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The Cytoplasmic Inclusions of the Germ-Cells:
 Part X. The Gametogenesis of *Saccocirrus*.¹

By

J. Brontë Gatenby, B.A., D.Phil. (Oxon.), D.Sc. (Lond.),
 Professor of Zoology and Comparative Anatomy, University of Dublin.

With Plates 1-4 and 1 Text-figure.

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1. INTRODUCTION.

IN this paper I have attempted to shed further light on that difficult problem, the oogenesis of an Annelid; while *Saccocirrus*, as Goodrich has shown (26), is an Archi-annelid closely related to *Protodrilus*, it is probably much like other Annelida so far as its oogenesis is concerned.

I was influenced to undertake a study of the oogenesis of an Annelid by three special reasons, namely, Schaxel has described 'chromatin' emission in *Aricia foetida*, a Polychaete; Hempelmann and Buchner have both described most peculiar peri-nuclear extruded bodies at one period of oogenesis of *Saccocirrus*; and, finally, *Saccocirrus* is an example of precocious entry of the sperm into the young oocyte: we know that the head of the sperm lies quiescent till the oocyte is ripe, and I believe that this would offer the opportunity of observing whether the tail of the sperm entered the oocyte, and (if so) whether it took any special part in fertilization.

The behaviour of the cytoplasmic inclusions has been the object of special attention, and I have also gone into the question of the spermatogenesis, and showed in these stages the presence of peculiar yolk-spheres which are divided out between the daughter-cells at the maturation divisions. It is extraordinary the way such metaplastic bodies are shepherded into two groups during the divisions of the spermatocyte.

This is the concluding part of this present series of papers.

2. PREVIOUS WORK.

The precocious entry of the spermatozoon into the young oocyte was discovered independently by three observers—F. Hempelmann, and by van Gaver and Stephan jointly. Hempelmann's paper appeared a short time before that of the two other observers.

Hempelmann has contributed two papers on this subject, one in 1906 (34), and one as recently as 1912 (35). His main conclusions are to be found in the latter paper. He shows that the sperms enter oocytes just after the end of the bouquet stage of the prophases of the heterotypic division. Only one spermatozoon enters one egg.

Just after the last stage of the prophases of the heterotypic division, Hempelmann describes peculiar granules which originate from the nucleus and pass into the cytoplasm, eventually leading to the formation of yolk. These granules are at first quite solid, grow large, and gradually become vacuolated here and there, so as to form a large number of small granules lying in a single vacuole. It is just after this has taken place that the egg cytoplasm becomes filled with yolk; speaking of these curious peri-nuclear granules, Hempelmann says, 'so scheint es mir kaum zweifelhaft, dass diese Zellbestandteile mit der Dotterbildung in Beziehung gebracht werden müssen'. With regard to the origin of the peri-nuclear granules Hempelmann writes, 'Auch der Nucleolus beteiligt sich wohl mehr oder weniger an dieser Dotterbildung, denn gelegentlich zeigt er sich umgeben von kleinen, sich ebenso wie er selbst mit den

gebräuchlichen Kernfarbstoffen intensiv färbenden Kügelchen, die wohl aus ihm hervorgegangen sind, und die an den Rand des Keimbläschens rücken, um wahrscheinlich aus diesem in das umgebende Plasma auszutreten'.

Hempelmann recognizes only one sort of plasma granule — 'yolk', originating from the peri-nuclear 'Tröpfchen'. According to him, the entry of additional spermatozoa into the oocyte is prevented by the formation of a membrane; the spermatogenesis has been studied by Hempelmann, who described the tripartite 'Nebenkern' of the spermatid; his material was not preserved carefully enough to allow of his giving a good account of the formation of the male gamete.

Van Gaver and P. Stephan have published two short notes on *Saccocirrus papillocerus*. In their second paper these observers state, 'Nôtre désaccord fondamental avec Hempelmann a trait à l'époque de la pénétration du spermatozoïde; pour nous, le spermatozoïde arrive dans l'oocyte dès que celui-ci est différencié en tant qu'oocyte, lorsque sa taille est encore extrêmement minime et avant toute formation de vitellus à son intérieur. Nous n'osons pas affirmer que l'action du spermatozoïde soit la cause initiale du développement de l'oocyte, mais nous avons constamment trouvé un de ces éléments dans les ovules en voie d'accroissement et d'élaboration vitelline'. In addition, van Gaver and Stephan believe that polyspermy and assimilation of spermatozoa by the cytoplasm take place in *Saccocirrus*. They find 'désintégration des têtes des spermatozoïdes et, par suite, leur assimilation par l'oocyte'. The articles of van Gaver and Stephan are not illustrated by figures.

The latest observer to attack these problems was Paul Buchner (3). He showed that the whole tail of the sperm may occasionally enter an oocyte, and break up to give rise to a number of peripheral droplets, while the head of the sperm remains quiescent. The yolk-formation he describes as taking place by a partial breaking up of the nucleolus, pieces of which wander to the periphery of the nucleus—particles of yolk appearing simultaneously apparently from the nucleolar

fragments which pass through the nuclear membrane. The droplets from the sperm tail shrink up and leave the periphery of the oocyte quite clear, while the nucleolar fragments grow and multiply and fill the egg with yolk. Buchner is not altogether happy in his work on *Saccocirrus* oogenesis, and is very cautious with regard to yolk-formation. He ends his research with the statement, 'Die Dotterbildung von *Saccocirrus* verdiente wohl eine eingehendere, mit Hilfe aller zu Gebote stehenden Färbungen und Reaktionen ausgeführte Analyse'.

No observer has hitherto given any account of Golgi body or mitochondria in the oogenesis of *Saccocirrus*, and this form has up to the present time resisted the efforts aimed at a satisfactory interpretation of the problems which its oogenesis presents.

3. TECHNIQUE AND MATERIAL.

Sections of a large collection of *Saccocirrus* were prepared according to the plans given in my recent paper on technique (13). The Mann-Kopsch and Champy-Kull methods were particularly valuable. All the other techniques in current use were tried. The material used was sent to me from Plymouth, two lots in the month of June, another in July.

The worms were cut into pieces after having been killed by being dropped whole into a capsule full of fixative. Great difficulty was experienced in making successful preparations of the Golgi apparatus. All the first trials were unsuccessful, but for some unknown reason all the latter attempts succeeded completely both with Mann-Kopsch, Cajal, and Da Fano methods (13).

I have to thank Dr. Allen, F.R.S., of the Plymouth Biological Laboratory, for sending me the material desired by me.

Professor E. S. Goodrich, F.R.S., of Oxford, kindly placed at my disposal some of his own material of *Saccocirrus papillocercus* from Naples, and sections cut from this proved very useful.

4. SPERMATOGENESIS.

(a) The Spermatogonium of *Saccocirrus*.

The spermatogonium is a small rounded cell with a spherical granular nucleus containing a karyosome. In Pl. 1, fig. 7, is drawn one of a group or rosette of spermatogonia: there was a spindle bridge as is common in such cases (SB). This cell was drawn from a Mann-Kopsch preparation, and the Golgi body (GA) shows as a number of black rods or batonettes surrounding an archoplasm or centrosphere. In most spermatogonia the mitochondria may be detected in the form of a cloud lying in the region of the Golgi body, as in Pl. 1, fig. 7, at M.

(b) The Spermatocyte.

Rosettes of spermatogonia grow synchronously to form groups of spermatocytes, and during the growth stages the mitochondria spread out through the cytoplasm, as in Pl. 1, fig. 1, M. The Golgi body grows too, and the number of its individual parts (Golgi rods, dictyosomes, or batonettes) also increases, as shown at GA. The nucleus at this period is often reniform. Possibly the most remarkable fact with regard to the spermatogenesis of *Saccocirrus* is the presence in many, if not all spermatocytes, of a group of true yolk-granules (dipin) quite separate from either mitochondria or Golgi body: in Pl. 1, fig. 1, the yolk-granules are at Y, and form a special cell inclusion. By the Champy-Kull method the nucleus stains bluish, the mitochondria and Golgi rods go dark red, the cytoplasm is yellowish, and the curious collection of yolk-granules stain brownish green in the osmic acid of the Champy's fluid. In Pl. 1, fig. 1, at YC on the right, is drawn one of the yolk-cells which accompanied this spermatocyte, and the yolk-granules stained the same shade as the yolk-spheres (Y) in the spermatocyte.

In Pl. 1, fig. 2, is a Kopsch preparation which shows the effect of prolonged osmication. The Golgi body (GA) has reduced the OsO_4 heavily, the mitochondria do not show, and the yolk-spherules are at X; but besides all these there

is found a group of perfectly spherical granules at *x*. These went black-brown in the OsO_4 ; their true nature or origin was not ascertained, but it was thought that ultimately they formed a part of the spermatid, as will be shown later. The number of granules was about twelve in all the cases I could count.

(c) The Spermatocyte Divisions.

Pl. 1, fig. 4, is a first spermatocyte metaphase. The peculiar yolk-spherules have taken up a position at the middle of the spindle, and in the next stage (Pl. 1, fig. 5) the granules have become sorted out into two groups (*y*) subequal in size. Each spermatid (Pl. 2, fig. 8) receives about one-quarter of the number of granules in the spermatocyte. The behaviour of the mitochondria in the divisions is peculiar: they lose their granular state, and during the prophase break down to form threads as in Pl. 1, figs. 4 and 5, in the telophase (Pl. 1, fig. 5); the threads lie chiefly around the equatorial plate. The Golgi rods are difficult to follow through mitosis; at the prophase they lose their staining power, and it is only in certain cases that the cell at metaphase has distinguishable elements (*xy* in Pl. 1, fig. 4) which might be identified as Golgi elements. It must be admitted that no positive evidence has been adduced with regard to the Golgi elements during division of the Saccocirrus spermatocyte.

(d) The Newly-formed Spermatid.

In Pl. 2, fig. 8, is a newly-formed spermatid. The yolk-spherules (*y*) are on the right, while the mitochondria surround the nucleus; the Golgi elements are at *GA*, being scattered. In the next stage the mitochondria collect to one side of the cell, in proximity to the nucleus, the Golgi elements lying behind, as in Pl. 2, fig. 9; this cell was drawn from a Kopsch preparation, and in it no yolk-spherules could be identified. At *x* are what I consider to be two of the granules marked similarly in fig. 2. In nearly all Kopsch or Mann-Kopsch preparations the spermatid cytoplasm is seen to be formed of very coarse reticulum, as shown in Pl. 2, figs. 9 and 14.

(e) Spermateleosis.

The spermateleosis stages comprising those leading to the metamorphosis of spermatid into spermatozoon are very peculiar. The mitochondria, which in Pl. 2, fig. 9, lie grouped behind the nucleus, begin to run together as depicted in fig. 10. Three main centres for this coalescence exist, but here and there a few separate centres exist; these ultimately join up with one of the larger centres, till one gets such a stage as in Pl. 2, fig. 11, where three balls of mitochondrial substance are produced (mm), while the remainder of the free mitochondria are gradually fusing up. By the stage of fig. 12 the mitochondria have all fused to form three solid spheres generally somewhat unequal in size, and as well, often in staining affinity. In Pl. 2, fig. 14, the three spheres are viewed from below, their unequal size being apparent.

Leaving the mitochondria at this stage, the fate of the yolk-spherules and of the Golgi elements may be described; at such a stage as in fig. 10 of Pl. 2 the Golgi elements lie behind the zone of the mitochondria; as the mitochondria fuse up, the Golgi elements keep behind the most distal mitochondria as in Pl. 2, fig. 11, and, finally, when all the mitochondria have fused to form the three spheres, the Golgi elements lie close up behind as in Pl. 2, fig. 12. In the case of the yolk-spherules a somewhat similar change in position has been noted: in Pl. 2, fig. 10, the yolk-spherules are on the right of the cell, but by stages in figs. 11 and 12 they have moved back behind the mitochondrial spheres.

In many, but not all, examples there can be observed between the three mitochondrial spheres, a small, often round, often angular grain, as in Pl. 2, fig. 14, at x. In figs. 9, 10, 11, and 12 such bodies are also seen.

These bodies, I believe, are derived from the grains marked x in Pl. 1, fig. 2. They seem to form a part of the mitochondrial sheath of the sperm-tail.

The spermatid at such a stage as that of fig. 12 now begins to lengthen. In Pl. 2, fig. 13, the three mitochondrial spheres

have become drawn out to form a comet-tail body attached to the spermatid nucleus. The latter has begun to undergo the usual changes which may be seen in figs. 9, 10, 11, 12, and 13. In Pl. 2, fig. 16, the tail has further elongated, the yolk-spherules and Golgi elements are drifting down the length of the tail, while the nucleus is losing its reticular arrangement. This lengthening process now goes on till the fully-formed elongate Saccocirrus spermatozoon is produced.

Owing to the small size of the cells, and possibly to the unsuitability of the material, no satisfactory description can be given of the formation of the acrosome. This can be seen in Pl. 2, fig. 13, at *as*. In figs. 9 and 10, at *gx*, were bodies which might have some connexion with the formation of the acrosome, but of this I am unable to speak with certainty.

(f) The Fate of the Golgi Apparatus during
Spermateleosis.

If one examines a bundle of ripe or ripening spermatozoa in material prepared by the Mann-Kopsch method, it will be found that near the end of the tail of each sperm are rounded or angular bodies which are stained by the osmic acid: in Pl. 2, fig. 17, is drawn such a bundle of sperms, the bodies being marked *gax*; at a higher magnification, as in fig. 15, the bodies are seen to be very like the Golgi body drawn in the spermatogonium in Pl. 1, fig. 7, at *ga*. I cannot say for certain whether these bodies are derived from the original Golgi rods depicted in Pl. 2, figs. 8-13, but I should think that they are so derived, and that they have undergone some change during late spermateleosis. If these bodies are an integral part of the spermatozoon and not the degeneration products of the spermateleosis, one might expect to find some sign of them in the spermatozoa within the female: in Pl. 2, fig. 18, is section of the receptaculum seminis of a female, and drawn to the same scale as fig. 17 above. The same bodies are to be seen at the tails of the ripe spermatozoa.

5. THE FATE OF THE TAIL OF THE SPERMATOZOON DURING ENTRY.

Buelner (3) showed that the sperm-tail sometimes entered the egg, sometimes not. I have been able to confirm these observations. In Pl. 3, fig. 25, is a typical oocyte to illustrate this: the sperm-head (sp) is wrapped around the oocyte nucleus, while the remains of the tail of the sperm are, in this section, seen as four irregular chromophile bodies at spt. In Pl. 3, fig. 22, in the lower oocyte, the sperm-head is at sp, while the tail is cut across as two irregular bodies at spt. In the upper cell of fig. 22 the sperm-tail seems to be partly inside the egg (upper) and partly outside (lower).

While it is generally impossible to say whether these irregular masses (which we can positively identify as remains of the sperm-tail) are, or are not, inside the egg cytoplasm, when we examine eggs at a little later stage of growth, it is quite certain that in the majority of cases the sperm-tail fragments have not only entered the egg but have broken up to form a number of spherical, extremely chromophile, bodies at the periphery. In Pl. 3, figs. 24, and 23 at spt, the beads are noted all around the periphery of the oocyte.

If Mann-Kopsch preparations be examined for this, the beads appear a pale yellow colour as in Pl. 3, figs. 19 and 21, at spt. In some cases it certainly appeared that the number of beads derived from the remains of the tail of the sperm increased in number as the egg grew. This was probably what van Gaver and P. Stephan thought when they believed that the spermatozoa might have something to do with yolk-formation. I do not believe, however, that the beads take part in fertilization or yolk-formation, either directly or indirectly.

Later on they either disappear or become hidden by the formation of clouds of yolk or nucleolar deutoplasm (described below).

6. THE OOGENESIS OF SACCOCIRRUS.

The oogenesis has proved the most difficult problem that I had hitherto attacked, and at one time I despaired of ever

unravelling the intricate story of the origin and nature of the complicated granulations of the oocyte cytoplasm. After a year's work, and the making of a large number of preparations, I feel that this present account is the correct interpretation of the oogenesis. The egg cytoplasm of *Saccocirrus* contains four kinds of grains or formed bodies: (a) Golgi elements, (b) mitochondria, (c) true yolk, (d) nucleolar extrusions or plastin-deutoplasm.

These can all be distinguished one from another by some staining method, as described on p. 18.

(a) The Nucleolus during Oogenesis.

Both Hempelmann and Buchner noted the peculiar perinuclear bodies drawn in Pl. 3, fig. 23, NL, and concluded that they were in some way concerned with yolk-formation. Such a marked process as that depicted in this figure is unknown in any other animal; the history of the formation of these extraordinary attachments to the nuclear membrane is not at all easy to make out, and it is only after a study of material fixed in Champy-Kull and stained by Benda's crystal violet and alizarin that a satisfactory conclusion can be reached.

In Champy-Kull-Benda preparations the nucleolus stains a very characteristic orange-brown shade, while the mitochondria and chromatin are in shades of violet; true yolk (derived from the Golgi apparatus) is stained by the OsO_4 of the Champy's fluid. Now in such preparations the nucleolus of the young oocyte is found to be budding off small pieces, as shown in Pl. 3, figs. 24 and 25; these pieces appear to wander to the periphery of the nucleus and to pass through, but to remain plastered upon the outer surface of the membrane, as in Pl. 3, fig. 24, NL.

Some considerable variation in the exact method of this process is found: in certain cases the pieces broken from the nucleolus are coarse and easily distinguishable, as in Pl. 3, fig. 24, but in some other examples, of which fig. 25 is hardly typical, the broken-off pieces are so small that they are difficult to identify.

Occasionally, as in Pl. 3, fig. 22, the nucleolus may be seen to be differentiated at its periphery into a number of small stainable bodies which may represent the beginnings of the parts to be extruded.

In Pl. 3, fig. 23, is a cell showing the appearance of the nucleolar extrusions after staining in iron haematoxylin or Champy-Kull, while in fig. 21 is a cell treated by Mann-Kopsch and the nucleolar extrusions appear as pale yellowish bodies.

From the stages represented by Pl. 3, figs. 21 or 23 onwards, there is generally marked difficulty in ascertaining the exact fate of the nucleolar extrusions. This is due to the fact that just about this period a second process is set into motion; this consists of an appearance all around the nuclear membrane of a chromophil cloud, which in most preparations obscures the nucleolar extrusions; an exaggerated example of this is drawn in Pl. 4, fig. 35, from a silver nitrate Da Fano preparation, but the cloud does not show so darkly with Benda or iron alum haematoxylin.

At all events there begins at this period a peri-nuclear activity, which also corresponds with the change of the chromophilicity of the egg cytoplasm from a primary oxyphilia to a basophilia. Two other occurrences also tend to obscure the peri-nuclear nucleolar bodies at this period: around each body a clearly-defined vacuole often appears (Pl. 4, fig. 29, NLV, from a Mann-Kopsch preparation), and moreover the mitochondria near the nuclear membrane are now forming actively-growing and dividing clusters. With iron alum haematoxylin it is not possible to make sure as to the fate of the nucleolar extrusions, because these and the mitochondria stain in the same colour. With the Champy-Kull fixation and Benda stain I have found examples which, I believe, establish as a fact my view that the nucleolar extrusions first lose their connexion with the nuclear membrane and then either pass right away into the cytoplasm or immediately begin to break up into much smaller fragments. As with the mode of appearance itself of the nucleolar extrusions, so also the subsequent behaviour of these bodies is open to a good deal of variation.

With almost a suddenness a large number of nucleolar yolk-bodies appear in a ring surrounding, but some distance away from, the nucleus (Pl. 4, fig. 34, is a somewhat later stage).

The appearance of this ring of numerous nucleolar yolk-bodies corresponds more or less closely with the spreading out of the basophil peri-nuclear cloud referred to below (p. 17).

The next period sees the complete change of the cell from primary oxyphilia to a temporary basophilia: this activity is often shown plainly with the Mann-Kopseh osmium tetroxide method, of which Pl. 4, fig. 29, is an example; the whole appearance of the cell seems to change. Later the peri-nuclear vacuoles disappear, the cytoplasm becomes smooth, and the nucleolar yolk-bodies are the most noticeable element in the egg.

In Pl. 4, fig. 30, is an egg fixed for six weeks in formol-Flemming; the true yolk (derived I believe from the Golgi elements) has gone black with the osmic acid, while the nucleolar yolk-spheres are pale, in this case fuchsinophile, bodies; neither mitochondria nor Golgi elements appear in this preparation.

That the nucleolar deutoplasm or yolk-spheres go on dividing in the egg cytoplasm seems to me a very likely suggestion, but I was unable to prove that such was the case. How then otherwise can we account for the extraordinarily rapid increase of clouds of these alizarin-staining granules such as appear in Pl. 4, fig. 34? It seems certain that smaller nucleoli inside the nucleolus keep budding off extra-nuclear fragments (Pl. 4, fig. 34), but this would not account for the arrangement and rapid growth of clouds of granules such as those at *nr* in Pl. 4, fig. 34.

In Pl. 4, fig. 30, which was drawn from a very clear example where the nucleolar yolk-spheres were large, I could not see any of the latter undergoing binary fission; I am therefore disposed to believe that these cytoplasmic nucleoli bud off little pieces just as the larger nucleolus is doing in the cell drawn in Pl. 4, fig. 34; and then these little pieces themselves grow larger.

In Pl. 4, fig. 31, is a part of the cytoplasm of a nearly ripe

oocyte ; it will be noted that there are now enormous numbers of granules formed, and the majority of these are nucleolar deutoplasm derived from the original nucleolus of the oogonium.

(b) The Mitochondria.

In the young oogonia I did not find it possible to demonstrate mitochondria, but in all the oocytes just at or after the last stages of the pro phases of the heterotypic division, mitochondria are easily identified, especially after proper staining in iron alum haematoxylin. In Pl. 3, fig. 22, are two oocytes showing the fine grey-staining bodies which I have identified as mitochondria. These show more clearly in Pl. 3, figs. 24 and 25 ; the mitochondria do not appear to have anything to do with the nucleolus.

(c) The Golgi Apparatus.

The Golgi apparatus (Golgi body or element) was studied by the Cajal, the Da Fano, and the Mann-Kopsch techniques : of these the Mann-Kopsch technique was the most suitable. In young oocytes the Golgi apparatus consists of an excentric juxta-nuclear mass, as at GA in Pl. 3, fig. 20. This mass really lies around an archoplasm, as in Pl. 3, fig. 24, at AR. In Pl. 4, fig. 33, is an oocyte showing the Golgi apparatus on the right of the nucleus. Now in the youngest oogonia the Golgi body is isolated at one side of the cell, but quite early in the history of the progerminative oogonium it grows rapidly and begins to fragment ; the additional pieces so derived move out into the other regions of the cytoplasm, as has already happened in all the three cells drawn in fig. 20 of Pl. 3. In fig. 33 on the same plate (though the oocyte is drawn much older in so far as the extrusion of nucleolar deutoplasm is concerned) the Golgi body is still fairly isolated, being just in process of fragmentation. In some cases, as pieces break off from the original body, they pass away into the free parts of the cytoplasm and form remarkable nests or areas of proliferation, as in the cell in Pl. 4, fig. 28, at GA. While in certain cases the fragments of Golgi body scarcely retain their semi-lunar

spherical condition, in other examples this condition is retained, as in Pl. 3, fig. 19, which was a remarkably clear example; the neighbouring cell in fig. 21 showed this condition less well, while in fig. 33, most of the smaller fragments were of no special shape. In Pl. 4, figs. 28 and 29, the Golgi elements form a fine dust at the periphery of the cell. In all the cases, however, the ultimate result is the same—the apparatus breaks up into hundreds of irregular grains, as in Pl. 4, fig. 32, at GA.

If the Saccocirrus is prepared by the Maun-Kopsch method, and the sections mounted in balsam without any previous treatment, the cell-granules of the oocyte appear as in Pl. 4, fig. 31; here we find a confused mass of granules which have become either blackened or browned to different degrees. But if the sections on the slide be treated for several hours in turpentine all but the Golgi granules become decolourized or a light yellow in colour. In Pl. 4, fig. 33, the egg-granules were throughout the colour of those in fig. 31, but the slide was treated in turpentine and the colour extracted from everything except the apparatus, which is here seen to be fragmenting and spreading through the cytoplasm.

(d) The Formation of Fatty Yolk.

In the egg of Saccocirrus which has been centrifuged, a layer of fatty yolk of an oily type collects on the upper pole of the egg (see Text-fig. 1). The characteristics of this yolk are that it goes greenish or brown only after prolonged osmication and is rapidly destroyed by fixatives containing lipoid solvents.

Such fatty yolk is quite distinct from both nucleolar deutoplasm and mitochondria, but it is best shown by Kopsch techniques which demonstrate the Golgi elements so well. In Pl. 4, fig. 30, the fatty yolk is shown black and the nucleolar deutoplasm yellowish grey, after prolonged immersion in formol-Flemming.

From the method and time of appearance of the fatty yolk I believe it is formed from the Golgi bodies, but I admit it is impossible to make a trustworthy statement in such unfavourable material.

(e) Changes in Chromophility during Oogenesis.

Method I.—Fixation in saturated solution of corrosive sublimate, staining in Ehrlich's haematoxylin and eosin according to Scott's directions (55). The nucleolus of the oogonium is amphophil with distinct basophil preponderance, i. e. more blue than reddish purple. The chromatinic reticulum becomes oxyphil after a certain time, and remains so throughout oogenesis; the oogonial cytoplasm is oxyphil. During oogenesis at the period of the appearance of the peri-nuclear bodies (nucleoli, Pl. 3, fig. 23) the cytoplasm becomes basophil, especially near the nucleus. This basophily persists in a peri-nuclear position for a considerable time and spreads out, but gradually the entire cytoplasm again becomes completely oxyphil.

A typical somatic cell (e.g. gut, or epidermal) shows an oxyphil cytoplasm and basophil nucleus. The head of the sperm is basophil, the tail oxyphil.

Method II.—The same material stained by eosin and toluidin blue (in this order) offered a new point of view. Somatic nuclei were blue, the sperm-head and most of the epidermis cytoplasm blue also. The body-muscles and the tail of the sperms were red. The oocyte cytoplasm had an oxyphil ground, but the nucleolar deutoplasm was bluish. The nucleolus itself was generally amphophil, and, as in the case of the Ehrlich preparation, had a basophil central core and an amphophil cortex with basophil preponderance. In other cases the nucleolus was entirely oxyphil. The peri-nuclear bodies were completely blue.

While these results are in themselves of little importance from the point of view of the detection of peri-nuclear 'chromatin omissions', they show very clearly that at the time when the primary oxyphilia is changing to the basophilia there is great new activity in the region of the nucleus; this activity leads to the formation of new denser cytoplasm, and it will be noted below that there is a correspondence between the pictures given by methods explained above and with formalin silver-nitrate or chrome-osmium techniques.

(f) On Peri-nuclear Activity.

In Pl. 3, fig. 25, is drawn a young oocyte at a time when the nucleolar deutoplasm is being formed: this cell was prepared by Da Fano's cobalt-nitrate-formol-silver-nitrate method. It is remarkable for the fact that it demonstrates very clearly the extraordinary peri-nuclear activity at this stage of oogenesis. It is possibly this material which stains basophil as described on p. 16. In all my silver-nitrate preparations the oocyte at this stage shows this peculiar appearance.

The peri-nuclear cloud stains black or grey according as to whether the preparation has or has not been toned, while the nuclear reticulum and nucleolus are only faintly yellowish and may subsequently be stained bright red in safranin.

I look upon this cloud as the direct result of active protein metabolism around the nucleus: the protein is possibly forming under stimuli sent forth from the nucleus. There is no evidence that this cloud is chromatinic, for the Golgi silver-nitrate methods (Golgi, Cajal, Da Fano) do not impregnate chromatin in any cells I have studied. There is, in addition, no evidence of intra-nuclear specks or dust as described by Schaxel for *Aricia foetida*.

Later on this cloud disperses through the whole egg cytoplasm.

7. SOME CHROMOPHILITY REACTIONS OF THE FOUR CATEGORIES OF CYTOPLASMIC GRANULES.

The table given on p. 18 summarizes the differences which can be shown to exist between the four categories of cytoplasmic granules found in the egg of *Saccocirrus*. Only those methods which best show these differences are mentioned, but besides I used many other fixing and staining techniques (13).

The nucleolar yolk-spheres or deutoplasmic elements approach somewhat in their density to the mitochondria and tend to stain rather like them. Besides these methods quoted there are such tests as the use of alcoholic or acidified

(acetic) fixatives, which either wash away the fatty yolk or the mitochondria, or both, and leave the nucleolar deutoplasm. Then there are the formalin silver-nitrate methods which stain the Golgi elements. The table below shows the following :

Method 1 constitutes a difference between mitochondria and nucleolus and its derivatives.

Method 3 constitutes a difference between nucleolus and its derivatives and true fatty yolk (from Golgi elements).

Method 4 constitutes a difference between nucleolus and Golgi apparatus.

Method 5 constitutes a difference between Golgi apparatus and mitochondria, as also do Methods 1, 2, and 3.

TABLE.

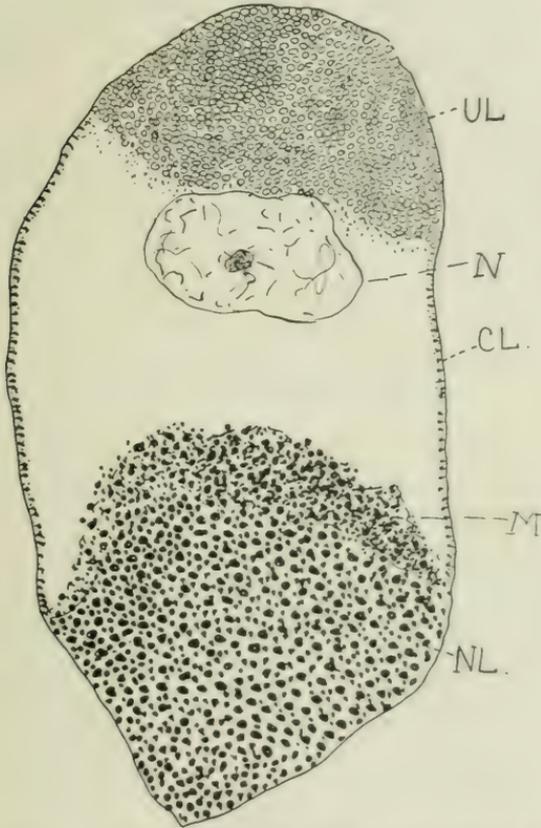
<i>Method Used.</i>	<i>Nucleolus and its Derivatives.</i>	<i>Golgi Apparatus.</i>	<i>Yolk.</i>	<i>Mitochondria.</i>	
Champy - Kull fixation. Benda's alizarin and crystal violet	Yellow-brown	Does not show	Black	Violet	1
Flemming without acetic acid, and iron haematoxylin	Black	Does not show	Greenish-brown	Black or grey	2
Formol-Flemming and Altmann's acid fuchsin	Reddish	Does not show	Black	Did not show plainly	3
Mann-Kopsch	Yellowish	Black, difficult to decolorize in turpentine	Black, easy to decolorize in turpentine	Did not show	4
Mann-Kopsch, Altmann	Reddish	Black, and as above	Black, and as above	Red	5

8. CENTRIFUGE EXPERIMENT ON THE OVARIAN OOCYTE.

Live specimens of *Saccocirrus* were placed in a tube and centrifuged for twenty minutes at 3,000 to 5,000 revolutions a minute. They were afterwards thrown into capsules of fixatives of various types. The centrifuged egg shows three layers, viz. an upper cap, a clear subcentral zone, and

a large lower zone. The upper cap is formed of delicate granules which I think are fatty yolk and probably of the Golgi elements; these granules will go yellowish green

TEXT-FIG. 1.



Centrifuged oocyte of *Saccocirrus*. Shows an upper layer of greenish oily yolk (UL), clear middle layer with nucleus (N), lower layer principally nucleolar deutoplasm (NL) with an upper layer mainly mitochondrial (M). At CL are the cortical lamellae of the egg membrane. (Chrome-osmium and Benda stain.)

after prolonged osmication. The middle layer generally shows two zones—an upper just beneath the fatty yolk and staining in crystal violet, or iron haematoxylin, and looking much like thickened cytoplasmic reticula; then there is the

large lower area formed of the heavy nucleolar deutoplasm, forming by far the largest separate part of the centrifuged oocyte.

These areas are shown in Text-fig. 1. The nucleus generally lies in the middle layer. The mitochondria appear to be lighter than the nucleolar deutoplasm and take up a position in an area above the latter, *m* in Text-fig. 1, but are also found throughout the lower area.

Around the exposed periphery of the egg, the cortical lamellae are beautifully apparent, especially in Benda preparations (*cl* in Text-fig. 1). It is from these lamellae that the substance of the fertilization membrane is produced. See also lamellae in Pl. 4, fig. 27, *cl*.

9. CELLS FOUND IN THE MALE, INTERMEDIATE BETWEEN SPERMATOCYTE AND OOCYTE.

In the coelom of the male *Saccocirrus* are found large cells packed with yolk-spheres; these large cells often fill up all the coelomic space in the mid region of the body, excepting for the areas occupied by developing spermatozoa.

Occasionally one finds large isolated cells lying completely surrounded by and shut in between the large yolk-cells. These isolated cells were once young spermatocytes, which, during growth of the yolk, have become shut off. That this is so is indicated by an examination of a sufficient number of *Saccocirrus* males.

Now these isolated cells are sometimes remarkable for the fact that they show a rough resemblance to oocytes at the stage of nucleolar extrusion. In Pl. 1, fig. 3, is drawn such a cell. The group of yolk-granules is at *y*, several groups of fine mitochondria are at *m*, *m*, while the nucleus is found to be in the process of extruding large peculiar nucleoli, *NLX*. In this one section the nucleus showed four pieces being extruded, two other nucleoli inside the nucleus, and one piece on the lower right of the cell already detached from the nucleus; an examination of the larger nucleoli in fig. 3 shows that they

have a stout triangular base quite like the bodies in Pl. 3, fig. 23.

When I came to examine my first preparations illustrating the oogenesis of *Saccocirrus*, I did not immediately notice the fine mitochondria drawn in Pl. 3, figs. 22 and 23, and I was temporarily led to believe that the nucleolar extrusions might represent the mitochondria. But before I had made more and better preparations, as the result of my experience on this new material, my belief in this view that the nucleoli might represent mitochondria was shaken by finding such cells as that in Pl. 1, fig. 3, in which I noted both mitochondria and bodies which, I concluded, represented the extruded nucleoli of the oocyte.

With regard to the probable reason for the appearance of nucleolar extrusions in a spermatocyte, I believe that it is due to the fact that such cells are packed away among yolk-cells which bring about conditions simulating the metabolism of the egg-cell.

10. DISCUSSION.

(a) General.

The oogenesis of *Saccocirrus* is likely to be typical of several other Annelida, and possibly of Polychaeta such as *Chaetopterus* and *Nereis*, judging from Lillie's figures of centrifuged ova of these genera.

A graphic representation of the oogenesis of *Saccocirrus* would be as follows :

Oogonium.	Full-grown Oocyte.
1. Nucleolus .	{ 1. Nucleolus.
	{ 2. Nucleolar deutoplasm or nucleolar yolk-spheres.
2. Golgi elements .	{ 3. Definitive Golgi elements.
	{ 4. Yolk-spheres (fatty).
3. Mitochondria .	5. Mitochondria.
4. Chromosomes .	6. Chromosomes only.

The part of this scheme about which I feel some doubt is the metamorphosis of the Golgi element into a yolk-sphere ;

that such takes place in Ascidians and Molluses is now quite certain, but the egg of *Saccocirrus* does not provide such clear opportunity for study as that of *Limnaea* or Ascidians. Nevertheless, I believe that I have made sufficiently clear observations on a large amount of material to justify the above interpretations. It is only after the dispersal and breaking up of the Golgi body that true fatty yolk puts in an appearance, and in many cases it seems that Golgi elements can be traced step by step as the eggs grow, metamorphosing into fatty yolk. The extruded nucleolar material has nothing to do with this, and I do not think that the mitochondria are concerned in the process.

When we take the case of extruded nucleolar material in *Saccocirrus* it is difficult to understand why the bulk of the formed reserve granules in the egg should be of nucleolar origin. If one studies the cytoplasm of the egg in a number of different examples of oogenesis, one sometimes finds that the nucleolus supplies the bulk of reserve material (*Saccocirrus*), sometimes the Golgi apparatus (*Patella* or *Limnaea*), sometimes the mitochondria, as in certain insects. In each of these cases, however, analysis of the entire reserve materials in the egg cytoplasm leads one to a similar conclusion for each example, namely, that reserve material in eggs of invertebrates consists of protein and fat or lipin (or both these). Then, of course, most eggs contain glycogen.

In some examples, as in the sponge *Grantia*, the main bulk of reserve material seems to be delicate vacuoles of lipin originating from the ground plasma.

(b) The Preciseness of the Modern Technique for the Cytoplasmic Inclusions and for Chromatin.

Some observers apparently unacquainted with the finer usages of modern cytological technique have written doubtfully of the preciseness of such methods. In all the cytological problems that I have attacked the difficulty I have met with lies not especially in the discrimination between yolk, Golgi elements, mitochondria, nucleolar deutoplasm, and

glycogen, for this was generally easy, but in the identification of chromatin; the problem was whether basophile chromatic material was chromatinic; this is the great problem of cytology at the present time.

I believe that I may be forgiven for holding an optimistic view with reference to our present and future understanding as to the behaviour of the Golgi elements and mitochondria during oogenesis: I think that the works of Jan Hirschler, Weigl, Nussbaum-Hilarowitz, Rio-Hortega, and my own series of papers on the cytoplasmic inclusions have gone far to shed a clear light on the subject, but I do at present feel much puzzled over the nuclear phenomenon in oogenesis.¹

One is driven onward trying to avoid the pool of Charybdis, formed by the chromosome theorists who will not admit of true chromatinic extra-nuclear extrusions, and the rock of Scylla, which in my mind is constituted by the fact that it is at times difficult to believe that the so-called extra-nuclear extrusions are not chromatin. This special matter is further discussed below under the heading of 'The Supposed Chromatinic Nature of Extruded Nucleolar Material'.

