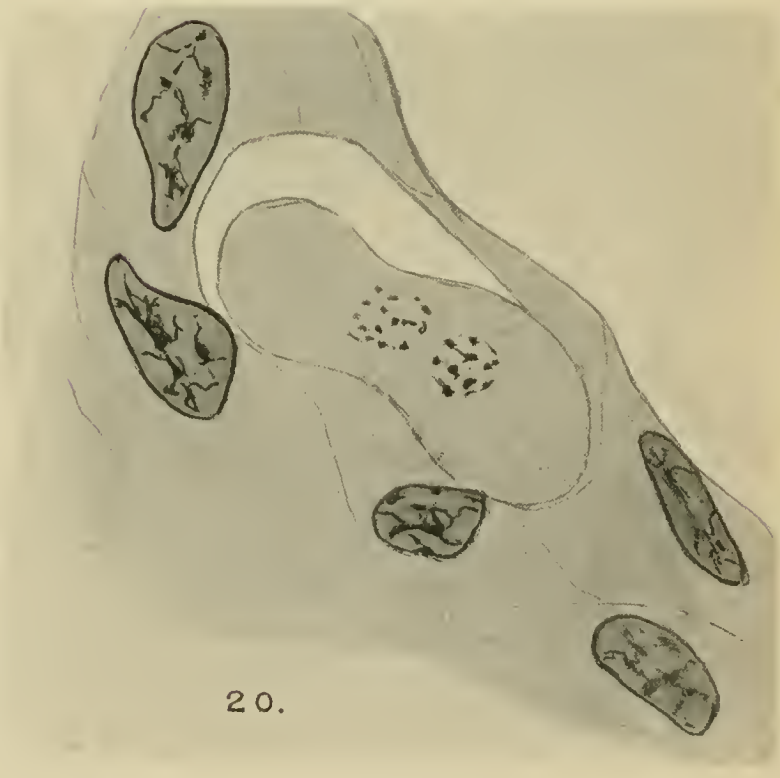

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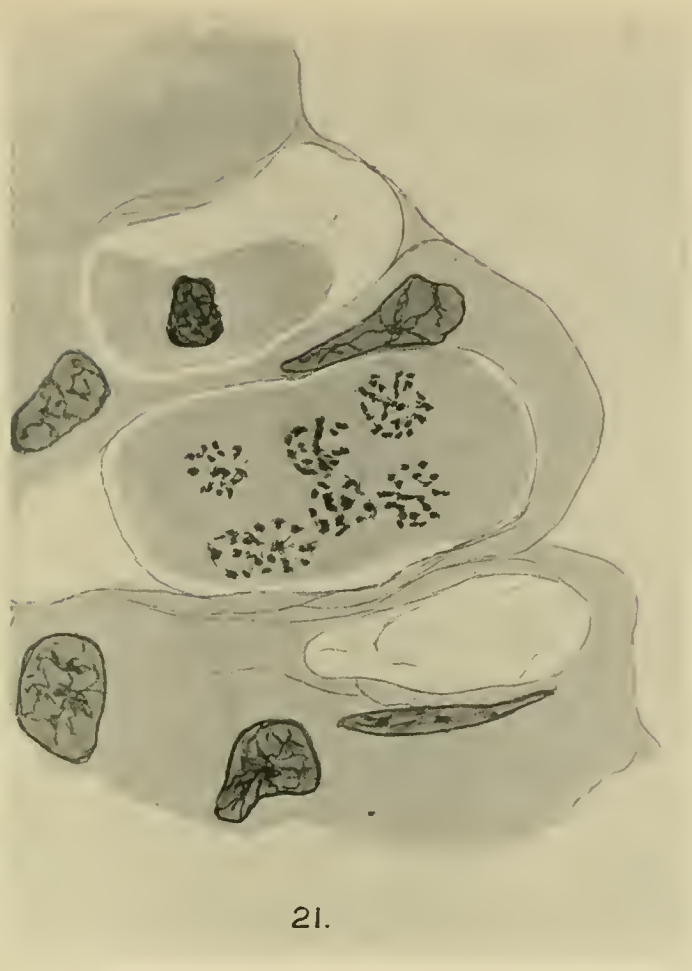

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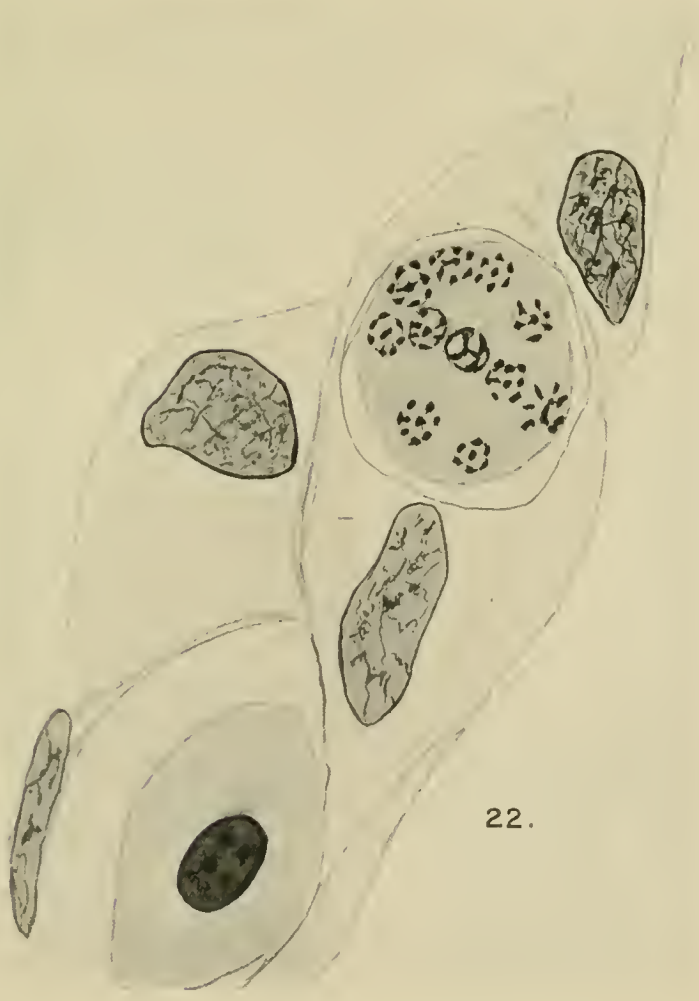

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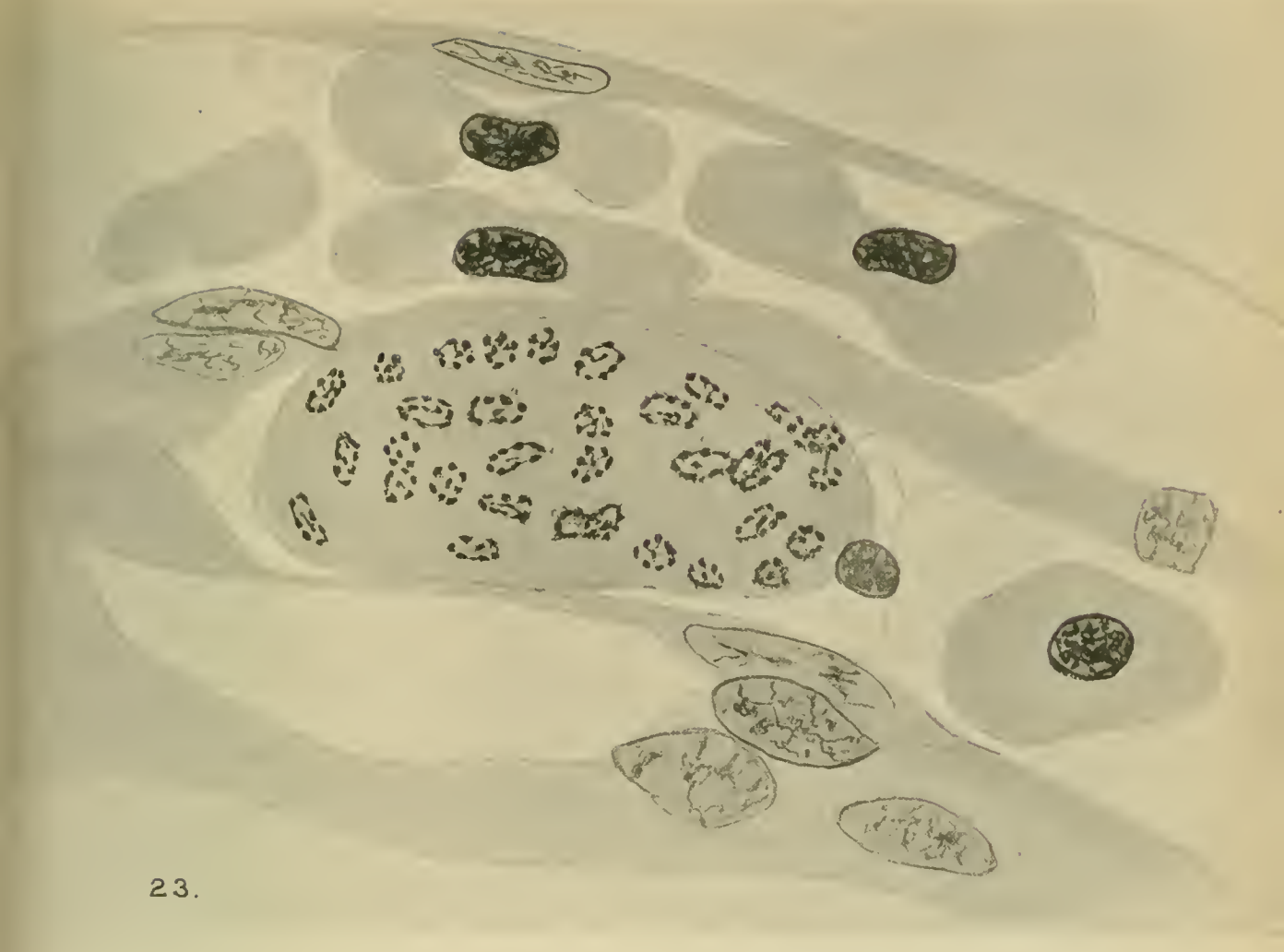

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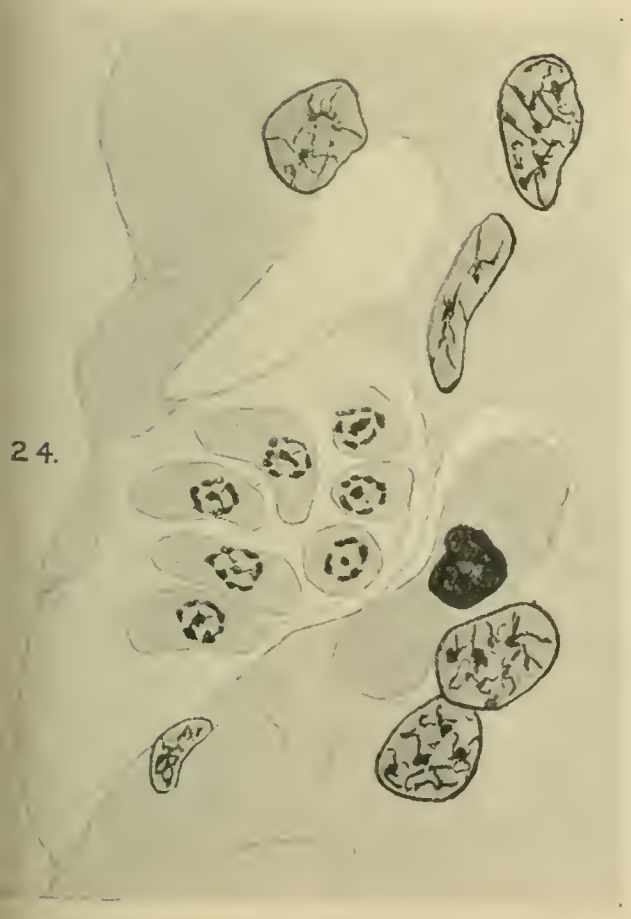

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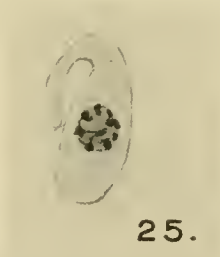

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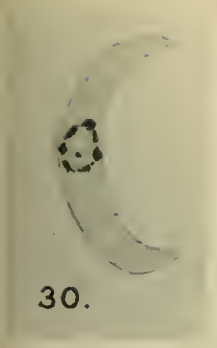

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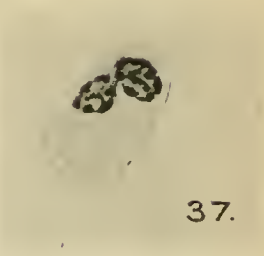

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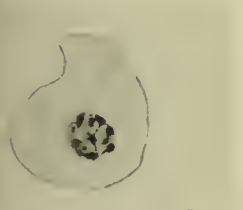

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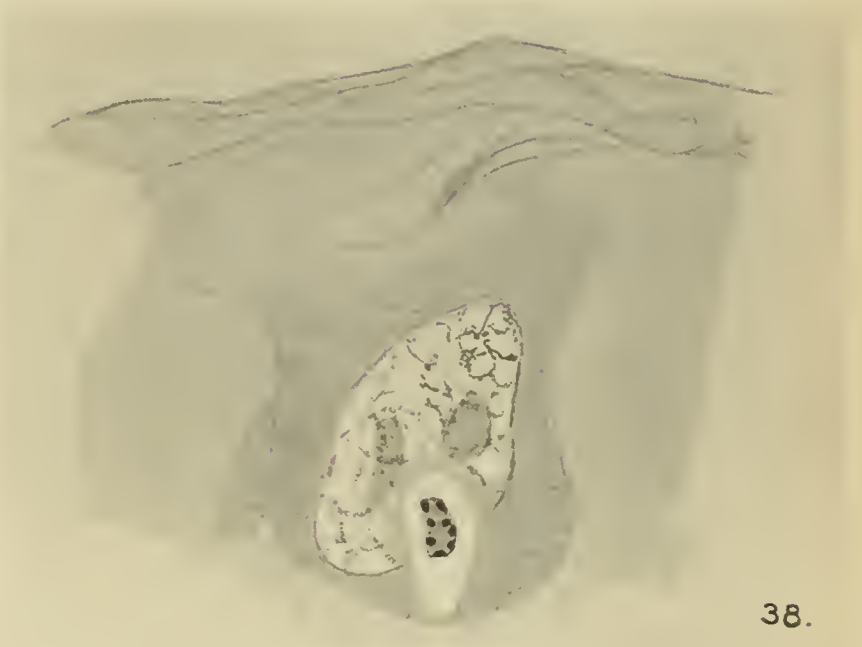