In all probability were it not for the ingenious, and one must say believable theories of chromosome workers of Morgan's, Wilson's, or McClung's schools, one would have no hesitation in saying that the extra-nuclear extrusions were chromatin, even though they frequently do not stain quite like the chromatin of the 'resting' nucleus. When one takes the case of the secondary nuclei of the Hymenopterous egg it is very difficult to avoid the conclusion that such granules are chromatin.

This is a matter to which I have given a good deal of attention. Quite recently I have again gone over my *Apanteles* material, and I have found an example which shows the chromatin filaments at the diplotene stage of the prophase of the heterotypic division, while the nucleolus is separate and shows buds, some of which have already passed into the cytoplasm to form minute secondary nuclei.

¹ Mr. R. J. Ludford's recent work has gone far to clear up parts of this obscure ground ('*Jour. Roy. Micr. Soc.*', 1920-21).

While this helps towards a disposal of the view that the chromosomes, at this period at least, are drawn upon to provide material for the formation of secondary nuclei, it does not dispose of the questions as to the nature and origin of the nucleolus which buds off the secondary nuclei.

It is possible to recognize several kinds of nucleolar activity in various examples of oogenesis.

Saccocirrus nucleolus	.	{ definite nucleolus. nucleolar deutoplasm.
Apanteles nucleolus	.	{ definite nucleolus. secondary nuclei (associated with yolk-formation).
Grantia nucleolus	.	{ definite nucleolus. mitochondria ('chromidia').

In certain other forms it is possible to recognize a process of nucleolar extrusion early in oogenesis, but which appears to lead to nothing (possibly in *Patella*).

The belief held by some observers that nucleolar extrusion may be looked upon as a process whereby the nucleus sends chemical messengers into the cytoplasm inducing growth to begin, is discountenanced, for *Saccocirrus* at least, by the very apparent fact that nucleolar extrusion is prone to much variation in the point of time and the rate that it takes place—as shown by comparing the sizes of the eggs in Pl. 3, fig. 23, and Pl. 4, figs. 29 and 33. The process is just beginning in the first-mentioned figure, and has finished in the smaller egg in the last-mentioned figure.

(c) On the Supposed Chromatinic Nature of Extruded Nucleolar Material.

If one fixes the testis or ovary of any animal in Zenker or Petrunkevitch fluid, and stains in Ehrlich's or Delafield's haematoxylin and eosin or Biebrich's scarlet, it will be noted that during the greater part of the development of the sperm the chromatin stains blue, or basophil; but there are certain periods when what we can only assume to be true chromatin,

as it is morphologically derived from preceding materials which stained like chromatin, will be found to stain oxyphil. or in the red stain. As Bayliss especially has shown clearly in his valuable 'Principles of Physiology', staining depends on a number of more or less obscure factors, and it is probably injudicious to lay too much weight on the results of staining fixed material. In many of the parasitic Hymenoptera the egg nucleus contains a large heavily-staining nucleolus which buds off fragments, which pass through the nuclear membrane into the egg cytoplasm, where they form what are known as secondary nuclei. With safranin and light green the nucleolus of the true egg nucleus stains red, and the nuclear (chromatinic) network a green colour. In the sponge *Grantia* the plasmosome of the oocyte partly passes into the egg cytoplasm to form bodies called by Jörgensen and Dendy 'chromidia': I have objected to the use of this term for such nucleolar fragments, both because we do not know that they are chromatinic and also because such 'nucleolar' extrusions appear to be identical with the mitochondria.

We must face the facts frankly: the chromosome theorists would object to the identification of 'nucleolar' extrusions as chromatinic in nature and as derived from the definitive chromosomes. I have shown above that staining tests are not conclusive; several others, and also I myself, have demonstrated that the secondary nuclei are derived from extruded fragments which in the case of such forms as *Myrmecina* or *Apanteles* are, I believe, to be regarded as of nucleolar origin. We find, therefore, that fragments of the nucleolus can form a true nucleus, with nuclear membrane, linin network, and nucleolus.

Seiler (55 a) described in Lepidopterous eggs what he has called a chromatin diminution process: the polar body spindle at metaphase is found to carry three groups of granules, the two outer being the chromosomes which have divided and are becoming separated, the middle group of granules being apparently derived from the ends of the chromosomes by a diminution process, well known in the somatic mitoses of

the developing *Miastor* egg (32). Just before his untimely death Professor L. Doncaster was examining this problem, and sent me some of his slides for examination and suggestions; all that I could do was to recommend the use of stains such as Auerbach and Pappenheim, and methyl blue eosin. Digestion tests and such other microchemical tests are impossible when one is working on the minute spindle in a very small egg. It certainly seemed to me that in the slides sent by Professor Doncaster the intermediate bodies were derived from the ends of the chromosomes as in *Miastor*.¹ Here again, however, we are faced with the same difficulty with regard to staining test, as I have pointed out with reference to the nucleolus: we are not justified in saying that a substance is chromatin simply because it selects methyl green from the Pappenheim or Auerbach stains; no one would care to say that the head of the spermatid was not chromatin, yet at certain periods it will select the red stain from the Pappenheim or Auerbach fluid. To my mind it is useless to declare that the head of the sperm at such stages is not true chromatin, but has only changed its chemical nature; the head of the sperm is derived from chromosomes before it reaches the egg and breaks up into chromosomes when it has penetrated into the egg. The spermatid nucleus takes the red stain from the Pappenheim or Auerbach fluid possibly because the arrangement of its surface or internal substance is more favourable to the molecules of the red stain, and unsuited for the absorption of the green stain.

The facts of the matter are that we know very little about the relationship between the nucleolus and the chromosomes, both during mitosis and during interkinesis; the same remark applies when we come to the subject of the microchemical nature of the nucleolus. I believe that a good step towards the elucidation of the first-mentioned problem has been taken by H. M. Carleton.

This observer has shown that the nucleolus of certain vertebrates contains an argentophil core, or is related more

¹ I have often wondered why this work of Prof. Doncaster was not edited and published.

or less closely to a body which under certain conditions becomes densely black in Cajal's formalin silver-nitrate technique for the Golgi apparatus. During mitosis Carleton has shown that the argentophil core which he calls a nucleolus (Haeckel) does not lose its individuality but divides, and may be found among the two chromosome groups of the telophase. I have been enabled to go through the preparations made by Carleton and can vouch for the correctness of his description; moreover, I possess preparations of the gut-cells of *Saccocirrus*, of the follicle-cells of *Stenobothrus*, and of many tissues in *Rana*, all of which show a typical nucleolus. What is very important is that Carleton has shown that the nucleolus may be associated with either a 'karyosome' or a 'plasmosome' type of nucleolus. These remarks will serve to indicate the importance of work carried out on the nucleolus, especially with Cajal's formalin silver-nitrate method; Da Fano's cobalt-nitrate method also serves to bring out the nucleolus in some forms.

Interpreting the work of Carleton on the nucleolus, and also in the light of Cajal's figures of various mammalian tissues and my own materials of invertebrates, I believe that the nucleolus, term used generally, might be morphologically independent of the chromosomes during the germ-cell cycle; the nucleolus during interkinesis might exist as a compound body consisting of a core which is argentophile and sometimes chromophile to other stains, and this core might act as the centre for the proliferation of a more extensive body which functions as the plasmosome or karyosome of the 'resting' nucleus; furthermore, during mitosis this outer region proliferated from the argentophil core possibly becomes lost, to be reformed in the next interkinesis. How far these suggestions will be found correct is impossible to say at present, but many of the facts we know now point in the direction I have indicated. Moreover, this view would coincide with the already-formed theories of the chromosome worker.

The nature of the nucleolus is mainly proteid, maybe even in some cases nucleo-proteid, but its functions appear to be different from those of the chromosomes. The nucleolus, like

the chromosome, Golgi element, and mitochondrion, is capable of growth and binary or multiple fission.

Buchner, in his paper on the secondary nuclei of parasitic Hymenoptera, among the other conclusions, comes to the two following: accessory nuclei are to be traced back at the beginning, as naked chromatin (*sic*) granules lying in the cytoplasm. From these granules develop enchylema, nuclear membrane, and linin network, while the granule itself becomes the nucleolus of the accessory nucleus. Buchner has used safranin and light green and iron haematoxylin as stains; he labours under the delusion that what stains in a basic dye must necessarily be chromatin. He states that the chromatin granule which induces the formation of karyolymph, linin network, and nuclear membrane, later becomes a 'nucleolus'. Buchner figures the oocyte of *Bombus* and *Myrmecina* showing the nucleoli of the head nucleus as red granules (safranin) and a more or less faint chromatin (?) network green ('lichtgrün'). The accessory nucleus also shows a red nucleolus and a green network. Buchner and others have concluded that the red-staining substance of the head nucleus, which becomes extruded through the nuclear membrane, is chromatin. As I have mentioned before I do not believe that one should lay too much weight on the staining tests (and Buchner has not tried several of the stains I should like to have seen used), but the points which must be emphasized are, firstly, that it is proven that the nucleolus of many hymenopterous insects does fragment and partly pass into the cytoplasm; and secondly, that these fragments do form secondary nuclei, exactly similar in certain species, to the head or principal nucleus. Call the red-staining body inside the head nucleus what one may, plastin or chromatin, plasmosome or karyosome, it is a fact that fragments of it can give rise to secondary nuclei.

There is some temptation to use the facts which have recently been described in parasitic Hymenoptera, and in this paper, with reference to the behaviour of nucleoli, as support for a 'binuclearity' hypothesis of some kind. In a recent paper

on the giant germ nurse-cells of *Testacella* (4) I ventured to interpret certain of my results in this manner, and it must be said that the case of the secondary nuclei is very suggestive.

There are three possible modes of general interpretation—either the nucleolus represents a second chromatin of some kind, but separate from the chromosomes, or it derives its chromatin from the chromosomes, or there is some cell substance other than chromatin which has the attribute of forming bodies similar to the ordinary nuclei, except for the presence in them of true chromatin. Whether the power of production of a nucleus-like body is to be looked upon as a proof of the chromatinic nature of a granule is unknown.

(d) On the Special Part played by the
Nucleus during Oogenesis.

Recent studies on the cytoplasmic inclusions of the germ-cells have revealed the fact that all such units possess both Golgi elements and mitochondria, and that these two categories of formed elements take a prominent part in the upbuilding of the egg cytoplasm. No one has claimed a nuclear origin for the Golgi body, and in my work I have found a complete Golgi apparatus in the earliest germ-cells which have been studied—in molluses, insects, birds, amphibians, and mammals. The case of the mitochondria is different; several observers have claimed that they have found the mitochondria to originate from the nucleus during early stages of oogenesis or spermatogenesis. I had never seriously believed these accounts, and still doubt most of them; but in my own studies on the sponge *Grantia* I was led to identify the 'chromidia' of Jörgensen as the representatives of the mitochondria; now Dendy firstly, and then I, have shown that the 'chromidia' of Jörgensen are nucleolar in origin. I still have some doubts as to whether true mitochondria do not exist in *Grantia*, but my efforts to demonstrate other granules which might be mitochondrial have so far not met with success; therefore I can but assume tentatively that in the case of *Grantia* the mitochondria are of nuclear origin.

It is important to notice that careful modern work on oogenesis confirms certain previous accounts of the extrusion of nucleolar material into the egg cytoplasm, and puts on a definite basis of truth the claim that the nucleus takes a part in the development of the cytoplasm.

All such positive evidence which we possess in this direction applies to the behaviour of the nucleolus, and I do not believe that we are able to point to any circumstances which would lead us to conclude that the chromosomes take a part, though I think that such is the case. Probably the only significant fact upon which we can fall back lies in the formation of flocculent threads and reticula from the chromosomes after the prophases of the heterotypic division, and just before the real inception of the growth period of oogenesis. But this might just as well be interpreted as preparation by the chromosomes for their own growth by means of substances absorbed from the egg cytoplasm.

With regard to this difficult matter of the relationship between nucleus and cytoplasm during oogenesis, I believe that zoologists may be able to ascertain new facts if they develop and use more constantly the various silver-nitrate techniques, which give pictures unobtainable by other methods.

(e) Schaxel's Chromatin Emission.

From time to time in these papers I have referred to Schaxel's work on chromatin emission in a number of invertebrates which he has studied. Criticisms which have already been brought forward by me, in conjunction with Woodger, are that Schaxel has not worked at his material by proper methods, and he has not attacked the problem from the point of view of the cytoplasmic inclusions. Furthermore, he has not established that his granules are chromatin or that they are emitted through the nuclear membrane. With corrosive fixation, &c., and Ehrlich's haematoxylin, the granules are found to be basophil, which probably proves nothing with regard to their microchemical nature. A new phase in the problem of Schaxel's work was introduced by Miss van Herwerden, who, by treating

Strongyloentrotus eggs in a 'nuclease' procured from spleen and pancreas, succeeded in dissolving away Schaxel's granules, which did not appear when the eggs were subsequently treated by methods which fixed and stained the granules in eggs not treated by the enzyme solution.

This work has been especially referred to by some recent writers, who consider that weight should be attached to Miss van Herwerden's statements.

With certain precautions, which were incomplete, she prepared a proteolytic enzyme from spleen, according to the directions of Sachs (52). Now I submit that her enzyme solution was probably a mixture of several enzymes, 'nuclease' possibly, but also lipolytic enzyme as well. The fact that cell granules disappear under treatment by such a solution proves nothing with reference to their precise chemical nature. These granules were possibly mitochondria whose proteid basis was washed away by some protease, which would cause them to disappear as definite granules—or what is more likely, Miss van Herwerden's 'nuclease' contained a lipoclastic body which swept away the limin content of the mitochondria.

Until an expert on enzymes prepares solutions whose contents are known and whose reactions towards various organic materials are completely worked out *in vitro*, until the microchemistry and origin of bodies in question are better understood, then and then only should one place any weight on such work by enzyme action as that of Miss van Herwerden on Schaxel's 'chromatin' granules. It should be noted carefully that Schaxel's granules do not produce bodies resembling nuclei, as happens in *Apanteles*, &c.; one should not without good reasons call any haematinophilous body chromatin: even if his granules are extruded from the nucleus, they might just as well be nucleolar as chromatinic; and he might with advantage try other methods.

Zoologists should note carefully that an espousal of Schaxel's views seems to necessitate either the further adoption of a binuclearity hypothesis or the rejection of the chromosome theory.

For if Schaxel's granules are chromatin, using the word in the sense that they are made of the same sort of material as the chromosomes, either they must have originated from the latter—have been budded off from them—or there must be two kinds of chromatin in the egg nucleus.

I cannot see how the adoption of the first alternative will allow one still to hold that the present-day chromosome theory is likely to be true; and the very behaviour of the nucleolus in *Apanteles* shows that there is a body other than the chromosomes which can produce a nucleus.

By placing one's belief in the second of the two alternatives—in some form of 'binuclearity hypothesis', one could also make many of Schaxel's observations fit in with the more theoretical aspect of the question.

While my mind is as open as it well could be in view of my own observations, I do not at present feel that Schaxel has attacked the problem in the best way, and I refrain from definitely accepting any of his views till some other observer carefully reinvestigates his claims and uses all the best and latest cytological techniques.

Perhaps it should be mentioned that the above remarks do not commit me to the espousal of any 'binuclearity hypothesis', though I feel that there is some good evidence for such a postulation.

(f) Centrifuge Experiments in Annelid Development and what they demonstrate.

It has been shown in this paper that the major part of the granules of the egg of *Saccocirrus* is derived from nucleolar material extruded from the nucleus. If these nucleolar extrusions represent Schaxel's chromidia or the granules which form the secondary nuclei in parasitic Hymenoptera, and if they are of chromatinic nature, and not merely metaplasm or yolk, one might expect them to play some special part during embryonic development. They might even represent organ-forming materials.

But apparently this is not the case: Lillie (43) has given

some figures of centrifuged eggs of *Chaetopterus* and *Nereis* which lead me to believe that in these animals the egg contains fatty yolk (or oil) and nucleolar deutoplasm as in *Saccocirrus*. In *Chaetopterus* he finds the layers in the centrifuged egg to be a grey cap, upper (the 'fatty yolk' of this paper), a clear area in the middle, and a lower layer of 'yolk' (my 'nucleolar deutoplasm' and mitochondria); these areas correspond with the layers in the centrifuged *Saccocirrus* egg (p. 18).

Now, speaking of these layers in developing embryos and of formative stuffs in general, Lillie remarks: 'So far as they (formative stuffs) are to be identified with the visible substances segregated by the centrifuge, it would appear to be indicated by experiments that they can play no specific rôle in differentiation, because in centrifuged eggs they may occupy variable positions in the embryo.' This view coincides with that of Morgan (quoted in my previous paper (17)) and with Miss Beckwith's study on *Hydractinia*. Any physiological derangement during the development of centrifuged eggs seems to be due either to mechanical difficulties of massed yolk or to absence of nutriment.

It is interesting to note, too, that Morgan came to his conclusion partly as a result of work on Echinoderm eggs, where Schaxel finds an emission of 'chromatin' granules.

(g) The Probable Part played by Mitochondria and Golgi Apparatus in Heredity.

Modern cytologists tend to become divided into two groups—those working on the nucleus and those working on the cytoplasm. Nearly all modern text-books dealing with Heredity and Sex treat exclusively of the part played by the chromosomes in the mechanism of Heredity, and most observers are satisfied to accept the view that ultimately the nucleus is the seat of the substances which contribute to bring about the phenomena of Heredity. 'Die Mitochondrien sind die protoplasmische Vererbungssubstanz' is a statement which serves to show us that the chromosome theorist is not alone in this field. In the germ-cell cycle the chromosomes have been

shown by Van Beneden, Boveri, Wilson, Morgan, Montgomery, McClung, Doneaster, and many others, to go through certain definite changes, which have been found to correspond with many of the peculiar phenomena of sex and heredity in breeding experiments. The main facts ascertained with regard to the chromosomes are briefly as follows :

1. They are constant in number in any one species.
2. In ordinary cell-division each chromosome is halved so that each moiety is a complete replica of its fellow.
3. In the formation of the germ-cells there is a process whereby the ripe gamete comes to have the halved or haploid number of the chromosomes.
4. The male and female pronuclei in fertilization are practically equivalent, and possess the same number of chromosomes (overlooking the x and y chromosomes).
5. In the formation of the ripe spermatozoon no visible part of the chromatinic substance is rejected.

In the cytoplasm of the animal cell it has been shown that two important categories of formed protoplasmic elements exist: namely, mitochondria and Golgi elements. The purpose of this section is to compare and contrast the behaviour of these protoplasmic bodies with the chromosomes of the nucleus. Under the first heading—'That the chromosomes are constant in number in any one species'—we may compare and contrast the Golgi body and mitochondria. While it is not generally possible to gain absolutely explicit evidence by examining the mitochondria in most animals, it is nevertheless true that in some forms the mitochondria are so few and so large that definite counts may be made. As examples I give the following: (*a*) In *Paludina* the typic spermatid may contain from four to seven spheres. Four is the commonest number. These spheres are subequal in size in those spermatids which contain four spheres and in those which contain seven. (*b*) Wilson (30) has shown the same variation to apply in *Centrurus*, and Retzius (25) also in a variety of Molluses. (*c*) It was shown (9) that in *Helix aspersa* the mitochondria in one spermatocyte

or spermatid were often remarkably different in size and number from those in another example. It is thus clear that the mitochondria are not usually of markedly definite number or size in the germ-cells or somatic cells of any given species. With reference to the Golgi apparatus the same applies. In *Helix aspersa* (9) and in other Molluscs it was shown that the dictyosomes or Golgi bâtonnets could vary in number considerably.

Moreover, examination of preparations of this apparatus in any somatic cells, as well as germ-cells, gives the impression that the Golgi elements are variable to an extreme.

The statement—'In ordinary cell-division each chromosome is halved'—may now be used as a basis for comparison and contrast with what occurs in the mitochondria and Golgi apparatus. In many cases it is difficult to get quite complete evidence as to whether a mitochondrion does divide during cell-division, but the general impression one gathers after examining cells in division is that the mitochondria are sorted out whole and haphazardly. In special cases, e.g. *Centrurus* (30) and *Paludina*, it is possible that the elongate mitochondria are halved transversely but not longitudinally. In by far the majority of animals it seems tolerably clear that the process of chondriokinesis or distribution of the mitochondria (or chondriosomes) between the daughter-cells is haphazard, and not in any way comparable to the process of karyokinesis. This result has been arrived at by a number of independent workers, and may be taken as established.

The Golgi body in the dividing cell consists of rods or granules (dictyosomes); in most cases these dictyosomes keep around the zone of the amphiaster, often stuck on the asters themselves, and, as with the mitochondria, the observer is impressed with the fact that the whole train of events in dictyokinesis, or the distribution of the dictyosomes between the daughter-cells, is extremely haphazard and much less precise than with the process of karyokinesis. That this is so can easily be shown to be the case in the molluscan germ-cell; in the spermatid of *Limax maximus* the Golgi apparatus generally

consists of two dictyosomes ('Nebenkern' bâtonnets); but in other cases there may be three, and one never finds a spermatid with a single bâtonnet. It is therefore certain that during dictyokinesis the Golgi elements are not always sorted out equally. That the Golgi rod is divided or halved like the chromosome is unlikely from this evidence, described in detail elsewhere (9 a): in *Limax agrestis* the spermatocyte has a Golgi apparatus formed of some eight dictyosomes or bâtonnets. This cell divides twice to give rise to four spermatids, but each of the latter only contains two of the Golgi bâtonnets; this shows that in dictyokinesis the bâtonnet is not divided like the chromosome.¹

With regard to the fact of the maturation of the germ-cells and the reduction of the chromosome number, nothing comparable can be found in either mitochondria or Golgi elements of germ-cells. In the egg the polar bodies rarely contain mitochondrial granules or Golgi elements, and never in such quantity as to suggest a special reduction in number. In the case of the male germ-cells the same applies: the first and second maturation divisions (chondriokinesis) in the male are of the same type, and while they bring about a halving, and then a rough quartering of the original number of mitochondria in the spermatocyte, this process is not of the same nature as the reduction of the chromosomes. The same remark applies to the Golgi elements in dictyokinesis of the male germ-cells during maturation.¹

In the last stages of gametogenesis in the male no chromosomes are lost: the case of the mitochondria and Golgi apparatus is instructive, for in many Mollusca it has been shown that possibly all the Golgi elements, and much of the mitochondrial matter, are lost during spermateliosis, being sloughed off the tail of the sperm (9, 9 a). Such seems to occur with the mitochondria in Mammalia; Regaud shows that the bead of sloughed off protoplasm of the sperm of rats may contain mitochondria (24), though the main bulk of the granules forms part of the sperm. In other words, the chromatin of the

¹ See also Ludford and Gatenby, 'Proc. Roy. Soc.', vol. 92, 1921.

sperm is the only part which is meticulously guarded during spermatogenesis of all animals.

That the male and female pronuclei contain the same number of chromosomes (leaving out the special x or y chromosomes) is a notorious fact. The sperm never contains as many mitochondrial granules as the egg, and in only one case (*Ascaris megalocephala* (32)) has it been shown that at the time of fusion of the ♂ and ♀ pronuclei, the number of mitochondria of the ♂ gamete are about the same as those of the ♀. The above comparisons show conclusively that of all the cell elements the chromosome is the only one whose behaviour is precise and coincident with the expected conduct of bodies directly engaged in the processes of heredity, the results of which, as breeding experiments show, are often of previously calculable exactitude.

As direct bearers of any important or precise factors of heredity, the Golgi body and mitochondria appear to be ruled out by their inexact and variable behaviour in the germ-cell cycle. The chromosomes, and the chromosomes alone, fulfil the necessary conditions.

11. SUMMARY.

Spermatogenesis.

1. The spermatogonium is of the usual type, containing both mitochondria and Golgi apparatus (Pl. 1, fig. 7).

2. The spermatocyte contains the same inclusions as the spermatogonium, but in addition there is to be found, in a large number of cases, a group of granules generally lying near the Golgi elements and giving the microchemical reactions of true yolk, i. e. turning greenish yellow in chrome-osmium fixatives, not staining in haematoxylin or acid fuchsin, and generally dissolved out by strong lipid solvents (Pl. 1, fig. 1, y).

3. Nurse-cells often accompany groups of spermatogonia. The nurse-cells contain large quantities of yellowish yolk, as well as fuchsinophil bodies, possibly mitochondrial in nature (Pl. 1, fig. 1, yc).

4. In the spermatocyte there is another group of granules to be found, especially in Kopsch (OsO_4) preparations. These are individually much larger than the four members of the group of yolk-granules, and are about ten to sixteen in number; they go brownish in OsO_4 . These larger granules have been traced into the spermatid, where about three or four are present, and appear to form the sides of the mitochondrial part of the sperm-tail (Pl. 1, fig. 2, x, and Pl. 2, figs. 11 and 12, x).

5. During the spermatocyte division prophases, the group of yolk-granules is found to take up a position near the equator of the spindle (Pl. 1, fig. 4, y), and subsequently becomes divided into two smaller groups in later stages of the division (Pl. 1, fig. 5). This process occurs in both maturation divisions, so that each spermatid contains about one-quarter of the yolk-granules of the spermatid. The mitochondria, as is generally the case during cell-division, become altered in such a way that they form a tangled mass of thread-like bodies, which are subequally sorted out into two portions, one in each daughter-cell (Pl. 1, figs. 4 and 5). The larger group of granules which were thought to form part of the tail-sheath were not found during mitosis.

6. The newly-formed spermatid contains the usual inclusions plus the group of yolk-granules (y in Pl. 2, fig. 8). At this stage the sperm-sheath granules are occasionally found, and occur much more often in later stages (Pl. 2, fig. 9, x).

7. The spermatocytosis stages, or metamorphosis of spermatid into spermatozoon, are remarkable for the manner of formation of the tail-sheath. The mitochondria become grouped behind the nucleus and around the outgrowing axial filament, while the Golgi elements and yolk-granules take up a position behind the mitochondria (Pl. 2, fig. 9). Tail-sheath granules are usually found in the vicinity (x in Pl. 2, fig. 9).

8. The mitochondria, hitherto single and all approximately equal in size, now begin to run together, like rain-drops, forming groups of larger and smaller granules (Pl. 2, fig. 10, MM and M). This process goes on till only three large subequal spheres are left (figs. 11, 12, and 14), and then these spheres begin to elongate

to form the mitochondrial tail-sheath (Pl. 2, fig. 13 and 16). The tail-sheath granules are seen at x in Pl. 2, figs. 11, 12, and 14.

9. During these stages the Golgi elements tend to become thrown downwards along the length of the sperm (Pl. 2, fig. 16), and this also occurs with the yolk-granules. In a bunch of ripe sperms within the body of the male *Saccocirrus* small granules are always found on the lower region of the sperm-tails, and there seems to be good evidence that such elements are derived from the Golgi apparatus (Pl. 2, figs. 17 and 18). If the receptaculum seminis of the female is examined, such granules are also found on the tails of the sperms (Pl. 2, fig. 18, GAX).

Fertilization.

10. *Saccocirrus* is an example of precocious entry of the spermatozoon into the unripe oocyte (Pl. 3, fig. 22). The nuclear head of the sperm alone enters the egg completely at first, while the tail remains plastered on the surface of the young oocyte (Pl. 3, fig. 22, head at sr, fragments of tail at spt). It is very difficult therefore to say whether these sperm-tail fragments are or are not inside the oocyte cytoplasm at this period. Later on, however, it is quite easily observed that the elements of the sperm-tail do enter the egg, break up further, and form large numbers of spherical granules (consecutive stages given in Pl. 3, figs. 21, spt, 22, spt, 24, and 25).

11. In many cases one cannot help believing that these beads, derived from the remains of the sperm-tail, grow in number and in size (cf. Pl. 3, figs. 23 and 25).

12. These beads always remain in the periphery of the egg, but do not seem to take any noticeable part either in the formation of yolk or in any process of fertilization. Careful examination of the periphery of many oocytes reveals the fact that the granules are of two types, one going yellowish in OsO_4 , the other going black, as shown in Pl. 3, fig. 21. It was thought that the black granules might have something to do with the black granules noted on Pl. 2, fig. 18, GAX, which were considered to be derived from the Golgi apparatus.

13. The peripheral granules of both types later disappear or become hidden by the formation of clouds of nucleolar deutoplasm.

Oogenesis.

14. By staining, fixing, and by centrifuge experiments it can be shown that the full-grown oocyte of *Saccocirrus* contains four distinct kinds of formed 'yolk'-granules, i.e. Golgi elements, mitochondria, true yolk, nucleolar extrusions or nucleolar deutoplasm. (See their fixing and staining reactions in a table on p. 18.)

15. The most numerous and chemically most resistant granules are neither mitochondria nor Golgi elements, but are derived from nucleolar extrusions. At a very early stage the oocyte nucleolus buds off pieces which pass through the nuclear membrane, but at first remain stuck on its outer surface (stages in Pl. 3, figs. 23, 24, and 25, XL). At one stage these nucleolar derivatives form an extraordinary picture, being stuck all over the nucleus in the form of pyramidal bodies, whose base adheres to the nuclear membrane (Pl. 3, fig. 23). The nucleolar derivatives stain intensely in haematoxylin or fuchsin (fig. 23), but only go yellowish in osmic acid (fig. 21).

16. Later on these pyramidal granules lose their connexions with the nuclear membrane, but, remaining quite near, become the centres of numbers of large vacuoles which appear (Pl. 4, fig. 29). Inside these vacuoles the nucleolar granules partially break up, and subsequently, after the absorption of the vacuoles, the granules move out further from the nuclear membrane and form clouds of granules in the egg cytoplasm (Pl. 4, figs. 30 and 34). The marked vacuolar stage in the history of the egg seems to occur with suddenness, and is not discoverable at this period in all eggs of this size (Pl. 4, fig. 29). It is just about this stage that great activity is noticeable around the periphery of the nucleus, as shown in Pl. 4, fig. 35, by formol-silver nitrate technique. The nucleolar deutoplasm forms dense clouds of heavy granules throughout the entire egg cytoplasm.

17. If the ovary of *Saccocirrus* be prepared by a silver

nitrate or osmic acid Golgi-body method, an appearance such as shown in Pl. 3, fig. 19, is seen. Large numbers of crescent-shaped bodies, such as were noted in the spermatocyte (Pl. 1, figs. 6 and 7), occur throughout the cytoplasm. In younger oocytes the bodies of the Golgi apparatus are densely packed and placed to one side of the cell (Pl. 3, fig. 20, GA). Such Golgi elements eventually divide rapidly, and spread out, as fine crescents or slightly elongated rods, through the cytoplasm of the full-grown oocyte (Pl. 4, fig. 32, GA).

18. If the ovary be treated by a chrome-osmium method, and stained in iron alum haematoxylin or acid fuchsin, fine mitochondria become visible (Pl. 3, figs. 23, 24, 25, M). Such mitochondria are difficult and sometimes impossible to see in the youngest oocytes and the oogonia.

19. In chrome-osmium preparations there are also to be seen fine true yolk-spheres, characterized by the fact that they go yellow-green in the fixative and do not stain in haematoxylin or fuchsin.

20. By centrifuging the oocyte, three layers appear, viz. an upper layer formed of true yolk (greenish), a middle clear protoplasm layer, and a lower layer mainly formed of nucleolar deutoplasm, with a mixture of mitochondria.

21. In many oocytes an enigmatic body, much like a secondary nucleus, was noted (Pl. 3, fig. 23, XX, and Pl. 4, fig. 30, XX).

22. The oogonial cytoplasm is oxyphil, and during oogenesis becomes basophil, and then again oxyphil in the full-grown oocyte (p. 16).

Intermediate Cells.

23. In Pl. 1, fig. 3, is a cell found in a male Saccocirrus, and it shows characters intermediate between an egg and a spermatocyte (p. 20).

Discussion.

24. The above facts are discussed on p. 21, and also the probable part played by mitochondria and Golgi bodies in heredity (p. 33).

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13. EXPLANATION OF PLATES 1-4.

ILLUSTRATING PROFESSOR J. BRONTÈ GATENBY'S PAPER ON THE GAMETOGENESIS OF SACCOCIRRUS.

EXPLANATION OF LETTERING.

AS, acrosome. AR, archoplasm or centrosphere. CH, chromosomes. CMO, vitelline membrane. CL, cortical lamellae of egg. GA, Golgi apparatus (‘Nebenkern’), Golgi body or element. GAX, body supposed to be a part of the Golgi apparatus. GX, body believed to be forming the acrosome. M, mitochondria. MM, macromitosome or forming middle-piece (mitochondria) of sperm-tail. N, nucleus. NL, nucleolus, or fragments of latter forming nucleolar deutoplasm. NLX, nucleolar bodies homologous with the true nucleolar deutoplasm of egg. NLV, vacuoles around the nucleolar extrusions. SB, spindle-bridge. SPT, sperm-tail. SPZ, spermatozoa. SP, sperm inside young oocyte. V, vacuoles in ground protoplasm of oocyte. X, bodies believed to form a part of the skeleton of the sperm-tail, on each side of the macromitosomal (mitochondrial) spheres. XX, nuclear-like bodies sometimes found in oocytes, possibly secondary nuclei. XY, bodies near asters, possibly part of the Golgi apparatus. Y, yolk-spheres. YC, yolk-cell or nurse-cell.

Scale of Figures.—On Pl. 1, all figures, except number 6, are drawn to the scale indicated in the middle of the plate. The scale for fig. 6 is near the drawing.

On Pl. 2, all figures, excepting 17 and 18, are drawn to the scale in the middle of the plate, the scale for figs. 17 and 18 being near by.

All figures on Pl. 3 are drawn to the scale on the right-hand side. On Pl. 4, all figures, excepting number 34, are drawn to the scale given below fig. 29.

Techniques Used.—M.K., Mann-Kopsch osmium tetroxide method. CH.K., Champy-Kull chrome-osmium acid fuchsin toluidin blue and aurantia method. D.F., Da Fano's cobalt nitrate formol-silver nitrate method.

PLATE 1.

Fig. 1.—Full-grown spermatocyte and part of nurse- or yolk-cell on left. In the spermatocyte the Golgi apparatus (GA), the mitochondria (M), and the group of yolk-spheres (Y) are to be seen. The nurse-cell contains

large yolk-spheres (γ) and smaller fuchsinophile bodies, possibly mitochondrial in nature (\mathfrak{M}). CH.K.

Fig. 2.—Younger spermatocyte from a Mann-Kopsch preparation, showing at x a number (about two) of largish spheres, believed to be identical with the same bodies marked x in figs. 9, 10, and 11, and which seem to take some part in the formation of the tail skeleton of the sperm. At γ are the yolk-granules, and at GA the Golgi apparatus; compare this with fig. 1, in which the apparatus is formed of delicate slightly-curved rods. In fig. 2 the rods are heavily impregnated with OsO_4 , and possibly owing to a shrinkage of the centrosphere, they have become much more curved. The mitochondria do not show. M.K.

Fig. 3.—Cell of the spermatocyte series but showing a modification; the mitochondria are small, like those of the egg (fig. 24), and peri-nuclear bodies are present at NLX (compare with egg in fig. 23). At γ is a group of yolk-spheres. CH.K.

Fig. 4.—Metaphase of second spermatocyte division. Note alteration in shape of mitochondria (\mathfrak{M}), which from their previous granular structure (fig. 1) have become filiform. At γ the group of yolk-spheres has become grouped near the spindle preparatory to being sorted out into two groups as in the next figure. At XY are bodies supposed to be Golgi elements stuck on the poles of the asters.

Fig. 5.—Telophase of second spermatocyte division, the equatorial plate, is forming, and the mitochondria, still filamentous, are grouped near in a special manner, being most numerous near the forming cell-wall. At γ are the yolk-spheres, now sorted out into two groups. CH.K.

Fig. 6.—Spermatocyte, for comparison with the oocytes in fig. 20. M.K. Scale above.

Fig. 7.—Spermatogonium drawn to same scale as spermatocyte in fig. 1. Shows Golgi apparatus consisting of from eight to ten dictyosomes or rods, a spindle-bridge at SB , and the mitochondria at \mathfrak{M} , grouped near the centrosphere. M.K., counter-stained in Altmann.

PLATE 2.

Fig. 8.—Newly-formed spermatid, showing the Golgi apparatus somewhat scattered on the right and the mitochondria surrounding the nucleus. The yolk-granules form a compact group at γ . The centrosome would be on the right side of the nucleus. CH.K.

Fig. 9.—Later spermatid after the outgrowth of the axial filament. The spheres at x are probably of the same nature as those drawn in Pl. 1, fig. 2. The mitochondria have now become grouped behind the nucleus at \mathfrak{M} , while the Golgi elements (CA) and yolk-spheres (γ) have drifted to the bottom of the elongating cell. The cytoplasm, as in many Kopsch (OsO_4) preparations, is coarsely fibrillar. At GX is a body believed to be forming the acrosome. M.K., counter-stained in Altmann.

Fig. 10.—Later stage. The mitochondria have begun to run together to form a number of larger spheres (MM). At x is one of the large granules seen in fig. 9 and in Pl. 1, fig. 2, while at GX is the same body mentioned in the description of fig. 9. The yolk-granules (Y) form a fine group to one side of the cell. The nucleus in this cell is still spherical, in this being less advanced than that of fig. 9, which is depressed. CH.K.

Fig. 11.—Later spermatid, nucleus now depressed on one side, or cap-shaped. The macromitosomal spheres (MM), 'Nebenkern' of some authors, are larger, not all the same size, and there is still a collection of unused mitochondria at M. The acrosome is seen as a thickened edge of the nucleus at AS. Other parts as before, except that notice should be taken of the fact that the Golgi elements (GA) have become drawn up below the macromitosomal spheres. CH.K.