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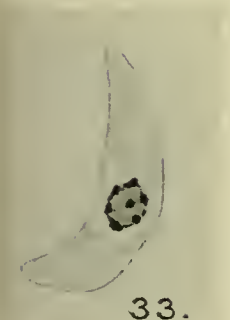

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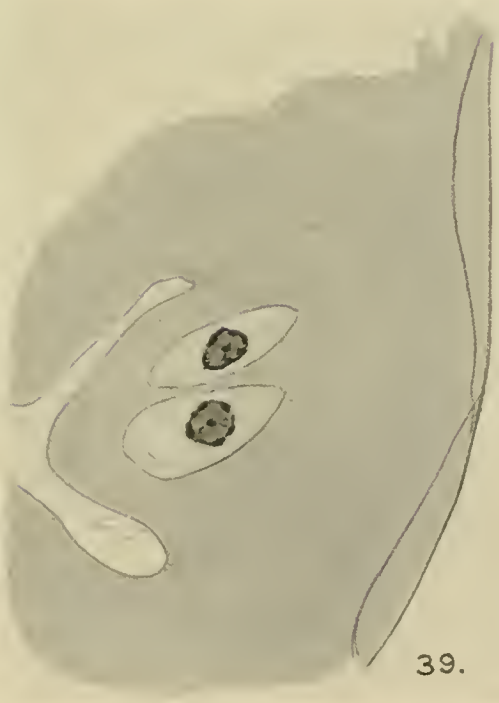

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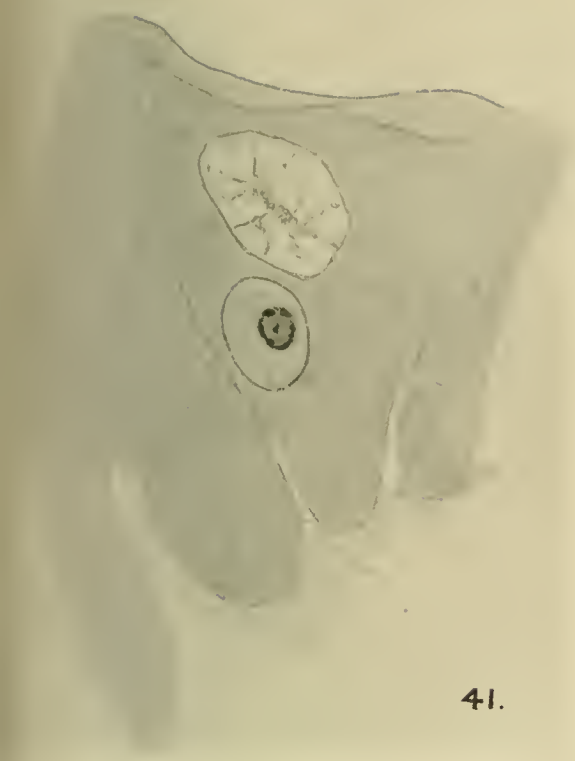

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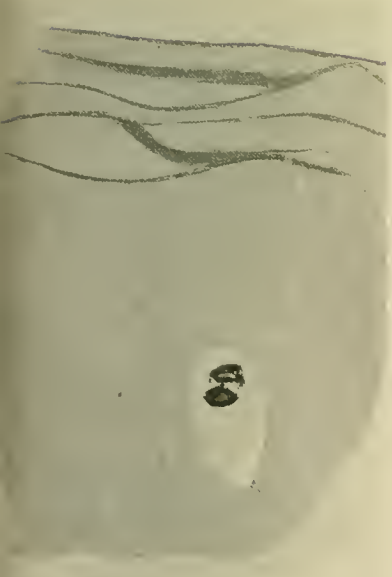

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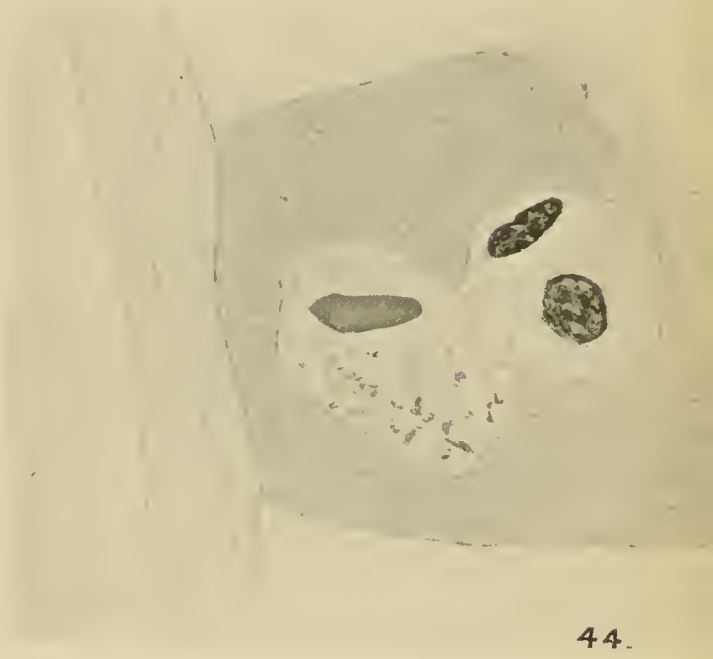

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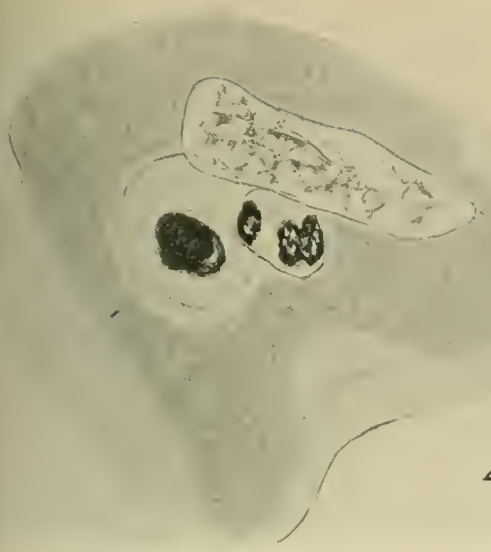

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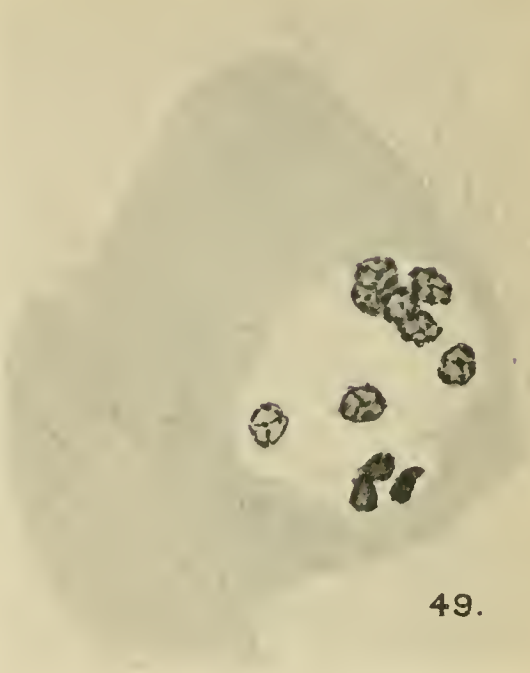

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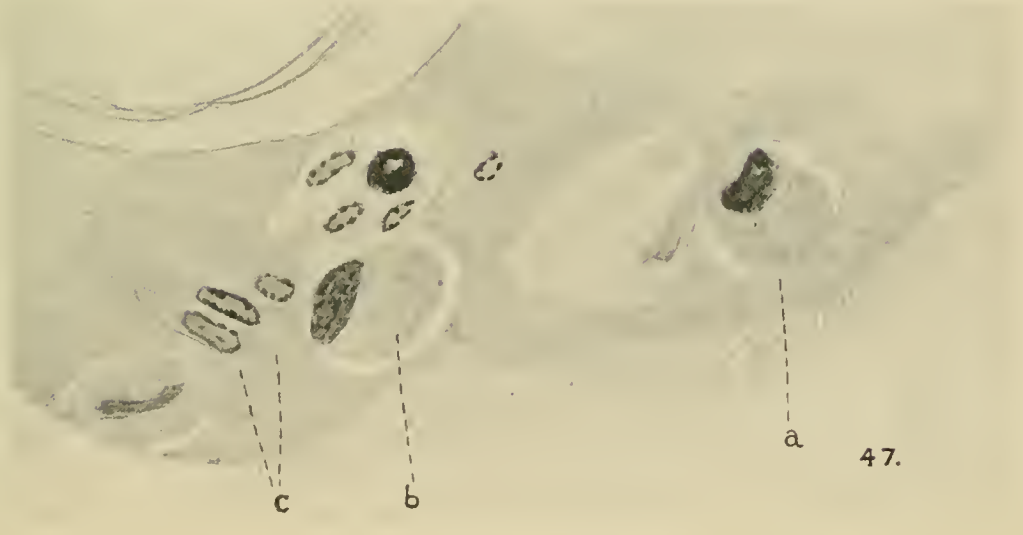

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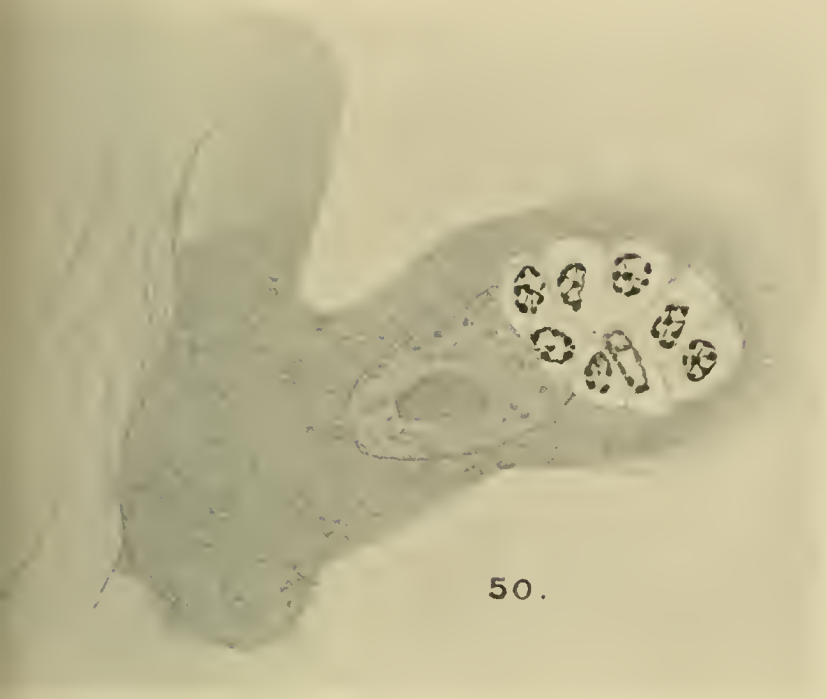

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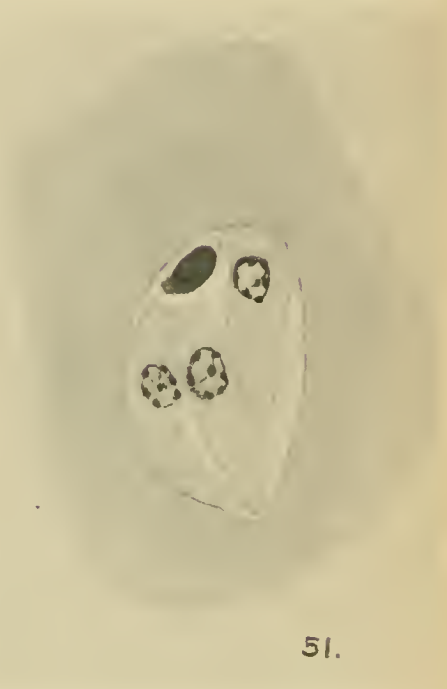

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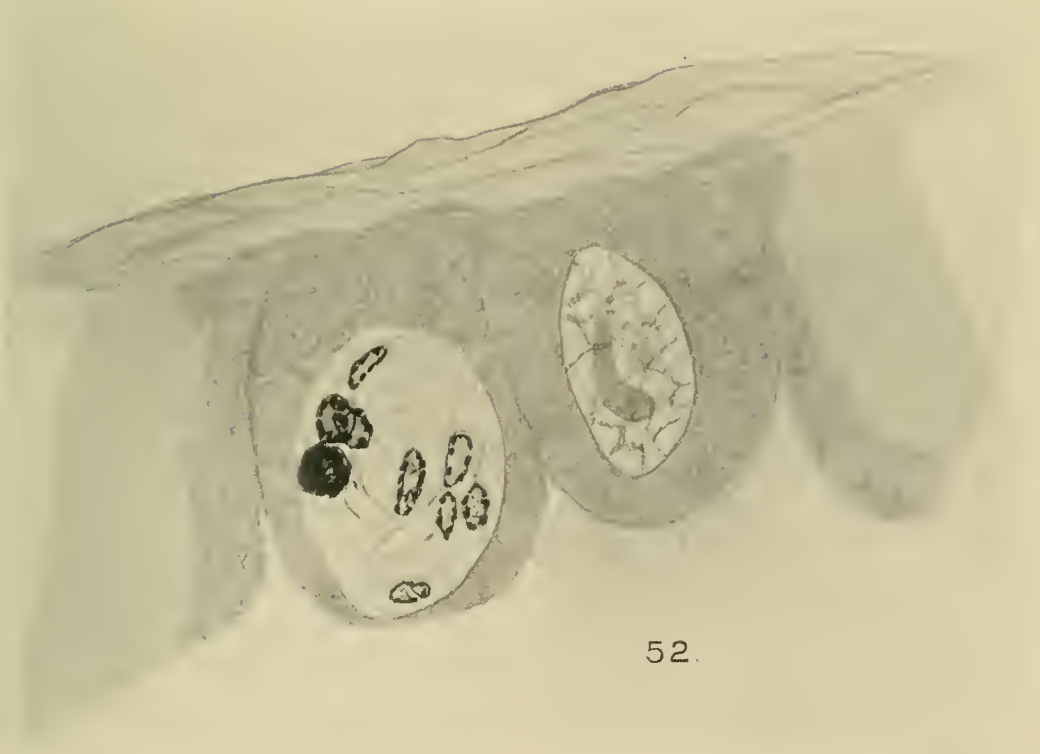