Fig. 12.—Later stage, all mitochondria have run into the macromitosomal spheres (MM), only two of which are shown. Golgi apparatus still drawn up below the mitochondrial spheres. The cytoplasm is stringy as is often found in osmic-acid preparations. Nucleus further depressed and shrinking in size. M.K., counter-stained in Altmann.

Fig. 13.—Forming spermatozoon, showing elongated macromitosome (MM) and other cell inclusions. CH.K.

Fig. 14.—Macromitosome or mitochondrial spheres, at the stage of fig. 12, but viewed from below. Note skeletal granules at x, and unequal size of spheres. CH.K.

Fig. 15.—Part of tails of fully-formed sperms, at a higher magnification than in fig. 17, to show the bodies marked GAX, which are thought to be Golgi elements. M.K.

Fig. 16.—Forming spermatozoon, at a stage later than that drawn in fig. 13.

Fig. 17.—Bundle of ripe sperms from coelom of male; refer to fig. 15. M.K.

Fig. 18.—Receptaculum seminis of female, to show the presence of the special granules (GAX) on the tails of the spermatozoa, SPZ. M.K.

PLATE 3.

Oogenesis of Saccocirrus.

Figs. 19 and 20.—Four oocytes prepared by the Mann-Kopsch-Altmann method to show Golgi apparatus. The peculiar peripheral granules derived from the sperm-tail (SPT) are shown well. At SR is the head of the spermatozoon, and at NL a peri-nuclear thickening marking partly the nucleolar extrusion, and also as well the peri-nuclear activity, which seems to be something apart from nucleolar extrusion (note also Pl. 4, fig. 35).

Fig. 21.—A later stage showing Golgi apparatus and advanced nucleolar extrusion, NL. The peripheral granules in such Kopsch preparations appear to be of two sorts—those staining quite black, and those yellowish.

Fig. 22 (on the left-hand bottom side of the plate).—Shows two young oocytes, just after entry of spermatozoon (SP). The mitochondria are at M, nucleolar extrusion is just beginning, while the remains of the sperm-tail (SPT) are seen lying, some inside, some outside the oocytes. CH.K.

Fig. 23.—Older oocyte, showing remarkable appearance of nucleolar extrusion (NL), the mitochondria (M), and the sperm-tail remains, which appear to have grown to form numerous spheres around the periphery of the oocyte, at SPT. Other vacuoles (V) are to be seen, which may be the negative image of the non-impregnated Golgi apparatus. At XN is a body thought to be a secondary nucleus of some kind. CH.K. and iron haematoxylin.

Figs. 24 and 25.—Stages earlier than previous figure. At AR is a centrosphere or archoplasm; in fig. 25 the sperm-tail remains are just passing into the cytoplasm, while in the other figure they have begun to break up into spheres. CH.K.

Fig. 26.—Part of egg cytoplasm, in half-grown egg, showing three categories of granules, true yolk at Y, nucleolar deutoplasm at NL, and the fine mitochondria at M. CH.K. and iron haematoxylin.

PLATE 4.

Fig. 27.—Nearly full-grown egg, showing structure of periphery. At CHO is an outer egg-membrane (vitelline membrane of some authors), and at CL are peculiar columns, the cortical lamellae of Lillie. The small granules are mitochondria (stained violet); the large, nucleolar deutoplasm (stained yellow). CH.K. fixation followed by Benda's stain.

Figs. 28 and 29. Two oocytes from a Mann-Kopsch preparation, to show the remarkable change which comes over the oocyte at the period of nucleolar extrusion (fig. 29).

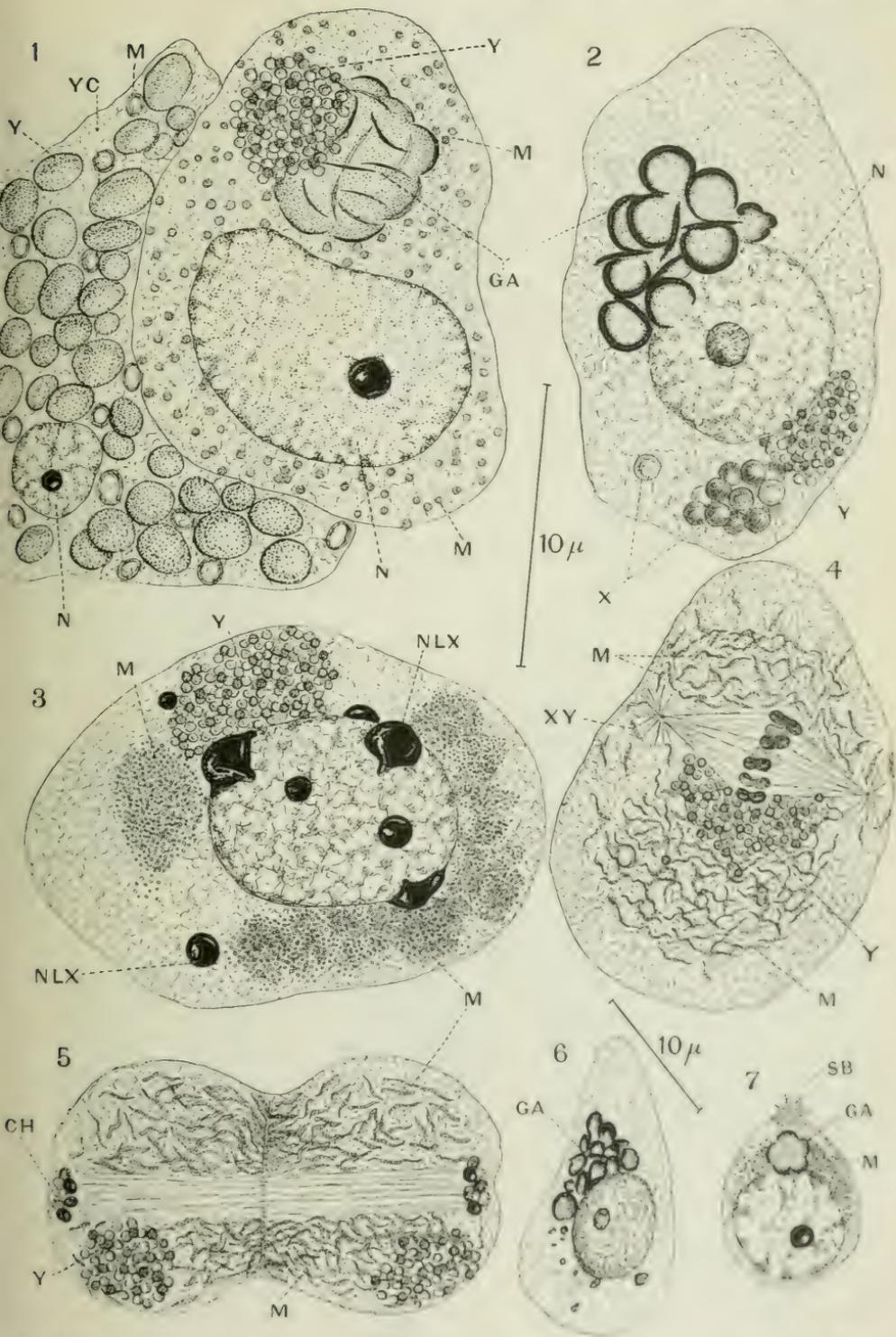
Fig. 30.—Young oocyte, after period of main nucleolar extrusion, showing the yolk-granules black and the nucleolar deutoplasm yellowish. At XN is another case of 'secondary nuclei'; compare also fig. 23. Taken from a *Saccocirrus papillocereus* (Naples) which had been immersed for six weeks in formol-Flemming.

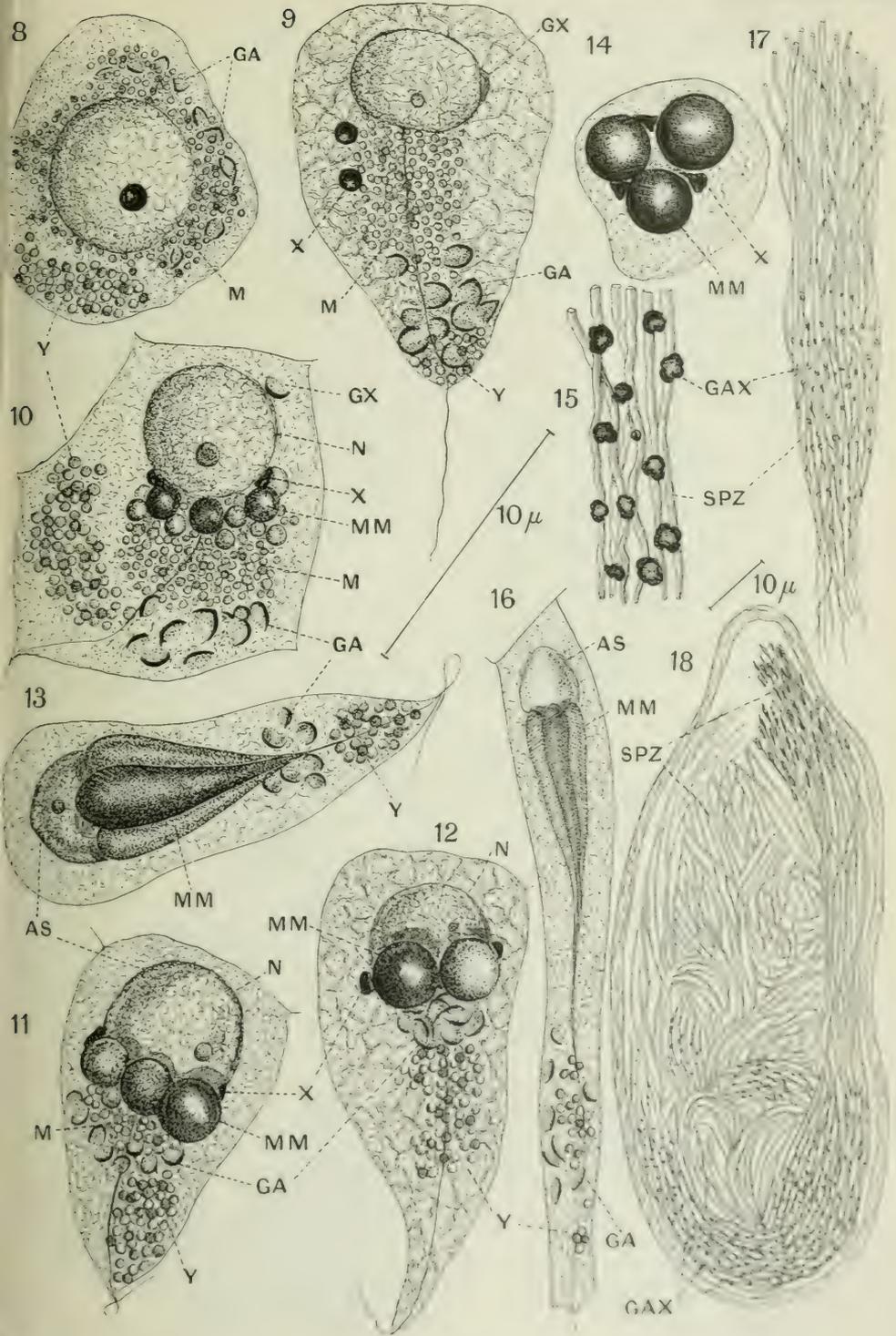
Figs. 31 and 32.—Mann-Kopsch preparation of nearly ripe egg. Fig. 31 shows appearance of cytoplasm before soaking sections in turpentine, and fig. 32, after treatment for about three hours in turpentine. In fig. 32 the Golgi elements alone resist decolorization.

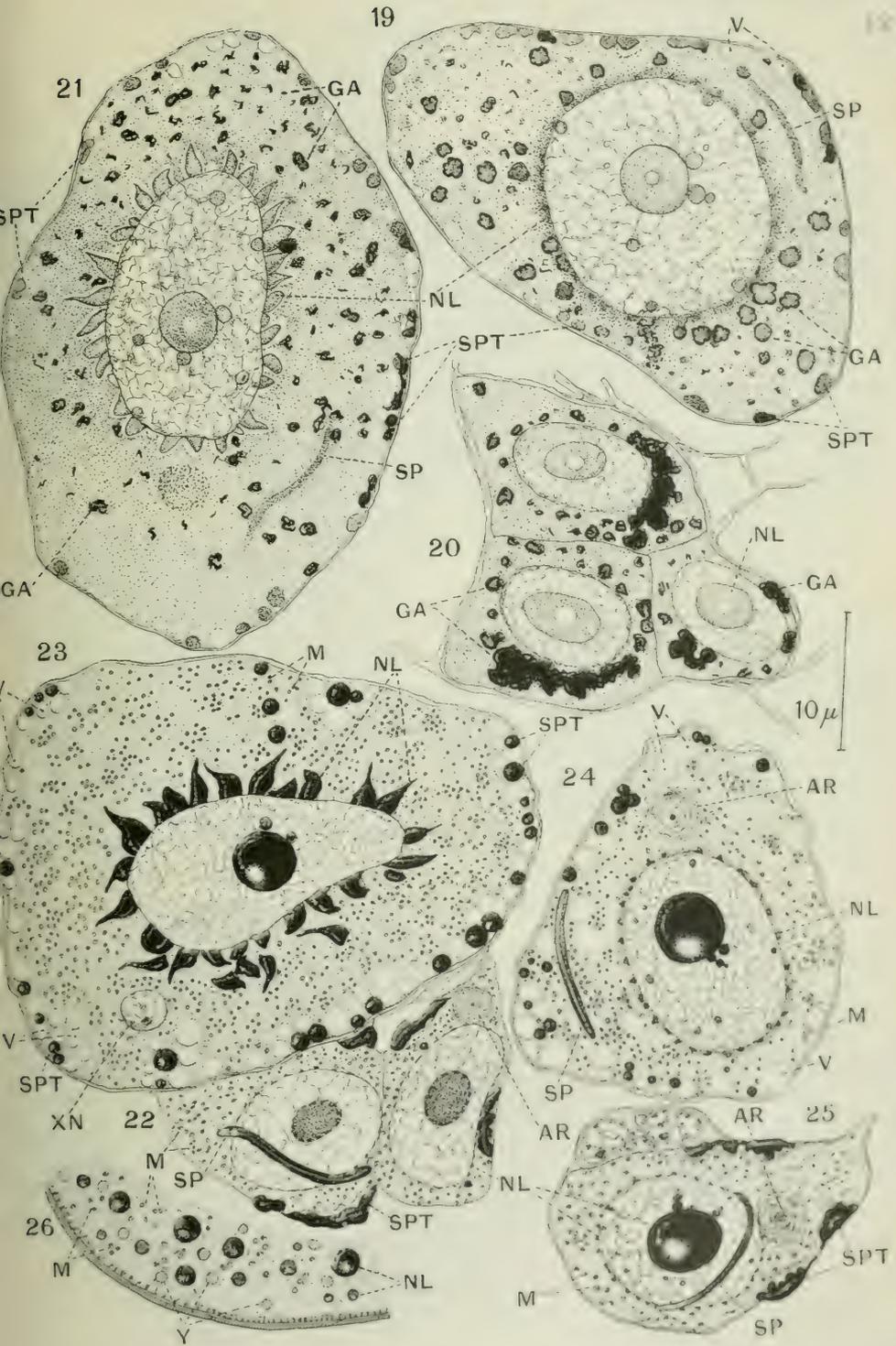
Fig. 33.—Mann-Kopsch preparation, decolorized in turpentine, to show spreading out of the Golgi apparatus. On the left of the figure is another example of the breaking up of the apparatus; compare also fig. 28.

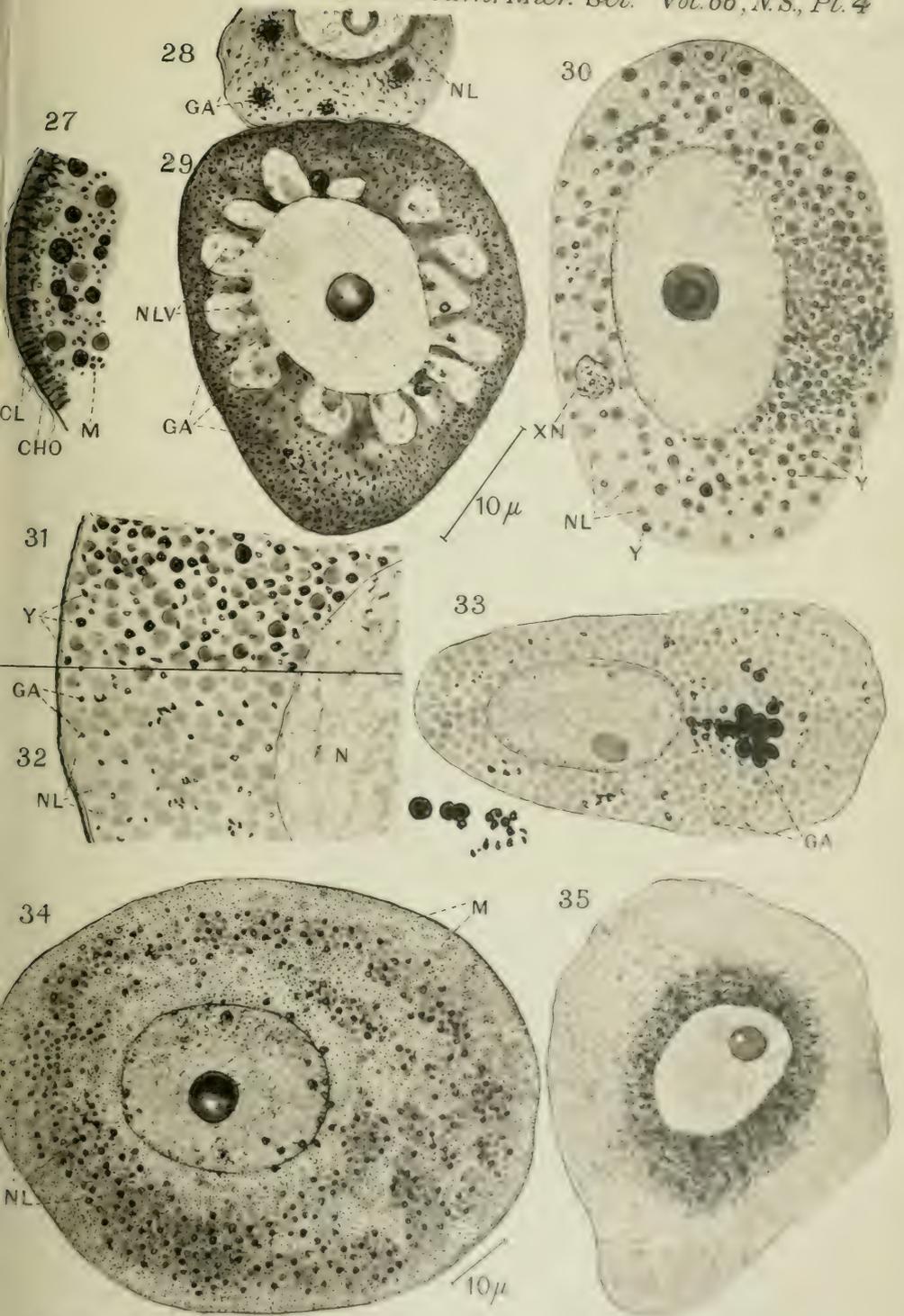
Fig. 34.—Nearly ripe egg fixed by Champy-Kull and stained in Benda. Mitochondria were violet, nucleolar deutoplasm yellow-brown.

Fig. 35.—Da Fano preparation to show peri-nuclear activity. Cobalt nitrate-formalin followed by silver-nitrate reduction.









On the Development of the 'Enteronephric' type of Nephridial system found in Indian Earthworms of the genus *Pheretima*.'

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With Plates 5-7 and 8 Text-figures.

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1. INTRODUCTORY.

IN a previous paper in this journal (1) I described a new type of nephridial system, which is found in Indian earthworms of the genus *Pheretima* and which I called 'enteronephric'.

The essential feature of this system is that the numerous septal and pharyngeal nephridia (all micronephridia) are connected with an elaborate system of ducts, which open, not on the surface of the skin but into the lumen of the intestine and other regions of the gut (buccal cavity and pharynx). These nephridia of the 'enteronephric' type co-exist in *Pheretima* with the integumentary nephridia, which are exceedingly numerous on the inside of the body-wall, and open on the surface of the skin through separate nephridiopores, like ordinary *Oligochaete* nephridia. Although in my paper I referred very briefly to the possible physiological significance of the discharge of excretory fluid into the gut of this worm, I did not enter, for want of embryological data, upon any discussion concerning the morphological significance of the discovery of the 'enteronephric' type of nephridial system, in relation to the commonly accepted view, due mainly to Goodrich (9 and 10), that all *Oligochaete* nephridia 'develop centripetally as it were, and quite independently of the coelom and are probably derived from the epiblast'.

While little doubt could be entertained, from a study of the disposition of the nephridial system of the adult worm, with regard to the ectodermal origin of the integumentary nephridia, it was difficult to believe that the septal and pharyngeal nephridia also had a similar origin for two reasons. In the first place, these nephridia have not only no connexion with the body-wall but are connected instead with the intersegmental septa, which are mesodermal structures; and they open, through an elaborate system of ducts, presumably mesodermal, into the lumen of the gut, the wall of which is partly mesodermal and partly endodermal. In the second place, the septal nephridia differ from the integumentary ones in that the former possess open 'funnels', which are absent in the latter. Although no solenocytes or 'flame-cells' have been found on the integumentary nephridia, the presence of a coelomic funnel in one case and its absence in the other might lead one to ascribe a different origin to the two sets of structures. In fact the connexions of the septal nephridia and their ducts

in the adult worm seemed to negative the ectodermal theory of the Oligochaete nephridium, and to point to a mesodermal origin of these nephridia of the new type.

It thus became evident that interesting results would be obtained from a study of the course of development of the nephridial system of this worm, and accordingly I undertook to investigate the problem and the following pages embody the results obtained by me.

The work was carried out in the Department of Comparative Anatomy at Oxford, under the general supervision of Professor E. S. Goodrich, to whom I am very much indebted for the keen interest he has all along taken in my work and for his valuable help and advice.

2. HISTORICAL.

The question of the origin of nephridia in Oligochaetes has engaged the attention of many distinguished observers. The early investigators, like Kowalewski (11), regarded the nephridium as a tube connecting the coelom with the exterior, and believed that a nephridium arose by a growth of the septal wall of the coelom, that it gave rise to a chain of cells projecting backwards, which eventually fused with the ectoderm and then became hollowed out, so that the whole nephridium is to be looked upon as a 'tail' of the coelom. Moreover, since the first trace of a cavity appears in the region of the funnel and is a prolongation of the body-cavity, the cavity of the nephridium might be said to be part of the coelom. Bergh (6) derives the whole nephridium, including the funnel, of *Criodrilus* and *Lumbricus* from a single large cell, the 'funnel-cell', lying close to the epiblast, between each successive pair of solid mesoblastic somites. The origin of this 'funnel-cell', from which the whole nephridium develops, has been a matter of considerable dispute. In a later paper on the subject (7) Bergh denies the origin of the 'funnel-cell' from the nephric row in *Criodrilus* and *Lumbricus*, and asserts that the funnel and the body of the nephridium have a separate and different origin in *Rhynchelmis*, the upper lip of the funnel

arising not from the 'funnel-cell' ('Trichterzelle') but from a peritoneal cell.

This view of the mesodermal or the so-called 'intraperitoneal' origin of nephridia is in strong contrast with that held by Hatschek, Wilson, Meyer, and Vejdovsky (in his later work), which ascribes an ectodermal or a 'retroperitoneal' origin to the main body of the nephridium and traces the 'funnel-cell' to the primary nephric row. According to this view the 'funnel-cell' arises from the primitive cell-row, or nephric cord, formed by the repeated division of one of the teloblasts on either side. In the earlier stages, this teloblast and the nephric cord to which it gives rise lie on the surface of the embryo; thus the 'funnel-cells' are epiblastic in origin. From the nephric row one cell enlarges and enters into connexion with each successive segment; these large cells, arranged metamERICALLY outside and between each pair of somites, are the so-called 'funnel-cells'. In some worms, like *Dendrobaena* and *Lumbricus*, the 'funnel-cells' give off the chain of posterior cells whilst separating from the nephric row, thus remaining for some time in connexion with it. In other cases, such as *Criodrilus*, the 'funnel-cells' appear to separate first (9).

This view of the superficial origin of nephridia was strongly supported by Goodrich's work (10), in which he showed that in certain Polychaetes (e.g. *Nephtys*) the nephridia do not open into the coelom at all, but terminate internally in a bunch of solenocytes which project into the coelom. He regarded the nephridium as essentially an ectodermic structure comparable with the excretory tube of a Nemertine or of a Platyhelminth. According to him the excretory organs of Oligochaeta are 'true' nephridia, i.e. tubes originally blind which have acquired secondary communications with the coelom, as distinguished from the 'coelomoducts', the term he uses for purely mesodermal structures. He points to the co-existence of the genital duct (which is a wide short coelomoduct) and the nephridium in the same somite, in *Lumbricus*, as evidence that the two structures cannot be homologous with one another (12).

The question as to which category (epiblastic or mesoblastic) the Oligochaete nephridia belong has recently been attacked by Staff (15), by renewed researches into the mode of their development in *Criodrilus*.

Staff found 'that in *Criodrilus lacuum* the mother-cells of the nephridia appear in the ectoderm at the hinder region of the embryo, and here act as teloblasts, giving rise to strings of cells by continuous budding off of smaller cells in front of them, like the mesodermic teloblasts situated internally to them. There are on each side four rows of such ectodermal teloblasts, and the rows of cells to which they give rise become wedged in between the ectoderm and the coelomic mesoderm. The strings of cells destined to give rise to the nephridia are broken into groups, and one group is pushed into each septum which divides one coelomic sac from another. Here each group grows and gives rise to a chain of cells, and this cell-chain becomes hollowed out and forms a tube. Its most internal cell projects into the coelomic cavity between the coelomic cells forming one side of the septum, and forms the greater part of the coelomic funnel of the nephridium. The lower lip of the funnel is constituted by one huge cell belonging to the coelomic wall' (12).

According to Staff, therefore, the nephridia develop from the 'retroperitoneal' cell-row, lying lateralwards to 'primitive muscle-fibres' in the manner that this breaks up in segmentally-arranged cell-groups, which project into the body-cavity and are covered over with the peritoneum. The whole nephridium is really ectodermal. The result of Staff's investigation, therefore, is to uphold Goodrich's view.

The earthworm *Pheretima* (*Perichaeta*), the development of which I have studied for the purpose of this paper, has all along been held to possess a branched 'plectonephric' nephridial system, a term which has become inapplicable to the system in *Pheretima* on our further knowledge of it gained recently (1). The development of this latter type of nephridia has been investigated by Beddard (3) in *Octochaetus multiporus*, by Vejdovsky (16) in *Mega-*

scolides australis, and by Bourne (8) in *Mahbenus imperatrix* and *Perichaeta pellucida*.

The earthworm *Octochaetus* (*Acanthodrilus*) possesses, in the adult condition in the interior of its body, eight tufts of nephridia in each segment, but a much larger number of external orifices for these nephridia. The funnels are present on these nephridia in the hinder region only and not in the anterior region (4). During development, according to Beddard (3), the embryo possesses a paired series of organs in each segment, which, as Vejdovsky thinks, are probably the equivalents of the pronephridia of *Lumbricus*. These paired nephridia of the embryo are, however, provided with well-developed ciliated and functional nephrostomes.

Beddard was not able to follow these paired nephridia to the condition obtaining in the adult, and his work is very incomplete; but he thinks that the nephridia of the embryo are converted into those of the adult, firstly by a temporary cessation of function (?) in a part of the nephridium—the portion nearest the funnel—which is produced by the disappearance of the lumen, and secondly by the active growth of this part of the nephridium, as well as other parts, and by the formation of a fresh series of apertures to the exterior.

Our knowledge of the development of nephridia in the Australian earthworm *Megascolides* is fairly complete. In the adult condition of this worm the diffuse network of minute excretory tubules is reinforced by the existence of larger paired tubes, one pair to each segment: and these large paired nephridia appear to be in connexion with the smaller tubes. We have, therefore, both the 'meganephric' and the 'plectonephric' systems existing side by side in the same worm. Vejdovsky (16) has found that 'in this worm also, during development there is to begin with a pair of nephridia to each segment; these have a funnel, and from the funnel leads a straight duct not perforate; here and there the cells become larger and finally form loops; these loops ultimately increase in size and become complicated coils, the connective point of the original tube degenerating into a mere strand of

connective tissue. The last step is the absolute severance of the connexion. Thus it appears, firstly, that the nephridial system of this worm originates from a pair of pronephridia to each segment; and, secondly, that this becomes broken up into a large number of nephridia, of which one only—the large paired nephridium—retains the funnel' (4).

The development of nephridia in *Mahbenus imperatrix* described by Bourne (8) is remarkably similar to that of the nephridia in *Megascolides* described by Vejdovsky (17). The only difference is that while in the former the funnel is at no stage well developed, is probably never functional, and afterwards entirely degenerates, in the latter the funnel is retained by one pair of nephridia. In fact, the resemblance in the development in the two forms is so great that there is a remarkable similarity between Vejdovsky's diagram (Pl. 32, fig. 5) showing the development of nephridia in *Megascolides* and Bourne's diagram (Pl. 5, fig. 39) showing the same in *Mahbenus*.

From the foregoing account of the history of our knowledge of the development of nephridia in earthworms we arrive at three more or less definite broad conclusions. The first is with regard to the fundamental problem of the ultimate origin (ecto- or mesodermal) of the Oligochaete nephridium. As we have seen, there is an overwhelming amount of evidence to show that the nephridia in Oligochaetes are certainly ectodermal.

Secondly, in all forms with the so-called 'pletonephric' system, studied so far, this adult condition is preceded in the embryo by a condition of paired pronephridia in each segment. In the third place, the adult condition of diffuse micronephridia is derived by the breaking up into separate loops of the embryonic pair of pronephridia, the original funnel either being retained by one of the nephridia in each segment or degenerating altogether.

The present work on the development of nephridia was undertaken to find an answer to the following questions:

1. Are all the three types of nephridia in *Pheretima* ectodermal in origin?

2. If they are ectodermal, how do the septal nephridia with their ducts come to lose all connexion with the body-wall and be associated with the septa and the gut, which are mesodermal and endodermal structures respectively?

3. Is the adult condition of nephridia preceded by a 'meganephric' or paired condition in the embryo?

4. If so, how is the adult condition derived from the embryonic condition?

5. Do facts of development throw any light on the phylogeny of the Oligochaete nephridial system?

3. THE COCOON.

The egg-capsules or cocoons of *Pheretima* do not differ in any essential particular of structure from those of *Lumbricus*, *Allolobophora*, or *Acanthodrilus*, previously described by Vejdovsky (16) and Beddard (3); but I am recording here my observations on the cocoons of this worm to bring out their special characters.

I have no observations to offer on the mode of formation of this structure in *Pheretima*, but I have no reason to doubt that it is formed in much the same way as in all the other genera where cocoon-formation has been carefully studied, and that the clitellum alone is concerned in its production.

Although the cocoons vary somewhat in size, they are very much smaller than those of *Lumbricus*. On an average they are about 1.5 to 2 mm. by 1.8 to 2.4 mm., i.e. about one-third the size of the cocoons of *Lumbricus*.

The cocoons are light yellow or olivaceous in colour, the empty cases having a clear transparent olive colour. In form they are more or less rounded in shape and give a distinctly swollen appearance, the two ends being drawn out into very short fibrous appendages.

My observations on the time of egg-laying are based on two species of *Pheretima*, namely *P. posthuma* and *P. rodricensis*. The cocoons of the first species were found by me at Allahabad (India) in spring and summer months

(March to June) out of doors in moist places in the surface layers of the soil in abundance, but during the rains (July and August) they were very rare. My friend Mr. B. K. Das has since informed me from Allahabad that he has been able to collect cocoons of earthworms (not necessarily of *Pheretima*) in the months of November, December, January, and February; and he rightly suspects that egg-laying continues almost throughout the year. Of course the number of cocoons found in the winter months is very small, since the surface layers of the soil get very dry on account of the prolonged drought, and the worms go deep into the soil and are themselves difficult to obtain.

As regards the cocoons of *P. rodricensis*,¹ my observations are based on worms kept in captivity in garden-pots in a hot-house. In order to make sure of the specific identity of my cocoons I kept worms of this species in sterilized earth, to which decaying leaves previously sterilized were added from time to time. From a number of garden-pots containing these worms I could obtain cocoons in any number containing embryos at various stages of development throughout the year. The statement is usually made in text-books that 'egg-capsules are formed in spring or early summer and the young worms grow mainly during the summer months. Sometimes large clusters matted together may be found in autumn packed away under clods or in banks where there is a favourable condition of moisture'.² Wilson (18) says, 'egg-laying seems in special cases to continue throughout the year, though it is most active in the spring and summer months. I have found the capsules of *Lumbricus foetidus* out of doors in nearly every month of the year, but in mid-winter they are only found in decomposing compost-heaps where the temperature is maintained at a tolerably high point'. From these authorities and from my own observations I am inclined to believe that the time of egg-laying depends

¹ I am indebted to Col. J. Stephenson of the University of Edinburgh for identification of this species.

² Osborn, 'Economic Zoology', New York, 1908, pp. 110-11.

very largely on the external conditions—temperature, moisture, and the richness of soil. My garden-pots containing the worm were kept quite damp; the temperature of the hot-house was always about 60° F. and the soil was frequently ‘manured’, so to speak: and it is no wonder, therefore, that under these artificial conditions cocoons were obtained at all times of the year. In nature these conditions are best fulfilled in spring and early summer, and hence we get the greatest activity in egg-laying in these months, although it seems that it does not stop altogether at other times of the year.

I have opened hundreds of cocoons of *Pheretima* and feel justified in considering, as a rule, there is only one embryo in a cocoon. Occasionally one comes across two embryos in a cocoon of a very young age, and only once did I see three embryos in one cocoon. In fig. 24, I have tried to represent three typical stages of the embryo of *Pheretima* in their natural size (the external segmentation of the body, although complete throughout, cannot be made out with the naked eye and is therefore not represented).

The rate of development is very much slower in *Pheretima* than in *Lumbricus*. Wilson (18) found that in laboratory cultures the young worms (*Lumbricus*) made their escape from the capsule in about two or three weeks. Beddard (3) judges that the shortest time in *Acanthodrilus* can hardly be less than five or six weeks. In *Pheretima* the rate is even slower than that in *Acanthodrilus*, and I cannot put the shortest period at less than eight weeks in this case.

Beddard (3) found that the albuminous fluid filling the cocoon in *Acanthodrilus*, as in *Lumbricus rubellus*, was milky and opaque while the shell was transparent; in *Pheretima*, however, the albuminous substance of the cocoon is perfectly clear and transparent like its shell, so that under a binocular microscope I could always see, by transmitted light, the embryo inside the cocoon without opening it, and it was thus very convenient to be able to know roughly the size and age of the embryo before opening it.

Vejdovsky and Beddard speak of two perfectly distinct membranes forming the shell of the cocoon. I have not been able to see these two membranes in the case of *Pheretima* cocoons, the shell of which seems to me to be single-layered.

4. GENERAL OUTLINE OF THE DEVELOPMENT OF NEPHRIDIA IN PHERETIMA.

The three sets of nephridia of *Pheretima*, namely, the integumentary, the septal, and the pharyngeal arise in the embryo at successive stages of its development. In order to elucidate, therefore, the development of the whole nephridial system consisting of these three distinct series of nephridia and their ducts, it is necessary to examine a large number of embryos of widely different ages. The work is rendered laborious and difficult on account of three facts: firstly, that each type of nephridium develops independently of the other—these several types are not derived one from the other; secondly, that the nephridia of the three series develop at different ages and in different positions in the embryo; and thirdly, that each series consists of numerous nephridia that go on developing for a long time even after the embryo has left the cocoon. But before going into the details of each stage of nephridial development, I shall provide here an outline sketch of the development of the elaborate excretory system of this worm.

Leaving aside the transitory excretory cells the earliest beginnings of permanent nephridia appear in this worm, as in *Lumbricus* (18), *Rhynchelmis* (16), and *Criodrilus* (15), as teloblasts lying on the surface of the embryo, ventral to the mesoblastic bands and in front of the mesodermal pole-cells. While these teloblasts form part of the surface epiblast in very young embryos (300 μ long), they soon sink below the surface and come to lie between the definitive ectoderm and the mesoderm. Strings of cells are budded off from and in front of these ectodermal teloblasts, and it is these cell-rows (nephroblasts) that form the material foundation ('Anlage') from which are derived all the future nephridia.

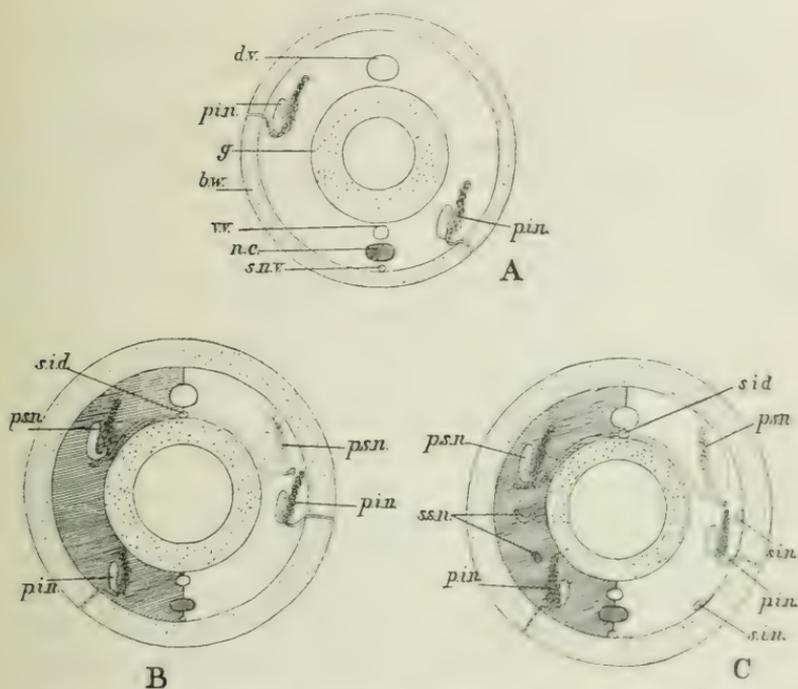
These strings of nephridial cells aggregate later into groups that lie opposite and a little posterior to the places where the intersegmental septa join the body-wall. These groups of cells, situated underneath the mesodermal peritoneal membrane ('somatopleure'), proliferate to form masses of cells, which, as they grow, begin to project into the coelomic cavity. These constitute the nephridial rudiments. They carry with them in their growth the sheet of peritoneal membrane, which now forms an enveloping sheath over these unformed nephridia (fig. 9). In longitudinal sections of an embryo, about 4 mm. in length, these embryonic nephridia are seen for the most part as solid clup-shaped masses, lying in the anterior part of each coelomic chamber, a pair in each segment of the body except the first two. While the first two segments are devoid of nephridia and the greater part of the embryo possesses solid nephridial masses, some of the anterior segments (seventh and eighth, for example) have fully-formed nephridia with the characteristic shape and the intra-cellular canals of the adult organ.

In preparations of whole embryos of suitable age, flattened after opening them through the mid-dorsal line, we can see the rudiments of these primary nephridia as elongated masses lying posterior to the septa towards the hind end of the embryo; but, as we examine the segments in front, we get the nephridia in all stages of development in the same embryo, since development proceeds antero-posteriorly. We may note here that these nephridia have no connexion with the septal partitions, and consequently a 'septal funnel' is never formed at any stage of development of this primary pair of integumentary nephridia.

At this stage of development (4 mm. long) the embryo exhibits a typical meganephric or paired condition like that of the adult *Lumbricus*, having a pair of 'true' ectodermal nephridia in each segment (Text-fig. 1 A). This marks the first stage in the development of nephridia in *Pheretima*, which comprises the developmental history from the first appearance of teloblasts up to the formation of a pair of primary integumentary nephridia in each segment.

In the second stage that follows we have the appearance and development of the primary pair of septal nephridia in each

TEXT-FIG. 1.



Diagrammatic representation of the three stages of development of the nephridial system in *Pheretima*. *A* represents a diagrammatic section of an embryo about 4 mm. in length, showing the paired condition of nephridia (meganephric stage). *B* represents a stage at which the embryo has two pairs of nephridia, in each segment, a primary integumentary pair of the first stage and a primary septal pair. *C* shows the formation of secondary septal and integumentary nephridia. In *B* and *C* the intersegmental septum is shown on the left half. *b.w.*, body-wall; *g.*, gut; *d.v.*, dorsal blood-vessel; *v.v.*, ventral blood-vessel; *s.n.v.*, subneural vessel; *n.c.*, nerve-cord; *p.in.*, primary integumentary nephridia; *p.s.n.*, primary septal nephridia; *s.in.*, secondary integumentary nephridia; *s.s.n.*, secondary septal nephridia.

segment of the body behind the first fourteen. As these nephridia begin to appear before all the integumentary ones of the first stage have attained to their full development and

size, we have the later development of integumentary nephridia going on side by side with the appearance and growth of septal nephridia, so that we have an overlapping, so to speak, of the first and second stages. The rudiments of septal nephridia appear in two rows, one on each side of the dorsal vessel. The latter in embryos is single anteriorly but double for the greater part of the posterior portion, and the earliest rudiments of the septal nephridia recognizable in whole preparations lie on both sides of this double dorsal vessel (Text-fig. 4). But while the integumentary nephridia vary in their topographical position from segment to segment, lying close to and away from the nerve-cord alternately, the septal ones lie in two straight rows, nearer the mid-dorsal than the mid-ventral line.

As their name implies, the septal nephridia develop on the intersegmental septa and, in sections, can be seen to lie just internal to the commissural that connects the dorsal with the subneural blood-vessel. As a septal nephridium develops, the pre-septal portion elongates to form a long narrow tube ending in the funnel, the body of the nephridium, lying in the coelomic cavity behind the septum, develops the limbs and loops of the adult organ, while the terminal duct elongates to run along the septum, parallel and internal to the commissural vessel, to meet its fellow into the supra-intestinal duct mid-dorsally.

These pairs of nephridia of the second stage differ from the primary integumentary nephridia in that the former develop on the septal wall and have no connexion with the body-wall from the very beginning, and that they develop a septal funnel. Thus we see that septal nephridia are not derived from integumentary ones, and have no connexion with them except that, as will be shown later, both types can be traced to the same source.

When the embryo has developed a pair of septal nephridia in each segment we get to the end of the second stage. At this stage the embryo possesses, in each of its typical segments, two pairs of nephridia, an integumentary pair and a septal one, the former opening to the exterior on the body-wall and lying

alternately dorsally and ventrally, and the latter opening into the supra-intestinal duct and lying dorsally throughout (Text-fig. 1 B). The vertical ducts leading from the supra-intestinals to the lumen of the gut at each intersegmentum are also formed at the end of this stage.

In the third stage we have the development of secondary nephridia, integumentary and septal. These begin to appear at a rather late period of development of the embryo, when it is almost fully formed and is about to come out of the cocoon. The circlets of setae are completely formed in all the segments of this age, and while rudiments only can be seen of septal and integumentary nephridia towards the posterior end, we find them in various stages of development anteriorly.

These secondary nephridia of both types appear independently of the primary pairs of their segments. The integumentary ones appear earlier than the septal, since towards the posterior end we find segments with rudiments of secondary integumentary nephridia but with no traces of secondary septal ones. In both cases these nephridia in their initial stages are lumps of cells having no connexion with the primary nephridia.

The septal secondary nephridia appear immediately ventral to the primary pair, and develop very much in the same way as the primary pair, their terminal ducts running dorsalwards on the septa and meeting the ducts of the primary nephridia. Some of these nephridia develop a pre-septal funnel like the primary nephridia, but others, as shown in fig. 15 a, develop the funnel in the same segment in which they lie. In this way we get two kinds of nephridia, one kind with pre-septal funnels and the other with funnels in the same segments as the nephridia. Subsequent pairs of nephridia develop similarly, and we get the formation of the septal canals by the union of the terminal ducts of secondary nephridia with those of the primary ones.

The secondary integumentary nephridia first appear on the body-wall as masses of cells lying beneath the somatic layer of the coelomic epithelium. They do not arise strictly in pairs

like the primary nephridia, but have a more or less scattered arrangement. They may appear either on the dorsal or on the ventral side of the primary nephridia (Text-fig. 1 c). They arise in connexion with the setal sacs, and one can very often see a string of cells running from the rudiment of a secondary nephridium in the coelom to the epidermis alongside a setal sac.

TEXT-FIG. 2.

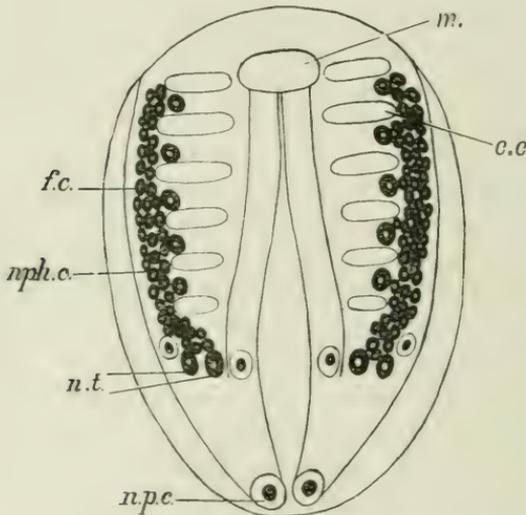


Diagram showing the formation of nephridial rows of teloblasts and the intersegmental position of large 'funnel-cells'. *m.*, mouth. For lettering see Text-fig. 3.

The pharyngeal nephridia of the fourth, fifth, and the sixth segments make their first appearance in a manner similar to the primary integumentary nephridia, although the former lag behind in development, the integumentary nephridia growing faster than the pharyngeal. Amongst the pharyngeal nephridia themselves the nephridia of the sixth segment develop faster than those of the fifth, and these, in their turn, faster than those of the fourth. The first pair in each of these segments originates from the ectodermal nephridial row, and has a long terminal duct which meets the wall of the pharynx ventro-

laterally. Secondary pharyngeal nephridia are formed distal to the primary ones as buds on the ducts of the primary pairs of nephridia (fig. 19 and Text-fig. 3).

5. DEVELOPMENT OF THE PRIMARY INTEGUMENTARY NEPHRIDIA.

Although I observed short intracellular canals in certain ectodermal cells of very young embryos (gastrulae) in the living condition, and believed these cells to be of the nature of larval excretory cells, I could not definitely locate them in preserved and stained embryos, nor could I examine and arrive at any definite result about these cells in sections. I shall, therefore, confine myself to the development of permanent nephridia alone.

As already indicated, the first set of nephridia to make their appearance in *Pheretima* are the ectodermal primary nephridia, a pair in each segment. At a stage of development when the embryo has fifty to fifty-five clearly-defined segments and is about 4 mm. long, we can easily see some of the anterior segments (seventh and eighth, for example) possessing a pair of fully-formed nephridia opening on the surface of the body-wall. Each of these segments, at this stage, resembles in this respect a segment of the adult *Lumbricus*, and we may even call this stage of development of the nephridial system of *Pheretima* the 'meganephric' stage.

The early history of these nephridia is very similar to that described in *Lumbricus*, *Criodrilus*, and other worms by previous writers. In an advanced gastrula in which the mesodermal bands are well formed and in which cavities are beginning to appear, we can recognize the earliest beginnings of nephridia. On examining such an embryo, when it is still a rounded sphere and has not begun to elongate, as for example the one shown in fig. 1, which is about $140\ \mu$ in diameter, we see the mesoblastic bands diverging from the two large mesodermal pole-cells lying at the future posterior end. These bands lie along the two sides of the ventral surface of the embryo, and, on careful focusing, we can also see the begin-

nings of five or six coelomic cavities in each of the two bands. On examining, however, the surface epiblast covering these mesoblastic bands ventrally, we can distinguish four rather large and rounded cells on each side, called the teloblasts. These teloblasts lie a little way in front of the pole-cells: the three ventral ones lie six or seven cells in front of the pole-cells as seen in longitudinal sections (fig. 2), while the fourth, the lateral teloblast, lies a little farther forward than the rest. In these very young embryos (figs. 1-4) the teloblasts form part of the surface epiblast, but can be easily distinguished from the adjacent epiblastic cells both by their larger size and by the fact that their nuclei are free from granules surrounding the nucleolus and thus give an appearance of greater transparency as compared with the nuclei of the other cells. Of these four teloblasts on each side the one near the mid-ventral line is the neuroblast, going to form the nerve-cord of the adult, the two lying outside the neuroblast are the nephroblasts, which go to form the nephridia, while the outermost and dorsal is the lateral teloblast which lies just outside and dorsal to the coelomic sac on each side at this stage of development of the embryo (fig. 4). In a series of transverse sections of an embryo, about $300\ \mu$ in length, from which figs. 3, 4, and 6 are taken, we can follow these four teloblasts forwards as they bud off rows of cells in front. The rows of cells in front of the teloblasts can be followed for a long way in young embryos. Concerning the nephridial cells in continuation with the nephroblasts, we have to note that while the nephroblasts are large cells and occupy the whole thickness of the epiblast, the nephridial cells in front are small and come to lie deep in the ectoderm. They can be seen distinctly marked off by a sort of boundary line from the definite epiblast, which is very thin at places where these nephridial cells occur. These cells are thus embedded in the ectoderm, as shown in figs. 3, 4, and 6, but they have not yet formed a separate layer of their own.

The next step in the development of the nephridia, which is slower than that of the nerve-cord, is that the nephridial

teloblasts and the rows of cells in front of them sink beneath the ectoderm and come to form a separate and distinct layer of their own, between the ectoderm on the outside and the mesodermal lining of the coelomic cavities on the inside. In longitudinal sections of young embryos (fig. 5) this layer towards the posterior end gives the appearance of a kind of string of nephridial cells. The large nephridial teloblast together with a row of smaller cells lying in front of it form the definitive nephridial layer. The transition from the previous stage can be well appreciated by comparing the position of the nephroblasts and the nephridial cells in figs. 2, 3, and 4, where they are superficial, with the deeper position occupied by them in figs. 5 and 7. This nephric cord is single-layered in the beginning and remains so for a long time at the posterior end, but its cells soon begin to multiply and proliferate opposite and behind the intersegmental septa which divide one coelomic sac from another, so that we get groups of these nephridial cells situated at intersegmental intervals. The cells of these intersegmental nephridial groups multiply here beneath the peritoneal lining of the coelom, and the cells tend to travel backwards towards the middle of the segment. Some of these nephridial cells push their way into the septa between the two apposing walls of the adjoining coelomic chambers (figs. 7 and 13).

We thus get these intersegmental nephridial masses segregating into two separate groups, one keeping its 'retroperitoneal' position while shifting backwards and multiplying rapidly, the other consisting of very few cells which make their way into the septa and lie between the two sheets of peritoneum forming the two faces of the septa. In earlier stages—or what amounts to the same thing, in the posterior segments of the embryos—we can see, in longitudinal sections, one nephridial group in each segment lying at the posterior of the two angles formed by the septum with the ventral body-wall (fig. 8). In later stages, or in the more advanced anterior segments, the segregation into two groups becomes quite evident. The group consisting of a few cells caught in between the two septal

sheets, leads, to anticipate matters, to the development of septal nephridia, which we shall speak of in the next part of the paper, while the other group consisting of a number of cells lying beneath the peritoneum and immediately posterior to each intersegmental septum, is the rudiment of the primary pair of integumentary nephridia. We shall now consider the details of development of these integumentary nephridia.

This group of cells beneath the peritoneum is the 'retro-peritoneal' group of Meyer (13) and forms the forecast of the whole primary nephridium of the first stage. The cells of this group separate away from the septum, divide and proliferate so as to bulge out as solid masses into the coelomic cavities, as shown in fig. 8, c. They carry with them their peritoneal covering which forms a thin sheath round these solid nephridia. The growth is not only vertical but also horizontal, and the nephridial rudiment besides increasing in thickness and projecting into the coelom also extends laterally, so that in a preparation showing the body-wall of an embryo flattened we get a pair of deeply-staining elongated solid masses of cells lying immediately behind each septum as shown in fig. 11, A, and Text-fig. 4.

By what steps this elongated ridge lying behind each coelomic septum develops into an adult nephridium I have shown in fig. 11 (A-F). The earlier stages, in which the nephroblasts and their derivatives multiply, form masses of cells at septal places which segregate further into two groups, a smaller one, the cells of which push their way into the intersegmental septa, and a larger one, the cells of which move backwards and form the so-called 'retroperitoneal' group of cells, which forms the elongated solid ridge bulging into the coelomic cavity, can all be followed in a few series of longitudinal and transverse sections; but once the nephridial rudiment has reached the size and shape of the elongated mass, shown in fig. 11 A, we can follow its further development best in whole embryos that have been opened in the mid-dorsal line, their endoderm with food-yolk removed, and the remaining portion mounted flat.

The ridge-shaped mass of nephridial cells grows in the middle, and we soon get a sort of papilla-like protuberance; this papilla elongates further into a long loop, having its two limbs close together. At this stage, while the two ends of the loop forming the proximal part are attached to the body-wall, the loop itself forming the distal part lies free in the coelomic cavity (fig. 11 c). This loop now elongates further, and, side by side with the elongation of the loop, we find its two limbs getting more and more closely pressed together so as to form one compact lobe. A bend appears, at this stage, towards the base of this lobe, and we now get two more or less distinct divisions of the embryonic nephridium, the one distal to the bend (fig. 11 d) and lying free, and the other proximal to the bend and connected with the body-wall. The distal portion is now a compact structure and goes to form the short straight lobe of the adult nephridium. Although it has visibly lost its double character, we must note that it is really double morphologically, having been formed by a close apposition of the two limbs of the loop.

The proximal portion of the developing nephridium, the part connecting the bend with the body-wall, is now the seat of further growth. In this portion the double nature of the nephridial loop persists for a time (fig. 11 d), but, soon after, the two limbs of the loop at its proximal end, which are really the two opposite ends of the original ridge, come closer together and elongate further. This further elongation of the part proximal to the bend results in the formation of a twist, a little way from the bend, resulting in a condition of the nephridium represented in fig. 11 e. Elongation and twisting go on further until we get the long twisted loop fully formed, with the number of twists characteristic of the adult nephridium. The straight lobe and the twisted loop having been fully formed, the proximal end connecting the nephridium with the body-wall becomes narrow and slender and forms the terminal duct of the nephridium. This duct has meanwhile grown through the thickness of the body-wall, and opens to the exterior in front of the row of setae occurring in the middle line of each segment.

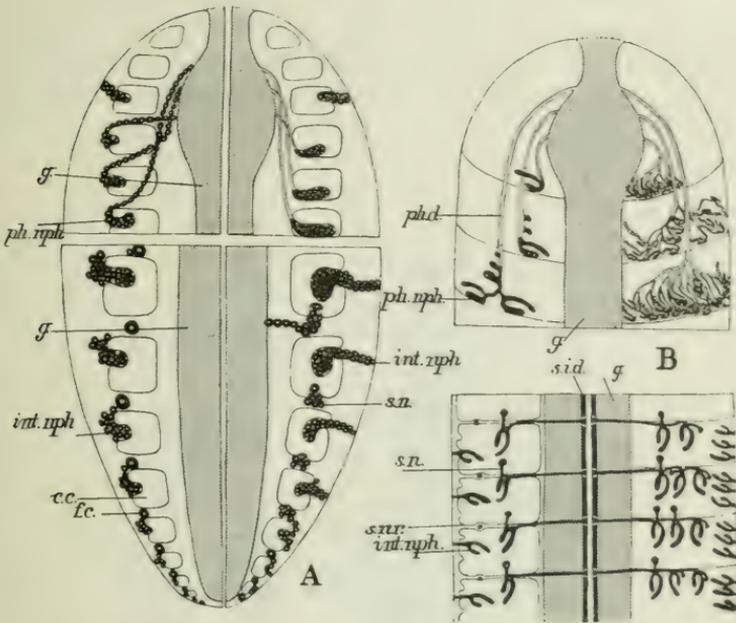
In this fully-formed integumentary nephridium we have to note the absence of either a coelomic funnel or a solenocyte or 'flame-cell'. During the course of development, when the two ends of the elongated nephridial ridge come close together, one end develops into the terminal duct opening to the exterior, while the other remains blind and does not develop any structure at all. These nephridia develop an intra-cellular canal and cilia like the septal ones; and, no doubt, the excretion in their case takes place by means of the diffusion of the coelomic fluid through their permeable walls.

6. DEVELOPMENT OF THE PRIMARY SEPTAL NEPHRIDIA.

When the embryo has acquired a pair of integumentary nephridia in each segment—fully developed in the anterior and in various stages of development in the posterior segments—the second set of nephridia, i.e. the septal, make their appearance. These form the second pair of nephridia in the body segments of *Pheretima* (Text-fig. 1 B). The first fifteen segments of the embryo do not develop this second set of nephridia, which appear only in segments behind the first fifteen. Unlike the primary integumentary nephridia the septal primary nephridia appear on both sides of the dorsal vessel instead of the nerve-cord. They form two rows, one on each side of the dorsal vessel, at a distance of about $160\ \mu$ from it in an embryo 9 mm. in length. The alternate or scattered arrangement characteristic of the integumentary nephridia of the first stage does not obtain in these septal ones, which occur in two straight rows.

These nephridia of the second set have no connexion with the body-wall, but appear from a very early stage, as their name implies, as outgrowths on the intersegmental septa. They can then be first recognized in whole mounts as masses of cells on the septum, projecting on its posterior surface. These nephridial masses on the septa can be recognized with certainty at the earliest in embryos, about 8 to 9 mm. in length, which have been opened in the mid-ventral line, their yolk removed and the rest including the endoderm mounted flat.

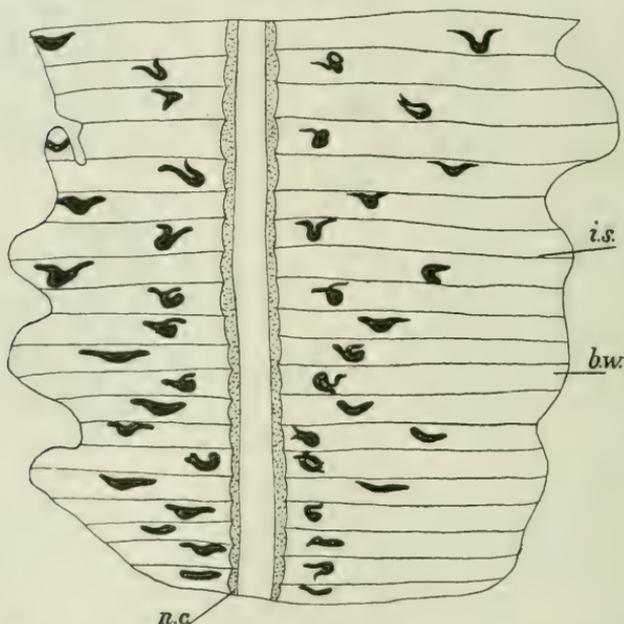
TEXT-FIG. 3.



A series of three diagrams showing the common origin and development of the three types of nephridia in *Pheretima* embryos. In *A* (left half) the integumentary nephridia are seen pushing themselves into the coelomic chambers while the 'funnel-cells' are travelling dorsalwards between the adjoining coelomic chambers. The origin of the pharyngeal nephridia and ducts is also shown. The origin of the pharyngeal nephridia and ducts is also shown. The right half shows the nephridia at a more advanced stage of development. Septal nephridia are developing between the adjoining peritoneal sheets, while the ducts of the pharyngeal nephridia are formed even before the nephridia themselves are fully formed. In *B* (on the left) is shown the development of secondary nephridia, while the right half shows more or less the adult condition of nephridia in the worm. *n.t.*, nephridial teloblasts; *nph.c.*, cells of the nephridial row; *c.c.*, coelomic cavities; *int.nph.*, integumentary nephridia; *s.n.*, septal nephridia; *s.n.r.*, rudiment of a septal nephridia; *ph.nph.*, pharyngeal nephridium; *ph.d.*, duct of pharyngeal nephridia; *g.*, gut; *s.i.d.*, supra-intestinal excretory duct; *f.c.*, funnel-cells.

In such preparations of embryos (Text-fig. 5, part of an embryo about 9 mm. long) we can follow these nephridial rudiments antero-posteriorly. The dorsal vessel in embryos of this age is double in the posterior portion, and consists of two lateral vessels lying on the sides of the gut. Anteriorly,

TEXT-FIG. 4.



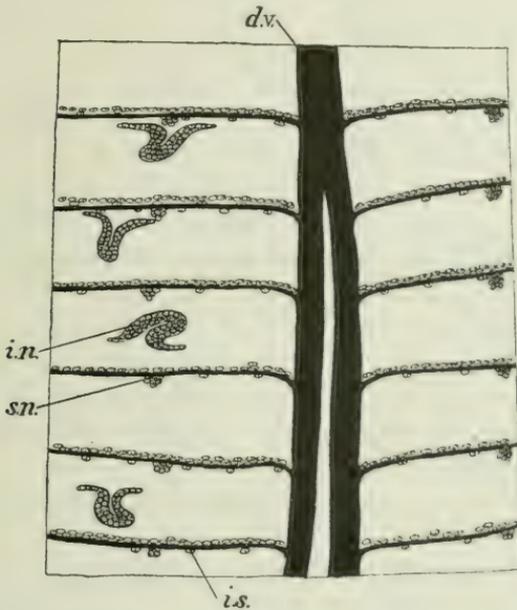
Portion of an embryo mounted flat after removal of the gut, showing the relative position of the developing integumentary nephridia in successive segments. *b.w.*, body-wall; *i.s.*, intersegmental septa; *n.c.*, nerve-cord.

however, the two dorsal vessels converge and fuse to form one single vessel, and we find it as such in the mid-dorsal line in the anterior part of the embryo. Following the nephridial masses from the septum 15/16, we can trace them backwards a good way beyond the point where the two converging vessels meet to form the single dorsal vessel (Text-fig. 5). The further development of these septal nephridia can be followed in whole mounts of embryos, 9 to 18 mm. in length, flattened

after being opened from the ventral and not the dorsal side.

As the nephridial mass grows in size we can soon distinguish the two ends of the growing nephridium, as shown in Text-fig. 6. One end grows inwards along the septum towards the dorsal vessel, beneath which it meets its fellow of the other

TEXT-FIG. 5.



Portion of the whole mount of an embryo 8 mm. in length, showing the rudiments of the septal nephridia on each side of the dorsal blood-vessel. *d.v.*, dorsal blood-vessel, double behind; *i.n.*, integumentary nephridia; *s.n.*, septal nephridia; *i.s.*, intersegmental septa.

side to form the supra-intestinal excretory duct; we can call this end the centripetal end. The other end of the nephridial rudiment at this stage is away from the dorsal vessel, and proliferates to form a mass of cells which project in front of the septum to form the beginnings of the 'funnel' of the nephridium. This pre-septal portion of the nephridium soon attains to a considerable size, and is a prominent feature of the

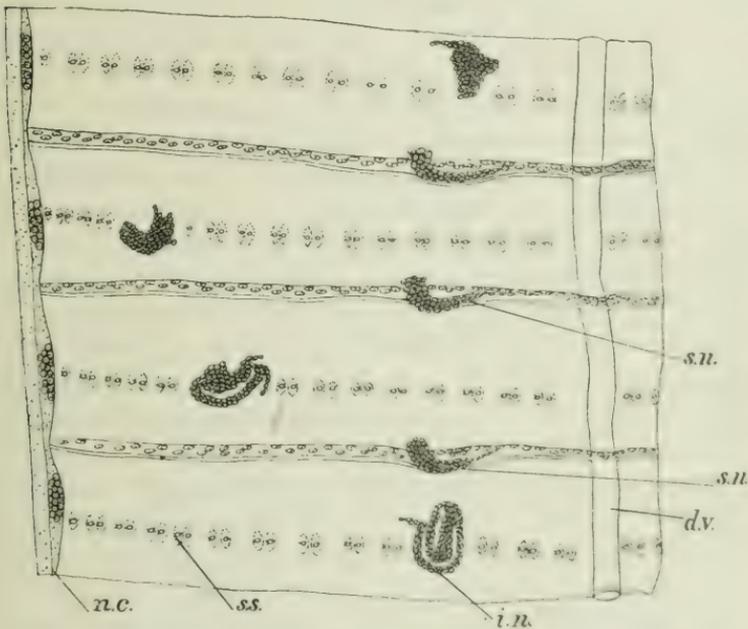
septal nephridia at all stages of their development (fig. 12). While these two ends of the nephridium—the ‘centripetal’ end and the ‘funnel’ end—are growing and differentiating, the portion of the nephridial mass between the two ends also grows and forms a papilla-like projection behind the septum (fig. 12 B). This papilla elongates to form a loop, the two limbs of which come close together; a bend appears, and the portion distal to the bend forms the rudiment of the short straight lobe of the adult nephridium. This stage of the development of the septal nephridium is represented in fig. 12 c. The two ends of the nephridium are attached to the septum while the body of the nephridium, consisting of a newly-formed straight lobe (*s.l.*), distal to the bend, and a growing region proximal to the bend, between the latter and the attached ends of the nephridium, lies free in the coelomic cavity. As this growing region elongates (fig. 12 D) the two limbs come close together, and, as a result of elongation, twists appear, which grow to form the spirally twisted loop of the adult nephridium (fig. 12 and Text-fig. 7). We thus get the main body of the nephridium, consisting of the short straight lobe and the long spirally twisted loop, fully formed. Histological differentiation, along with the formation of intracellular canals with cilia lining them at intervals, completes the development of a nephridium.

As will be seen by comparing the foregoing account of the development of a septal nephridium with that of an integumentary nephridium described in the last section, the successive steps of growth in the two cases are very similar if not identical. The chief difference lies, of course, in the fate of the two ends of the nephridium. In the case of a septal nephridium one end grows out to be pre-septal and is differentiated to form the ‘funnel’, the other end forms the terminal duct which runs along the septum, parallel to the commissural vessel, and joins its fellow to form the supra-intestinal duct; on the other hand, in the integumentary nephridium the ‘funnel’ end is blind, and the terminal duct opens on the surface of the skin.

We have now followed the development of a septal nephri-

dium from a stage when it consists of a mass of cells on the septum (fig. 12 A) to a stage when it has attained to its adult structure (Text-fig. 7). But in order to assign these septal nephridia to one of the three primary germ-layers we must trace the ultimate origin of this septal mass—the unformed septal nephridium. We must note that an intersegmental

TEXT-FIG. 6.



Portion of the whole mount of an embryo showing septal nephridia at a more advanced stage of development than those in fig. 13. s.s., setal sacs; other letters as above.

septum is morphologically double and results from a coalescence of the two layers of peritoneum covering the two faces of the septum, and that although this double character of a septum is not discernible in sections of an adult worm the two layers of peritoneum can easily be distinguished in longitudinal sections of embryos. The question naturally arises as to whether this nephridial mass arises by a proliferation of one or more cells belonging to the two layers of peritoneum forming the septum.

like the testes and ovaries, or whether the mass arises by multiplication and growth of one or more cells lying between the two adjoining sheets of a septum. Is the septal nephridium intra-peritoneal or inter-peritoneal; or, in other words, is it mesodermal or ectodermal? This is the fundamental morphological question to be answered.

We have already noticed that during the course of development of the primary integumentary nephridia the mass of nephridial cells lying opposite and behind the intersegmental septa, underneath the coelomic epithelium, segregates early on into two groups—one forming the 'retroperitoneal' group of cells and developing into an integumentary nephridium, and the other consisting of a few cells that push their way into the septum between its two layers of peritoneum. This second group, which is directly traceable to the original nephric row and has thus the same source as the integumentary nephridia, is in fact the primordial rudiment of the septal nephridia.

In a series of longitudinal sections of an embryo about 6 mm. long, we can trace how a cell from this primordial group travels through the septum to take up its final position in the row of septal nephridia on each side of the dorsal vessel. If we examine, in this series, a septal nephridium on one of the anterior septa—say the twentieth—we find that it lies at a little distance from the dorsal vessel immediately internal to the commissural vessel (fig. 14 c). This incipient nephridium and the commissural vessel are both situated between the two sheets of the septum, one below the other (fig. 14). As we trace this nephridial rudiment backwards we find that it retains the same relative position with regard both to the dorsal and the commissural vessels. We can thus trace the nephridial rudiment, consisting of a few cells as it lies dorsally on each side of the dorsal vessel on one of the anterior septa, back through successive segments to the posterior end of the worm, where the nephridial rudiment lies ventrally on each side of the nerve-cord, and is just beginning to push its way into the edge of a septum. On examining the sections shown in figs. 13 and 14 two fundamentally important facts come out.

The first is the inter-peritoneal situation of the rudiment of a septal nephridium, i. e. in other words, the septal nephridia are not derived from one of the cells belonging to the peritoneal lining of the septa but take their origin from cells lying between the two sheets of peritoneum forming a septum. The second is that these rudiments can be traced directly to the original nephric row. Since the original nephric row is ectodermal in origin we have established the ectodermal origin, in the last analysis, of the septal nephridia.

In all descriptions of previous work on the development of nephridia in earthworms, mention is made of a 'funnel-cell' ('Trichterzelle'), a term which is used in at least two senses. It is either used for a single large cell which is separated off very early from the nephric row and forms the forecast of the whole nephridium, or it is used for the most internal cell of a series or group of cells which go to form the whole nephridium. and, in this case, the 'funnel-cell' gives rise only to the funnel of the nephridium. The term is used in the former sense by Bergh in the case of *Criodrilus* and *Lumbricus* (6 and 7). and in the latter sense by Staff in the case of *Criodrilus* (15).

During the development of nephridia in *Pheretima* also we can distinguish a large cell which is probably the equivalent of the 'funnel-cell'. So far as the development of the integumentary nephridia are concerned, the 'retroperitoneal' group of cells, which give rise to them, contains no 'funnel-cell' in it, nor, as we have seen, do we get a funnel formed in the adult integumentary nephridia. But with regard to the septal nephridia we can distinguish a cell larger than others at almost all stages of their development. In fig. 6, which represents part of a transverse section of the posterior end of a young embryo, we can distinguish one large cell in connexion with the septum on each side. Further, during the passage of this cell to its dorso-lateral position, one cell can always be distinguished by its very large size as compared with the surrounding peritoneal cells. Finally, when the rudiment of the septal nephridium consist of a group of three or four cells lying on the septum on each side of the dorsal vessel,

one of the cells of this group is larger than the rest (fig. 14 B), and we can infer that this large cell is the so-called 'funnel-cell'. It would seem, therefore, that this large cell, as it pushes itself into the septum, is the forecast of the whole nephridium, and is a 'funnel-cell' in the sense in which Bergh uses it. And later this large cell divides and gives off cells smaller in size than itself; and while these smaller cells go to form the body of the nephridium the large cell develops into the funnel and becomes a 'funnel-cell' in the sense in which Staff uses it.

7. DEVELOPMENT OF THE SECONDARY NEPHRIDIA, SEPTAL AND INTEGUMENTARY.

At the end of the second stage of nephridial development, as we have seen, a typical segment of the embryo contains two pairs of nephridia—an integumentary and a septal. Soon after, rudiments of other nephridia, both septal and integumentary, begin to appear. These rudiments of secondary nephridia (all nephridia appearing after the first pair, septal and integumentary, have been grouped together under the term 'secondary') can be seen both in sections and in whole preparations of embryos of suitable age as deeply-staining masses of cells on the septa and the body-wall. In order to study the development of these secondary nephridia, two sets of embryos should be selected—the first set, consisting of those embryos which are fully formed and are about to hatch out of their cocoons: these show the secondary nephridia at a fair degree of development; the second set, consisting of those embryos which are not fully formed and would take some time before they are ready to hatch out: these show secondary nephridia in their very early rudimentary condition. It may be difficult, in the beginning, to distinguish embryos belonging to these two sets, but when one gets familiar with them after opening a number of cocoons, one can always distinguish them with a fair degree of accuracy. A surer method of distinguishing the embryos of two sets externally is to examine the setal line. In fully-formed embryos the circlelets of setae are

complete, and, on examination of the embryo under low power, we can see the setae; but in younger embryos, although setal sacs and muscles can be made out in sections, the setae are not yet formed and so cannot be distinguished externally.

(a) Secondary Septal Nephridia.

The secondary septal nephridia arise very much in the same way as the primary septal pair. They appear ventral to the primary septals and, like the latter, appear in pairs. It is very difficult to say whether this paired origin is maintained throughout the development of all the septal nephridia, but it is certain that the first two secondary nephridia arise in pairs. We may also note that these nephridia appear later than the secondary integumentary nephridia, since in the posterior segments of embryos with well-developed secondary nephridia in their anterior portion, though we can make out the rudiments of secondary integumentary nephridia, the septal ones have not yet been formed.

The group of cells forming a very early rudiment lies, as in the case of the primary pair, between the two peritoneal sheets of a septum and is consequently 'inter-peritoneal'. One of the cells of this group is larger than the rest and corresponds, in all probability, to the 'funnel-cell'. As regards the original relations of this secondary pair we have to note, in the first place, that their rudiments lie ventral to and at some little distance from the primary nephridia, and, secondly, that until the nephridium is almost fully formed and has developed its long terminal duct there is no connexion between this and the primary nephridium, nor are there any stray cells lying on the septum between these two nephridia. The obvious inference is that the secondary nephridia do not arise by a process of budding or the like from the primary nephridia, but do so *de novo* at their place of origin. Whence do the rudiments of these nephridia come?

In describing the ultimate origin of the primary septal nephridia we traced their beginnings to a group of cells which pushed their way into each septum, and which, in their turn,

could be traced further to the original nephric row derived from the ectoderm. This group of cells pushing its way into the septum forms the primitive material foundation of all the septal nephridia. The primary pair is formed from one of the cells of this group, travelling dorsally on each side. More cells move into the septa and give rise to the other nephridia (secondary septals). That this does actually happen is shown firstly by the fact that there are always a number of cells lying into the septum at its junction with the body-wall, even after the rudiments of the primary pair of nephridia are well formed dorsally; and, secondly, by the fact that we very often come across cells lying interperitoneally within the septa at a little distance dorsal and inwards to the group of cells referred to above (the group pushing its way into the septum), these cells having apparently been detached from the fundamental group and being on their way to their final place of settlement and growth.

We thus conclude that although the secondary septal nephridia do not originate as buds from the primary ones and are completely independent of them as regards their origin, they can be traced to the same source as the primary nephridia, i. e. the intersegmental group of nephridial cells, which form a store-house, giving origin to the rudiments of all the septal nephridia, primary as well as secondary.

Coming now to the later development of the secondary nephridia, the chief point of interest is the topographical position of the funnel. The usual position of the funnel is always pre-septal, and we have seen that it is so with regard to the funnels of the primary pair of septal nephridia. But in the adult *Pheretima* (1) I have described the funnel as lying in the same segment as the rest of the nephridium, and there was thus an incongruity between the two facts of structure. This led me to a close examination of the funnels of the developing nephridia in the embryos, and also to a re-examination of the position of the funnel in the adult worm. While, on the one hand, it came out that all the primary nephridia have a pre-septal funnel, in the secondary nephridia,

on the other hand, both conditions prevail—the funnel is pre-septal in some cases and post-septal in others. Both conditions are represented in fig. 15 A and B. In the adult *Pheretima* it was found that while a large majority of nephridia have their funnels in the same segment there are some with pre-septal funnels. The statement that all the septal nephridia have funnels in their own segments is therefore not quite universally true, as I thought before. We may note, however, that in the case of those nephridia which have the funnel in the same segment, all that happens is that the 'funnel-cell' and the cells going to form the body of the nephridium project in the same direction, either pre-septal or post-septal.