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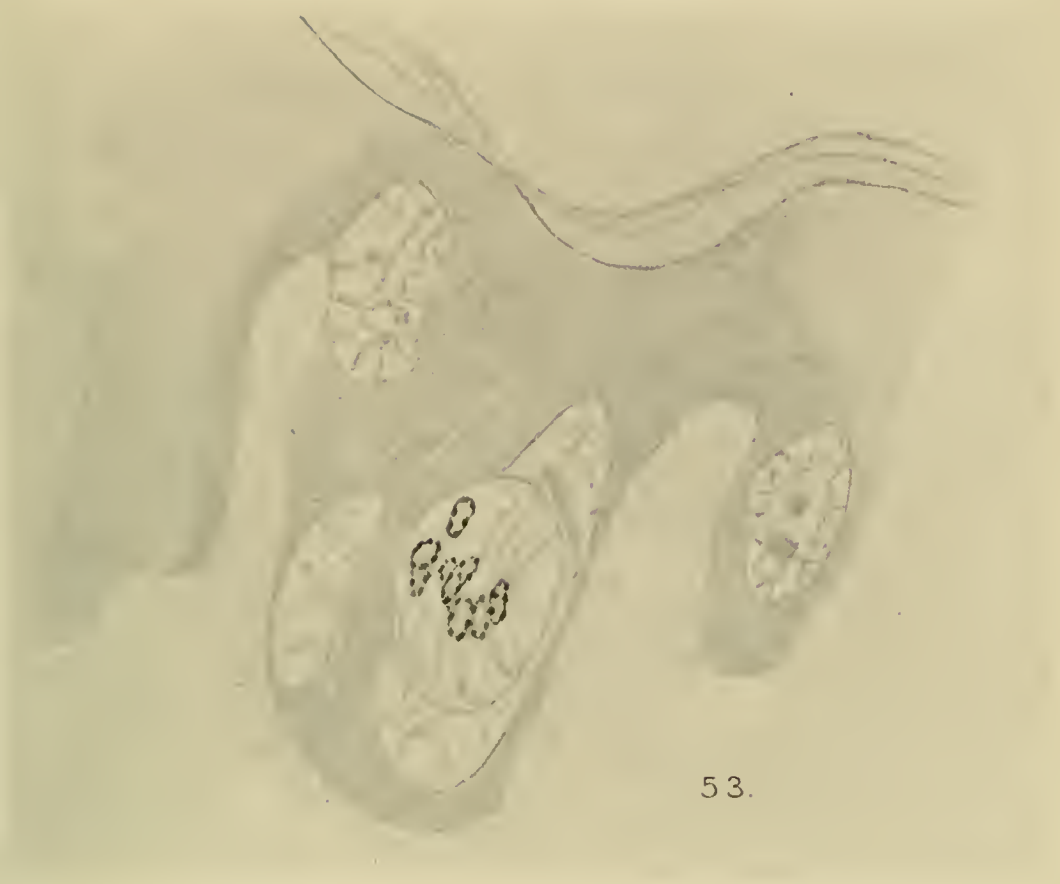

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On the Origin and Migration of the Stinging-Cells in Craspedote Medusæ.

By

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With Plates 42 and 43 and 5 Text-figures.

Contents.

1. Introduction.

Lewis Murbach (1), in 1894, definitely established the fact that the stinging-cells of the Hydromedusæ have the power of active movement in the tissues by the formation of pseudopodial processes from the cnidoblasts.

These observations were confirmed by K. C. Schneider[1] (2) who published a detailed account of the development of nematocysts in Agalmopsis and other Siphonophora, and in his paper stated emphatically that:

"Alle Nesselzellen der Siphonophoren enstehen an locali-

[1] Schneider, as early as 1890, pointed out the fact that developing nematocysts were excessively rare in the tentacles of Hydra, and suggested that they might be formed on the body of the animal. He did not, however, pursue the subject any further (*vide* "Bibliography," 3.)

sierten Bildungsherden, von denen aus sie in einem bestimmten Entwickelungsstadium als Wanderzellen auf die Verbrauchsstätten überwandern."

The subject has been recently revived by Jovan Hadži (4) in a remarkable paper in which he records his observations on the thread-cells of marine hydroids. Hadži's results are of the greatest interest, as he was able to examine the living tissues as well as preserved material. His main conclusions are as follows :

(1) The thread-cells of hydroids are not formed "in situ" but in the ectoderm of the cœnosarcal branches, where, on account of the thick perisarcal investment, they can obviously not become functional.

(2) When completely developed, except for accessory structures such as the cnidocils and the stalks, they migrate to the important nematocyst batteries on the tentacles. This migration can take place in two different manners. In simple forms, e.g. Campanularia, the thread-cells move actively by means of their pseudopodia, making their way between the ectodermal cells of the colony. In Tubularia however, they adopt a quite different method of locomotion : from the ectoderm of the cœnosarc they force a way through structureless lamella and endoderm into the cavity of the hollow stem, whence they are carried by the current caused by the flagella of the endoderm cells to the hydranths. Here the thread-cells re-enter the tissues and migrate actively by their own movements to the ectoderm of the tentacles.

In a recent paper, whilst describing the structure of the Egyptian lacustrine medusa, Mœrisia lyonsi (5), I called attention to the fact that large nematocysts were to be found in abundance among the endoderm cells of the manubrium. Being at a loss to account for their presence in this position I cut sections of a large number of specimens, careful examination of which convinced me that I was dealing with a case similar to that investigated by Hadži in Hydroids. As this phenomenon has not been described previously in

Medusæ, I have endeavoured in this paper to give as complete an account as possible of the origin and distribution of the nematocysts of this form.

The material used for this investigation was collected by Dr. Cunnington and myself in Lake Quruu, and was carefully fixed either with osmic acid or with hot corrosive sublimate. Sections were cut by the ordinary paraffin method and a number of stains were tried, the best results being obtained with hæmatoxylin followed by eosin; this produced an excellent double-stained effect, the eosin bringing out the nematocysts and rendering them most conspicuous. Borax carmine followed by picro-indigo-carmine was another good differential stain and iron-hæmatoxylin was useful when examining sections of the developing Medusæ. The work in connection with this paper was carried out partly in the Morphological Laboratory at Cambridge and partly in the Zoological Laboratory of Birmingham University. I wish to express here my sincere thanks to Professor F. W. Gamble, who very kindly read through my manuscript and made many valuable suggestions.

2. The Stinging-cells of the Adult Medusa of Mœrisia.

As mentioned above, a striking feature of the anatomy of this medusa is the presence of numerous thread-cells[1] in the endoderm at the base of the manubrium. At first it seemed possible to account for their occurrence in this unusual position by assuming that these stinging-capsules were used ones taken in by the jelly-fish together with its food. On careful consideration this view was found to be quite untenable, for—

[1] The nomenclature of the different parts of the stinging-cells is somewhat cumbrous and complicated; moreover, the various names have been used very loosely. In this paper I have employed the terms thread-cell or stinging-cell for the whole structure comprising the nematocyst (the actual stinging capsule), and nematoblast (the cell in which the former is embedded, and of which the cnidocil and the stalk are parts).

(*a*) The nematocysts found in the endoderm are always undischarged.

(*b*) Favourable sections show them to be accompanied by their nematoblasts.

(*c*) The nematocysts are never to be found near the free margins of the endoderm cells, but, for the most part, between the more basal portions of these cells near the structureless lamella.

These thread-cells can, obviously, not become functional in this position, and the only possible explanation of their occurrence here is that they are making their way from their place of origin to some battery where they can be of use.

At this point it may be well to review the distribution of stinging-cells in the ectoderm of the manubrium. The chief battery is situated around the mouth-opening; here the thickened ectoderm forms a circular lip crowded with nematocysts, and constitutes a powerful organ of offence (Pl. 42, figs. 3 and 4). The ectoderm of the remainder of the manubrium proper consists of a single layer of low, closely fitting epithelial cells with occasional isolated nematocysts; it is to be noticed that here, as well as on the oral lip, interstitial cells are completely absent. At the base of the manubrium is the broad stomach, the ectoderm of which is considerably thickened and forms the conspicuous gonad.

Interstitial cells and developing thread-cells being absent from the more distal parts of the manubrium, the question arises—Where are the nematocysts of the oral battery formed, and how did they attain their position in this region? An answer is, I think, afforded by the study of the distribution and arrangement of the nematocysts in the manubrial endoderm. The greatest number of these are to be found just below the region of the gonad, where, in most specimens, numerous thread-cells are to be met with among the large digestive cells of the endoderm. In this position one can usually find a number of dark-staining interstitial cells, some of which contain rudiments of stinging-capsules, and are obviously nematoblasts (Pl. 42, figs. 1 and 2).

In the more distal parts of the manubrium we find nematocysts to occur less abundantly, and their position in the endoderm is very regular, the longer axes of the capsules being parallel with the structureless lamella and their broader ends directed towards the mouth of the medusa (Pl. 42, fig. 3). Previous authors have shown this orientation to be characteristic of migrating thread-cells, and we must come to a similar conclusion; namely, that they are making their way from the base of the manubrium to the oral battery. This view is confirmed by an examination of the tissues of the mouth region, where one can often find thread-cells actually forcing their way through the structureless lamella to the oral battery. Here they take up their definitive position and develop accessory structures, e.g. cnidocil and stalk, from the nematoblast. A stinging-cell occasionally turns aside before reaching the oral region (Pl. 42, fig. 4), and passing through the lamella, forms one of the isolated nematocysts to be met with in the more proximal parts of the manubrial ectoderm.

The route followed by the thread-cells of the medusa is readily explained. These structures, when the nematocysts are completely developed, are of considerable size, whereas the ectoderm of the manubrium is very low, and, moreover, forms a very definite epithelium of closely fitting cells, between which the large stinging-cells could scarcely force a passage. We need, therefore, not be surprised that they adopt the much easier way between the large and loosely packed cells of the endoderm.

From the above account it appears, therefore, that in Mœrisia the nematocysts of the oral battery of the medusa are developed in the endoderm at the base of the manubrium; this does not necessarily imply that the nematoblasts are themselves endodermal in origin, as will be explained in the section of this paper which deals with the development of the medusa-bud.

In addition to that surrounding the mouth opening, the main nematocyst batteries of the medusa are situated on the

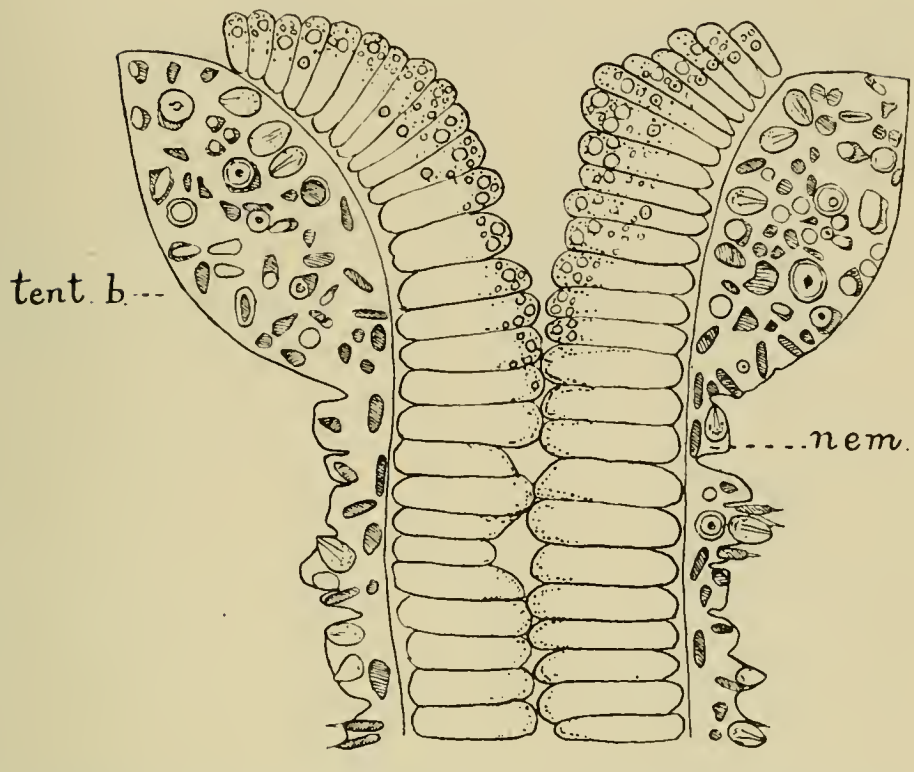
four perradial tentacles suspended from the umbrella edge. These tentacles are slender and of great length when fully extended; at their bases they are swollen to form the very conspicuous ocellar bulbs, each of which bears on its exumbrellar surface a bright red eye-spot. The tentacles are hollow, their cavities being continuous with that of the circular canal; the ectoderm is thickened at regular intervals to form conspicuous transverse rings crowded with nematocysts, and becoming very noticeable and almost bead-shaped when the tentacles are fully extended.