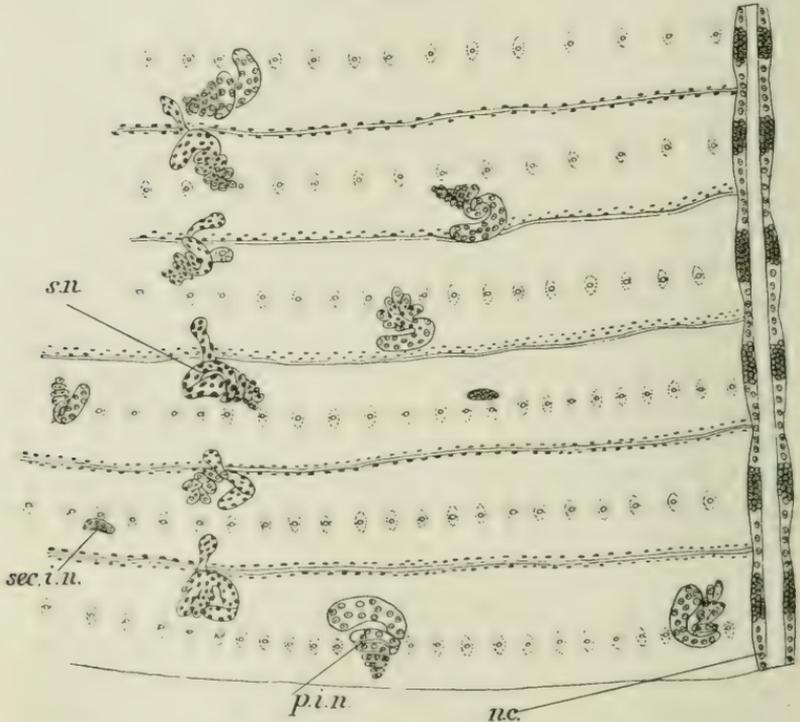
When the first secondary nephridium is fully formed, its terminal duct running along the septum meets that of the primary nephridium dorsal to it, and, similarly, the ducts of all the succeeding nephridia join those of the preceding ones, and that is how we get the formation of the septal excretory canal running parallel and internal to the commissural vessel (dorso-sous-nervien, 2). We may note that, like the septal nephridia themselves, the septal excretory canal is also inter-peritoneal.

(b) Secondary Integumentary Nephridia.

The secondary integumentary nephridia, in their early rudimentary condition, can be seen in older embryos about to hatch out of the cocoon. In whole mounts, as shown in Text-fig. 7, they can be distinguished as small solid deeply-staining masses on the side of and between the setal sacs. The setal sacs at this stage have not yet developed full-grown setae in them, and rudiments of secondary nephridia in sections (fig. 17) can be made out lying beneath the coelomic epithelium between the inner ends of two adjoining setal sacs. They arise at almost any place on the body-wall, like the primary nephridia; in some segments they are found near the dorsal vessel, in others on each side of the nerve-cord (fig. 17). Whether the secondary nephridia develop from some of the nephridial cells, lying beneath the somatic peritoneum, that

have been left over from the original nephridial masses, or whether they arise from epidermal cells that become nephridial at the time and migrate inwards, I cannot be sure. On

TEXT-FIG. 7.



Portion of a whole mount of an embryo about to hatch out of the cocoon, showing the fully-formed primary septal nephridia and the primary and secondary integumentary nephridia. *s.n.*, septal nephridia; *p.i.n.*, primary integumentary nephridia; *sec.i.n.*, secondary integumentary nephridia; *n.c.*, nerve-cord.

examining Text-fig. 4 it would seem as if the nephridial substance (cells potentially nephridial) is spread over the whole of the body-wall, and any part of it might become active and form a nephridium, and hence the appearance of nephridia at all sorts of places on the body-wall. If that be the case, and if, as shown in fig. 8, nephridial cells extend on each side of the definite nephridial rudiments, we probably get these

secondary nephridia formed from the stray nephridial cells lying beneath the peritoneal membrane of the body-wall. But, as in fig. 17, we have to account for a string of cells; which is not always seen, running from the nephridial rudiment to the ectoderm. It may be that it is a secondary formation leading from the nephridial rudiment to form the terminal duct.

8. DEVELOPMENT OF THE PHARYNGEAL NEPHRIDIA AND THEIR DUCTS.

The development of the pharyngeal nephridia of the fourth, fifth, and sixth segments can be followed in all its stages in the same embryos which show the development of the integumentary and septal nephridia. The pharyngeal nephridia appear at the same time as the primary integumentary nephridia, but are rather slower in growth than the latter. At a stage of development when the embryo is 5 to 6 mm. long and the integumentary nephridia of some of the segments behind the first six (e. g. seventh, eighth, and ninth) are almost fully formed and have developed their intra-cellular canals, the pharyngeal nephridia are seen as deeply-staining compact masses of cells lying on the body-wall, a pair in each segment, one on each side of the nerve-cord. They develop from the same source as the primary integumentary nephridia, i. e. from the nephridial cells belonging to the original ectodermal nephric row; but their manner of development is different from the other two types. While in the case of the integumentary nephridia the terminal duct is very short and appears rather late in development, forming a lumen at the same time with the rest of the nephridium, the ducts of the pharyngeal nephridia develop very early. In an embryo 5 mm. in length the nephridia of the fourth, fifth, and sixth segments are small club-shaped solid masses produced into long solid strings of cells leading anteriorly to the lateral walls of the pharynx (fig. 18). The terminal ducts are thus formed earlier than the bodies of the nephridia themselves. This is still more marked at a later stage in an older embryo in which the

ducts of the pharyngeal nephridia are seen to have acquired a lumen, while the nephridia themselves have not yet developed their adult form and are still solid. These ducts are intracellular, but are surrounded by the muscular tissue of the strands passing from the pharynx to the body-wall for part of their length.

The usual order of antero-posterior development is reversed in the case of pharyngeal nephridia. In the two stages of development mentioned above, the nephridia of the sixth segment are advanced further in development than those of the fifth, and the latter than those of the fourth segment.

There is only one pair of nephridia anterior to the pharyngeal ones, i. e. the one belonging to the third segment, the first two segments of the embryo being anephrous. This most anterior pair, although integumentary in character, follows the pharyngeals in their time and rate of development.

The ducts in these early stages are very thin with intracellular lumen, and are therefore to be looked upon as the elongated terminal ducts of the primary pairs of pharyngeal nephridia rather than as outgrowths from the walls of the pharynx. Three successive thickenings on the lateral pharyngeal wall mark the places of entrance of the ducts into the pharyngeal lumen. It is a remarkable fact that not only do the terminal ducts acquire a lumen before the formation of the canals in the nephridia themselves, but that they also open into the cavity of the pharynx long before the nephridia are able to function at all.

The pharyngeal nephridia, like the integumentary ones, do not develop a 'funnel', but we have to note that at an early stage when the terminal ducts have been formed and the nephridia are developing their adult structure, the pharyngeal pair come into connexion with the intersegmental septa not in front of but behind them. The dorsal blood-vessel and the lateral oesophageals act as the afferent and the efferent vessels to these nephridia, and the branches of these vessels near their points of origin and entrance into the main vessels lie on the septal supports.

Secondary pharyngeal nephridia arise in a way different from that of the secondary septal and integumentary ones. They do not appear independently of the primary pair but develop as buds on the nephridial ends of the pharyngeal ducts. In fig. 19 are seen three buds in the fifth and two in the sixth segment ; while in the fourth the primary nephridium itself is not fully formed yet. As these buds develop into fully-formed nephridia, their terminal ducts, longer than those of the other types of nephridia, remain continuous with the primary pharyngeal duct, or rather open into it. Thus we get a large number of pharyngeal nephridia forming big tufts and having their terminal ducts opening into these primary ducts. The primary ducts themselves, although originally very narrow and intra-cellular, enlarge and acquire a muscular investment which makes their walls thick and tough as they are in the adult condition.

9. COMPARISON WITH THE DEVELOPMENT OF 'MEGANEPHRIC' AND THE SO-CALLED 'PLECTONEPHRIC' TYPES OF NEPHRIDIA.

I have referred in brief to the known facts of development of these two types of nephridia in the historical part of this paper. So far as the 'meganephric' type of nephridia are concerned, we can compare them only with the primary integumentary nephridia of *Pheretima*, a pair in each segment. The obvious differences between the meganephridia of *Lumbricus* and the primary pair of integumentary nephridia in an embryo of *Pheretima* are the larger size of the former and the presence of a 'funnel' in them. In his recent memoir on the development of nephridia in *Criodrilus*, as already mentioned on p. 53 (15), Staff derives the nephridium from the 'funnel-cell' and the retroperitoneal group of cells behind each septum, both being ultimately derived from the nephridial string of cells between the ectoderm and the mesoderm. In *Pheretima*, as we have seen, the primary integumentary nephridia develop from the 'retroperitoneal' cells alone and there is no 'funnel-cell' taking part in their

formation, and that is why they do not develop any funnel at all. The septal nephridia, on the other hand, develop from the intersegmental nephridial mass of cells which possesses a 'funnel-cell', and so we get septal nephridia with funnels developing from this source.

This meganephric, or rather the paired condition in the embryo, is superseded by, and is assimilated into, the adult condition which is 'enteronephric', so far as the septal and pharyngeal nephridia are concerned; but is 'micronephric' and diffuse so far as the integumentary nephridia are concerned. It cannot be called 'plectonephric'. If the place of opening of the nephridia be taken into consideration we can divide the nephridia into two groups: those that open to the outside on the surface of the body-wall and may be termed 'dermo-nephric' or 'exonephric', and those that open into any part of the gut and may be called 'enteronephric'. The former category will include the ordinary 'meganephridia' of *Lumbricus*, as well as the integumentary micronephridia of *Pheretima* and the plecto-nephridia of other worms. The latter term, i.e. enteronephric, will comprise the septal and pharyngeal nephridia of *Pheretima*, the 'pharyngeal tufts', the 'peptonephridia' or 'salivary glands' of such forms as *Megascolides*, *Periscolax*, and *Enechytraeids*, and the anal nephridia of *Octochaetus* and *Allolobophora antipae* (14).

Comparing the nephridial development of *Pheretima* with that of the plectonephridia of *Megascolides* (17) and *Mahbenus* (8), we have to note that, while in the latter the whole system results from the breaking up and branching of the first pair of nephridia, in *Pheretima* there is no such branching and breaking up, and all the nephridia, septal and integumentary, appear independently of one another. Moreover, the first pair of nephridia in *Megascolides* and *Mahbenus* have 'funnels' which persist in *Megascolides* but degenerate in *Mahbenus*, while *Pheretima* has no 'funnels' even on its initial pair of integumentary nephridia.

In my previous paper (1), from a study of the structure of

adult nephridia, I came to the conclusion that each nephridium is a separate and discrete structure, that there is no network of any kind connecting one nephridium with the other, and that it is a mistake to describe the nephridial system of *Pheretima* as a 'coelomic network' or as 'plectonephric', implying the idea of a reticular connexion between the nephridia. This conclusion is now confirmed by the evidence we have from the embryology of the excretory system of this worm. We now know that each nephridium, integumentary or septal, originates independently of the others, and, therefore, even the embryonic connexion found in *Megascoides* and *Mahbenus* is wanting in this worm.

10. PHYLOGENY OF THE OLIGOCHAETE NEPHRIDIAL SYSTEM.

Before any developmental facts were known with regard to the excretory organs of *Oligochaetes*, it was commonly held 'that the paired nephridia (meganephridia) of most *Oligochaeta* were formed by reduction from a network such as now exists in *Perichaeta* and many other genera' (4). But after the embryology of the excretory system in *Octochaetus* and *Megascoides* had been elucidated, and it was found that a meganephridial condition preceded the diffuse condition, this view had to be given up. In speaking of the phylogeny of the system, Beddard (4) says that 'it does not follow that the diffuse nephridia are the outcome of a branching and specialization of the paired nephridia; what the developmental facts prove is that both paired and diffuse nephridia are formed out of similar pronephridia; that in fact both kinds of excretory organs are equally ancient'. This view was definitely formulated by Vejdovsky (16), who says, 'Es hat daher meiner Ansicht nach sowohl das "Plecto- als Meganephridium" gleiche genetische Bedeutung. Beiden muss ein einfacher, paarig in jedem Segmente sich anlegender Strang—das Pronephridium—vorausgehen, aus welchem erst secundär seitliche Wucherungen entstehen, die sich als zahlreichere oder spärlichere Nephridiallappen erweisen. In grosser Menge bilden

sich offenbar die Lappchen bei den mit "Plectonephridien" versehenen Oligochaeten und in den vorderen Segmenten von *Megascolides*. In den hinteren Segmenten des genannten Riesenregenwurmes reduciren sich die Lappchen an einige grossere, welche der Lage nach den Schlingen am Nephridium von *Lumbricus* entsprechen.' Vejdovsky's conclusions are based on his researches on *Rhynchelmis*, in which a definite pronephridial stage precedes the permanent nephridia, and on the development of nephridia in *Megascolides*, in which a paired pronephridial condition precedes the permanent plectonephric system.

The facts of nephridial development recorded in this paper do not lend themselves to Vejdovsky's interpretation of the evolution of the excretory system. In the first place, we cannot distinguish in the development of *Pheretima* a pronephridial stage as distinguished from a stage of permanent nephridia. What we do get is a paired condition in the embryo which goes to form part of the adult system and is not entirely superseded by it. Secondly, the paired nephridia themselves are not transformed, as they are in *Megascolides*, into the diffuse system of the adult, but numerous nephridia arise independently to be added to the primary integumentary nephridia. In the third place, the adult *Pheretima* does not show the condition referred to by Vejdovsky in some other worms ('Riesenregenwurmes'), where the anterior segments have numerous nephridia but the posterior ones show the paired meganephric condition.

Since the diffuse and paired forms of the excretory system occur in genera which are so nearly related, Beddard (3) thinks there can be no profound gap between the two kinds of organs. But when one takes into account the fact that in the family *Perichaetidae*, *Pleionogaster* possesses nephridia of the diffuse type all opening to the exterior, *Megascolex* has a pair of large nephridia in each segment in addition to the small scattered nephridia, while *Perionyx* and *Diporochaeta* have only large paired nephridia, it becomes very difficult to think of and offer an explanation for the intermediate

evolutionary stages between the condition in *Perionyx* (exonephric) and that in *Pheretima* (enteronephric). Although we can derive the diffuse condition of *Pleinogaster* from *Perionyx*, through such forms as *Megascolix* showing an intermediate condition, we cannot ignore the fact that the gap between the 'exonephric' (p. 86) and 'enteronephric' conditions is very deep indeed.

A few facts in the embryology of the nephridial system, however, seem to throw some light on the possible evolution of the enteronephric system. I have already shown (p. 67) that the primary integumentary nephridia have a common source of origin in the nephridial masses lying opposite the intersegmental septa. We have also seen that, while the integumentary nephridium has no 'funnel-cell', it is represented in the rudiment of each septal nephridium, and consequently the former lacks and the latter possesses a 'funnel' in the adult condition. It is possible to suppose that the first great step in the process of evolution of the enteronephric system was the severance of the connexion between the 'funnel' and the 'body' of the nephridium. That this severance has probably taken place in *Pheretima* is supported by very strong evidence from the embryology of the nephridia of *Octochaetus* (3), *Megascolides* (17), and *Mahbenus* (8). In all these forms there is a paired meganephric condition in the embryo, and each nephridium is provided with a well-developed funnel. In the transformation of this embryonic into the adult condition the part to degenerate first is always the duct following the funnel. In *Octochaetus* (3), Beddard found that the change took place by the disappearance of the lumen in the portion nearest the funnel. Vějdovsky (16) has found in *Megascolides* that the paired embryonic nephridia have a funnel from which leads a straight duct without lumen, and that this duct joins the nephridial loops. During development the connecting part of the original tube (i. e. the straight solid duct) first degenerates into a mere strand of connective tissue and finally breaks up entirely. But the funnel remains and forms part of the large nephridium in the

adult. Bourne (8) found a similar condition in *Mahbenus*, the funnel, however, in this case degenerating entirely.

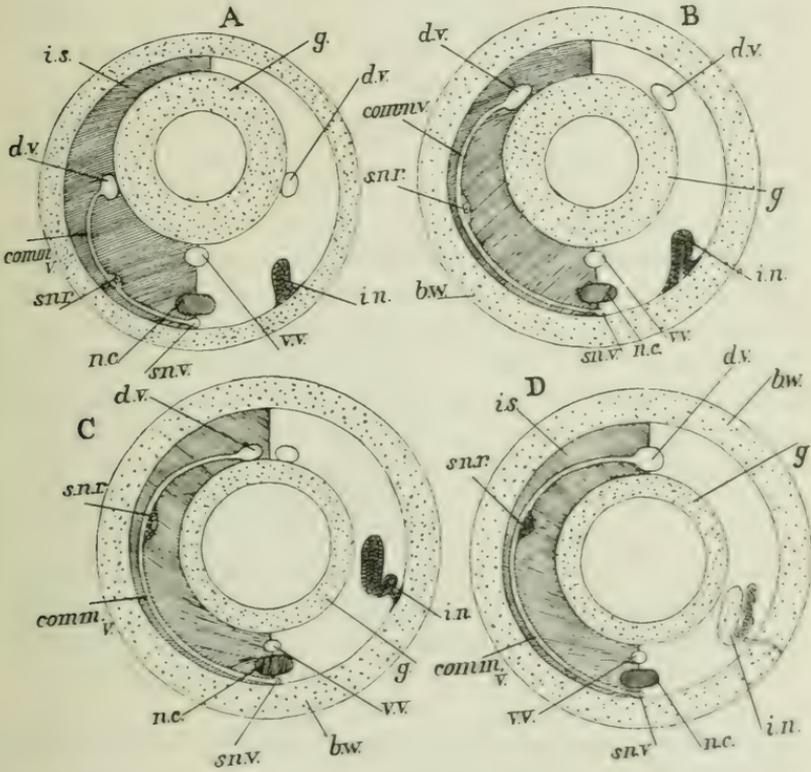
It is easy to derive the condition in *Pheretima* from what takes place in *Megascolides*. In the latter the funnel, with part of the tube following it, develops into the large paired nephridium with funnel, a pair to each segment, while the body of the nephridium gives rise to a network of minute excretory tubules. In *Pheretima* the separation of the funnel from the body of the nephridium is carried a little farther and takes place early in ontogeny. The result is essentially the same as in *Megascolides*, i. e. the formation of two kinds of nephridia: in this case the larger septal nephridia with funnels and the smaller integumentary ones without funnels. The evolution has taken place along the same lines in *Octochaetus* and *Mahbenus* also, but in these two genera the degeneration and disappearance of the portion following the funnel has likewise affected the funnel which also degenerates, and that is why we get only one type of nephridium without funnel in these two cases, although Beddard found some nephridia with funnels towards the posterior end of *Octochaetus*, along with those without funnels.

In *Pheretima* the 'funnel-cell' along with some other nephridial cells separate off early from the main nephridial mass, and while the integumentary nephridia develop at once from the main nephridial mass the development of the septal ones from the 'funnel-cell' comes a little later. We tacitly assume, of course, that the 'funnel-cell' itself by division is capable of giving rise to the whole nephridium, and this is what actually takes place (cf. figs. 13 and 14). Why the funnel gets separated off from the main body of the nephridium during development in *Octochaetus* and *Mahbenus*, and why the separation begins so early in *Pheretima*—whether it took place in phylogeny before or after the septal nephridia had acquired their openings into the gut—are questions difficult to answer.

How does this 'funnel-cell' travel dorsalwards and take up

a dorso-lateral instead of its usual ventral position? That it travels dorsalwards can be seen easily from figs. 13 and 14. and that this is very unusual can be realized from the fact that

TEXT-FIG. 8.



A series of four diagrams showing how the commissural vessel elongates with the migration dorsalwards of the dorsal vessel and how the nephridial rudiment on the septum is carried to its dorso-lateral position. *d.v.*, dorsal vessel (double in A, B, and C); *v.v.*, ventral vessel; *n.c.*, nerve-cord; *s.n.v.*, subneural vessel; *i.s.*, intersegmental septum; *i.n.*, integumentary nephridium; *s.n.r.*, rudiment of a septal nephridium; *b.w.*, body-wall.

ordinarily the funnel in a nephridium is the most ventral part of it and lies almost next to the ventral nerve-cord. But although away from the nerve-cord, the position of the primary septal nephridia is not very unusual if we bear in mind the

scattered arrangement of the integumentary nephridia (Text-fig. 3). By comparing the position of the septal and integumentary nephridia in Text-figs. 5 and 6 the discrepancy does not seem to come to very much. But that there is a shifting dorsalwards, however little (and in many septa a great deal), admits of no doubt. This can be explained, however, easily, if we take into account the facts of development and growth of the septa and the structures connected with them, e.g. the commissural vessel and the dorsal vessel. In the series of diagrams in Text-fig. 8 I have tried to illustrate the gradual growth and formation of the commissural which connects the subneural and dorsal vessels. The dorsal vessel is formed by the progressive backward concrescence of the two lateral vessels, which, as a rule, lie at the dorsal edge of the advancing mesoderm bands. The commissural, which appears very early and connects the subneural with the lateral (the semi-dorsal so to speak), lies from the very beginning between the two sheets of the mesodermal septa. From an examination of figs. 13 and 14 it becomes clear that the 'funnel-cell' or the rudiment of the septal nephridium is closely associated with the commissural vessel, lying in the same intraseptal cavity with and ventral to the blood-vessel. It would seem that in the process of migration of the lateral vessels to the mid-dorsal position the commissural vessel must keep pace and travel dorsalwards. Further, in the general growth of this vessel dorsally, the 'funnel-cell' or nephridial rudiment closely associated with it also travels dorsalwards, and hence results the dorso-lateral position of the primary septal nephridium.

That the commissural vessel and the rudiment of the septal nephridia lie in the same hollow of the septum between the two sheets of mesoderm in the embryo is clear from figs. 13 and 14, and is evidence of the close association of these two structures. That there is some morphological relationship is also shown by the fact that, in the adult worm, the septal nephridia are only present on those septa that have a commissural vessel. In the first fourteen segments of the worm there are no septal nephridia and no commissural vessels either.

Once the 'funnel-cell' reached its dorso-lateral position, it developed into a nephridium with a funnel (a septal nephridium); but it would seem that the terminal duct of the nephridium had, so to speak, lost its original course, having been removed from the body-wall and having been caught in the 'tunnel' of the septum containing the commissural vessel. The terminal duct followed the course of the vessel and travelled towards the mid-dorsal line, where, on meeting its fellow of the other side, it formed the supra-intestinal excretory duct. It would be a case of induced development and growth, stimulated by the course and development of the commissural vessel. When we have once got a septal nephridium formed in the dorso-lateral position, its terminal duct would tend to find a way out. But since the way to the body-wall is blocked, the terminal duct lengthens out and follows the course of the commissural vessel. Examples of this kind of 'dependent differentiation', a term due to Roux, are found in the experiments of Lewis and Spemann. Lewis has shown that a lens will be formed from any patch of ectoderm taken from some other part of the body and grafted over the optic cup during the development of the eye in *Amblystoma* and some species of frogs. Spemann and Lewis have also found that in the absence of contact between the optic cup and the ectoderm the cornea is not developed, that is to say, the overlying ectoderm does not 'clear' (lose its pigment), it does not thin out, and Descemet's membrane is not formed.¹

Once the supra-intestinal duct is formed in the mid-dorsal line above gut, the only possible way to discharge the excretory fluid is to have communications with the gut in each segment. This tendency of the nephridial ducts to open into the gut has been noticed in other worms also. Rosa (14) found in one species of *Allolobophora* (*A. antipae*) that all the nephridia of the posterior region of the body, instead of opening on the exterior, communicate with a pair of longitudinal canals which open posteriorly into a median vesicle communicating with the rectum. It would appear that in outline

¹ Jenkinson's 'Experimental Embryology', pp. 271-7.

the condition of nephridia in the posterior region of the body of *Allolobophora antipae* is remarkably similar to that in the whole length of the body of *Pheretima*. On the formation of communications between the supra-intestinal ducts and the lumen of the gut, the essentials of the 'enteronephric' system are completed.

The formation of secondary septal and integumentary nephridia is not difficult to explain. The septals are, no doubt, traceable to the same source as the primary nephridia, i.e. to the cell-group making its way into the intersegmental septum. The primary nephridium already has a pre-septal funnel, while, of the succeeding secondary ones, most have a post-septal funnel, there are some with a pre-septal funnel. It is possible that the original 'funnel-cell' has something to do with the pre-septal or post-septal position of the funnel. If the funnel is formed from the original 'funnel-cell' or a derivative of it, we get a pre-septal funnel; but if the funnel is formed from one of the other cells which takes up the character of the 'funnel-cell', a post-septal funnel results.

As regards the secondary integumental nephridia, their separation from the primary nephridia and from one another has gone much deeper and further than that shown in the developing nephridia of *Megascolides* (17) and *Mahbenus* (8). They are coeval in origin, but not connected in any part of their development.

Both the buccal cavity and the pharynx, forming that portion of the alimentary canal which lies in the first four segments of the body, belong to the stomodaeum; and since the latter is morphologically external, the pharyngeal nephridia opening into the stomodaeum may be said to open on the ectoderm. But the facts of development do not help us in understanding the possible course of evolution of these nephridia. We cannot, for example, assume that these nephridia are really integumentary, but have come to occupy their present position and relationship on account of the anterior portion of the worm being formed into an 'introvert' or a stomodaeum. This assumption does not work in giving us the adult structure

from the hypothetical original condition of these nephridia. The development of secondary pharyngeal nephridia as distal buds on the pharyngeal ducts is remarkable.

11. MATERIAL AND TECHNIQUE.

The material for this work consisted of cocoons of *Pheretima* containing embryos of various ages. In the Kew Gardens, where I got my supply of these worms from, they are found along with two or three other genera in the soil of the Lily House, and, if the cocoons were collected from that soil, it would be difficult to distinguish the cocoons of one genus from another. Accordingly, to be quite sure of the specific identity of my material, I tried the isolation and culture method, which I briefly describe below.

I took common garden soil and sterilized it for two or three hours to kill all organisms in it, specially the cocoons of other worms, eggs of insects, &c. This earth was mixed with sand, and finally I added to this mixture a quantity of decaying leaves which had also been previously sterilized. Four garden pots, the bottom holes of which were closed with corks, were filled with the sterilized soil and about fifteen worms of this species of *Pheretima* were kept in each pot. These pots were kept in a hot-house with an average temperature of 60° F.; the earth in the pots was kept moist and sterilized decaying leaves were added from time to time. After the first two months I was able to get cocoons in this way almost throughout the year, and was always sure that the cocoons were of *Pheretima* alone and of no other worm.

Various methods were tried to sift out the cocoons from the earth, but the least troublesome, and therefore the best, is to put a heap of earth in a fine sieve and to stir the earth while keeping the sieve in a bucket of water. The earth passes through the sieve and settles down at the bottom of the bucket, while the cocoons are left in the sieve along with the large pebbles and pieces of stone. The cocoons can be easily found and picked up with a wet paint-brush.

Since the cocoons of *Pheretima* have a transparent shell, the embryo inside a cocoon can be easily seen under a binocular microscope by transmitted light. It is therefore easy to know the age and size of the embryo before opening the cocoon. Since in a given lot of earth the cocoons are of all ages, we can at once select an embryo of the desired age, provided we have a large number of cocoons. As a rule there is only one embryo in each cocoon, but we sometimes meet with two or even three.

The cocoons are opened in salt solution by means of a pair of sharp needles under the binocular microscope. Very early stages (blastulae and gastrulae) were mounted whole in clove oil after staining with paracarmine. Two pieces of hair were placed below the coverglass, which enabled the rounded embryo to be rolled under the coverslip in order that it could be examined from all sides.

Embryos of about 4 to 6 mm. in length were used both for whole mounts and sections for the study of integumentary nephridia. The embryos while in salt solution were always narcotized by ether and fixed either with Bouin's fluid or corrosive-acetic or Petrunkevitch. The latter solutions were found preferable to Bouin, since this fluid hardens the food-yolk very much and makes it brittle for section-cutting. Serial longitudinal sections of a few embryos of suitable age enables one to follow the development of integumentary nephridia fairly completely. A series of transverse sections, $5\ \mu$ in thickness, of an embryo about $300\ \mu$ in length, was very useful in following the very early stages of development, e.g. the teloblasts and their development. For the later stages of development of primary nephridia, as represented in Text-fig. 4, embryos fixed in Bouin or corrosive are slit open by means of a sharp needle along the mid-dorsal line, the roll of albuminous material filling the gut is removed, and with a little care the endoderm itself is removed. What is left is the body-wall with the nephridia attached to it. This is stained with paracarmine, flattened out, and mounted whole.

Older embryos, about 10 mm. or more in length, are fixed

in the same way and opened by a mid-ventral incision; the albumen is removed but not the endoderm: these, when mounted flat, show the septal nephridia in all stages of development, as represented in figs. 14 and 15. Since they lie in two rows, one on each side of the dorsal vessel, it is best to open the embryos from the ventral side and look for the nephridia on each side of the dorsal vessel. In order to avoid any displacement of the septal nephridia it is best to leave the endoderm on; but it is necessary to brush carefully the inside of the embryo with a fine camel-hair brush, so that all yolk-material sticking to the inside of the gut is removed and the preparation rendered quite transparent to show the septal nephridia to the best advantage.

The most difficult part of the task, however, was to trace the initial stages of development of the septal nephridia—to find out whether the ‘rudiment’ (‘Anlage’) of the septal nephridia arose as a multiplication of one or more cells belonging to the walls of the adjoining coelomic sacs (the septa), or it was formed by a group of cells between the two contiguous sheets of the intersegmental septa. For this purpose it was necessary to have serial longitudinal sections of the dorsal and dorso-lateral parts of an embryo of suitable age, i.e. one in which one could expect to find the septal nephridia in very early stages of development. The difficulty in getting such sections arises from the fact that the gut in embryos of this age is so enormously distended with food-yolk as to squeeze out of existence altogether the coelomic cavity between the body-wall and the gut dorsally. Consequently it becomes impossible to distinguish, in sections of such embryos, the septa and the nephridial masses on them. Many series of sections were cut of embryos which had been flattened out, after being cut open ventrally and the food-yolk removed. In these series, although the coelom could be distinguished in some of the segments, it was obliterated in others, and no accurate and reliable observations could be made. In many of the embryos part of the posterior end was cut off before fixation to allow the food-yolk to ooze out and thus let the wall of the gut shrink away from the

body-wall, restoring the coelomic cavity on the dorsal side. I did not meet with much success even by this method. At last I was lucky in finding two embryos, one of which was just of the right age (about 6 mm.) and in both of which the gut was narrow and the coelomic cavity all round very spacious. A complete series of longitudinal sections of one of them gave me all the stages of development of the septal nephridia, and I was able to establish, firstly, that the septal nephridia develop between the two peritoneal sheets (inter-peritoneal), and, secondly, that they could be traced to their final septal position from their first place of origin in the primary nephric row in the body-wall.

12. SUMMARY.

1. The three kinds of nephridia—integumentary, septal, and pharyngeal—appear at successive stages of development of the embryo: the integumentary preceding the septal and pharyngeal, both of which develop simultaneously.

2. All the three kinds can be traced back to the original row of nephridial cells of ectodermal origin. Thus all the different nephridia are ultimately derived from one common origin.

3. The primary pair of integumentary nephridia are the first to appear from a 'retro-peritoneal' group of cells. The rudiments lack the 'funnel-cell', and consequently a 'coelomic' funnel is never developed in these nephridia. They open to the exterior on the body-wall.

4. These primary integumentary nephridia do not appear in the same position in successive segments of the embryo, but are irregularly distributed all over the body-wall.

5. The septal primary nephridia can be traced back to a group of nephridial cells, including the 'funnel-cell', which make their way into each septum between its two adjoining peritoneal lamellae.

6. The primary septal nephridia have always a well-developed pre-septal funnel, and appear along a straight line on both sides of the dorsal vessel. They appear after the primary

integumentary pair has reached a fairly advanced stage of development.

7. The secondary nephridia of both the integumentary and septal types are not budded off from the primary nephridia, but the rudiments of all have a common origin and separate early. They resemble the primaries in every respect, except that in the case of the septal secondaries the funnel is either pre-septal or post-septal.

8. The terminal ducts of the primary septal nephridia form the dorsal portions of the septal excretory canals on the septa, and the canals of both sides form the supra-intestinal duct on meeting the mid-dorsal line above the gut. The segmental ductules establishing a communication between the supra-intestinal duct and the lumen of the gut appear soon after the formation of the supra-intestinal ducts.

9. The primary pharyngeal nephridia of the fourth, fifth, and sixth segments develop from a 'retro-peritoneal' group of cells like the integumentary ones, and have long ducts reaching the wall of the pharynx. Secondary nephridia are formed as successive buds on the ducts, anterior to the primary nephridia.

10. The possible phylogenetic stages in the evolution of the 'enteronephric' type of nephridia are as follows: (1) the severance of the connexion between the septal funnel and the body of the nephridium; (2) migration of the severed portion, i.e. the 'funnel-cell', together with some other nephridial cells from a ventral to a lateral position in the embryo; (3) the growth of this severed portion into a septal nephridium and the acquisition by the latter of an opening into the gut; (4) the elongation of the terminal ducts of all septal nephridia towards the mid-dorsal line (induced by the course of commissural vessels) and the formation of continuous supra-intestinal ducts. It is problematic whether the severance of the connexion between the funnel and the body of the nephridium took place before or after the connexion of the nephridium with the gut.

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EXPLANATION OF PLATES 5-7.

Illustrating Dr. K. N. Bahl's paper 'On the Development of the "Enteronephric" type of Nephridial System found in Indian Earthworms of the genus *Pheretima*'.

Fig. 1.—Whole mount of a very young embryo ($146\mu \times 130\mu$) seen in a ventro-lateral aspect, showing the mesodermal pole-cell *M.P.C.*, the neural and nephridial teloblasts *N.N.T.*, and the mesoderm band *m.b.* $\times 500$.

Fig. 2.—Longitudinal section of an embryo 245μ in length, showing the relative position of the mesodermal pole-cell and one of the teloblasts. The ectodermal origin of the teloblast is clearly indicated. *t.* the neural teloblast; *c.c.*, coelomic cavities in the mesoderm; *arch.*, archenteric cavity; *end.*, endodermal cells with yolk-granules. $\times 500$.

Fig. 3.—Ventral portion of a transverse section from the posterior end of an embryo 300μ long, showing the origin of the teloblasts in the ectoderm. The teloblasts are still embedded in the ectodermal layer, but are sharply marked off from the definitive ectodermal cells. On the right are seen two teloblasts, the ventral one being the neural and the lateral one the nephridial. On the left are seen three cells still embedded in the ectoderm which lie in front of the teloblasts and have been budded off from them. *n.t.*, neural teloblast; *np.t.*, nephridial teloblast; *c.t.*, cells budded off from teloblasts; *m.s.*, hollow mesodermal somites with coelomic cavities, *c.c.*; *ect.*, ectodermal cell. $\times \text{cir. } 620$.

Fig. 4.—Ventral portion of the section just in front of the one shown in fig. 3, showing the gradual demarcation of the neural and nephridial cells from the definitive ectoderm. *lat.t.*, lateral teloblast; *n.c.*, neural cells; *nph.c.*, nephridial cells. $\times \text{cir. } 664$.

Fig. 5.—Ventral portion of a longitudinal section through the hind end of an embryo about 3 mm. long, showing the nephridial string of cells breaking into the cell-masses destined to give rise to the nephridia. *M.P.C.*, position of mesodermal pole-cell; *nph.t.*, nephridial teloblast; *nph.l.*, nephridial layer of cells; *m.l.*, layer of mesoderm with coelomic cavities; *ect.*, ectoderm. $\times 545$.

Fig. 6.—Transverse sections of an embryo 300μ long, from which figs. 3 and 4 have been drawn, showing the 'funnel-cells' in section between the two adjoining coelomic cavities, i.e. in the septa. *n.c.*, nerve-cord; *nph.c.*, groups of nephridial cells; *f.c.*, funnel-cells; *ect.*, ectoderm. $\times 400$.

Fig. 7.—Portion of a longitudinal section of an embryo, showing the nephridial groups of cells pushing into the intersegmental septa. Other letters as before. $\times 640$.

Fig. 8.—Five consecutive longitudinal sections of a series taken from the hinder portion of an embryo about 4 mm. long, showing the development of primary integumentary nephridia, and their relations with the intersegmental septa. *nph.*, nephridia (integumentary); *nph.c.*, nephridial cells pushing their way into the septa; *i.s.*, intersegmental septum; *c.c.*, coelomic cavity; *ect.*, ectoderm.

Fig. 9.—A portion of a longitudinal section of the same embryo as in fig. 8, showing the developing integumentary nephridia. Letters as in fig. 8. $\times 1,200$.

Fig. 10.—Portion of a longitudinal section of an embryo, showing the semblance of a 'funnel', the only series in which a 'funnel' is seen. *f.*, pseudo-funnel. $\times 1,160$.

Fig. 11 (A-F).—A series of diagrams showing the developing stages of an integumentary nephridium, taken from whole mounts of embryos with endoderm and albumen removed. Nephridia lie posterior to the septa. *i.s.*, intersegmental septa; *s.l.*, short straight lobe of the nephridium; *t.l.*, the twisted loop. $\times \text{cir. } 630$.