On examination of sections and maceration preparations of these organs, one is again struck by the almost complete absence of nematoblasts or other interstitial cells, and we are driven to the only possible conclusion, namely, that the stinging-cells have developed elsewhere and have migrated to the batteries on the tentacles. The large, eye-bearing bulbs at the bases of the tentacles immediately suggest themselves as possible nematocyst "factories," and sections of these structures show that such a function must be assigned to them (Text-fig. 1).

An ocellar bulb consists of a mass of thickened ectoderm crowded with small, irregularly shaped cells and nematocysts in various stages of development. The fully formed thread-cells are devoid of cnidocils or other accessory structures, and the capsules are never orientated so as to lie at right angles to the surface; we must, therefore, conclude that they do not become functional in this region. In the centre of the bulb the nematocysts lie in all directions, but near the base of the tentacle we find a distinct tendency for these organs to be arranged with their longer axes parallel with the structureless lamella, a position, as mentioned above, characteristic of migrating thread-cells.

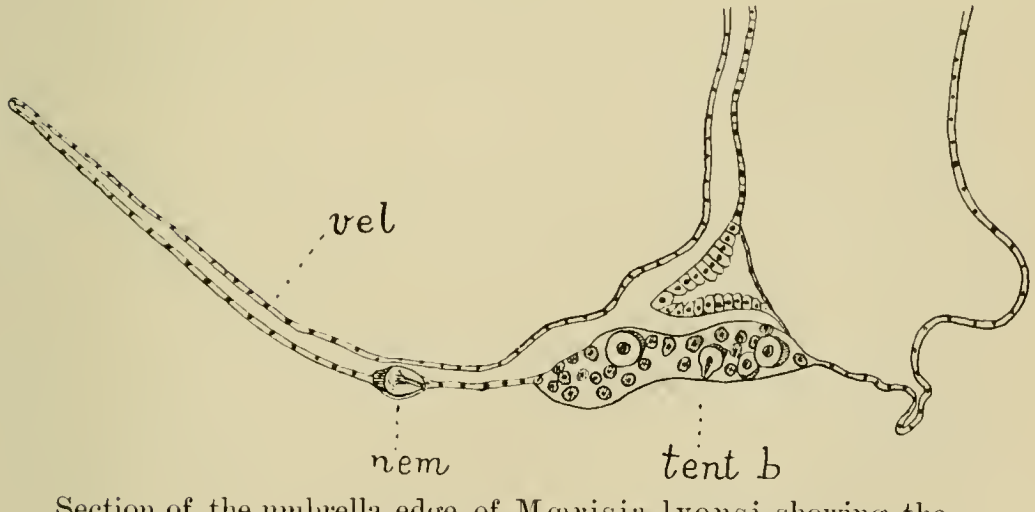
The above-mentioned facts lead us to the conclusion that the stinging-cells of the tentacles, like those of the oral battery, are not developed "in situ," but migrate into these organs from "factories" situated in a more central position on the medusa, in this case from the ocellar bulbs, whence a

TEXT-FIG. 1.



A longitudinal section through the ocellar bulb and the base of a tentacle of Mœrisia lyonsi. *tent.b.* Ocellar bulb. *nem.* Nematocyst migrating into the tentacle.

TEXT-FIG. 2.



Section of the umbrella edge of Mœrisia lyonsi showing the velum (*vel*) and part of an ocellar bulb (*tent.b.*) *nem.* Nematocyst migrating towards the edge of the velum.

continual stream of thread-cells are being poured forth. The majority of these are obviously on the way to their tentacular batteries, although occasionally one may wander into the velum, as shown in Text-fig. 2.

3. Migrating Stinging-cells in Other Medusæ.

In the preceding paragraph I have attempted to prove that the conspicuous bulbous swellings which occur so constantly as the bases of the tentacles of craspedote Medusæ have an important function besides that of bearing the ocellar sense-organs. In such craspedote Medusæ as are devoid of tentacle-bulbs, e.g. the Trachomedusæ and Narcomedusæ, we find that the edge of the umbrella is provided with a special thickened ring of ectoderm, containing stinging-cells, sometimes known as the "nettle-ring." Further, those forms in which the tentacles take their origin some distance from the margin of the bell on the exumbrellar surface are provided with special bands of nematocysts, called peronia, which connect the above-mentioned nettle-ring with the bases of the tentacles. These facts make it very tempting to assume that the marginal ring of nematoblasts replaces the ocellar bulbs in function, and reference to the figures of this organ, given by various authors, seems to show that this assumption is probable correct. It is a point which requires special investigation, and I will at present merely refer to the evidence which is at my disposal.

The Hertwigs' most accurate figure of the umbrella edge of Carmarina (**6**, Pl. iv, fig. 5)[1] shows the nettle-ring to be packed with thread-cells without definite orientation; at the base of the tentacle, however, a number of nematocysts are drawn arranged in such a manner that there can be little doubt that they are migrating from the marginal ring to the batteries on the tentacle. I have examined sections through the tentacles of a medusa of the same genus, and

[1] I should like to express my indebtedness to Dr. S. F. Harmer, F.R.S., for calling my attention to this figure.

these showed the same orientation of nematocysts as in the specimen figured by the Hertwigs. I have figured one of these sections (Text-fig. 3) chosen from a series in the Cambridge Morphological Laboratory; comparison with that of Mœrisia (Text-fig. 1) is very instructive.

TEXT-FIG. 3.

A longitudinal section through the base of a tentacle of Carmarina sp. *vel.* Velum. *nem.* Nematocyst migrating through the ectoderm of the tentacle.

Günther's figure of Limnocnida (7, fig. 6) shows that a similar migration of thread-cells must occur in that medusa.

4. The Development of the Medusa of Mœrisia.

As shown above, the nematocysts of the main stinging batteries of Mœrisia are formed in two quite distinct positions in the medusa: (*a*) The manubrial endoderm, (*b*) the ectoderm of the ocellar bulbs.

In order to properly understand the origin of these

different situations of the stinging-cell factories it is necessary to examine the development of the medusa in some detail.

Until recently the accepted view of the development of the gonophores of the Hydromedusæ was based essentially on L. Agassiz's observations on Syncoryne mirabilis, published in 1862 (8). His account of the process was confirmed by Hertwig (9), Weismann (10), and almost all later workers on the same subject, and is essentially that to be found in the majority of modern text-books. The following description of the development of the medusa of Bougainvillea is taken from one of the latter (18), and represents the prevailing ideas on the subject:

The medusa-bud makes its first appearance as a simple hollow bud formed by the evagination of the two layers of the mother-polyp. Multiplication of the ectodermal cells at the apex results in the production of a lens-shaped mass of small cells which sinks below the level of the superficial ectoderm, pressing the endodermal wall in front of it into the shape of a cup. This mass of ectoderm is called the entocodon (Glockenkern), and a cavity which appears in its interior is the rudiment of the subumbrella cavity. It is followed by an invagination of the superficial ectoderm, the wall between the new cavity thus formed and the subumbrella cavity being the future velum. Growth of this subumbrella cavity results in an approximation of the endodermal walls of the cœlenteron, and these ultimately fuse into an endoderm lamella except where the circular and radial canals are to lie. The upgrowth of the manubrium from the floor of the subumbrella cavity, the formation of the tentacles and the perforation of the velum and manubrium complete the formation of the medusa.

A. Goette (11) has recently made a thorough examination of the development of the gonophores of Podocoryne carnea and a large number of other hydroids, and has published a long and elaborate paper on the subject. As the result of his investigations this author concludes that the current views on the origin of these structures are quite

erroneons, and states that carefully cut series of sections of developing medusa-buds show that a double-walled cup of endoderm is not present at any stage; moreover, the four radial canals arise from four unconnected pouches of endoderm which grow out separately, although simultaneously, from the cœlenteron of the bud, and are completely independent of the entocodon. The endoderm lamella is formed later by the lateral extensions of the solid edges of these pouches, which finally fuse with one another. Again, an invagination of the superficial ectoderm does not take place and the forecast of the velum is present at a quite early stage, and is then represented by the flattened apex of the bud, where the superficial ectoderm and the distal wall of the entocodon come into contact with one another.

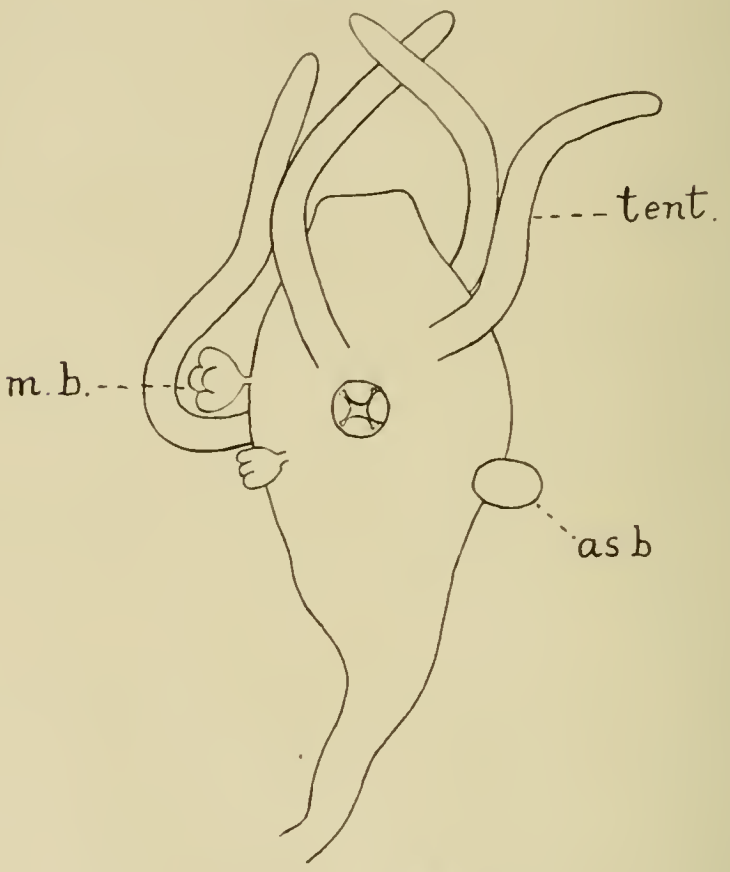
Goette's paper has not received (at any rate in this country) the attention which so important a communication deserved, and the only confirmation of his results is that of his pupil, Walter Richter (13), who, acting on his professor's advice, worked out the development of the gonophores in Rhizophora, Physalia, and other Siphonophora and described a similar origin for these structures in this division of the Hydromedusæ.

In my account of the anatomy of the hydroid stage of Mœrisia I did not go into this subject with any detail, but merely stated that the development of the medusa-buds seemed quite typical, the growth of the cavity in the entocodon causing the approximation of the endodermal walls of the bud.

The examination of a large series of sections during my investigation of the origin of the nematocysts has shown me that this statement was erroneous, and that the development of the medusa of this form agrees very closely with that of Podocoryne carnea as desribed by Goette. My error, like that of other writers on the same subject before Goette, was due to the use of optical sections, and partly to the examination of single sections of the buds instead of complete series.

In Mœrisia lyonsi the medusa-buds are to be found scattered irregularly on the broadest region of the hydranth between the bases of the tentacles (Text-fig. 4), thus differing in position from the asexual lateral buds, which are restricted in the majority of cases to the more proximal parts of the body.

TEXT-FIG. 4.



Outline sketch of a hydranth of Mœrisia lyonsi to show developing gonophores and a small asexual bud. × 30. *tent.* Tentacle. *m.b.* Gonophore with conspicuous ocellar bulbs. *as.b.* Asexual lateral bud.

The ectoderm of this region is somewhat deeper than in other parts of the hydroid, the boundaries of the large musculo-epithelial cells are difficult to detect, and the whole tissue is crowded with interstitial cells, for the most part nematoblasts, containing nematocysts in various stages of development. The endoderm consists of large vacuolated

digestive cells, between which are numerous characteristic gland-cells with coarse granular contents which stain deeply.

The first indication of a developing medusa-bud is to be traced in the ectoderm, an accumulation of interstitial cells causing this layer to project slightly outwards. The endoderm soon begins to take part in this bulging out of the tissues, and owes its increase in area chiefly to the proliferation of the large cells, but partly also to the accumulation of interstitial cells, which are to be found in the endodermal tissue in the region of a developing bud. These cells I believe to be ectodermal in origin, for favourable sections show occasional interstitial cells to migrate from the ectoderm through the structureless lamella into the endoderm. In this way a hollow, double-layered bud is formed (Pl. 43, fig. 5) by a process which cannot be called one of simple evagination, but in some respects resembles that of the formation of the early stages of the lateral buds in Hydra, as recently described by J. Hadži (**14**).

As long ago as 1891, W. B. Hardy (**15**) showed that in the early development of the gonophores of Myriothela phrygia there was a certain mixing up of endodermal and ectodermal cells to form a kind of blastema, and it seems probable that further investigations will prove that the production of a bud from the body of a hydroid is by no means so simple a process as has been made out by some authors.

The entocodon is next formed by the proliferation of the ectoderm at the apex of the bud, and consists of a small-celled plug of tissue between ectoderm and endoderm. Four pouches of endoderm are arising simultaneously from the cœlenteron; from them the radial canals of the adult are to be derived. Reference to fig. 6 will show that there is nothing of the nature of a double-walled endodermal cup in the bud, one side of the obliquely cut section showing a radial pouch, the other the contact of the entocodon with the superficial ectoderm.

It is to be noticed that this superficial ectoderm has not

changed in character and is identical in structure with that covering the hydranth, consisting of large epithelial cells, interstitial nells, and nematoblasts, with occasional nematocysts.

The independent origin of the four radial pouches of endoderm is still more obvious in figs. 7, 8, and 9, which are three sections in different planes of a slightly later stage. In the transverse section (fig. 7) the entocodon is seen to be roughly square in section, being in contact with the superficial ectoderm at the four corners (interradii); the four perradial pouches are thus completely separated from one another. A median longitudinal section (fig. 8) through the perradii at this stage shows, of course, two of the endodermal pouches separated by the hollow entocodon. As pointed out by Goette, it is from the examination of such a section, independently of others of the series, that the idea arose that a double-walled cup of endoderm was formed by the growth of the entocodon. A tangential section taken a short way on either side of this median section will naturally show a single pouch only, as illustrated in fig. 9. In this stage the forecast of the manubrium is already conspicuous, and is, of course, clothed externally by the proximal wall of the entocodon.

The four endodermic pouches continue their growth outwards to the very tip of the bud, and at their terminations push out the ectoderm, causing the formation of four perradial bulbous projections, which are the forecasts of the ocellar bulbs. A section, therefore, taken through a perradius gives rise to the false idea of an invagination of ectoderm towards the entocodon (Pl. 43, fig. 10). The four bulbs are very conspicuous features of the external anatomy of the medusa, even at this relatively early stage of development (Text-fig. 4).

The formation of the endoderm lamella is exactly as described by Goette for Podocoryne carnea; the central part of each endodermal pouch becomes a radial canal, the large cells at the edges growing out to form two solid wings of endoderm, which meet similar projections from the other

pouches at the interradii (Pl. 43, fig. 7, *r.p.e.*[1]). The ring-canal is formed by the fusion of the distal ends of the radial pouches at the bases of the bulbous swellings referred to above.

Up to this point the histology of the two layers has been quite constant; the superficial ectoderm has retained its original character and remains crowded with interstitial cells of all kinds, in striking contrast with the small-celled regularly arranged tissues derived from the entocodon. The endoderm lining both the cœlenteron and the radial pouches consists of large clear cells, with somewhat indefinite outlines and containing numerous large nutritive spheres, which stain deeply with iron-hæmatoxylin; a few irregularly shaped interstitial cells are to be found, most numerous between the endoderm cells lining the manubrium.

In the last stage of the development described above we found all the organs of the adult medusa already well defined, with the exception of the tentacles. From this point onwards the more important changes are to be found in the structure of the umbrella, which now grows rapidly, especially in the region between the ocellar bulbs and the base of the manubrium, so that the superficial ectoderm loses its characteristic features, as noticed above, and gives rise to a low, small-celled epithelium covering the external surface of the bell. The endoderm behaves in a somewhat similar fashion. The ocellar bulbs, however, remain unaltered; the endoderm still consists of large irregular cells with nutritive spheres; the ectoderm is still crowded with interstitial cells, thread-cells, and nematoblasts, the latter increasing rapidly and forming new nematocysts, both large and small (Text-fig. 5).

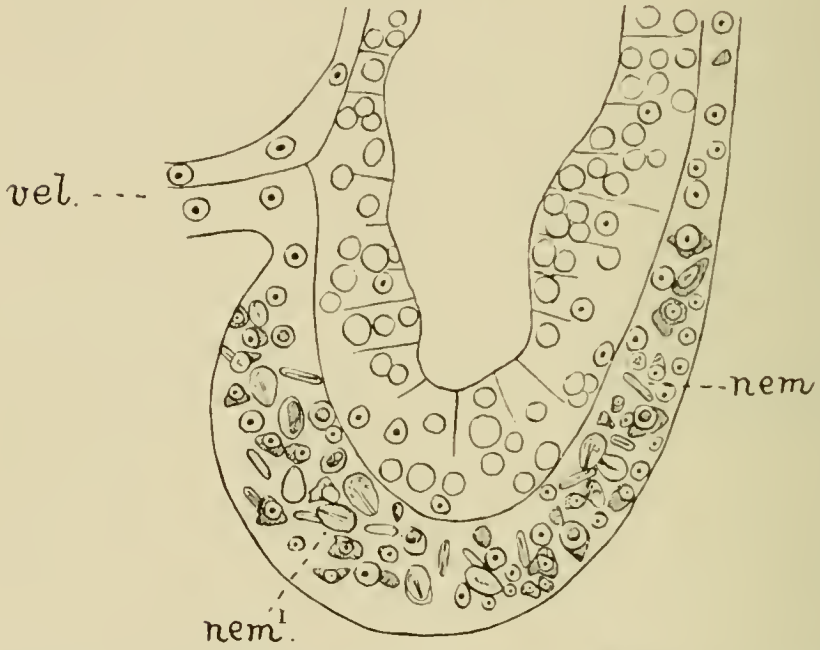
The ocellar bulbs give rise to the tentacles, their main function being obviously that, already mentioned on p. 768, of supplying these organs with stinging-cells.

In the preceding paragraphs I have tried to emphasise the fact that beyond an increase in the actual number of cells, the ectoderm of this region has remained practically unchanged throughout the development of the gonophore. The

tentacular nematocysts of the fully-formed medusa thus arise in the interstitial cells derived from the ectoderm of the parent hydroid.

The ocellar bulbs are, of course, retained throughout the life of the medusa, and, as mentioned above, keep on supplying the tentacles with stinging-cells; they are no doubt especially active during the regeneration of these organs. This explains the constant presence of such swellings at the

TEXT-FIG. 5.



A longitudinal section of an ocellar bulb of Mœrisia lyonsi just before the development of a tentacle. *vel.* Velum. *nem.* Small nematocyst. *nem.*[1] Large nematocyst.

bases of the tentacles of the Hydromedusæ, as well as their early appearance and relatively enormous size in the developing medusa-buds. The function of bearing the ocellar sense-organs must be a secondary one, for such bulbs are conspicuous in the formation of medusæ which do not possess ocelli, e.g. Podocoryne carnea, concerning which Goette (11, p. 19) remarks:

"Bald nach der Fertigstellung des Velum verdickt sich das Ectoderm jedes Randwulstes dicht über dem Velum zu

einem vorspringenden Polster, das den Ocellarbildungen andrer Medusen entspricht, aber, wie schon die älteren Beobachter (Allman, **16**; Grobben **17**) feststellten, keine Ocellen entwickelt."

In a young medusa of Mœrisia a short time before its liberation the manubrium is still without a mouth opening, and is clothed externally by a single layer of low ectodermal cells (Pl. 43, fig. 11), the internal lining consisting of large clear endoderm cells containing nutritive spheres and occasional irregularly shaped interstitial cells. The latter become more numerous as development proceeds, and some can be clearly recognised by their enlarged nuclei to be sex-cells. These at a later stage, no doubt, become transferred to the ectoderm of the stomach region, and by their further division form the gonad. Owing to the absence of individuals of the right age, I am unable to state how the transference of sex-cells from one layer to another takes place. I have never met with them migrating through the structureless lamella, and it is quite possible that the transference is a passive one, similar to that described by Goette in the male gonophores of Hydractinia (**11**, p. 70). In the youngest free-swimming medusæ examined by me the endoderm of the slightly swollen stomach had lost its small cells, and was separated by a very thin lamella from the ectoderm, which contained a few rows of developing sex-cells.

The endoderm slightly distal to this region had retained a number of interstitial cells, some of which prove to be obvious nematoblasts and contained developing nematocysts. These are, of course, the rudiments of the fully formed stinging-cells, which, as described in the first part of this paper, are to be found in the endoderm, just below the stomach of the adult medusa, and which later migrate to the battery at the oral extremity of the manubrium.

From this we must infer that the nematoblasts of the manubrium arise in the endoderm of the developing gonophore in exactly the same way as do the sex-cells; like the latter they are able to migrate through the tissues of the medusa.

When we remember the similar origin of the two kinds of cells from undifferentiated interstitial cells, we need not be surprised that they both possess the same powers of active movement.

That the thread-cells are identical in origin with the sex-cells is further emphasised by the fact that in exceptional cases part of the testis of Mœrisia can give rise to a nematocyst battery instead of producing sperm-cells, as shown in Pl. 43, fig. 12.

Both kinds of cells are first to be recognised in the endoderm of the medusa-bud; this does not necessarily imply that they originate in that layer; in my account of the early development of the gonophore, I showed that interstitial cells of the ectoderm occasionally migrate through the structureless lamella of the hydranth and become incorporated among the proliferating cells of the endoderm. It is probable that these cells or their derivatives give rise to the sex-cells and nematoblasts.

In my description of the anatomy of Mœrisia lyonsi (5), I mentioned that exactly the same types of nematocysts were to be found in the medusa as in the hydroid; in this paper I hope to have proved that they are not only identical in structure, but actually originate from the same cells. This fact is one which might be of use in systematic work on the Hydromedusæ, where the assignment of Medusæ to hydroids is often only a matter of inference; a careful comparison of the nematocysts of the two stages should be of great value in this connection.

5. General Conclusions.

(1) The stinging-cells of the medusa of Mœrisia lyonsi are not developed "in situ" on the principal batteries, but migrate to their final positions on the oral lip, or on the tentacles.

(2) The stinging-cells of the oral battery are formed in the endoderm of the manubrium, just below the stomach; those

of the tentacles in the ectoderm of the conspicuous ocellar bulbs at the terminations of the radial canals.

(3) There is reason to believe that the bulbous swellings at the bases of the tentacles have this function throughout the craspedote Medusæ. In the sub-divisions Trachomedusæ and Narcomedusæ, they are probably replaced by the thickened ring of thread-cells on the margin of the bell.

(4) The development of the gonophores of Mœrisia takes place in the manner described by Goette for other hydroids. There is no double-walled cup of endoderm at any stage, the radial canals and the endoderm lamella being derived from four separate pouches of endoderm, which grow out simultaneously from the cœlenteron of the simple bud.

(5) The stinging-cells of this medusa are developed from cells, which, like the sex-cells, arise directly or indirectly from the ectoderm of the parent hydranth.

Birmingham,
June 19th, 1910.

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EXPLANATION OF PLATES 42 AND 43,

Illustrating Mr. C. L. Boulenger's memoir "On the Origin and Migration of the Stinging-cells in Craspedote Medusæ."

PLATE 42.

Explanation of Lettering.

ect. Ectoderm of the manubrium. *end.* Endoderm of the manubrium. *gl.c.* Gland-cell. *nem.* Endodermal nematocyst. *nem.*[1] Nematocyst of the oral battery. *nem.*[2] and *nem.*[3] Migrating nematocysts. *s.l.* Structureless lamella. *test.* Testis.

Fig. 1.—A longitudinal section of the proximal part of the manubrium of the medusa, Mœrisia lyonsi, to show the nematocysts in the endoderm.

Fig. 2.—A transverse section through the same region.

Fig. 3.—A longitudinal section of the distal part of the manubrium showing the oral battery and a stinging-cell (*nem.*^{3}) migrating through the endoderm towards it.

Fig. 4.—A similar section showing a stinging-cell (*nem.*^{2}) making its way through the structureless lamella to the ectoderm.

PLATE 43.

Explanation of Lettering.

c.b. Cavity of the medusa-bud. *c.e.* Cavity of the entocodon, i.e. subumbrella cavity. *c.m.* Cavity of manubrium. *ect.* Superficial ectoderm of the developing gonophore. *end.* Endoderm of the same. *ent.* Ectoderm of the entocodon. *g.c.* Gland-cell. *i.c.e.* Interstitial cell of the endoderm. *nem.* Small nematocyst. *nem.*^{1} Large nematocyst. *n.s.* Nutritive sphere of the endoderm. *r.p.e.* Radial pouch of endoderm. *r.p.e.*^{1} Lateral solid entension of the same, which later forms the endoderm lamella. *s.c.* Sex-cell. *tent.b.* Tentacle-bulb. *test.* Testis. *v.* Velum.

Fig. 5.—Longitudinal section of an early stage in the formation of the gonophore of Mœrisia lyonsi (cf. text, p. 775).

Fig. 6.—Tangential longitudinal section of a young bud showing the entocodon and a single radial endoderm pouch.

Fig. 7.—Transverse section of an older gonophore to illustrate the complete independence of the four radial pouches. The entocodon already has a large cavity (subumbrella cavity), and at *r.p.e.*^{1} can be seen the solid extension of the edge of a pouch which later forms the endoderm lamella.

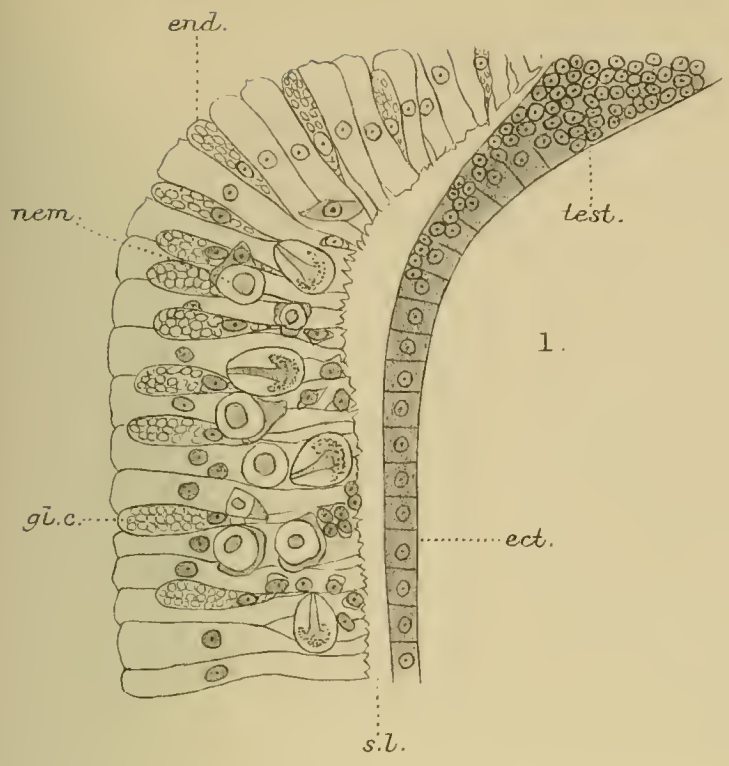
Fig. 8.—Radial longitudinal section through a similar (slightly younger) bud, showing two radial pouches of endoderm separated by the entocodon.