Fig. 12 (A-E).—A series of diagrams from whole mounts showing the exact mode of development of a primary septal nephridium. *f.*, the pre-septal funnel; *s.l.*, straight lobe; *t.l.*, twisted loop; *t.d.*, terminal dust; *c.v.*, commissural vessel; *i.s.*, intersegmental septum. $\times 530$.

Fig. 13.—Longitudinal sections of the posterior end of an embryo about 6 mm. in length, showing how the 'funnel-cell' travels from its original position in the nephridial layer of the body-wall to its final position on the septa on each side of the dorsal vessel. 1-6 are sections of septa from the same series showing the change in position. *c.v.*, commissural vessel; *f.c.*, funnel-cell; *v.lat.*, ventro-lateral branches of the ventral vessel; *c.c.* coelomic cavities. $\times \text{cir. } 1,250$.

Fig. 14 (A-D).—A series of septa in longitudinal sections showing the early development of a septal nephridium ventral and close to the commissural vessel and between the two sheets of mesoderm forming a septum. *b.w.*, body-wall; *c.v.*, commissural vessel; *nph.r.*, nephridial rudiment. $\times \text{cir. } 1,250$.

Fig. 15.—Two intersegmental septa with nephridia on them from a whole mount of an embryo about to hatch out of the cocoon, showing the developing secondary nephridia of both kinds, one with pre-septal funnel and the other with post-septal funnel. *psn.*, primary septal nephridium; *sec.s.n.*, secondary septal nephridium with post-septal funnel; *sec.s.n'*, secondary septal nephridium with a pre-septal funnel. $\times 450$.

Fig. 16.—Part of a transverse section of an embryo, showing a rudiment of a secondary septal nephridium. *c.v.*, commissural vessel; *b.w.*, body-wall; *n.c.*, nerve-cord; *d.v.*, dorsal vessel; other letters as in fig. 15. $\times 330$.

Fig. 17.—Portions of two consecutive sections of an embryo, showing

the development of secondary integumentary nephridia. *sec.in.*, secondary integumentary nephridia; *ep.*, epidermis; *circ.m.*, layer of circular muscle-fibres; *long.m.*, layer of longitudinal muscles; *s.s.*, setal sacs. $\times 440$.

Fig. 18.—Longitudinal section of the anterior portion of an embryo about 4.5 mm. in length reconstructed from serial sections, showing the early development of pharyngeal nephridia and their ducts (both being solid at this stage). *ph₄*, *ph₅*, *ph₆*, pharyngeal nephridia of the fourth, fifth, and sixth segments with their respective ducts; *phx.*, pharynx; *i.g.₇*, integumentary nephridia of the seventh segment; *b.c.*, buccal cavity; *c.g.*, cerebral ganglion; *ph.gl.*, pharyngeal gland-cell.

Fig. 19.—Longitudinal section of the anterior portion of an embryo about 6 mm. in length, showing the primary pharyngeal nephridia and the buds of the secondary ones arising on the ducts. *d.v.*, dorsal vessel; *lat.oe.v.*, lateral oesophageal vessel.

Fig. 20.—Three embryos of typical ages of *Pheretima rodricensis*, shown in natural size. *A* is an embryo about 4 mm. in length, from sections of which figs. 5, 8, 9, and 22 are taken. *B* is an embryo about 6 mm. in length, from sections of which figs. 17, 18, and 23 are taken. Whole mounts shown in figs. 11, 12, 13, 14, and parts of 15 are taken from embryos of about this age. *C* is an embryo about to hatch out of the cocoon about 17 mm. in length. Figs. 16, 19, 20, and 21 are taken from whole mounts or sections of embryos of this age. Nat. size.

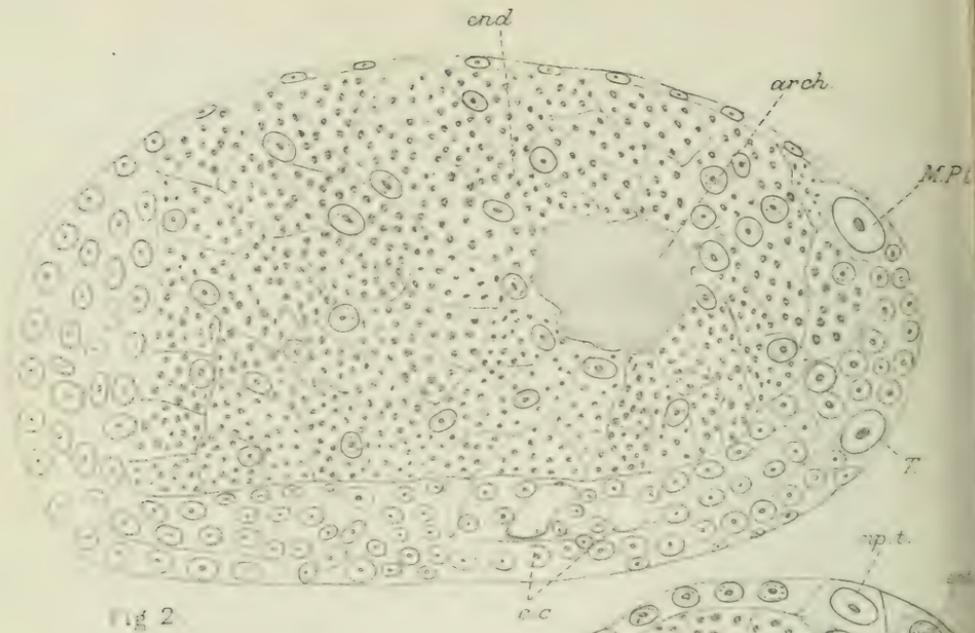


Fig. 2

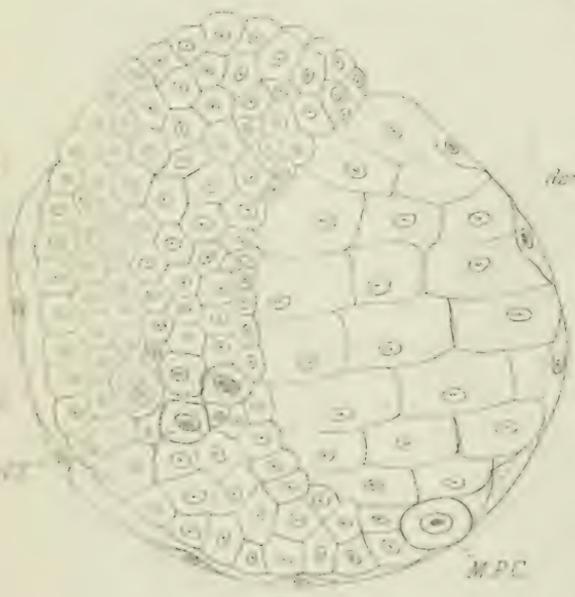


Fig. 1



Fig. 3

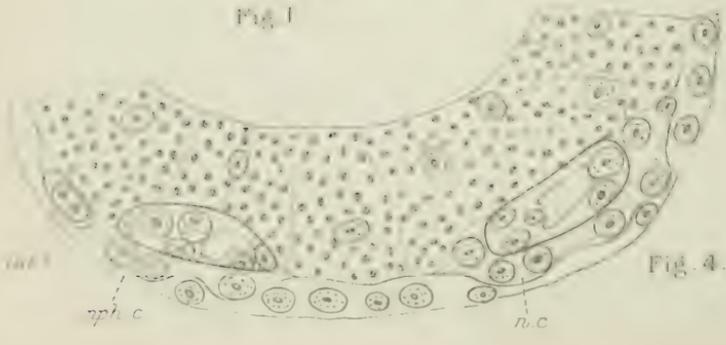


Fig. 4

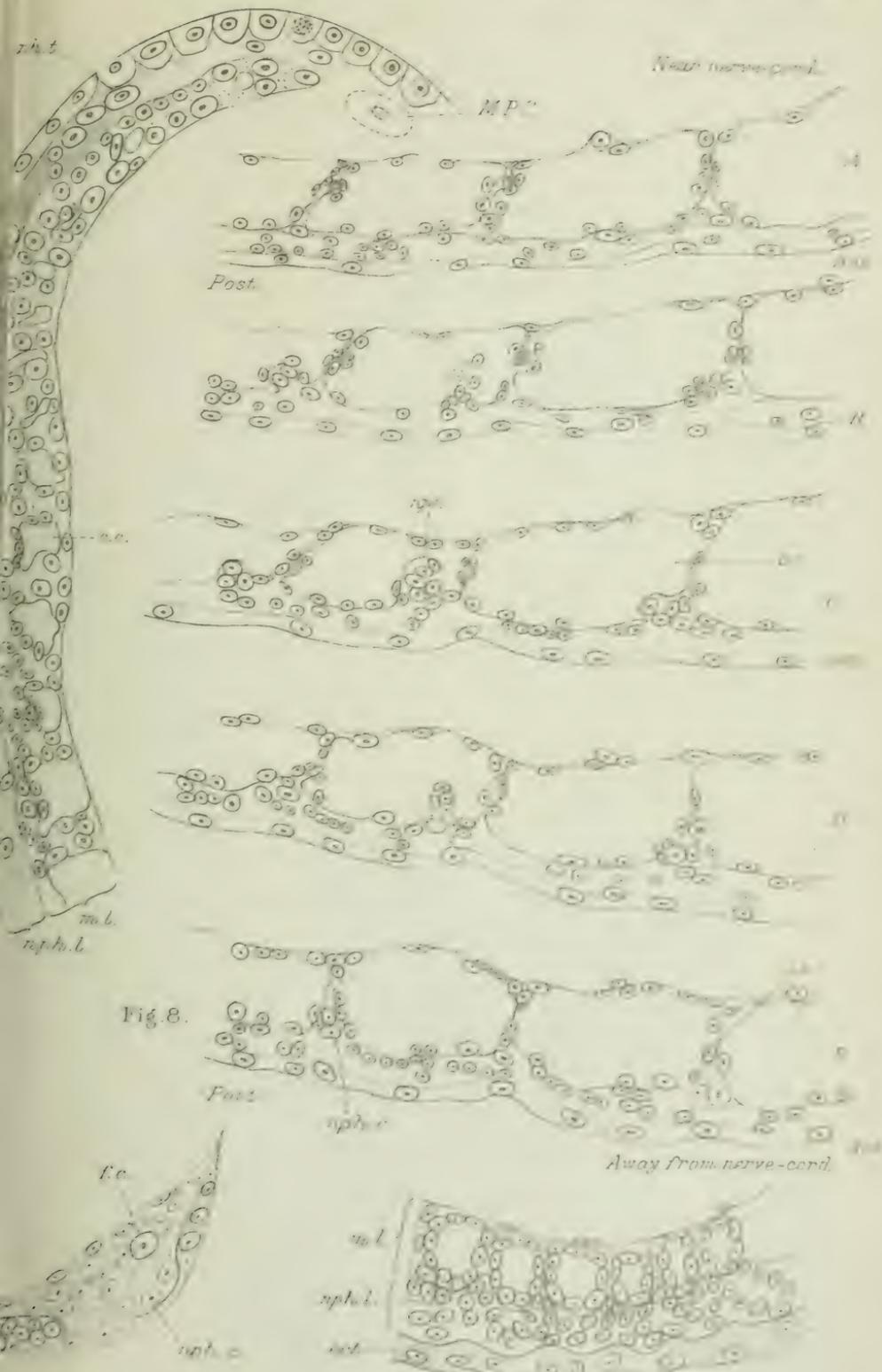
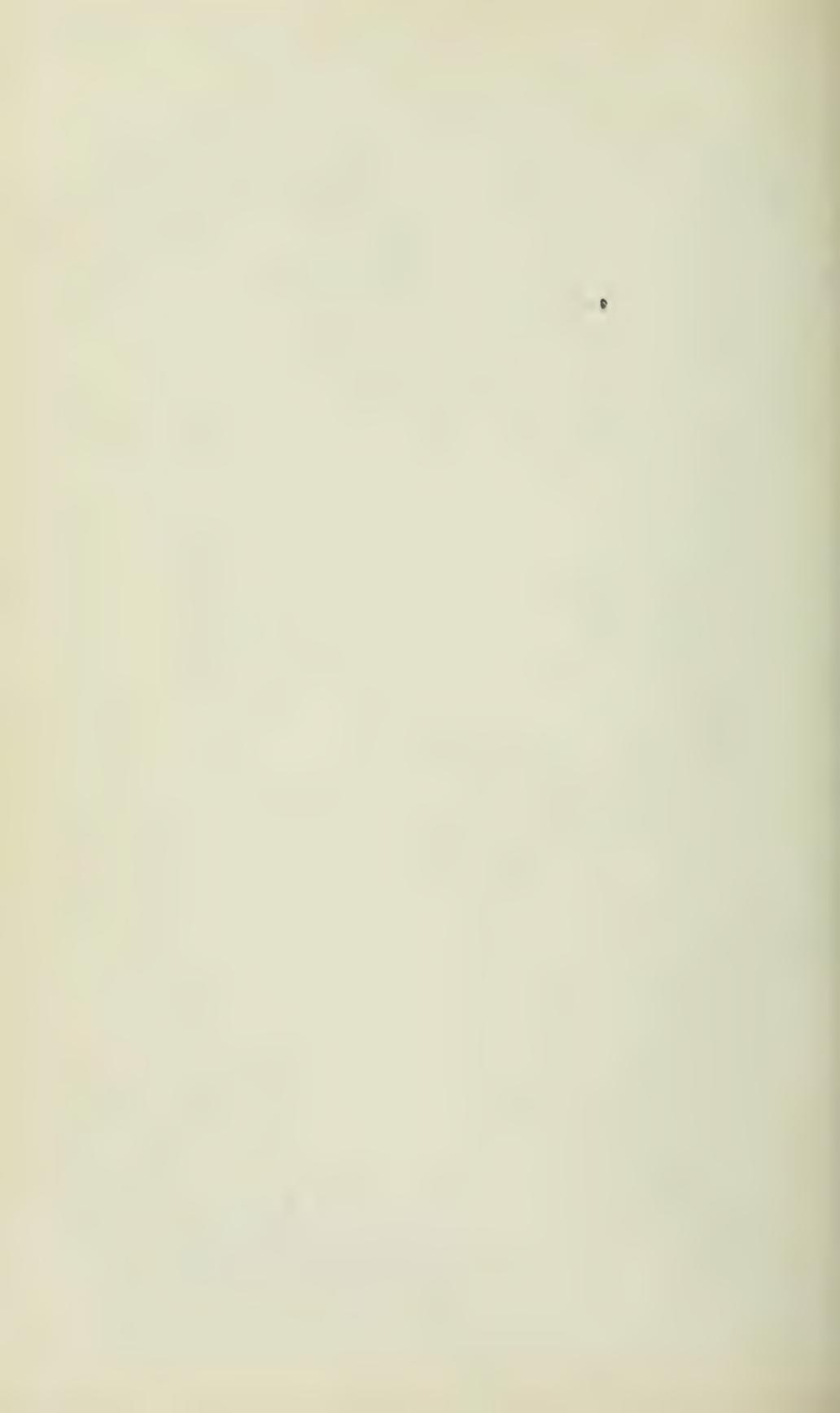


Fig. 7



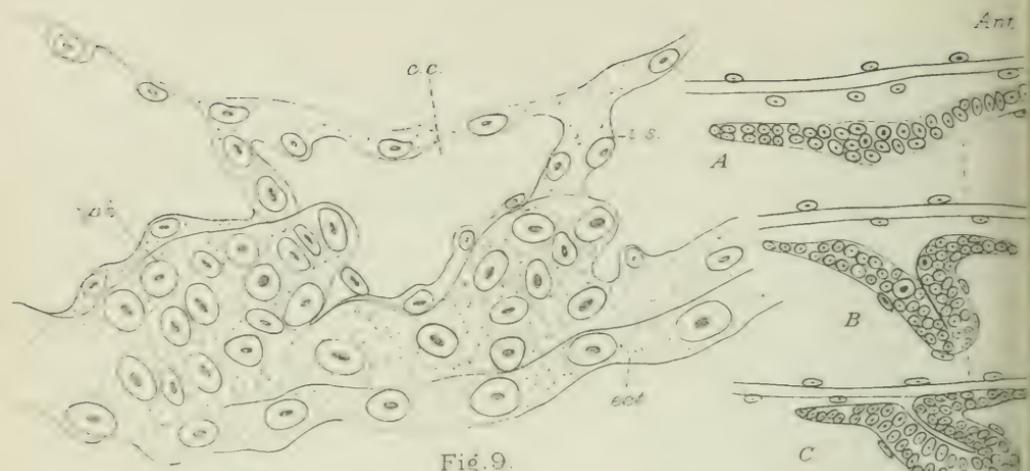


Fig. 9.

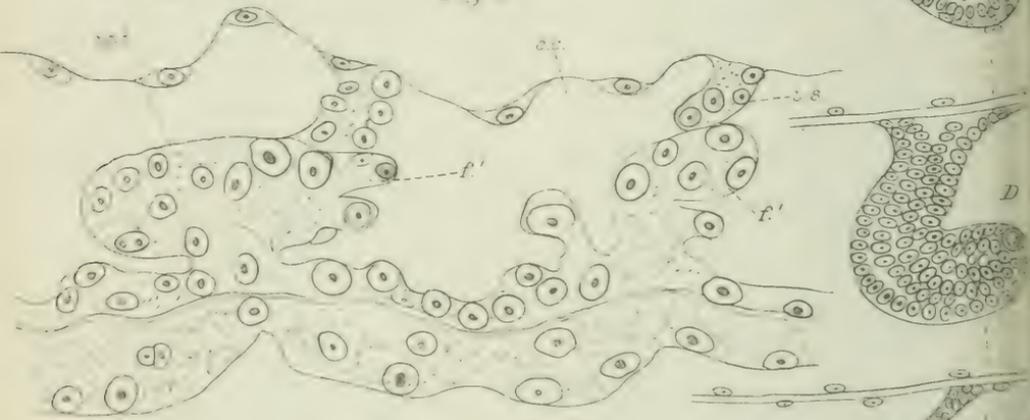


Fig. 10.

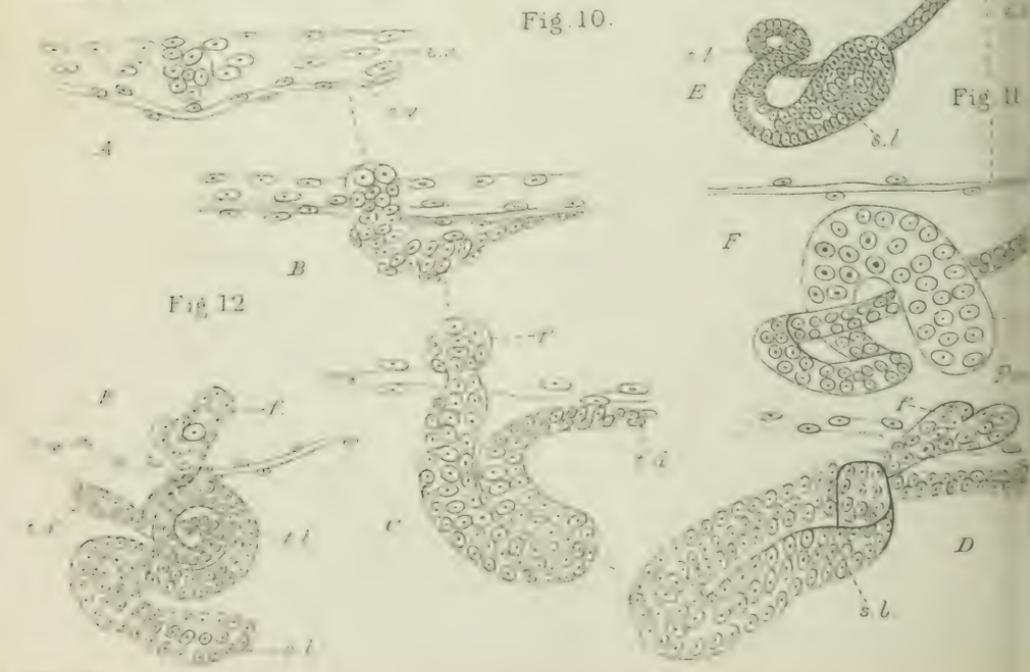


Fig. 11.

Fig. 12.



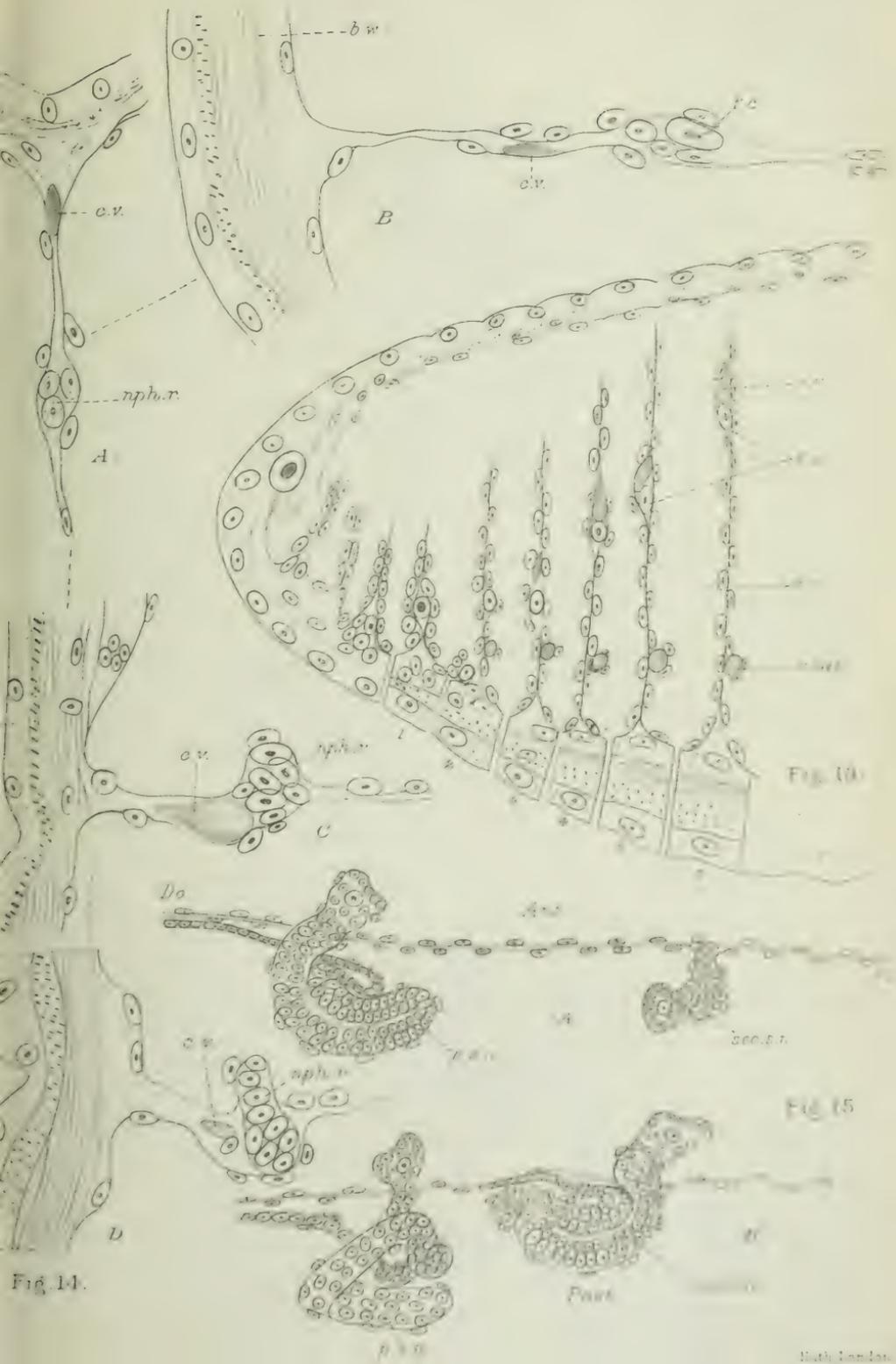




Fig. 17.



Fig. 16.



Fig. 18.

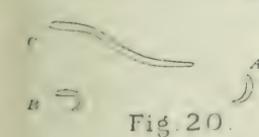


Fig. 20.

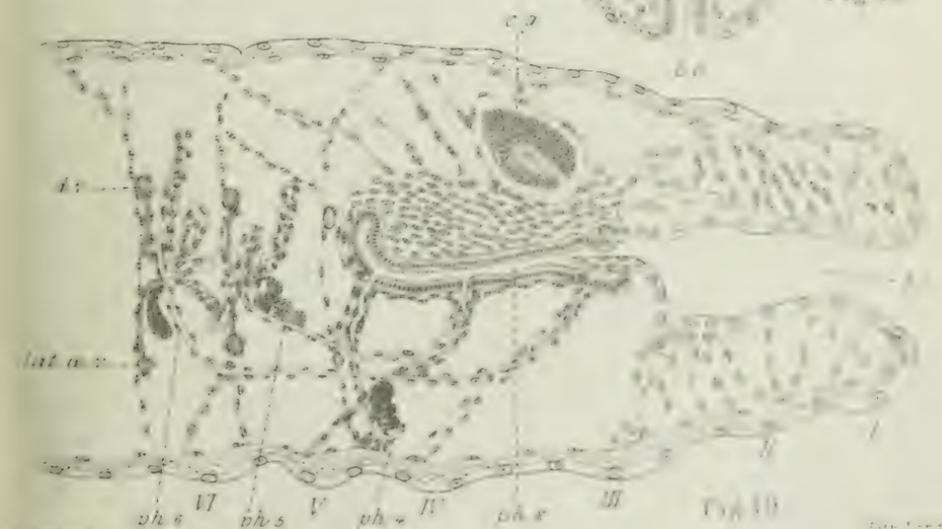


Fig. 19.

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The Occurrence of *Situs inversus* among artificially-reared *Echinoid* Larvae.

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With 3 Text-figures.

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1. INTRODUCTION.

A REMARKABLE case, where a hydrocoele and its associated structure had developed only on the right side instead of on the left side of the body, as in normal specimens, came under my notice among the artificially reared larvae of *Echinus miliaris*.

Cases of situs inversus viscerum are not very rare in nature, and are frequently met with under artificial conditions. Spemann (29, pp. 400-14), in his most interesting experimental studies on Triton larvae, has made an exhaustive survey on cases of situs inversus. According to him (p. 401) the cases may be classified into two categories, though the distinction between these two may not be clean-cut. The one comprises such cases where an 'inverting' factor affects an individual very early in its ontogeny, it may be even before fertilization, so that the 'microstructure' of the egg undergoes a change at once and completely. Those Gasteropods with reversed spiral belong to this category. Conklin (3, p. 585) suggested as its cause the reversal of the polarity in the egg.

To the second belong those cases where that factor acts much later in the embryonic development, a little while previous to the time when any visible asymmetry of organization occurs. It affects only a single but decisive part, and in consequence of the abnormal development of that part all the other adjoining organs will assume the inverse situs. There are many interesting instances of this: thus, for example, a chick embryo heated on its left side (Dareste, Warynski and Fol), a Triton embryo with a portion of the medullary plate cut out and replaced in the inverted position (Spemann), an egg or embryo which has been constricted along its median plane partially or completely so as to give rise to either a double monster or twins (Spemann; compare Bateson, 2, p. 560, and Morrill, 18, p. 267), and two halves of embryos with different rate of growth grafted together (Spemann) can likewise produce the situs inversus. Cases of such partial situs inversus have also been interpreted in a most satisfactory manner, as has also the striking fact that generally the abnormality is exhibited by the right-hand members of double monsters of Triton (and trout) and by the right-hand member of twin Triton larvae.

Turning now to the case of the reversed Echinus larvae, I have tried to propose tentatively an interpretation. This

case is, it seems to me, more or less related to, but distinct in some respects from, the above-mentioned second category (see p. 141). The idea came to my mind after the experiments had come to an end, and it needs further test with special reference to this question.

The experiments were made during the early summer of this year (1920) in the Zoological Department, Imperial College of Science and Technology, London. It is my pleasant duty to tender my hearty thanks to Professor E. W. MacBride for his kind supervision and unceasing encouragement throughout the time during which the work has been carried out. The writing of the manuscript was done in the Natural History Department of the British Museum. My cordial gratitude is also due to Sir Sidney F. Harmer, Director of the Department, for his kind permission to work there and to use the library.

2. DESCRIPTIONS OF THE LARVAE WITH INVERSE SITUS.

It must at the outset be stated with regret that the descriptions of internal structures as here given are founded on a very few specimens which I could preserve and section. As will be seen in the table (p. 115) the total number of reversed larvae I found was more than 150, but with the hope of getting as many metamorphosed young as possible I did not kill and preserve many of them. The observation on the early stage when the right hydrocoele makes its appearance, i.e. the earliest visible sign of the abnormality, is also lacking. About half a dozen metamorphosed young were obtained, but all the rest died off gradually without affording me any opportunity of following the internal changes which had taken place.

External Characters.—Eight larvae with the inverse situs were first found on May 31, when they were eleven days old. The 'larval' body was quite normal both in size and shape: two pairs of larval arms, post-oral and antero-lateral, both symmetrical and fairly long; postero-dorsal arms still very short; the posterior part of the body-rod beginning to degenerate, with its club-shaped end separated from the rest

and lying near the hind end of the body. Both the ventral and dorsal epaulettes were already separated from the ciliary bands, the anterior transverse part of the latter showing a peculiar twist which indicated the future position of the paired pre-oral arms.

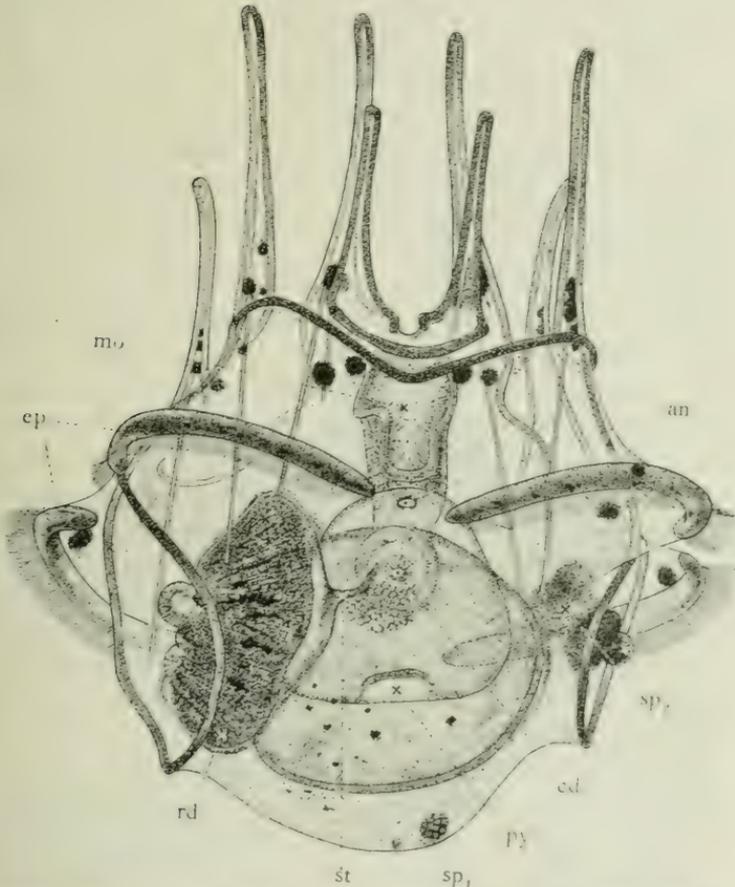
A hydrocoele, stone-canal, and amniotic invagination were all situated on the right side, whilst no such organs were found on the left side. No special attention was paid to such a slight asymmetrical distortion in shape of the stomach as was often noticed by Runnström in some abnormal larvae (24, 25).

Similar larvae were found later to be fairly numerous, and were transferred to a separate jar where they were allowed to develop further. There was found no difference in the rate of growth between normal larvae and these abnormal ones. When fully grown (Text-fig. 1) the abnormal larva possessed four pairs of well-developed arms, a large echinus-rudiment (*rd*) on the right side, from which five primary tentacles often protruded and moved actively. Whether a pair of pedicellariae really appeared on the left side as is the case with *Strongylocentrotus* (see p. 136) I cannot assert at present, though it seems to me to be highly probable. As to those paired calcareous structures which appeared on the left side, as seen in the text-figure (*sp*₂), I am almost certain that they were groups of spines.¹ The unpaired spine which should appear in normal cases at the hind end, a little to the right of the median line, was here found shifted to the left side (*sp*₁).

No less than half a dozen of such abnormal larvae passed metamorphosis when a month old. As to the external feature of these young sea-urchins one can find no difference from

¹ While dealing with the living larvae I thought without the slightest doubt that the paired calcareous structures always found on the left side were really pedicellariae. Text-fig. 1, which is the only drawing made of this stage from life and the only evidence now available, shows that they are situated inside the loop of the ciliary band. This position coincides precisely with that of the groups of spines as described by Runnström (27, pp. 21-2, figs. 21-3). In this particular specimen at least there were present no true pedicellariae (see p. 138).

TEXT-FIG. 1.



Full-grown larva of *Echinus miliaris* with inverse situs.
Ventral view. $\times 75$.

an, anus; *cd*, constriction between larval oesophagus and stomach; *ep*, ventral and dorsal epaulettes; *mo*, larval mouth; *py*, constriction between larval stomach and intestine; *rd*, echinus-rudiment formed on the right side; *sp₁*, rudiment of posterior unpaired spine situated a little on the left to the median line; *sp₂*, a pair of groups of spines formed on the left side.

normal young. All the sets of primary unpaired and first-paired tentacles, pedicellariae, pointed and square-ended spines, were formed precisely as in the young which had metamorphosed from normal larvae. It was hoped that they would develop further to the stage when the asymmetrical arrangement of the organs, above all the peculiar coil of the intestine, would be more pronounced. Unfortunately, however, they were all lost after ten days, probably being destroyed by a tiny Gasteropod which had been carelessly put into the jar together with some Corallinae.

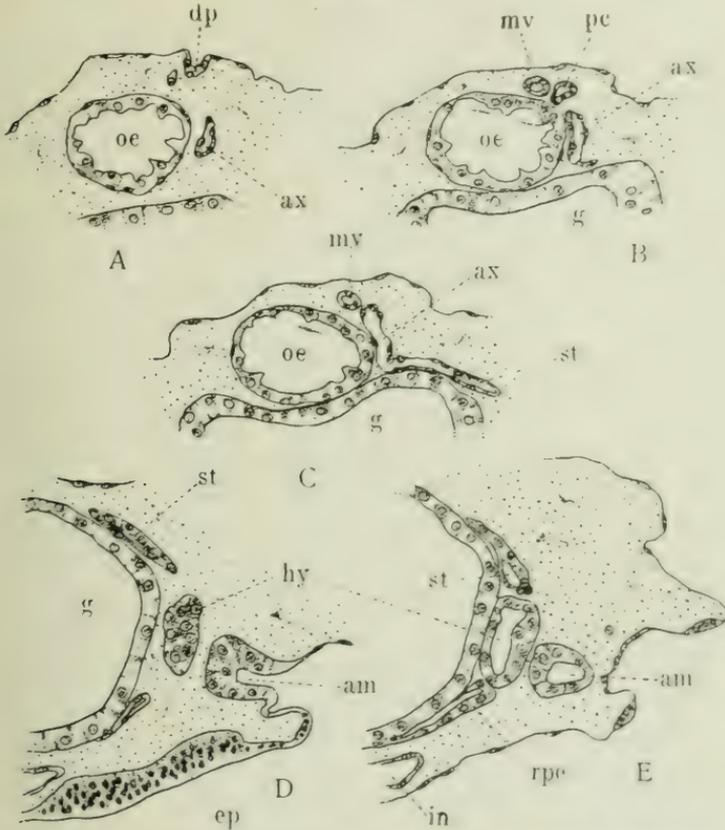
Internal Structures.—So far as the internal anatomy of the larva is concerned, the following short account is all we can learn. The transverse sections of the larva (Text-fig. 2) are exactly the mirror-images of those of normal larvae, so that one cannot distinguish them from sections of a normal larva mounted upside down. An eleven-day-old larva has the pore-canal (*pc*) still distinctly opening on the right side of the mid-dorsal line (*dp*), a madreporic vesicle (*mv*) lying close to the canal, situated at its median side but without any communication whatever with it. The canal then leads to the thin-walled axial sinus (*ax*) which lies close to the oesophagus (*oe*). The stone-canal (*st*) connects the axial sinus and the hydrocoele just as in the normal case. The hydrocoele (*hy*) situated on the right side of the stomach (*y*) has just begun to produce lobes, and an amniotic invagination (*am*) has already appeared. No traces of hydrocoele, stone- and pore-canals were found on the left side. From want of material it is not known on which side of the posterior coelom the genital stolon would be formed.

Thus, with doubtful exception of the pedicellariae and genital stolon, the internal organs as well as the external characters showed perfectly the inverse situs in every detail, so far as I could examine. With regard to the pedicellariae and genital stolon I refrain from expressing a definite opinion. We may expect to find some aberrant types as might be suggested from further descriptions of double-hydrocoele larvae.

Similar cases previously known.—So far as I am

aware, the similar cases among Echinoid larvae have only been recorded twice by Runnström. He described two such larvae of *Strongylocentrotus lividus* reared at Monaco.

TEXT-FIG. 2.



Transverse sections of an eleven-day-old reversed larva of *Echinus miliaris*. $\times 300$.

am, amniotic invagination; *ax*, axial sinus; *dp*, dorsal pore; *ep*, ciliary epaulette; *g*, stomach; *hy*, hydrocoele; *in*, intestine; *mv*, madreporic vesicle; *oe*, oesophagus; *pc*, pore-canal; *rpc*, right posterior coelom; *st*, stone-canal.

[Case A.] Runnström, 1912 (23), pp. 2-3, 'no. 1'; 1918 (26), pp. 419-20.