Fig. 9.—Tangential longitudinal section of the same medusa-bud; only a single pouch is shown.

Fig. 10.—Longitudinal section of an almost completely developed medusa to show the bulbous swellings at the termination of the radial pouches.

Fig. 11.—Manubrium of the same medusa under a higher magnification.

Fig. 12.—Section through the testis of an adult medusa, part of which has given rise to a stinging-cell battery.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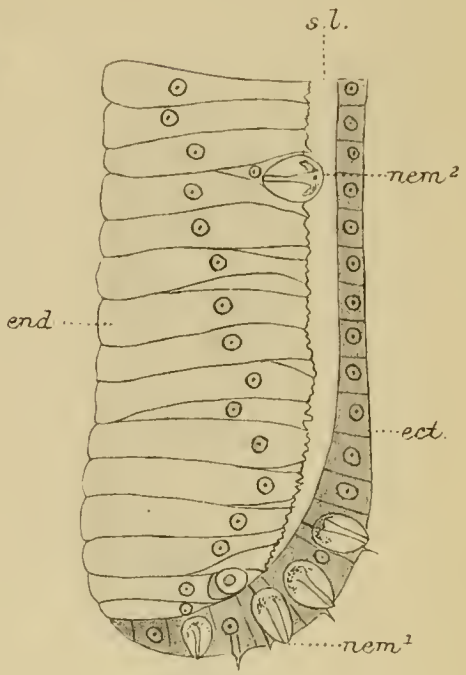
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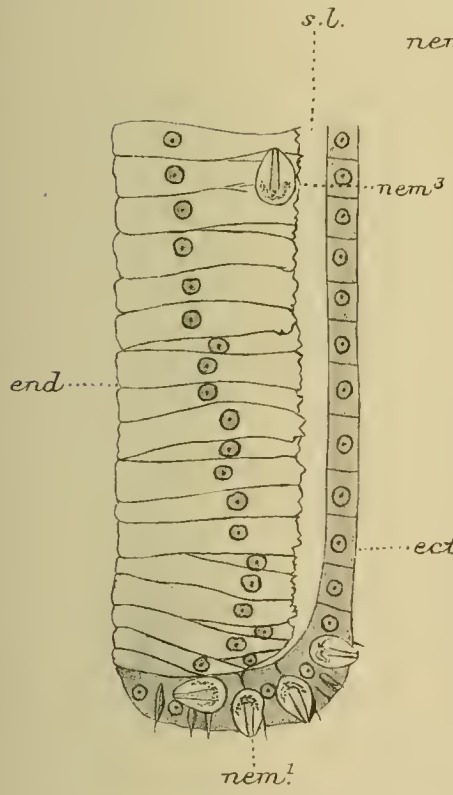
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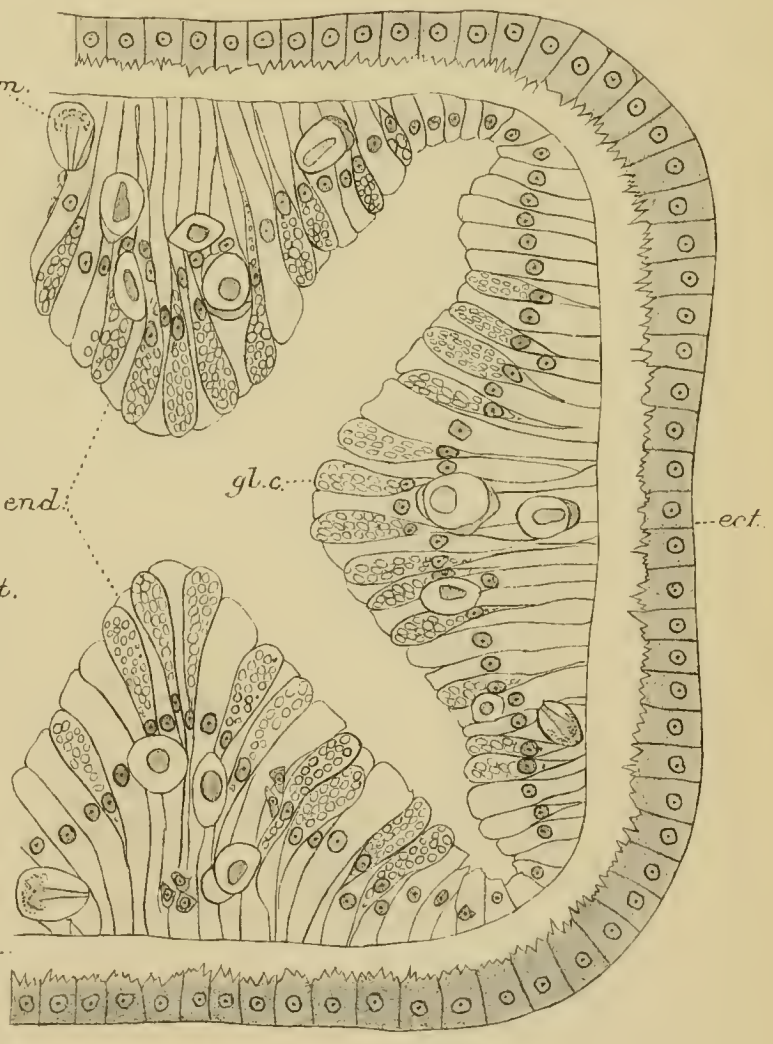
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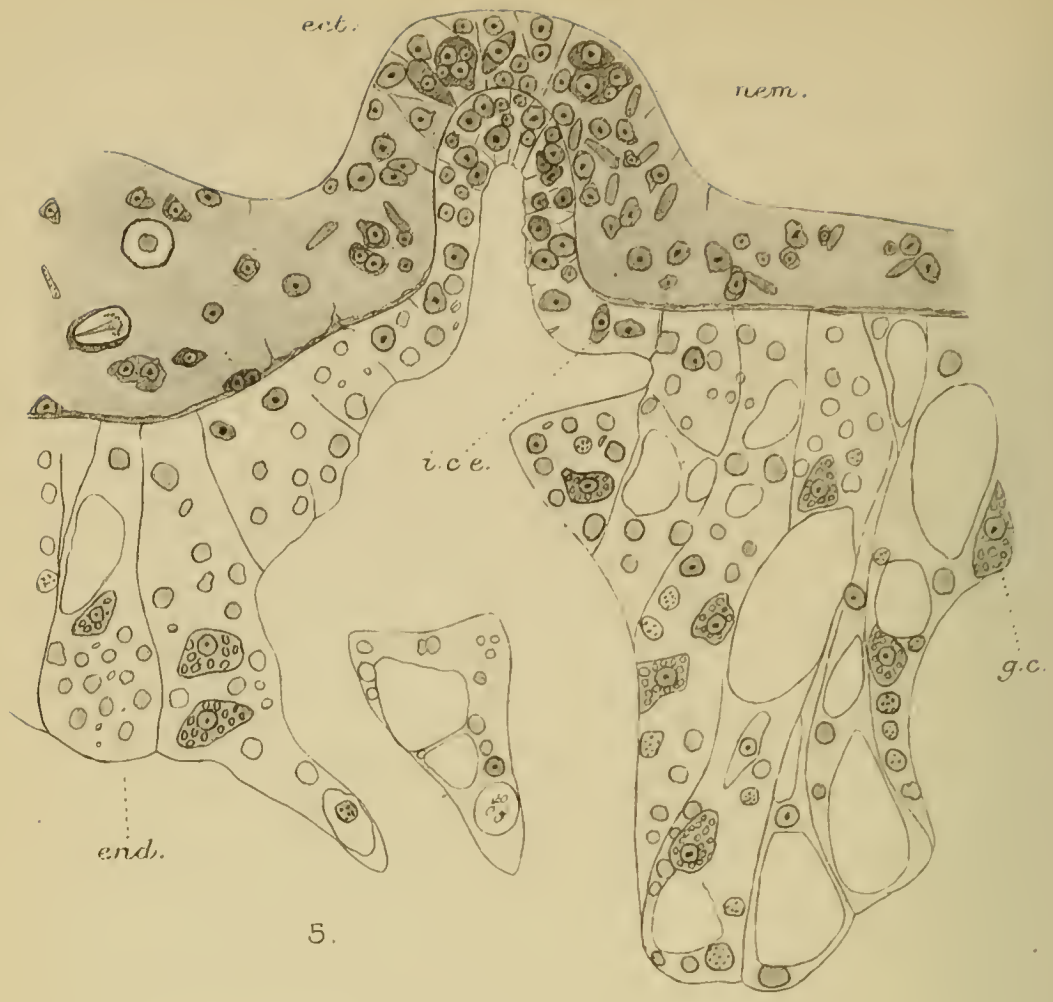

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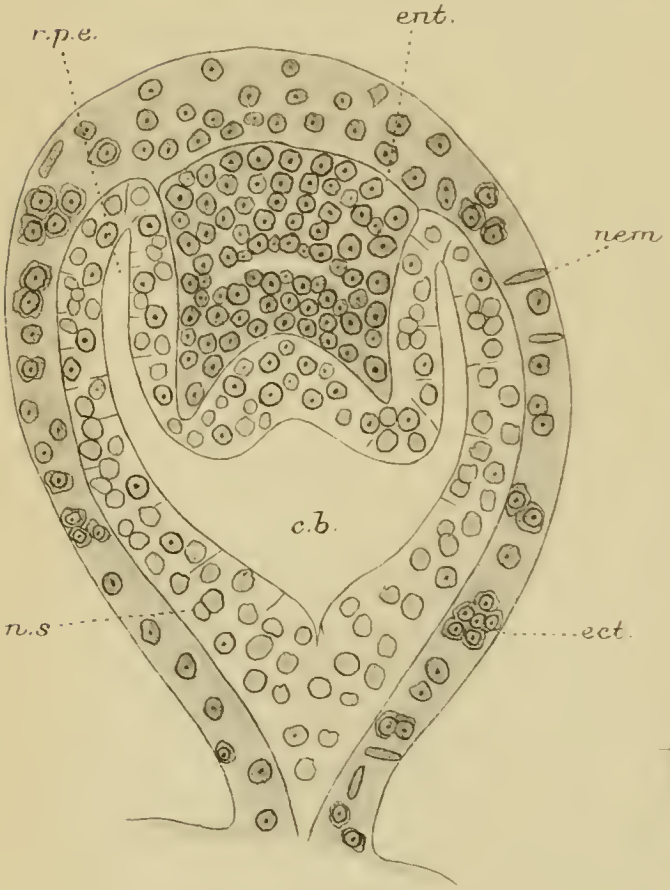

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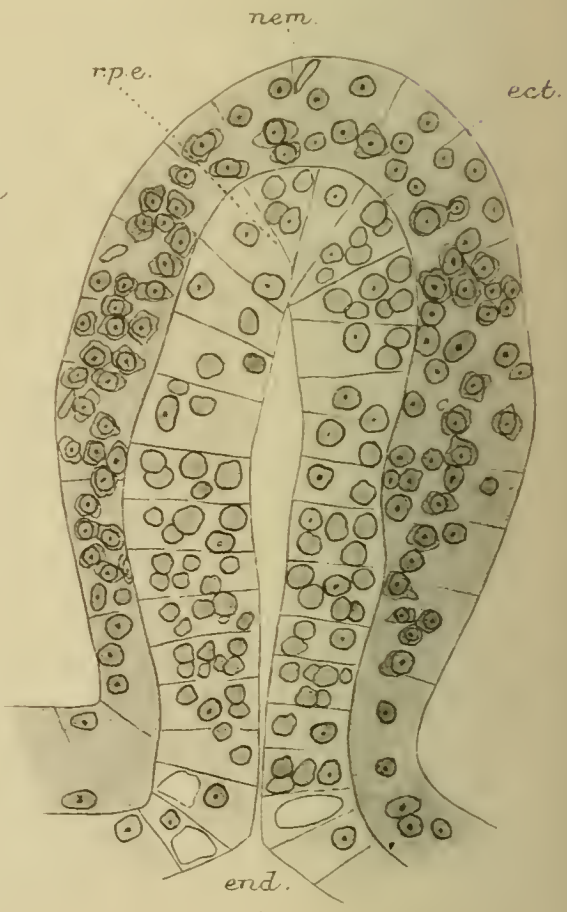

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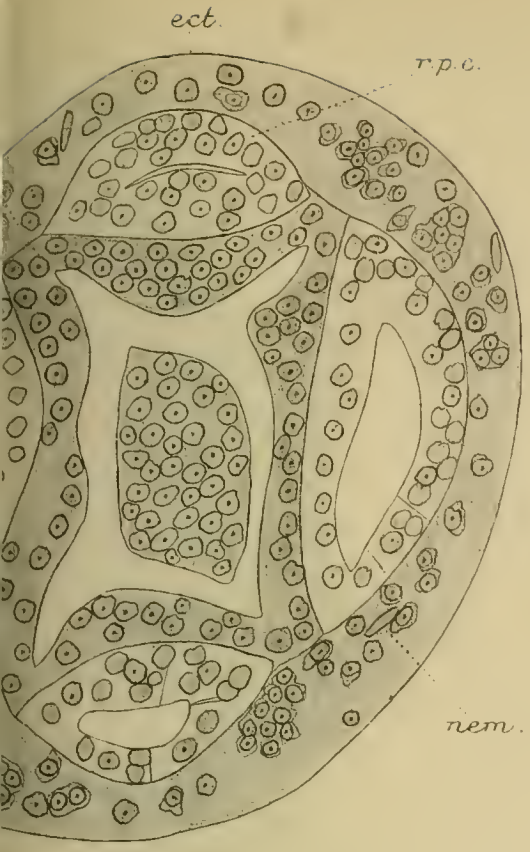

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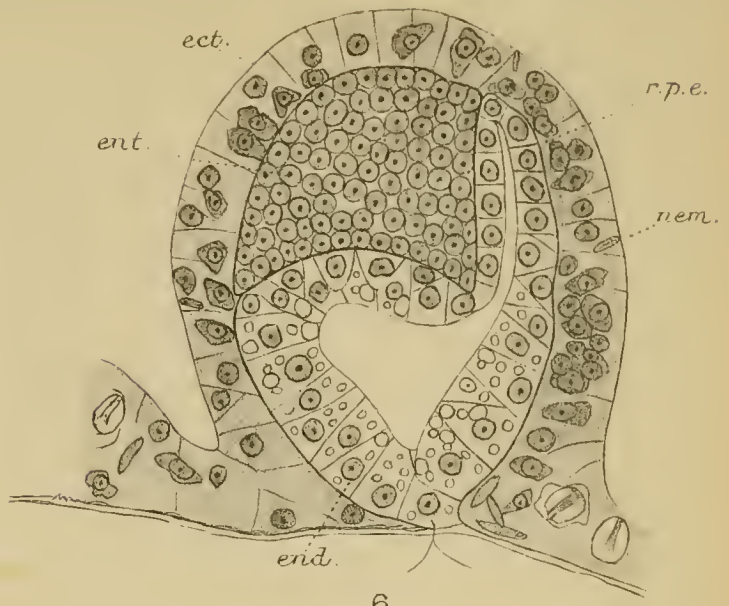

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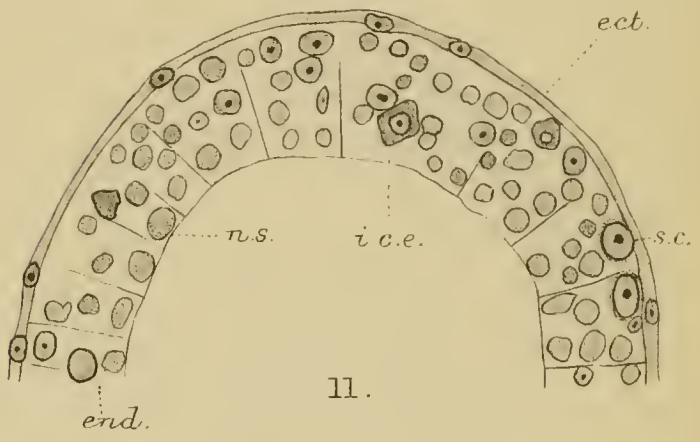

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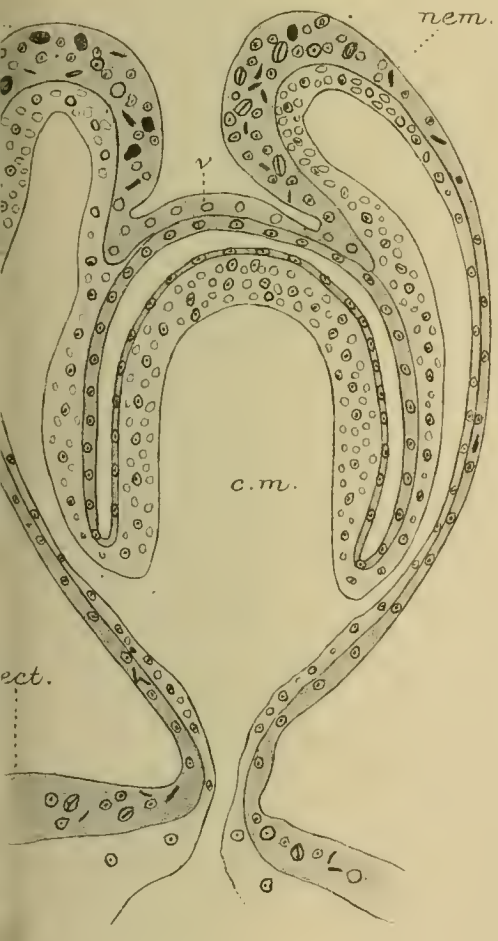

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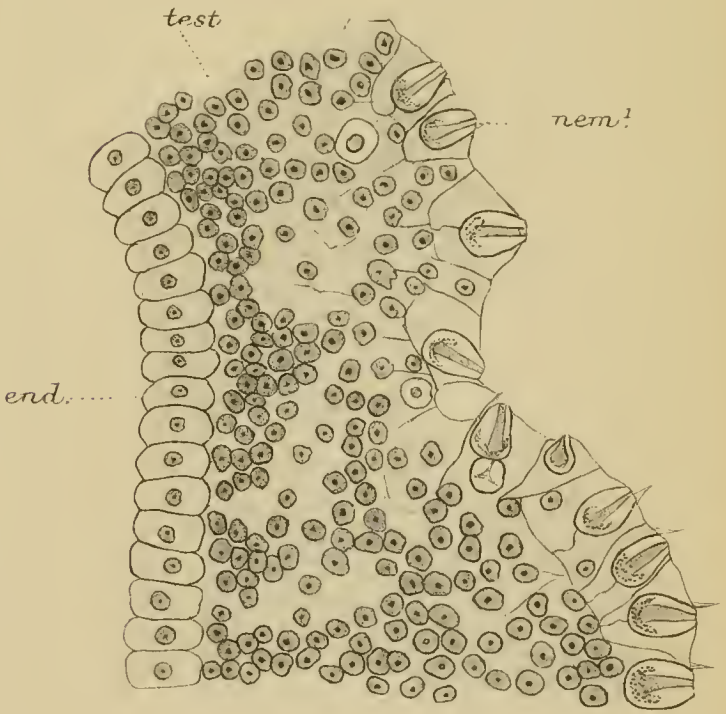

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The Researches of Bouvier and Bordage on Mutations in Crustacea of the Family Atyidæ.

By

W. T. Calman, D.Sc.,

of the British Museum (Natural History).

With 4 Text-figures.

Some six years ago Professor E. L. Bouvier ('04, '05)[1] called attention to the remarkable dimorphism of certain tropical river-prawns of the family Atyidæ, which he compared with the phenomenon of mutation described by de Vries in the vegetable kingdom. He pointed out that the case was especially noteworthy, not only because of the marked discontinuity and constant occurrence of the variations, but also because they affected characters regarded as distinctive of genera; and he drew the conclusion that these genera had originated by a process of mutation. M. E. Bordage has recently published ('08, '09A, '09B) the results of some observations and experiments on the living animals which seem to support Bouvier's views, and to indicate, at all events, a promising field for further investigations. At the suggestion of Sir Ray Lankester the following account has been prepared in the hope that it may induce some naturalists, who have the opportunity of studying the animals under natural conditions, to give attention to the matter.

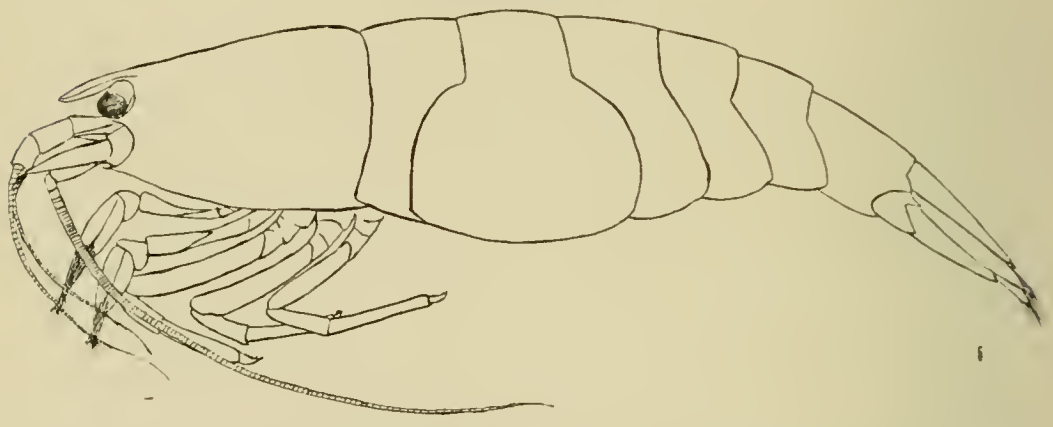
The Atyidæ (see Text-fig. 1) are a family of Decapod Crustacea belonging to the tribe Caridea (which includes most of our common prawns and shrimps), and are widely distributed in fresh waters in the warmer regions of the globe (see Ortmann

[1] The numbers refer to the list of papers on p. 796.

'94, and Bouvier '05). Some of the members of the family show very primitive characters, having, for instance, swimming branches or exopodites on all the thoracic limbs, as in the so-called "Schizopods." In this and in other features they resemble the deep-sea Hoplophoridæ, from which, or from some allied forms, most authorities are agreed in considering them to have been derived.

Other members of the family, however, are considerably specialised. In some characters this specialisation has proceeded along lines parallel to those followed in other series of the Caridea—for example, in the progressive disappearance

TEXT-FIG. 1.



Atya bisulcata. Ovigerous female of the Atya-form. × 3. From a specimen in the "Challenger" collection from Honolulu.

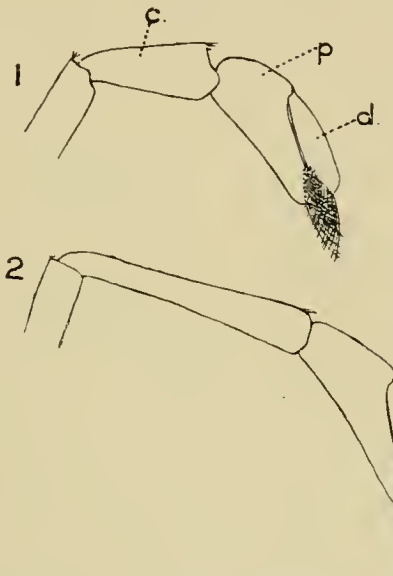
of the exopodites and, later, of the epipodites of the legs, and a diminution in the number of the branchiæ. In other characters specialisation has followed lines peculiar to the family, and this is especially the case with the modifications of the chelate first and second pairs of legs. In nearly all Atyidæ these limbs are comparatively small, not dissimilar in size, and have the fingers each tipped with a brush of long hairs (Text-fig. 1). Fritz Müller ('92) has described how these brushes are used in collecting pellets of mud on which the animal feeds.[1] Among the more specialised members of the

[1] I do not understand Bordage's statement that the chelæ are used for excavating burrows in the mud, for which their structure would appear to be ill-adapted.

family the characters used as distinctive of the genera are chiefly drawn from the modifications of the chelipeds, and some of these may now be considered in fuller detail.

In the very numerous species of the genus Caridina (Text-fig. 2) the chelæ themselves do not differ greatly, except in carrying brushes of setæ, from the typical form found in many other Decapods. The dactylus (*d.*) or terminal segment of the limb, forming the "movable finger," is opposed to a thumb-like process ("immovable finger") of the penultimate

TEXT-FIG. 2.



Caridina nilotica var. 1, 2, first and second chelipeds. *c.*, carpus; *d.*, dactylus; *p.*, palmar portion of propodus. × 40. From a specimen collected by Dr. W. A. Cunnington in the Victoria Nyanza.

segment or propodus. The proximal part of the propodus, expanded to contain the muscles moving the dactylus, forms what is known as the "palm" (*p.*) of the chela. In Caridina the two pairs of chelipeds differ in the form of the segment which supports the propodus, the "wrist" or carpus (*c.*). In the second pair it is more or less elongated and slender, and the propodus articulates with its distal end; in the first pair, on the other hand, it is short and broad, its distal margin is more or less concave (cf. Text-fig. 2, 1, and Text-fig. 4, *A'*), and the propodus articulates with its lower corner.

The species of the genus Ortmannia (formerly known as Atyoida) differ from those of Caridina chiefly in the fact that the carpus of the second pair resembles that of the first pair (Text-fig. 3, *B′*, *B″*), being short and broad, with its distal margin excavated and articulating with the propodus at its lower corner. It is to be noted that these characters are not equally well marked in all the species referred to Ortmannia; in some the second carpus is still, as in Caridina, somewhat longer than the first, and the excavation of its distal margin is shallow (as in Text-fig. 4, *B″*); in other species the carpus is nearly similar in the two pairs and so deeply excavated as to assume an almost crescentic form (as in Text-fig. 3, *B′*, *B″*). Associated with this excavation of the carpus is a shifting (already begun in Caridina) of the carpo-propodal articulation from the proximal end to the lower border of the propodus. Further, while in some species the chelæ themselves are quite similar to those of Caridina, in others the "palm" is much shortened, or, in other words, the articulation of the movable finger is carried backwards towards the base of the propodus.

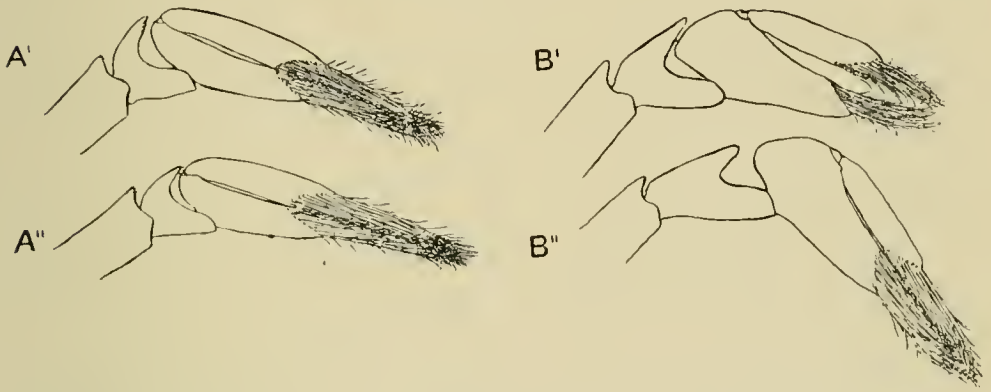
These modifications lead towards the conditions found in the genus Atya, which includes the largest and most highly specialised members of the family. In these the two pairs of chelipeds (Text-fig. 3, *A′*, *A″*) are quite similar, and the carpus is reduced by the excavation of its distal border to a narrow crescent, with the lower limb of which the propodus articulates. The propodus itself assumes a form unlike that of any other Decapod; the backward shifting of the articulation of the dactylus has been carried so far that the palm has entirely disappeared, and the chela is composed of two similar parts, hinged together at one end, like the legs of a pair of compasses.