Left: no hydrocoele developed, but instead of it a ventral primary pedicellaria was formed.

Right: echinus-rudiment well developed. Dorsal pore remaining at its original position on the right of the mid-dorsal line, no shifting towards the latter taking place.

[Case B.] Runnström, 1912 (23), pp. 7-10, 'no. 5'; 1918 (26), pp. 420-4, Taf. xiv, figs. 12-16.

Left: hydrocoele not formed, anterior coelom remaining rudimentary. Amniotic invagination formed as only a shallow depression, and afterwards disappearing. Pedicellariae present, the dorsal one being already formed while the ventral one was indicated by accumulated cells.

Right: anterior coelom consisted of two portions, one being the axial sinus communicating with stone-canal and the other representing the madreporic vesicle ('pulsating organ'), which displayed infrequent and irregular pulsation. The dorsal pore was not at first formed, though an ectodermal groove indicated it. After some days a pore opened anew, and the madreporic vesicle began to pulsate more frequently and regularly than before. The stone-canal became split into two parts, one short and still communicating with the hydrocoele whilst the other was longer and opened freely into the coelom.¹ These two then degenerated and a new stone-canal appeared, so that the hydrocoele regained its communication with the exterior. The hydrocoele produced two diverticula, one of the ordinary size and the other much larger. The amniotic invagination was not formed, but instead of it there were two ectodermal pits. These the author at first (23) interpreted as rudiments of the primary pedicellariae, but afterwards (26) corrected his former view and called them 'spine invaginations' (see p. 138).

In other classes of Echinoderms, Auriculariae with the hydrocoele on the right side only were noticed by Müller many years ago (19, pp. 101, 109, Taf. v, fig. 1). From communications with Dr. Th. Mortensen I have learnt that he found among the larvae of *Ophionotus hexactis* two specimens which had a right hydrocoele only. I here

¹ 'Cavité générale' and 'Körperhöhle' in the original descriptions. Under these terms the posterior coelom is probably meant.

express my thanks to Dr. Mortensen for the kind permission to note this discovery, which he has not yet published.

3. RESULTS OF THE EXPERIMENTS.

The purpose of our experiments made under the direction of Professor MacBride was to carry out a further test of the influence of high salinity on the production of double hydrocoeles (15, pp. 334-7, 341). Fresh specimens of *Echinus miliaris* were sent from Plymouth, ripe males and females were then selected from among them, and the eggs were fertilized. For detailed descriptions of the method we adopted I refer to MacBride's paper (15, pp. 326-9). Only a few details need be added here (see table on p. 115). 'Outside water' of Plymouth (1, p. 372) was always used in starting the culture, viz. the eggs were fertilized in it and then kept for a day in finger-bowls filled with clean 'outside water' ('finger-bowl period'). One-day-old larvae with pyramidal body and a pair of rudimentary post-oral arms were then transferred to Breffit jars, which had been filled with 'outside water' supplied with some *Nitzschia* ('Breffit-jar period'). Then some of them were treated for several days with 'hyper-tonic' sea-water, which had been synthetically prepared according to Allen and Nelson (1, pp. 369-71), and the salinity increased roughly to 3.7 per cent. while others were left untreated as controls. When about a fortnight old, the larvae were put into plunger jars, which had been filled with synthetic sea-water of normal salinity mixed with a small quantity of 'outside water' ('plunger-jar period'). The results of five more or less successful cultures are here shown in the table. They were offsprings of three different parents: culture nos. 1 and 4 belonging to the first, nos. 6 and 9 to the second, and no. 11 to the third. The larvae with the inverse situs were first discovered among no. 4, on May 31. Those fifty-four abnormal larvae of this culture were then kept separate in a Breffit jar. On June 19, thirty days after fertilization, some few among the normal ones of this culture were found just metamorphosed into tiny young sea-urchins, while

one of those fifty-four abnormal larvae also metamorphosed on the same day. Within ten days afterwards 127 normal larvae and six abnormal ones had metamorphosed to young sea-urchins from this culture. MacBride (11, p. 294) got the larvae of *Echinus esculentus* to metamorphose in forty-two to fifty days after fertilization, while Allen and Nelson (1, pp. 420-1) found the earliest metamorphosed young of *E. acutus* forty-two days after fertilization, of *E. esculentus*, forty-eight to sixty-eight days, and of *E. miliaris*, thirty-eight days. As compared with these records of regular sea-urchins our case was much quicker in development. On the other hand, the culture no. 11 and others from the same parents suffered from want of food seriously after the first week of their development, and when examined on September 3 they were, though seventy-six days old, all very far from metamorphosis, the 'larval' body fully developed, but the echinus-rudiment, if present, being very small. The culture no. 6, for some unknown cause, gave poor results. Most of the larvae died off very quickly, and the survivors showed various irregularities in shape.

The food supply was generally good during the first week or so, but afterwards in most cases it could not be continuous, and became unavoidably very irregular, owing to the unsuccessful culture of *Nitzschia*.

Now, from among the 'treated' larvae (nos. 4 and 9), which number 784 in all, there were found 88 inverse (11.2 per cent.) and 6 doubles (0.8 per cent.). In 'controls' (nos. 1, 6, and 11), on the other hand, from among 646 larvae, there appeared 69 inverse (10.7 per cent.) and 13 doubles (2 per cent.). This shows clearly that there is no noticeable difference in the rate of producing abnormalities between these two differently treated lots. We shall discuss this question later on (p. 143).

The results of Professor MacBride's experiments of producing the double hydrocoele (15) may here be cited briefly.

1914 (pp. 334-5). The larvae three or four days old were treated for ten or eleven days with 'hypertonic' sea-water

TABLE SHOWING THE RESULTS OF EXPERIMENTS.

Culture No.	Provisional name of culture.	Finger-bowl Period (beginning at the time of fertilization, kept in 'outside water').	Brefli-jar Period (feeding on Nitzschia begun).	Plunger-jar Period ¹ (water consisting of synthetic sea-water mixed with a small quantity of 'outside water').	Total number of larvae examined.	Number of larvae with inverse situs.	Number of larvae with double hydrocoele.	Number of larvae devoid of hydrocoele.
1	'Control'.	May 20—May 21 (1 day).	May 21—June 1 (11 days), still kept in 'outside water'.	June 1—June 7 (6 days).	450	46 (10.2%)	1 (0.2%)	Few.
4	'Treated'.	Do.	May 21—June 1 (11 days), treated with 'hypertonic' seawater for five days. May 22—May 27.	Do.	334	54 (16.2%)	0	16 (4.8%)
6	'Control'.	May 21—May 22 (1 day).	May 22—June 4 (13 days), still kept in 'outside water'.	June 4—June 7 (3 days).	30	1 (3.3%)	8 (26.7%)	Few.
9	'Treated'.	Do.	May 22—June 4 (13 days), treated with 'hypertonic' seawater for five days. May 22—May 27.	June 4—June 5 (1 day).	450	34 (7.6%)	6 (1.3%)	Few.
11	'Control'.	June 19—June 20 (1 day).	June 20—June 23 (3 days), still kept in 'outside water'.	June 23—Sept. 3 (72 days).	166	22 (13.25%)	4 (2.4%)	Fairly many.

¹ The end of this period means the time when the larvae were examined and recorded as in the adjoining columns. They were kept alive further, being either put back into the same jar as before, or transferred to Brefli jars until some of them passed metamorphosis.

Except in no. 4 the exact number of such larvae in each culture was not counted. In nos. 1, 6, and 9 the percentage did not seem much different from that given for no. 4.

which had been prepared by evaporating. A right hydrocoele appeared but the amniotic invagination failed to appear, and the larvae refused to develop further.

1915 (p. 335). From among the larvae treated as above the most promising ones were isolated and fed on abundant *Nitzschia*. One larva produced a five-lobed hydrocoele on the right side.

1916 (p. 335). In both groups, those kept throughout in 'hypertonic' sea-water and those put back in normal sea-water, after being treated for one to three days, were found some larvae with an unmistakable right hydrocoele provided with five tentacles.

1917 (pp. 335-7). 'Hypertonic' sea-water was prepared this time by adding common salt to sea-water. The fourth-day larvae were transferred to 'hypertonic' sea-water and allowed to remain in it for six days, after which period they were again put back in normal sea-water. The larvae with double hydrocoeles were about 2 per cent. in one jar, while at least 5 per cent. were in the other. Amongst hundreds of controls there was found only one specimen which had a double hydrocoele.

The result obtained in 1919 was so similar to that of the foregoing year that he thought it unnecessary to publish anything about it.

Before further discussing the causes and processes of formation of the abnormalities, let us stop for a moment to consider some questions which may naturally arise in the reader's mind. These are the questions of fundamental importance: (1) Is not the writer's discovery due to an error of observation? (2) Is not the occurrence of such abnormal larvae also common in nature for this particular species—at least in a particular season and at a particular place? (3) Is not the scantiness of records due to negligence on the part of previous observers? (4) Is not the so-called 'abnormal' condition hereditary?

(1) It is rather incredibly frequent to find that even careful observers make an error in the use of the so-called endless screw of the fine adjustment of some microscopes so as to confound the upper surface of the object with the under

surface, for instance, with the result that a minute spiral structure may be taken as turned in a wrong direction. In my case it will be quite sufficient to state that as the larvae were fairly large objects under the microscope, I used to focus by means of the coarse adjustment while examining them with respect to the symmetry relations.

(2) It is now impossible to compare our culture with the larvae belonging to the same species which might have been found in plankton near Plymouth in the early summer of the same year (1920). One may suppose that if quite a number of naturally-developed larvae were examined carefully there might also be found some such abnormal forms. I think one may safely say, however, that at least the occurrence of this abnormality in so high a percentage as more than 10 per cent. is really due to artificial conditions.

(3) In view of the fact that in our cultures such larvae with inverse situs were eight times as numerous as the doubles (157 : 19), I cannot help doubting that the previous workers, who were fortunate enough to discover a few double-hydrocoele specimens from among hundreds of larvae, would have overlooked those inverse forms which might have been more frequent. It is very desirable to know if situs inversus occurs also fairly frequently in other species of sea-urchins when artificially reared.

(4) As stated above, the five lots of cultures shown in the table were obtained from three different parents. It is highly improbable that such a remarkable case, if inheritable, was found in at least three individuals out of seventy sea-urchins (more than 4 per cent.) which had been sent from Plymouth.

From all these considerations I am driven to conclude that the occurrence of the abnormality is true, and can even be fairly frequent among artificially-reared larvae.

4. CHANGES WHICH MAY POSSIBLY HAVE TAKEN PLACE DURING EARLIER STAGES.

One of the most remarkable and well-known cases of situs inversus among animals is that of the snails with sinistral

shells. In some genera and species it is a normal character, while in others it is regarded as abnormal. As is well known, the sign of the reversal goes as far back as the segmenting egg, which shows its spiral cleavage in the direction contrary to that found in the eggs which will give rise to normal dextral snails. Conklin (3, p. 585) tried to interpret the phenomenon by assuming the reversal of the polarity in the egg, which change might have taken place in its very early stage. This hypothesis, though still lacking any satisfactory experimental evidence, is very simple and admirable; and besides this we have as yet no other explanation.

There is no reason to deny that a state similar to that occurring among sinistral Gasteropods may occur also among Echinoderms. But can we not find in our cases of *Echinus* larvae any other interpretation which is more plausible and more probable than this?

The Echinoderm egg has been known to be 'equipotent', or, in other words, the distribution of the organ-forming substances becomes established much later than in the eggs of most other groups. We owe to Runnström our knowledge of this question. In his series of experiments with *Strongylocentrotus lividus* (24, pp. 533-44, Text-figs. 7 a, 10) he showed that in this species embryos developing from half-eggs assumed normal characters later than did similar embryos of *Echinus microtuberculatus* and *Sphaerechinus granularis*. The larva developed 'probably' from the right half of the egg of *Strongylocentrotus* has its skeleton more strongly developed on the right side than on the left, and, moreover, the coelomic sac appeared only on the right side. Another of his experiments (28, pp. 471-3, Text-figs. 16 a, b) shows that when an early gastrula of *Solaster* sp. had been constricted along its median line, in the double monster so produced, no hydrocoele formed; but a dorsal pore appeared on its left side instead of on the right, forming a mirror-image of the dorsal pore of the left half. He thus confirms what Driesch observed in some few double monsters of *Echinus microtuberculatus*

in 1906 (4, p. 765). These results, considered in connexion with Spemann's Triton twins and double monsters and also with Morrill's double monsters of the trout referred to in a foregoing page (p. 106), lead us to expect that if successfully reared we might get an inverse larva from the right half of the egg in these Echinoderms also. Indeed, Spemann suggested this idea at the end of his work (29, p. 413). I may, however, only mention that our inverse larvae were all of normal size, and that there can be no doubt as to their having been developed from whole unseparated eggs. Gemmill's information of several cases of twin larvae of *Luidia sarsi* (6) is not uninteresting in this respect. Eggs of early cleavage stages were sent from Plymouth to Glasgow, and, according to him, the long-continued shaking during the transportation might have caused the blastomeres to dissociate and such twins resulted. His figures, especially of those 'side-by-side' doubles (Pl. ii, fig. 13; Pl. iii, figs. 19, 21), clearly show that there is no perceptible difference in structure between the two halves developed from partially-separated blastomeres, nor is there any sign in the right half of assuming a mirror-image of the left. We cannot, however, help doubting whether separation really took place during the long-continued shaking. Judging from the haphazard relative positions of the halves and from apparent differences in age between them in some cases, one may naturally suspect that the conditions observed resulted from fusion of two individuals. It is desirable to learn how the left side of a member will affect the right side of the other in artificially-grafted larvae. Results of both chemical (Goldfarb) and mechanical (Runnström) grafting of the eggs or embryos are unfortunately inadequate to solve the present problem.

5. VARIATIONS AMONG DOUBLE-HYDROCOELE LARVAE AND OTHER ABNORMALITIES.

Our attention will naturally turn to the double-hydrocoele larvae which appeared in cultures associated with the reversed larvae. To try to find if any relation exists between these

two kinds of abnormalities we may first examine those known cases of double-hydrocoele and other abnormal larvae, and then consider the behaviour of individual organs and the interrelations to be found between them.

I. Hydrocoeles formed on both sides.

(a) Right hydrocoele and its associated structures more or less incomplete.

[Case 1.] *Strongylocentrotus lividus*. Runnström, 1912 (23), pp. 3-5, 'no. 2'; 1918 (26), pp. 417-18, Taf. xiii, figs. 8 a, b. Reared at Monaco.

Left: anterior coelom large, divided into three regions: first, the ampulla to which the stone-canal opens; second, the main body of the axial sinus extending transversely to the right and communicating with the third region, the madreporic vesicle. The last-named vesicle exhibited no pulsating movement. Pore-canal and dorsal pore lacking. Stone-canal and hydrocoele well developed, the latter produced into five lobes. Amniotic invagination deeper than normal.

Right: anterior coelom smaller than that of the left, with pore-canal given out towards the epidermis, without, however, an opening to the exterior. Stone-canal showing a sign of degeneration, its anterior end beginning to be absorbed. Hydrocoele smaller than that of the left side. Amniotic invagination did not form on this side. Posterior coelom produced into an anterior process, which probably corresponds with genital stolon.

[Case 2.] *Strongylocentrotus lividus*. Runnström, 1912 (23), pp. 5-7, 'no. 3'; 1918 (26), pp. 413-14, Taf. xiii, fig. 4. Reared at Monaco.

Left: anterior coelom large, consisting of two regions, one on the left, connected with stone-canal, the other on the right, corresponding with madreporic vesicle. The latter became later separated from the former, and was not seen pulsating. Pore-canal absent. Stone-canal and hydrocoele well developed, the latter produced into five lobes. Amniotic invagination formed but remaining totally undifferentiated.

Right: anterior coelom smaller than that of the left side. A vesicle was seen to be produced from it, which latter the author interpreted with some doubt as the hydrocoele. All these parts were seen beginning to degenerate. Amniotic invagination formed very late, but soon disappeared. Pedicellariae not formed. The posterior coelom produced an anterior diverticulum, probably representing the genital stolon.

[Case 3.] *Strongylocentrotus lividus*. v. Ubisch, 1913 (30), pp. 440-3, Text-fig. v. Reared at Naples (Giesbrecht).

Left: axial sinus well developed with the pore-canal which opened externally by a dorsal pore. Madreporic vesicle ('dorsal sac') large, lying close to the axial sinus, but no communication between them existing at all. Echinus-rudiment fairly advanced. Genital stolon developed.

Right: axial sinus smaller than that of the left side, pore-canal only represented by a knob from wall of the former, and a fibrous tissue connecting the epidermis with this knob. Echinus-rudiment less advanced than that of the left side.

[Case 4.] *Strongylocentrotus lividus*. Runnström, 1918 (26), pp. 418-19, Taf. xiii, figs. 9, 10. Reared at Monaco.

Left: axial sinus, stone-canal, and hydrocoele all developed normally. Pore-canal and dorsal pore present, the latter later shifted its position towards the median line. Amniotic invagination formed.

Right: axial sinus well developed, with pore-canal and dorsal pore. The latter like its left fellow changed its position later towards the median line. Stone-canal formed later, its slight expanded posterior end representing the hydrocoele. No amniotic invagination formed.

[Case 5.] *Echinus miliaris*. MacBride, 1918 (15), p. 347, Pl. v, fig. 9. Reared in London.

Left: axial sinus fused with that of the right side and communicated with the exterior through a single pore-canal. Echinus-rudiment large.

Right: Echinus-rudiment smaller than the left one. No pedicellariae formed.

[Case 6.] *Echinus miliaris*. MacBride, 1918 (15), pp. 339, 343, 347, Pl. vi, fig. 11. Reared in London.

Left: axial sinus provided with a pore-canal and dorsal pore. Lobed hydrocoele and amniotic invagination developed normally.

Right: axial sinus with a pore-canal and dorsal pore. Hydrocoele smaller than that of the other side and no lobes were formed. Amniotic invagination absent. Two pedicellariae developed.

[Case 7.] *Echinus miliaris*. MacBride, 1918 (15), pp. 338, 339, 348, Pl. viii, figs. 18, 19. Reared in London.

Left: axial sinus fused with that of the right side. Madreporic vesicle situated between the compound axial sinus and the gut. Pore-canal and dorsal pore single. Echinus-rudiment well-developed. Stone-canal double, probably formed by the splitting of the string which had connected the hydrocoele bud with the anterior coelom.

Right: echinus-rudiment developed but, judging from the figures, it was smaller than the left one.

[Case 8.] *Echinus miliaris*. Culture 9, 'treated'. The larva was fifteen days old when found and killed.

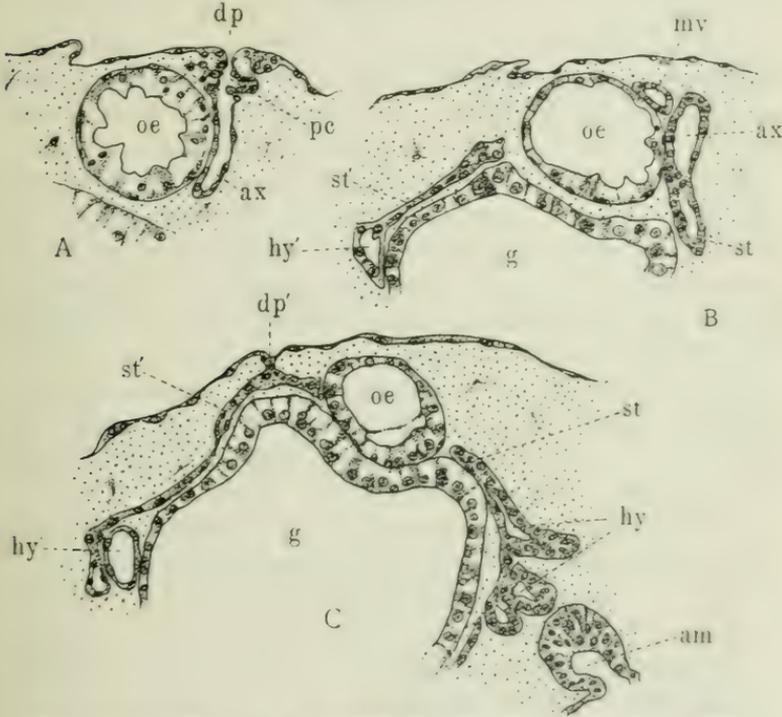
Left: axial sinus well developed with a pore-canal and dorsal pore. Madreporic vesicle rather rudimentary, but distinctly seen lying close to the pore-canal. Stone-canal normal, leading to the slightly-lobed hydrocoele. Amniotic invagination formed.

Right: axial sinus and pore-canal rudimentary leaving no visible lumen. Dorsal pore absent. No madreporic vesicle found on this side. Hydrocoele as large as that of the left side, but simply vesicular in shape. Stone-canal has in its posterior part a distinct lumen and its calibre is as thick as its left fellow; but the canal passes into a solid cell-string as it goes dorsad towards the vestigial axial sinus. No amniotic invagination formed.

(b) Left hydrocoele and its associated structure more or less incomplete. Such is found very rarely, and hitherto any

definitely-recorded case belonging to this category is lacking. By the kind permission of Professor MacBride I examined his preparations and found among twenty whole mounts only a single specimen with the right echinus-rudiment larger than that of the left side. [Case 9.]

TEXT-FIG. 3.



Transverse sections of a fifteen-day-old double-hydrocoele larva of *Echinus miliaris*, in which the water-vascular system of the left side has begun to degenerate. $\times 300$.

am, amniotic invagination; *ax*, axial sinus; *dp*, *dp'*, dorsal pores; *g*, stomach; *hy*, *hy'*, hydrocoeles; *mv*, madreporic vesicle; *oe*, oesophagus; *pc*, pore-canal; *st*, *st'*, stone-canal.

[Case 10.] *Echinus miliaris*. Culture 9, 'treated'. This larva was found to have double hydrocoele when fifteen days old, and then killed and examined by means of sections (Text-fig. 3).

Left: Axial sinus quite reduced, being represented by

a solid thickening at the dorsal end of the stone-canal, while the latter also has no visible lumen (*st'*). Pore-canal represented by a solid cell-mass (*pc'*). Hydrocoele simple and vesicular (*hy'*). No amniotic invagination.

Right: anterior coelom well developed (*ax*), with its external communication through a pore-canal (*pc*) and dorsal pore (*dp*). Madreporic vesicle with fairly distinct lumen (*mv*), with its wall in contact with the axial sinus, but no communication whatever existing between them. Stone-canal (*st*) formed normally, and hydrocoele (*hy*) well developed, being provided with five lobes. Amniotic invagination formed (*am*).

[Case 11.] *Echinus miliaris*. Culture 9, 'treated'. This was also fifteen days old when found and killed.

Left: no trace of axial sinus and pore-canal to be found. Stone-canal ending blindly at the anterior end, while posteriorly it opens to a small widened cavity of the hydrocoele. Amniotic invagination formed but small.

Right: axial sinus, pore-canal, dorsal pore- and stone-canal all well developed. Madreporic vesicle lying close to the pore-canal. Hydrocoele lobed. Amniotic invagination a little smaller than the left one.

(*c*) The hydrocoele and its associated structures of both sides equal in their state of development or nearly so.

[Case 12.] *Spatangoid pluteus* collected at Messina. Metschnikoff, 1884 (17), p. 64.

Echinus-rudiments with ambulacral feet and spines 'quite equally' developed on both sides.

[Case 13.] *Mellita pentapora*. Grave, 1911 (9), pp. 35-46, Text-figs. 1-3. Reared at Beaufort, N.C.

Axial sinuses, hydrocoeles, and amniotic invaginations of both sides very nearly equal in development and symmetrically arranged. Two pore-canals opened by a common dorsal pore situated on the mid-dorsal line.

[Case 14.] *Echinus miliaris*. MacBride, 1911 (14), pp. 237-41, Pl. xxiv, fig. 1. Reared in London.

Here also axial sinuses, hydrocoeles, and amniotic invaginations were found almost exactly in the same state on both sides.

There was only a single dorsal pore, and no trace of madreporic vesicle was found.

[Case 15.] *Echinus esculentus*. MacBride, 1911 (14), pp. 241-4, Pl. xxiv, figs. 2-4. Reared at Plymouth (de Morgan).

Echinus-rudiments fully developed when examined and drawn by the observer, the larva then being fifty-five days old. The *echinus*-rudiment of the right side very slightly smaller and less advanced than the left one. Two pedicellariae developed on the right side and a third appeared at the posterior end.

[Case 16.] *Strongylocentrotus lividus*. v. Ubisch, 1913 (30), pp. 440-3, Text-fig. r, Taf. viii, fig. 26. Reared at Naples (Giesbrecht).

Axial sinus well developed on both sides and almost the same in size, each beset with a pore-canal, which opened to the exterior separately by dorsal pores. Mid-dorsally-situated madreporic vesicle communicated through a narrow canal with the right axial sinus. *Echinus*-rudiments and stone-canals on both sides equally well developed.

[Case 17.] *Echinus miliaris*. MacBride, 1918 (15), pp. 338, 347, Pl. v, fig. 8. Reared in London.

Echinus-rudiments on both sides of almost equal size. Dorsal pores two, and no pedicellariae.

[Case 18.] *Echinus miliaris*. MacBride, 1918 (15), pp. 339, 347, Pl. vi, fig. 10. Reared in London.

Both *echinus*-rudiments nearly equal in size, the right one slightly smaller. Dorsal pore single. A single pedicellaria formed on the right side.

[Case 19.] *Echinus miliaris*. Culture 1, 'control'.

The larva when found was eighteen days old, and had a lobed hydrocoele, stone-canal, and amniotic invagination developed almost exactly in the same state on each side. Two pore-canals opened separately through a respective dorsal pore. The larva has been carefully fed on sufficient food, and the *echinus*-rudiments on both sides developed at equal rate. The two dorsal pores retained as before their side-by-side positions.

No asymmetry in shape of the stomach was to be found. The unpaired spine appeared at the hind end on the median line. The larva lived for forty-six days and was at the end of that period very near to metamorphose, but was missed suddenly, and hence no further information on the internal structures could be obtained.

[Case 20.] *Echinus miliaris*. Culture 9, 'treated'.

The larva was found and killed when it was fifteen days old. The flattened axial sinus, pore-canal, stone-canal, and lobed hydrocoele developed nearly symmetrically on each side. Only on the right side the pore-canal ended in a solid cell-mass, and no dorsal pore opened. Amniotic invaginations formed on both sides, the right one being smaller than that of the left side. No madreporic vesicle found.

(d) Two hydrocoeles formed on one side.

[Case 21.] *Echinus miliaris*. MacBride, 1918 (15), p. 339. Reared in London.

There were formed two hydrocoeles on the right side due to the splitting of the hydrocoele bud which had been formed at the hinder end of the anterior coelom. One of them normally developed and had associated with it an amniotic invagination. The other, smaller and situated posteriorly to it, also possessed well-developed lobes. There was, however, no amniotic invagination for the smaller hydrocoele.

II. Hydrocoele formed on the left side only as in normal larvae, but some abnormalities found in other associated structures.

(a) Amniotic invaginations on both sides.

[Case 22.] *Strongylocentrotus lividus*. Runnström, 1912 (23), p. 7, 'no. 4'; 1918 (26), pp. 414-15, Taf. xiii, figs. 5, 6. Reared at Monaco.

Left: anterior coelom extending along dorsal side to the right to form a canal, which had no external opening. It seems, however, that an opening existed in an earlier stage. Stone-canal ending blindly at the posterior end. Hydrocoele isolated, with five lobes, and a blind canal sent towards the

stone-canal. The author suggests that this hydrocoele may probably have differentiated from the posterior coelom of the left side. Posterior coelom was absent at first, but appeared later. Amniotic invagination formed.

Right: anterior coelom posteriorly situated, being very elongated and developed much more strongly than normal. Despite the absence of hydrocoele on this side a small and shallow amniotic invagination appeared later.

[Case 23.] *Strongylocentrotus lividus*. Runnström, 1918 (26), pp. 415-17, Taf. xiii, figs. 7 *a*, *b*. Reared at Monaco.

Left: anterior coelom undifferentiated and devoid of external communication. Pore-canal 'post-generated' and dorsal pore opened on the mid-dorsal line. Hydrocoele at first remained undifferentiated, but later, when amniotic invagination appeared, it began to develop again.

Right: hydrocoele not formed. Amniotic invagination appeared later, but soon degenerated.

(*b*) No amniotic invagination formed.

[Case 24.] *Strongylocentrotus lividus*. Runnström, 1918 (26), p. 424, Taf. xiv, figs. 17 *a*, *b*. Reared at Monaco.

The left anterior coelom represented by a widened end of the stone-canal, and a short wide pore-canal opening externally. Hydrocoele provided with four lobes, but owing to the absence of amniotic invagination its development was abnormal. One of the primary tentacles gave out a small branch which corresponds to one of the paired tentacles.

(*c*) Pore-canal and madreporic vesicle doubled.

[Case 25.] *Strongylocentrotus lividus*. Runnström, 1918 (26), p. 419, Taf. xiii, fig. 11. Reared at Monaco.

Both axial sinuses beset each with a pore-canal opening to the exterior by a dorsal pore. A pair of vesicular organs lying each near the pore-canal of each side were identified without doubt by the author as madreporic vesicles. The one on the left side acquired later a communication with the left axial sinus, while the other on the right side began to degenerate.

III. Hydrocoele absent from both sides.

[Case 26.] *Strongylocentrotus lividus*. Runnström, 1918 (26), p. 424, Taf. xiv, fig. 18. Reared at Monaco.

Amniotic invagination failed to be formed on the left side; and, instead of it and at the place where the former should in normal case be formed, a calcareous spine appeared.

[Case 27.] *Echinus miliaris*. MacBride, 1918 (15), pp. 339-40, Pl. vi, figs. 12-14; Pl. x, figs. 22-3. Reared in London.

Under this heading more than one specimen will be described together. Anterior coelom on neither side enlarged so as to form an axial sinus. On neither side was a hydrocoele discovered, nor was there any vestige of a stone-canal or a dorsal pore. Only in an exceptional case was there found a dorsal pore. In one case a madreporic vesicle was seen and figured (fig. 22). A group of pointed spines developed on each side within the loop of the ciliated band and another spine was found situated dorsal to this loop on both sides.

6. CONSIDERATIONS ON THE ORGANS AND STRUCTURES CONCERNED AND THE FACTORS CONCERNED IN THEIR DEVELOPMENT.

(a) Anterior Coelom.—This is formed separately on each side pinched off from the posterior coelom, the left one being earlier in its formation than the right fellow (see MacBride, 11, p. 298). Sometimes the two anterior coeloms unite to form a single sac on the dorsal side of the larval oesophagus (Cases 5, 7). The left one is connected with the pore- and stone-canals and remains as a distinct sac, called the axial sinus, while the right one normally remains as a simple sac and very often degenerates later.

(b) Madreporic Vesicle.—This is a minute round sac normally found a little on the left side lying close to the pore-canal, and often is stated to exhibit a rhythmic pulsation. MacBride (11, p. 299) discovered in *Echinus esculentus* that this vesicle was derived from the right anterior coelom,

at first as a solid thickened end of a string of cells given out from the posterior end of this coelom. Later (16, pp. 261-2) he confirmed this in *Echinocardium cordatum*, in which species the vesicle in question is unusually large. Runnström found a pair of madreporic vesicles in a larva of *Strongylocentrotus lividus* (Case 25), and, moreover, according to him, the one on the left side became later connected with the axial sinus of the same side. Perhaps other instances of the presence of a communication between the vesicle and one of the axial sinuses (Cases B, 1, 2, 16) may also be due to a secondary change. In the Case 2 the vesicle is seen later again separated from the coelom. Often this vesicle is absent (Cases 14, 20). v. Uebisch (30, p. 443) is of the opinion that the madreporic vesicle was not possessed by the ancestor of the sea-urchins, but that it represented the only remnant of the degenerated right anterior coelom having assumed a new but unknown function in the course of phylogenetic development. And, further, according to him, when the right anterior coelom made its unusual development the highly-differentiated and functioning madreporic vesicle could not be affected thereby and both of them existed side by side.

In the reversed and also in some double-hydrocoele larvae (Cases 10, 11) the madreporic vesicle was found on the right side, close to the right pore-canal. In the case where two such vesicles are present (Case 25) the right one may be the homologue of this. In neither case is its origin made clear. From want of sufficient material and from our ignorance of its function any definite statement will be premature.

(c) Pore-canal and Dorsal Pore.—The primary dorsal pore is formed from the left coelomic sac to communicate with the exterior before the latter becomes divided into the anterior and posterior coeloms. In the course of further development of the larva the pore shifts from its original position on the left side towards the mid-dorsal line. This shifting is preceded by the formation of a transverse groove of the ectoderm. Probably in connexion with this shifting process it is often the case that the canal gets temporarily or permanently obliterated (Cases B,

1, 2, 20, 22, 23). The cause is unknown to us; still, I think there is hardly any doubt as to its being due to artificial conditions. Too large a number of diatoms or bacteria in the vessel in which the larvae have been kept may cause this. Shortly afterwards the pore and canal can regenerate (Cases B, 23) and the revived development of the whole water-vascular system follows. In other instances no second pore was formed, and degeneration of the system soon set in (Cases 1, 8, 10, 11).

The presence of the right pore-canal side by side with the left is a constant and normal character in the larva of *Mellita pentapora* (Grave, 9, p. 42; and also his former paper, 1902, p. 58). The same is not common in *Echinus miliaris*; still, it has been recorded by MacBride in a larva which was otherwise quite normal (15, p. 339). Although the presence of two pore-canals is a very common occurrence among double-hydrocoele larvae (Cases 4, 6, 16, 17, 19, 20) it seems by no means to be a necessarily associated feature. In starfish the occurrence of the double dorsal pore has never been seen even among double-hydrocoele larvae (Gemmill, 5, p. 230; 7, p. 31; 8, p. 62). To such an important difference found between these two classes let us return later (p. 142). According to Runnström the formation of the dorsal pore and pore-canal seems to be a self-differentiation (25, p. 301).

(d) Stone-canal.—This is the part which at first connected the hydrocoele bud with the main body of the anterior coelom. This canal is sometimes found doubled, being caused from either its defective origin (Case 7) or abnormal regeneration (Case B). When degeneration takes place, probably due to the lack of communication with the exterior, it begins from that end which is adjacent to the axial sinus (Cases 1, 8, 11).

(e) Hydrocoele.—It is a well-known fact that the right coelomic sac has in normal larvae the potentiality of producing a sac which is homologous with the left hydrocoele. Such a special organ-forming substance seems to be located especially at the place where the coelomic sac has to divide later into the anterior and posterior coeloms. We see from MacBride's

work on *Ophiothrix fragilis* (13, pp. 578, 586) that this sac, homologous with the left hydrocoele, exhibits varying degrees of development among normal larvae, and in a few extreme cases it gives rise to a five-lobed hydrocoele (Pl. xxxvi, fig. 54; compare further those double-hydrocoele *Ophioplutei* described by Müller and Metschnikoff).

Whether this unusual development of the right hydrocoele is to be regarded as a case of atavism or as another kind of variation is a matter of choice. MacBride (14, pp. 240, 244) is of opinion that the free-swimming ancestor of the Echinoderm had a pair of hydrocoeles, equally developed on each side, the right one has, however, become atrophied as soon as the free-swimming habit was given up. The appearance in some abnormal larvae of a right hydrocoele is an atavistic feature. But, according to him, the appearance and further completion of the associated structures, such as amniotic invagination, set of spines and dental sacs, derived from the ectoderm and mesoderm respectively cannot be accounted for by atavism, because it is quite impossible to endow the ancestor with such a double set of highly-developed spines and Aristotle's lanterns. Therefore, he introduced the idea of the internal secretion, in that the abnormally-developed right hydrocoele must have given off some stimulating substances which caused both ectoderm and a part of the posterior coelom to respond, with the result that there appeared a second set of spines and dental sacs. He further discussed this theory in his second paper on the double hydrocoele (15, pp. 341-5). Some months earlier than the first of these papers Grave (9, p. 43) discussed the same idea and made the objection 'that such an explanation presupposes that the series of structures in question was present and in some way related in the normal development of the ancestral echinoderm, a supposition for which there is no basis in observed fact'.

Now, we may find no great difficulty in assuming that such stimulating power of the left hydrocoele has been acquired since the disappearance of the right hydrocoele, as v. Uebisch (30, p. 444) remarked in reply to Grave's objection. It

is necessary, however, to introduce another supposition to understand how the right hydrocoele in our abnormal case acquired that power of stimulating other tissues, which power was not possessed by the right hydrocoele of the ancestor. In short, even if we accept the view that the Echinoderm ancestor possessed a double hydrocoele, it seems to me that the atavistic interpretation has to encounter with such a difficulty as stated above.

The development of a right hydrocoele to such an unusual degree may then safely be regarded as a case of homoeotic variation. The examples of this kind of variation given by Bateson (2, pp. 721-35) should be classified at least into two different groups. One group contains the cases characterized by the appearance on one side of a wholly new structure, which is quite unknown in the animal's phylogenetic history, whereas a mirror-image of it is normally present on the other side. Gemmill's 'primary' homoeosis (8, p. 71) seems to be this. A tadpole of *Pelobates fuscus* with a second spiracle on the right side is an example, and if Runnström's view is accepted the appearance by self-differentiation of an amniotic invagination on the right side of the sea-urchin larva would be another. The second group comprises those cases where, in obviously paired organs, one member, which is normally vestigial, develops in certain circumstances to the same degree as its fellow. A double-tusked narwahl is the best illustration of this kind. Gemmill's term 'secondary' homoeosis perhaps denotes the same phenomenon. I feel very doubtful whether the case of our double hydrocoele should be placed under this latter category or under the first. The paired origin of the front teeth in the narwahl is quite obvious, while the presence of a pair of well-developed hydrocoeles in the Echinoderm ancestor will not be accepted unanimously by all zoologists.