Although, within each of the genera, there is some variation in the degree to which these characters are developed, this variation is so far discontinuous that all the known species could, prior to Bouvier's researches, be referred without much difficulty to one or other of the genera. If it be objected that such apparently trivial differences should

not be regarded as of generic value, it may be pointed out that, as a rule, though not in every case, they are coincident with other features which help to characterise, although they do not define, the generic groups; and further, there is no criterion by which the generic value of a character may be estimated, except that of its constancy throughout a group of species.

Bouvier's discovery may be shortly expressed by saying that certain species were found to be dimorphic and to oscillate, as it were, in a state of unstable equilibrium between one generic group and the next. Thus, Miss Rathbun ('01)

TEXT-FIG. 3.



Atya bisulcata. *A'*, *A''*. First and second chelipeds of the Atya-form. *B'*, *B''*, First and second chelipeds of the Ortmannia-form (Ortmannia Henshawi). × 7. From specimens in the "Challenger" collection from Honolulu.

had described a new species, Ortmannia Henshawi (Text-fig. 3, *B'*, *B''*), found in association with Atya bisulcata (*A'*, *A''*), on the island of Hawaii; Bouvier pointed out that this association was not accidental, but constant, that the two forms were indistinguishable, except by the characters of the chelipeds, and that they should be regarded as constituting a single dimorphic species. He found a similar phenomenon in the case of Atya serrata, described by Spence Bate from specimens obtained by the "Challenger" Expedition at the Cape Verde Islands, and since found in many localities on the islands of the Indian and Pacific Oceans. To the Ortmannia-form of this species Bouvier gave the name

O. Alluaudi. In both species the two forms were sharply distinguished, although in the Ortmannia individuals (especially in O. Alluaudi) a considerable amount of variation was observed in the relative proportions of the fingers and palm of the chelæ; the Atya-form, on the other hand, presented no noteworthy variation. In both species Bouvier found that the dimorphism was independent of age and sex; both forms were found through a wide range of size, although the Atya individuals were, on the whole, somewhat larger, and females of both were observed carrying eggs. In the case of A. bisulcata (O. Henshawi) both forms occurred in about equal numbers; in A. serrata (O. Alluaudi) there was some evidence that the relative proportions varied in different localities.[1]

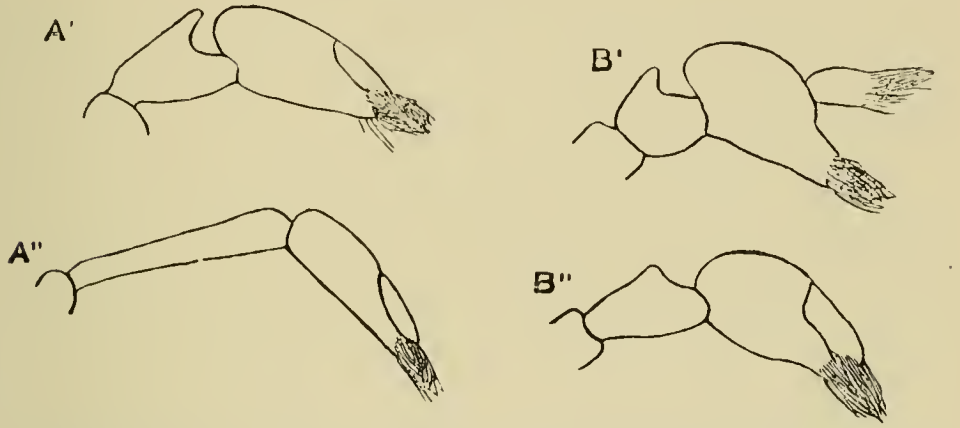
In one species of Caridina Bouvier found evidence of the existence of an analogous mutation leading to the genus Ortmannia. Among eleven examples of C. apiocheles (Text-fig. 4) (probably from the Seychelles), he observed one in which the carpus of the second pair of chelipeds (Text-fig. 4, *B″*), instead of being long and slender as in the typical individuals, was short, broad, and excavated distally, resembling that of the first pair, so that the specimen, had it occurred alone, would have been referred to Ortmannia. In this case, however, it remains to be seen whether the

[1] It may be of interest to give here the results of a preliminary examination of the material of these two species in the British Museum collection. In one lot of Atya bisulcata obtained by the 'Challenger' Expedition at Honolulu forty-two specimens are of the Atya-type and forty-six of the Ortmannia-type. Only one specimen cannot be referred to either, having three chelæ of the Atya-shape, while the fourth is distinctly of the Ortmannia-shape. There is a considerable amount of variation in the chelæ of the Ortmannia-individuals, and their terminal brushes of setæ are always much shorter than in the Atya-individuals. In a second lot of specimens from Hawaii only nine Ortmannia-individuals are found among thirty-eight Atya-individuals. Of the two type-specimens of Atya serrata from the Cape Verde Islands in the 'Challenger' collection, the larger is of the Atya-type while the smaller is a distinct Ortmannia.

occurrence of the mutation is a normal and constant feature of the species.[1]

Professor Bouvier discusses at length the possible explanations of these curious phenomena. He points out that it is impossible to continue to regard Atya bisulcata and Ortmannia Henshawi, for instance, as distinct and independent species; their constant association and their identity in all characters except those of the chelipeds forbid their separation, and it may be added that Professor Bouvier's proved skill and experience as a carcinologist give special weight to his opinion on this point. He also dismisses, and no doubt

Text-fig. 4.



Caridina apiocheles. *A'*, *A''*, First and second chelipeds of the typical Caridina-form. *B'*, *B''*, First and second chelipeds of the Ortmannia-form (O. Edwardsi). After Bouvier.

rightly, the suggestion that the phenomena are due to hybridisation; and he concludes that the facts he describes have their closest analogy in the "mutations" of de Vries.

[1] The question whether the genera implicated in these phenomena of mutation are to be retained as valid is of secondary importance, and hardly concerns more than the convenience of the systematist. If they are to be retained, however, it would seem that a good case exists for the re-instatement of the name Atyoida in place of Ortmannia. Miss Rathbun displaced Atyoida on the ground that the surviving type-specimens of Randall's Atyoida bisulcata, the type-species of Atyoida, have chelæ of the Atya-type. If, however, O. Henshawi, the type-species of Ortmannia, is only a form of A. bisulcata, the two genera are synonymous and the older name should be used.

Instead of being limited to comparatively trivial characters and giving rise to varieties or "petites espèces" as in de Vries's examples, the mutations of the Atyidæ affect characters of generic importance. Bouvier believes that the course of evolution from the more primitive Caridina to the specialised Atya has been discontinuous, proceeding at a single step from Caridina to Ortmannia and again from Ortmannia to Atya, and that the species mentioned remain in the condition of instability accompanying the transition from one to the other. It is also implied, although Bouvier does not dwell on the point, that these genera are polyphyletic and have originated independently in several regions of the globe.

There is still another possibility, not alluded to by Bouvier, that deserves mention here, namely, that the apparent dimorphism is due to heteromorphic regeneration of the chelipeds after mutilation. Many cases are now known among Arthropoda in which regenerated appendages depart from the normal type, and not infrequently revert to a simpler and more primitive form ("régénération hypotypique" of Giard). Although the chelipeds of many Atyidæ readily break off from the body in preserved specimens, it seems very improbable that this mutilation should happen so frequently in nature that 50 per cent. of the specimens collected would have regenerated limbs; nor is it less improbable that all four chelipeds would be removed simultaneously[1]; and the experiments of Bordage, described below, lend no support to this suggestion.

Professor Bouvier pointed out the desirability of testing his conclusions by observation and experiment on the living animals, and it was at his suggestion that Bordage undertook the researches of which the results are presented in his recent papers ('08, '09A, '09B). On the island of Réunion Ortmannia alluaudi, with its mutation Atya serrata, occurs abundantly in mountain streams at altitudes above 300 mètres. Owing to the high temperature prevailing at the

[1] Only one case has been noticed in which one of the chelipeds differed from the others (see above, p. 790, footnote).

coast (St. Denis), where the experiments were carried on, it was impossible to keep the animals alive in small aquaria, but after several failures Bordage succeeded in keeping living specimens in a small tank of masonry through which a current of water from the town supply was kept flowing. The inflow and outflow were guarded by fine wire gauze covered with muslin to prevent the escape of adults or larvæ, or the accidental introduction of additional specimens. A single ovigerous female of the Ortmannia form was placed in the tank, and in a few days numerous zoea larvæ were observed in the water. Only seven individuals survived to assume the perfect form a fortnight later, and these proved to be all, like the parent, of the Ortmannia-type. A second experiment, however, was more successful. Another ovigerous Ortmannia was placed in the tank (which had been emptied and cleaned out between the experiments) and the larvæ were hatched in due course. When they were about to pass into the final stage of their metamorphosis some weeks of torrential rain rendered the water-supply muddy and opaque, so that the young prawns were lost sight of. On cleaning out the tank, however, sixteen specimens were discovered among the mud, and of these ten were like the parent, while six were of the Atya-type. Bordage assures us that the precautions he took absolutely exclude the possibility of these young prawns having come from any source other than the eggs carried by the original female. In another experiment two females of the Atya-type produced twenty-seven young, all of which resembled the parents. Bordage states that he was unable to obtain fecundation of Ortmannia females by Atya males, while they bred readily with males of their own type.

These results are somewhat surprising, and can hardly be accepted as final without a good deal more experimental evidence. If the two forms do not interbreed, and if, as Bordage considers probable, the Atya-form always breeds true, it is evident that the Ortmannia-form would disappear (in the absence of a selective death-rate operating in its

favour) even more speedily than is required by the "loi de Delboeuf" to which Bouvier refers.

Bordage also made some experiments on the regeneration of the chelipeds. He found that after amputation of the chelipeds of an Atya, the regenerating limbs had at first the Ortmannia-form—that is to say, the propodus showed a distinct palmar portion. At the first moult after the operation, however, the Atya-form was assumed, the articulation of the dactylus having shifted to the proximal end of the propodus. It is not clear from the account given whether the chelæ were perfectly formed and movable before the first moult. Bordage regards this as a typical case of atavistic regeneration (régénération hypotypique), and he also cites a case described by Fritz Müller ('92) as showing that the regenerated second pair of chelipeds in Ortmannia potimirim have an elongated and slender carpus like that of Caridina.[1]

While Bordage's results are highly interesting and suggestive, they rest upon a very narrow basis of experimental evidence. There seems to be no reason to doubt his statement that young of the Atya-type were hatched from the eggs of an Ortmannia female, but it is based on the result of a single experiment carried out under unfavourable conditions, and no figures of the young prawns are given. The supposed inability of the Atya females to produce Ortmannia young rests also on the negative result of a single experiment and the simple statement that the two forms do not interbreed deserves to be examined in greater detail. It would be of interest to have further particulars as to the normal course of development in the two forms, and to know whether there is any trace of an Ortmannia stage in the development of the Atya-form of cheliped. The phenomena of regeneration also require more thorough investigation; it is possible that, as is

[1] It may be mentioned that a comparison of Müller's original figure with the copy given in Bordage's paper does not increase our confidence in the diagrammatic drawings which the latter author gives to illustrate his own observations.

known to be the case in other Decapods, the form of the regenerated limbs may differ according to the age of the individuals experimented on. While it is very improbable, for the reasons stated above, that the whole appearance of dimorphism can be due to regeneration, it remains to be tested whether the form of the chelipeds does really remain constant throughout the life of the individual. Apart from the possibilty of further experiments with the living animals, it would be of importance to get together sufficient material for a biometrical investigation into the degree of discontinuity in the variation and its incidence in relation to age, sex, and locality.

One of the most interesting features of these mutations, if Bouvier's interpretation of them be confirmed, is the direct way in which they bear on the problems suggested by a study of the Atyidæ from the systematic standpoint. This may be illustrated by an example. In Lake Tanganyika (Calman, '99 and '06) the collections of Mr. J. E. S. Moore, and, more especially, of Dr. W. A. Cunnington, have revealed the existence of numerous peculiar species of Atyidæ, which differ from all the other members of the family (with one exception to be mentioned presently) in having a reduced branchial formula. Thus the Tanganyikan Caridella resembles Caridina in most of its characters, except that it has no pleurobranchia on the last somite of the thorax, and Atyella differs in the same character from Ortmannia. I have pointed out elsewhere that while the reduction in the number of branchiæ may have occurred independently in each of the Tanganyikan genera, so that Caridella may be supposed to be derived from Caridina, and Atyella from Ortmannia, Bouvier's results suggest as a possible alternative that Atyella may have originated from Caridella by a mutation parallel to that by which, in other parts of the world, Caridina has given rise to Ortmannia. The latter hypothesis has recently received the support of Prof. Bouvier himself ('09A, '09B), in connection with his very interesting discovery that Atya Poeyi

of the West Indian Islands has the same branchial formula as Caridella and Atyella, and in fact only differs from the last-named genus in having chelæ of a distinctly Atya type. He refers the West Indian species to a new genus, to which he gives the name Calmania. He supposes it to have been derived from Atyella in the same way as Atya from Ortmannia, and he concludes that Atyella (and Caridella also) must formerly have existed in America. From this view I would venture to dissent. Even if the phenomena of mutation lead us to believe that similar forms of chelipeds may have been acquired independently in different localities, there is no greater difficulty in supposing that a simple suppression of the posterior pleurobranch may also have occurred more than once in the evolution of the family. In all the groups of animals composing the remarkable fauna of Tanganyika, there is reason to believe that many of the endemic genera and species have been differentiated within the limits of the lake itself; and until the Atyidæ with a reduced branchial formula are shown to have a much wider geographical distribution than is at present known, it seems impossible to believe in a direct affinity between the Tanganyikan Atyella and the West Indian Calmania.

It may be freely admitted that these phylogenetic speculations rest upon much less solid ground than do the conclusions drawn directly from experiment or based upon statistics; but unless we are to abandon all hope of rationalising the facts of systematic and geographical biology, some such hypotheses are, for the present, indispensable.

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