I do not believe that the development of a double hydrocoele has 'resulted in a larval organization better adapted to the conditions under which the existence of the pluteus is led', as Grave (9, p. 45) states in his discussion on the homoeosis.

We need not explain the cause of homoeosis in this way only. The chance by which the double hydrocoele is induced to develop seems to be quite unusual, as I will try to show presently. It is not at all a result of adaptation.

In his famous experiments on *Alpheus*, Przibram showed that if a large claw of this Crustacean is amputated a small claw will appear at the spot, whilst the small claw of the other side, which was not operated upon, will become a large claw. This phenomenon he calls 'compensatory hypertypy'. For more detailed information I refer to his later paper (22). A similar but slightly different idea can be applied in the case of double hydrocoeles. The right hydrocoele might have arisen as a result of compensatory hypertypy caused by the arrested state of development in the left hydrocoele. The differences from the case with *Alpheus* are that (a) the presence of a rudimentary right hydrocoele is not a normal feature, but no doubt the right anterior coelom has a potentiality of producing it, while the small claw of *Alpheus* is present constantly and quite functional, and (b) the left hydrocoele has not yet been fully developed but arrested in its early stage of development, while the large claw of *Alpheus* was removed after it had reached the full-grown state. With these differences kept in mind we may use Przibram's term in our case as well.

According to Runnström (25, p. 305) the further differentiation of the hydrocoele, left or right as the case may be, depends largely on the formation of an amniotic invagination. There was, however, an exceptional case (Case 24). Besides, from lack of a corresponding amniotic invagination and from obliteration of the dorsal pore, the hydrocoele and its associated structures will degenerate from hunger (Runnström, 25, p. 265; MacBride, 15, pp. 339, 340).

The presence of two hydrocoeles on one side was noticed by MacBride (Case 21), and interpreted as being due to the splitting of the hydrocoele bud. Another curious abnormality was described by Runnström (Case 22). There are, according to this observer, two possibilities as to the cause of such

an isolated hydrocoele: (a) it may have been separated from the end of the stone-canal, or (b) the posterior coelom may have given rise to it under the influence of the amniotic invagination. From the absence of posterior coelom, though one appeared afterwards, he thinks the latter more probable. In one of Runnström's larvae of inverse situs (Case B) we see another extraordinary feature in the right hydrocoele (23, p. 9: 26, p. 423). The hydrocoele was three-lobed, and close to it there were two curious structures. One was a round closed vesicle, the origin of which the author could not ascertain. The other was an ectodermal groove running nearly parallel to the stone-canal and lined with very actively-moving cilia. This groove at last became separated from the ectoderm, and together with the above-stated closed vesicle, united with the hydrocoele, remaining as a larger lobe of the latter. Runnström is of the opinion that in those pathological cases a hydrocoele or a part of it can be formed both from posterior coelom and ectoderm.

(f) Amniotic Invagination.—This is formed some days later than the appearance of the hydrocoele. It seems to me highly probable that this structure is homologous with the stomodaeal invagination of Holothurians. As early as 1906 MacBride (12, p. 615) pointed out that the larval stomodaeum of Holothurians reminds one 'of the amniotic cavity in the Echinopluteus'. This idea has since found another support in the fact that in Cucumaria the stomodaeal invagination is formed to the left of the mid-ventral line, as was first discovered by Newth (20, p. 634, Pl. i, fig. 6) and afterwards confirmed by the writer (21, pp. 379, 384, Pl. v, figs. 5 and 6). It is therefore quite improbable that the ancestral Echinoid had a pair of amniotic invaginations. MacBride (15, p. 343) never found in any single instance an amniotic invagination formed where no hydrocoele existed, and confirmed his former view (14, pp. 240-1) that the undifferentiated ectoderm can give rise to an amniotic invagination only under the influence of the hydrocoele. Runnström's view is diametrically opposed to this. He has shown us several

instances where an ectodermal invagination was formed at a place under which no hydrocoele had been developed (Cases B, 22, 23, and also 25, p. 271). He further made experiments to prove his view that the formation of the amniotic invagination is a self-differentiation and is not formed from stimulus of an underlying hydrocoele. He could produce a new amniotic invagination in a larva of *Echinus miliaris* from which the echinus-rudiment had been removed (27, pp. 9-11). In another of his experiments an amniotic invagination was seen to appear in each of two pieces of a larva where the normally-formed invagination had not been included; thus in this larva three amniotic invaginations in all were formed (pp. 13-14). It may be mentioned that in all of his cases the ectodermal invagination was very small and lined with flat epithelial cells. In another place he states (25, p. 302) that the invaginated ectoderm forms cylindrical cells only at the place where the hydrocoele wall comes to be in contact, while in the other part the cells remain flat. I myself understand by the term amniotic or 'echinid' invagination an ectodermal pit whose epithelial cells are from its first appearance high and cylindrical, even when fairly apart from the hydrocoele (Text-figs. 2, D, and 3, c, *am*). In this sense I cannot help doubting whether all of Runnström's structures deserve the name amniotic invaginations. He admits that the further development and differentiation of the amniotic invagination is conditioned by the presence of a hydrocoele, and that without it the former degenerates (25, p. 305). It is of interest to see that he pointed out that the rôle of an amniotic invagination could be played, to a less extent, by other ectodermal invaginations, such as that which he termed 'spine invagination' (Case B). According to him, if there was no amniotic invagination formed the stone-canal stopped developing when it had reached its normal length, and later gradual degeneration set in of the whole water-vascular system. But, as for the larva in question, the 'spine invaginations' were situated further back than normally an amniotic invagination is placed, and the stone-canal did not stop at the normal length.

but continued to lengthen until the hydrocoele reached those invaginations (26, p. 421). As to the nature of these invaginations let us examine again (p. 138).

(g) Posterior Coelom and Genital Stolon.—The anteriorly-prolonged end of the left posterior coelom shares the formation of the echinus-rudiment (MacBride, 11, pp. 304–5). This change takes place also on the right side in abnormal larvae where a right hydrocoele developed. In the normal case the genital stolon makes its appearance shortly before metamorphosis from the wall of the left posterior coelom (MacBride, 11, p. 309). How its right fellow behaves in abnormal larvae is still an open question. Runnström inclines to think that in two of his double-hydrocoele larvae (Cases 1 and 2) a rudiment of genital stolon was formed from the right posterior coelom. v. Uebisch (30, p. 445) concludes that the doubleness is not extended to all organs as shown from the fact that in his older double-hydrocoele larva (Case 3) the genital stolon was seen formed only on the left side. This conclusion cannot pass unchallenged because in this larva the right echinus-rudiment was much less advanced than the left, and also because the structure in question is not distinct until the larva reaches the height of its growth.

(h) Pedicellariae.—In normal Echinus larvae there appear a pair of pedicellariae on the right side, one being dorsal to the loop of the ciliary band, the other ventral to the same. In some imperfectly symmetrical double-hydrocoele larvae one or both of them appear on the right side only (Cases 6, 15, 18) or on both sides of the larva (MacBride, 15, p. 343). According to Runnström the reversed larvae of *Strongylocentrotus* had pedicellariae appearing on the left side (Cases A and B), and I am inclined to believe that it is also the case with our *Echinus*, though unfortunately any positive evidence is lacking at present. In the complete absence of hydrocoele from both sides no true pedicellariae appear (Case 27). Thus the relation between the pedicellariae and echinus-rudiment (or hydrocoele) is somewhat complicated. Probably the echinus-rudiment calls forth the forma-

tion of pedicellariae on the opposite side. It seems to me that they are not inhibitory to each other on the same side, because they can co-exist side by side. The fact, however, that in most of the double-hydrocoele larvae the pedicellariae are not formed may simply be due to lack of sufficient material, or that the echinus-rudiment, being more vigorous in development than the pedicellariae, wins the competition. MacBride assumes that 'the influences emanating from a hydrocoele not only tend to inhibit the formation of pedicellariae on the same side but to determine their formation on the opposite side of the larva' (15, p. 343), and that the hydrocoele can act as such even in its early stage. Thus, the fact that an echinus-rudiment and a pedicellaria or two can co-exist on the same side is explained by him in the following manner: 'If we assume that in these larvae the growth of both hydrocoeles has been arrested at an early stage, but after the stage at which the stimulus to form pedicellariae on the opposite side had already gone forth from them, and that then, after the formation of these organs on both sides had been determined, further nourishment became available and the left hydrocoele developed further, the structure of such larvae can be explained' (pp. 343-4). Runnström's case that some starved larvae, which had no hydrocoele, developed a pair of pedicellariae (25, pp. 269-70, Text-figs. 33-5) is now very difficult to understand. It is doubtful whether the hydrocoele was really absent in those larvae.

(i) Spine.—The larva of *Echinus miliaris* produces, when fairly grown, a rudiment of a spine at the hind end a little towards the right from the median line. This gives rise, as do some others which develop later, to a square-ended spine on the future abactinal side. This rudiment is found situated a little on the left side in reversed larvae (Text-fig. 1, *sp*₁), and in most of the double-hydrocoele larvae, in an almost median position. Such a different position of this spine is undoubtedly correlated with the different behaviour of the echinus-rudiment. Characteristic are the spines which develop in the larvae devoid of hydrocoele (Case 27). As already

stated there is a group of pointed spines and a solitary one on each side of the larva. Runnström found such a spine only on the left side (Case 26). I am much inclined to think that from want of regulating influence of the hydrocoele the rudiments of pedicellariae were developed in an aberrant way into some of those peculiar spines.

Runnström (27, pp. 21-2, figs. 21-3) discovered in the normal larva of *Echinus miliaris* a pair of small ectodermal invaginations formed inside the loop of the ciliary band on the right side. In each of these invaginations 2-3 spines belong to the Basalia 3 and 5 are later formed. He called the former 'spine invaginations' (26, p. 420). Spines undoubtedly identical with these have been seen by me on the left side of one of the reversed larvae (Text-fig. 1, *sp*₂). In *Strongylocentrotus lividus* these structures do not appear normally, still Runnström identified the pair of pits found in an abnormal larva with them (Case B). These may be an abnormal amniotic invagination divided into two. His descriptions and figures (23, p. 8; 26, pp. 420-1, Taf. xiv, fig. 13) are not quite satisfactory enough to substantiate his refusal to look on them as modified amniotic invaginations.

(j) Gut.—We know really nothing about the change the gut undergoes in accordance with the formation of the double echinus-rudiment or situs inversus. Normally the definitive stomodaeum appears at the centre of the floor of the epineural space (MacBride, 11, p. 307), and the rudiment of the oesophagus, as an outgrowth from the left wall of the stomach meeting the stomodaeum, appears later (p. 310). The adult mouth breaks through some days after metamorphosis, and the anus is formed still later (pp. 311-12). Runnström (24, pp. 544-52) found in the larvae developed from the eggs which had been treated with potassium-free sea-water some asymmetrical distortions in the larval stomach and formation of a new oesophagus on the left. He interpreted the phenomenon as the formation of the definitive oesophagus precociously indicated. It is quite conceivable that in the course of the

development of an echinus-rudiment, no matter on which side of the larva it may lie, the hydrocoele, working together with other ectodermal and mesodermal tissues, can induce this new structure to appear and thus the actinal part of the young sea-urchin be completed.

7. PROBABLE MECHANISM WHEREBY ABNORMALITIES ARE PRODUCED.

From those observed facts above considered the following conditions seem to concern the production of abnormalities of the hydrocoele and its associated structures.

1. Obliteration of the pore-canal. This seems to be a cause of the arrest of the further development of the water-vascular system and then a quick degeneration of the whole system follows.

2. Activation of the right anterior coelom of its latent potentialities of producing a hydrocoele, to compensate the degenerating left hydrocoele.

3. Regeneration of the pore-canal or fusion of the two axial sinuses. Both afford the left hydrocoele a renewed communication with the exterior, and the further development and differentiation of the water-vascular system thereby take place.

4. Development of a right amniotic invagination and the peculiar change of the anterior prolongation of the right posterior coelom. These changes seem to have been evoked by the stimulus of the unusual right hydrocoele. These three elements working together give rise to an echinus-rudiment.

From these data, if adequately combined, the following changes are quite possible.

Let us start from a young normal larva, in which hydrocoele, axial sinus, pore-canal, and dorsal pore are all formed on the left side. An amniotic invagination may already be formed on the left side. The right anterior coelom may have a pore-canal.

Now, the dorsal pore of the left side becomes obliterated, which fact is followed by the arrest of development and further

degeneration of the left water-vascular system. Two courses are here open : A. The right anterior coelom begins its unusual development to produce a right hydrocoele, which acquires communication with the exterior through a pore-canal. B. The right anterior coelom does not become active either from very weak disposition of the right anterior coelom or, more probably, from want of sufficient nutrition. The result is the total absence of hydrocoele from both sides.

The further fate of larvae in which the course of events has been that indicated by A will be one of the following three :

1. Appearance of a new dorsal pore on the left side which revives the power of the left hydrocoele to develop further. If well fed the hydrocoele on each side will continue to develop side by side so as to give rise to a double-hydrocoele larva.

2. Axial sinuses of both sides come in contact with each other and then unite, thus making the left hydrocoele regain its communication with the exterior and enabling it to develop further. The result is also a double hydrocoele.

3. No reappearance of a second dorsal pore nor fusion of the axial sinuses takes place. The left water-vascular system will then degenerate quickly, while the right one will develop like the normal left. A larva with situs inversus is the result.

In both the courses of events indicated by 1 and 2 the following three conditions may possibly arise, according to the different stages at which the right hydrocoele had arrived, when the recovery of the left hydrocoele took place :

- (a) The recovery of the left hydrocoele takes place before the right hydrocoele attains a size equal to the left. The period during which the hydrocoele is deprived of communication with the exterior is very short. Under such a condition the result is a larva whose left hydrocoele or echinus-rudiment is larger or more advanced than that of the right side. This is very frequently met with among double-hydrocoele larvae.

- (b) The left hydrocoele recovers at the time when the right one attained a size about equal to it. The larva developed under such a condition has two hydrocoeles or echinus-rudiments

equal in size. Such a case is less frequently met with than the former.

(c) The left hydrocoele recovers late when the right one is in a more advanced state than it. The period during which the hydrocoele is deprived of communication with the exterior is here very long. The result is that the larva has the left hydrocoele or echinus-rudiment smaller than the right. Usually the hydrocoele and its associated structures cannot remain unchanged for so long a time after being deprived of its external communication. This case is therefore met with very rarely.

The above may not be the only ways of reaching the respective results, but probably are the commonest. Many modifications are naturally conceivable: for instance, the right dorsal pore may be obliterated in its turn, which causes the degeneration of the whole water-vascular system of the right side and thus a normal larva will result secondarily (see Case 8).

Let us now compare this interpretation of the occurrence of the inverse situs in *Echinus* larvae with Spemann's case of *Triton* larvae (29, p. 407). Though equally caused by a 'defective' development of a single organ—alimentary canal in *Triton* and hydrocoele in *Echinus*—further results in which the other organs become affected are different in these two cases. Instead of displacement of other adjoining organs, the arrest in development of the left hydrocoele causes a new hydrocoele to appear on the other side and also a new set of associated structures as a consequence. The normal left hydrocoele can, if it regains its opportunity of further development, produce another echinus-rudiment, so as to give rise to a double-hydrocoele larva. Any parallel of such a feature is very improbable in *Triton* larvae.

There is no reason to expect that the above is equally applicable to the formation of double hydrocoele of other classes of Echinoderms. Conditions may be totally different. Let us, for instance, take the case of the double-hydrocoele larvae of starfishes. Normally in most species of starfishes the paired coelomic vesicles grow forwards, and their anterior

ends meet and unite in front of the larval mouth. The presence of two dorsal pores is very common, but the right one gradually atrophies (Gemmill, 5, p. 231), and still the right coelomic vesicle retains its communication with the exterior through the left dorsal pore. The hydrocoele becomes later differentiated from the middle portion of the spacious left coelomic sac. In the case of the double hydrocoele the right one is likewise formed from the middle portion of the right coelomic sac. Among the double-hydrocoele larvae of *Porania pulvillus* and *Asterias rubens* Gemmill found no case of the presence of double dorsal pores, in all instances the left pore only being present (5, p. 230; 7, p. 43; 8, pp. 62, 69). Thus it is evident that the obliteration of a dorsal pore has hardly any influence on the further development of the hydrocoele on the same side. Under such a different condition I suppose that the occurrence among starfish larvae of the situs inversus as we find in Echinoid larvae will be extremely unusual. Gemmill tried to explain the cause of the double hydrocoele chiefly by the supposition that, owing to the over-fed condition of the larva, its stomach becomes expanded and globular, so that the ventral horn of the left posterior coelom tends to fail to unite with the right middle coelomic region. The latter region, being thus left isolated from the posterior coeloms, produces a right hydrocoele (5, p. 244; 8, pp. 54-5). This interpretation in its turn cannot hold true in the case of those double-hydrocoele Echinoid and Ophiuroid larvae, in which no such extension of the left posterior coelom takes place normally (MacBride, 15, p. 326). The discovery by MacBride (10, pp. 368-70) of a double-hydrocoele larva in *Asterina gibbosa*, in which species the egg is heavily laden with yolk, is a serious objection to the hypothesis of excessive food. One feature is, however, certainly common in the double-hydrocoele larvae of the three different classes: namely, the temporary arrest in the development of the left hydrocoele in some way or other in an early stage. And this occurs more frequently under artificial conditions than in nature.

With regard to the occurrence of the reversed Auriculariae, as discovered by Müller (19, pp. 101, 109, Taf. v, fig. 1), the attempt to interpret the phenomenon by virtue of the compensatory hypertrophy is nearly hopeless. It is a widely-accepted fact that in Holothurians the right anterior coelom does not exist at any stage throughout life, whilst the hydrocoele is differentiated even before the coelomic sac divides into right and left halves (posterior coeloms). It is not easy to imagine that the right posterior coelom could ever produce a hydrocoele, when the normal hydrocoele happened to be arrested in its development. If this cannot be the case we must regard it as a result either of the change of polarity in the egg (according to Conklin, 3) or of twin formation (of Spemann's sense, 29).

8. EXTERNAL FACTORS AS CAUSES OF ABNORMALITIES.

From series of his experiments MacBride (15) came to the conclusion that the chief cause producing double-hydrocoele larvae of *Echinus* was the increased salinity of the water used for culture. Unfortunately, as I have pointed out in a foregoing page (p. 114), the result of our experiments of this year was quite different from our expectations. As shown in the table the number of double-hydrocoele larvae was greater in 'controls' than in 'treated', i.e. 2 per cent. and 0·8 per cent. respectively. As the double hydrocoele and situs inversus start, I believe, under the same condition, the figures of reversed larvae may also be used in this connexion. The occurrence of the reversed larvae was practically equal in both 'controls' and 'treated', i.e. 10·7 per cent. and 11·2 per cent., the difference being within the range of probable error.

Let us now turn to examine whether artificial synthetic sea-water had anything to do with the production of abnormalities. Culture 11 came into contact with the synthetic sea-water when the larvae were four days old, Culture 1 when twelve days old, and Culture 6 when fourteen days old. They were examined and counted seventy-two, six, and three days afterwards respectively. Though it is unsafe to draw any

decided conclusion from such few cases and numbers one can hardly see any effect of the synthetic sea-water on the production of doubles or reversed if allowed to act earlier in one culture than in others.

One might reasonably expect that the artificial treatment of the egg and sperm might have caused some disturbance from the normal development of the larva. This is of course quite possible, but I may only mention that it is curious to see that among such material as the sea-urchin egg so commonly used for study and demonstration in embryological work only a very few cases of the abnormalities in question have been noticed.

One of the most important factors which differ more or less from the conditions in nature is the food supply. The method of feeding marine larvae on diatom cultures, through which many different forms of pelagic larvae have been successfully reared, is relatively a recent introduction. The result is very often over-feeding. In an over-fed larva hypertrophy and other disturbances in growth is quite conceivable. From uneven distribution of food in the culture jar and from a different state in the health of larvae, over-fed and under-fed individuals may arise within one and the same jar. The obliteration of the normally-formed left dorsal pore, which seems to me a direct cause of the production of the double hydrocoele and situs inversus of the *Echinus* larvae, may be associated with the excess of diatoms and other minute organisms in the jar. Whether it is physiological or mechanical it is hard to decide at present.

Runnström (25, pp. 321-2) found that the larvae of *Strongylocentrotus* showed the degeneration of organs when over-fed on yolk. The echinus-rudiment was above all the most sensitive to the treatment and degenerated completely. Undigested yolk granules were found migrating everywhere, even scleroblasts were laden with them and the absorption of calcareous bodies followed. The effect of over-feeding on diatoms will naturally be very different from this. Though somewhat difficult to control (MacBride, 15,

p. 338) it is desirable to experiment on the effect of different amounts of diatom-food upon the development of the larval organs.

The effects of hunger were observed both by Runnström (25, pp. 254-321) and MacBride (15, pp. 339-40). The difference between the results of these two observers is remarkable. In every instance of Runnström's larvae showed extreme degeneration of skeletons, while in MacBride's case the larval arms were almost normal, owing to the well-developed state of the skeletons, but the hydrocoele degenerated and peculiar spines formed. Besides the differences in degree and duration of hunger, the stage at which the larvae were treated, &c., there must be still other complicated factors which caused such different results. For those starved larvae bacterial infection is no doubt another important cause of abnormal development (25, pp. 273-4). Grave (9, p. 36) remarked that among the larvae of *Mellita* only those well fed developed the echinus-rudiment.

As to the effect of other chemical and physical environments upon the development of the sea-urchin larvae we have those valuable results obtained by Vernon, Tennent, and others. But we know hardly anything with regard to the changes of coelomic vesicles and hydrocoele treated specially.

9. SUMMARY AND CONCLUSION.

1. Under artificial conditions more than 10 per cent. of the larvae of *Echinus miliaris* exhibited the situs inversus.

2. So far as I could examine, the internal as well as external structures of such abnormal larvae were mirror-images of those of the normal larva.

3. The young sea-urchins metamorphosed from such inverse larvae showed no abnormal features externally.

4. The manner in which such abnormal larvae departed from the normal development seems to be analogous to that in the case of 'compensatory hypertypy' in the claws of *Alpheus*.

5. In an early stage of the normally-developing larva it

happens sometimes that the left dorsal pore becomes obliterated. This seems to be associated with the shifting of the pore towards the mid-dorsal line. The hydrocoele, thus deprived of its communication with the exterior, ceases to develop and then degeneration of the whole water-vascular system sets in.

6. The right anterior coelom, on the other hand, is now evoked to realize its latent potentiality of producing a hydrocoele (homoeosis). The degenerating left hydrocoele gives place to a newly-appearing right hydrocoele.

7. The right hydrocoele stimulates its adjoining tissues to give rise together to an echinus-rudiment.

8. The external factor or factors which cause the obliteration of the dorsal pore could not be found. This probably is connected with the presence of too much diatom-food and other micro-organisms in the culture jar.

9. If a new dorsal pore is formed on the left side before the degeneration of the left hydrocoele sets in, the developing power of the latter will thereby be revived. If sufficiently fed a double-hydrocoele larva will result under such a condition.

10. If, while the left hydrocoele is arrested in its development and then degenerates, the right anterior coelom fails to develop a new hydrocoele presumably from want of sufficient food, a larva devoid of hydrocoele will result.

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11. APPENDIX.

To the examples of the reversed larvae (p. 112) a case in the starfish *Cribrella oculata* as figured and described by A. T. Masterman is to be added (see ' Trans. Roy. Soc. Edinburgh ', vol. 40, 1902). My thanks are due to Professor J. F. Gemmill, who has kindly called my attention to this paper. Compare also: Gemmill, " Notes on the Development of the Starfishes *Asterias glacialis* O. F. M.; *Cribrella oculata* (Linck) Forbes; *Solaster endeca* (Retzius) Forbes; *Stichaster roseus* (O. F. M.) Sars ", ' London Proc. Zool. Soc. ', 1916, p. 557.

With regard to the reversed larvae of *Ophionotus hexactis* a description can now be found in the following work: Th. Mortensen, ' Studies on the Development and Larval Forms of Echinoderms ', Copenhagen, 1921 (p. 182).

H. O.

NOTE BY PROFESSOR E. W. MACBRIDE ON MR. HIROSHI OHSHIMA'S PAPER ON 'THE OCCURRENCE OF SITUS INVERSUS AMONG ARTIFICIALLY-REARED ECHINOID LARVAE'.

The most interesting paper by my friend and pupil Dr. Ohshima, which appears in this number of the 'Quarterly Journal of Microscopical Science', calls for some comment from me. Dr. Ohshima refers to a paper published by me in the 'Proceedings of the Royal Society' in which I described a method for inducing the formation of a second (right) hydrocoele in Echinoid larvae by stimulating the larva at a critical period of its growth by exposure to hypertonic sea-water.

Dr. Ohshima states that an attempt which he made to repeat this experiment in my laboratory in 1920 resulted in failure. Nevertheless certain larvae with two hydrocoeles turned up, and he gives a different explanation of the cause for their appearance. I am convinced that the explanation which Dr. Ohshima gives is the right one to account for the phenomena which he observed in 1920; but I wish to emphasize the fact that his and my explanations agree in one most important particular, viz. we both feel convinced that the right anterior coelom of an Echinoid larva has the innate constitutional power of developing a right hydrocoele. This power I account for on the hypothesis that Echinoderms are derived from a free-swimming ancestor provided with sets of tentacles on the right and left sides of the body. Dr. Ohshima's explanation is that it is a case of 'homoeosis', but to use this term of Dr. Bateson seems to me to be merely restating the difficulty in other language without offering any explanation at all.

The fact that when the right hydrocoele does appear it appears in similar form to that exhibited by the left, and not in the condition in which the original right hydrocoele must have been when it was functional, is in my judgement to be accounted for by the assumption that the modifications which the left hydrocoele subsequently underwent have been pushed backwards in development according to the principle of tachygenesis till they now affect the earliest differentiated

organ-forming substance out of which the hydrocoele arises—and that this organ-forming substance gives rise to both hydrocoeles.

The results which I obtained in 1917 I was able to obtain under precisely similar conditions in 1919. Dr. Ohshima's failure to obtain them in 1920 may, I think, be attributed to several causes. I stated that for success several conditions were necessary, one of which was a vigorous culture of the diatom *Nitzschia*. For some unknown reason this was excessively difficult to obtain in 1920. Again and again our cultures died off and the larvae were checked in their development. Dr. Ohshima obtained a few 'doubles' both in the control and the 'treated' culture which were started in May, and a few more doubles in the control culture started in June. But the May cultures were not obtained from satisfactory females: they were obtained from masses of eggs in which only a small proportion developed, and they could not be described as vigorous cultures or likely to show a proper reaction to stimulation. The June cultures were vigorous, but, owing to the failure of the *Nitzschia* culture, the 'treated' culture died off completely, and the 'control' culture was for weeks in a condition of checked and stunted growth and only recovered later when the *Nitzschia* finally re-established itself. In a word Dr. Ohshima obtained his specimens with a right hydrocoele through the checking of the growth of the normal left one by starvation, whilst I obtained mine in 1917 and 1919 by stimulating the larvae in their early growth by the action of hypertonic seawater.

E. W. MACBRIDE.

The Behaviour of the Golgi bodies during nuclear division, with special reference to Amitosis in *Dytiscus marginalis*.¹

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With 4 Text-figures.

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1. INTRODUCTION.

DICTYOKINESIS, or division of the Golgi bodies during cell division, has recently been worked out by Professor J. Brontë Gatenby and the present writer in the male germ-cells of several animals (5). It was found that the distribution of the Golgi elements, or dictyosomes, to both halves of a dividing cell was a very haphazard process and was unaccompanied by any splitting of the dictyosomes such as occurs in the case of the chromosomes. In the cricket *Stenobothrus*, the dictyosomes

¹ Part of the materials used in this research was purchased by a Government Grant of the Royal Society, for which I express my thanks.

become scattered in the cytoplasm of the spermatogonium before cell division takes place, and they remain in this condition during meiosis, so that approximately a half become contained within each of the newly-formed cells. A different process, however, occurs in the Mammals, *Mus* and *Cavia*, and in the Molluses, *Helix* and *Limnaea*. The Golgi bodies in the spermatogonia of these types consist of a number of dictyosomes arranged around the archoplasm, inside which is the centrosome. As this latter organ divides, preparatory to the formation of the spindle, its two constituent parts separate and carry with them to both ends of the cell, approximately half of the archoplasm, still with the dictyosomes attached. During late prophase, the dictyosomes become temporarily detached from the archoplasm and scattered throughout the cell, and then at the late telophase they collect together again around the archoplasm.

The examples of dictyokinesis described in our previous paper were those which occurred concurrently with meiotic nuclear division. Professor Gatenby suggested to me the desirability of investigating the behaviour of the Golgi body during amitotic nuclear division, and in the present paper is described the behaviour of the apparatus during amitosis in the follicle cells of the ovary of the beetle, *Dytiscus marginalis*.

2. PREVIOUS WORK.

Deinceka (1) has described dictyokinesis in the dividing epithelial cells of Descemet's membrane and connective-tissue cells of the cornea, during both mitotic and amitotic nuclear division. He found that the Golgi body surrounded the archoplasm, and during mitosis divided into two parts so that each daughter-cell received a 'Netzapparat', as he calls it, but that in amitosis there is no division of the centrosome and no change in the Golgi body. These observations are quoted by Macklin (8) in support of his own conclusions derived from a study of nuclear division in cells of tissue cultures of the heart of the embryo chick, that amitosis involved

merely division of the nucleus, and not of the cytoplasm. He observed that binucleate and polynucleate cells were formed as the result of amitotic nuclear division. During such division the centrosome and archoplasm remained unchanged. The archoplasm could be seen in the living cell, but not the centrosome; but the latter was to be seen in fixed preparations stained with iron haematoxylin as two small black bodies embedded in the archoplasm. Mitochondria were visible in the living cells, but not the Golgi body.

Macklin's observations are of special interest in that they substantiate materially the evidence that has been accumulated against the view upheld by writers such as Meves (9), that mitosis can occur in an amitotically-formed nucleus. Binucleate cells were observed by Macklin to undergo mitosis, but in this process the nuclei which had been formed by amitotic division came together and their chromatin masses fused to form the chromosomes which underwent the usual stages of mitotic division. It is therefore concluded that amitosis does not imply division of the cytoplasm but only fission of the nucleus.

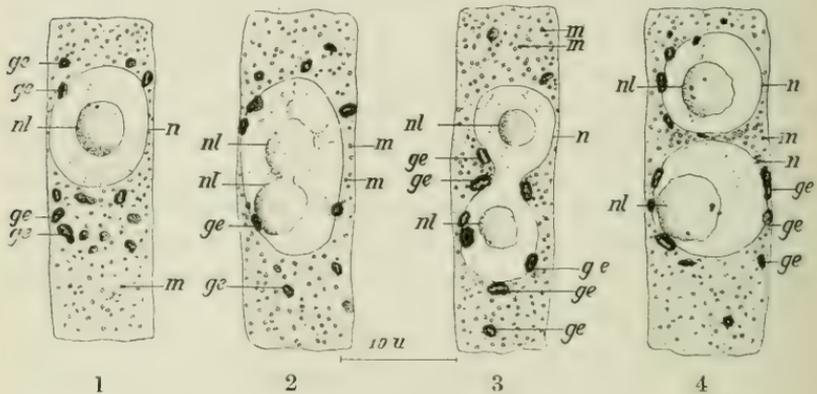
3. THE GOLGI BODY DURING AMITOSIS IN THE FOLLICLE CELLS OF THE OVARY OF DYTISCUS.

In common with most insects *Dytiscus* has an ovary laterally disposed on either side of the abdomen. Each ovary is composed of a number of tubules containing a single row of oöcytes in all stages of development, the most mature being at the distal end. The oöcytes are surrounded by the follicle cells, and between each oöcyte is a group of nurse or nutritive cells whose function is to provide nourishment for the developing oöcytes. At the proximal end of the ovuliferous tubules is a mass of undifferentiated cells from which arise three types of cells, viz. oöcytes, nutritive cells representing modified oöcytes, and the follicle cells in which the behaviour of the Golgi body during amitosis was studied.

It was found rather difficult to impregnate the body in the follicle cells of insects. After a number of unsuccessful attempts

the best results were obtained by adopting Cajal's method (4) with slightly longer fixation than he describes. Ovaries of *Dytiscus* were fixed for about eight hours in Cajal's standard fixative, and after rapid washing in distilled water were left in silver nitrate solution for three days. Prepared slides were stained by safranin or carmine. The Golgi body then appears as a number of black granules in a pink-coloured cytoplasm, the mitochondria when visible are usually brown in colour, and the nucleoli of the cells are red.

TEXT-FIGS. 1-4.



Follicle cells of the ovary of *Dytiscus marginalis*. The upper cell-wall is in each case in contact with the oöcyte wall. *ge*, elements of the Golgi body (Dictyosomes); *m*, mitochondria; *n*, nuclear membrane; *nl*, nucleolus (plasmosome).

In the text-figures are shown the various stages of amitosis. At fig. 1 is seen the so-called 'resting stage' of the cell. It will be observed that there is a single nucleolus within the nucleus, and scattered through the cytoplasm are the darkly-impregnated elements of the Golgi body, while the mitochondria are more or less evenly distributed in the cell. In the stage shown in fig. 2 the nucleus has elongated and the nucleolus is dividing into two. The Golgi elements still remain scattered in the cytoplasm, but it will be noticed they show a tendency to lie near the nuclear membrane—a tendency which is apparent in the other figures. At a later stage, as shown in fig. 3, the

two parts of the nucleolus have separated and the nucleus is greatly constricted, but the dictyosomes are still irregularly scattered: while in the cell shown in fig. 4, when the two parts of the nucleus are completely separated, the dictyosomes are still irregularly disposed around them.

It will be noticed that the nucleolus appears to play quite an important part in this process: its division seeming to initiate the division of the nucleus. This process has been verified by observations on material prepared by fixation in corrosive acetic and Bouin, and stained with Mann's methyl blue eosin (7). In such preparations the nucleolus stains oxyphil, and is apparently of the nature of a plasmosome. Its appearance is the same in both kinds of preparations. This type of amitosis, originally described by Remak, in which the nucleolus appears to play an important part, has been found by recent workers to be exceptional rather than typical, and Macklin, observing amitosis in living cells, says 'the division of the nucleolus has no direct relationship to nuclear division. It may, however, have to do with the size of the nuclear portions' (8).

The extent to which the dictyosomes are distributed in the resting follicle cell is subject to variation. In some cases, evidently owing to the large size of the nucleus in comparison with the width of the cell, the elements of the apparatus are crowded together towards its outer wall and appear in rare cases to be attached to an archoplasm; but this does not occur when the cell is dividing amitotically, and in no case has the separation of two distinct groups of dictyosomes, as occurs in mitosis, been observed.

4. DISCUSSION.

Gatenby has suggested (3) that the scattering of the Golgi elements during oögenesis is a means whereby it is able to exert a maximum formative influence upon the cytoplasm, as well as prepare for even distribution in the cells of the segmenting ovum. In a previous paper (6) I have described how the dispersing dictyosomes influence the formation of

yolk in the oöcyte of the Mollusc *Patella*. It would seem possible, therefore, that the spreading out of the apparatus in the follicle cells of an insect might be related to the high degree of metabolism existing in such cells.

Chun, quoted by Nakahara, regarded the division of the nucleus in amitosis as a means of increasing the nuclear surface as an aid to metabolic interchange between nucleus and cytoplasm; while Flemming pointed out that amitosis was especially associated with intense secretive and assimilatory activity, but he considers such cells as being on the way to degeneration (2). Recent work has shown that fragmentation of the nucleus does occur in pathological growths, in cells subject to faulty nutritive conditions, and in tissue cultures which have been left unattended for some time. Such fragmentation is regarded by Macklin (8) as an altogether different phenomenon from amitosis, but in the past there is no doubt that there has been confusion between the two.

Nakahara, who has made an investigation into the subject of amitosis in adipose cells of insects and an extensive survey of the literature of the subject, concluded that 'amitosis, occurring in secreting or reserve forming cells and in other cells of similar activity, may be for the purpose of securing an increase of the nuclear surface to meet the physiological necessity due to the active metabolic interchanges between the nucleus and cytoplasm. Apparently it is not a method of cell multiplication nor a sign of degeneration or senescence of cells, but, whenever it occurs, it seems to indicate an intense activity in the vegetative functions of the cell' (11). It is altogether in accordance with our present knowledge of the Golgi apparatus to assume that in such cells, as for example the follicle cells of insects' ovaries, the dictyosomes scattered in the cytoplasm would play a by no means unimportant part in the lipid metabolism.

In conclusion, I have to acknowledge my indebtedness to Professor J. Brontë Gatenby, of Trinity College, Dublin, for his kindness in reading through the manuscript of this paper.

5. CONCLUSION.

We may now recognize the following modes of behaviour of the Golgi bodies during nuclear division :

- (1) During karyokinesis the Golgi bodies may either,
 - (a) remain scattered in the cytoplasm and be approximately shared out amongst the two newly-forming cells, e.g. male germ-cells of *Stenobothrus* (5) ;
 - (b) divide into two masses surrounding the separating centrosomes and thus pass into each cell, e.g. (i) during meiosis in the male germ-cells of the Molluscs, *Helix* and *Limnaea*, and the Mammals, *Mus* and *Cavia* (5) ; (ii) during mitosis in the epithelial cells of Descemet's membrane and connective-tissue cells of the cornea (1).
- (2) During amitosis either they
 - (a) remain as a number of elements or dictyosomes arranged around the archoplasm, e.g. in mammalian epithelium (1), or they
 - (b) become irregularly scattered throughout the cytoplasm, as described in this paper in the follicle cells of insects' ovaries.

It is suggested that these differences are related to different conditions of metabolism existing in cells exhibiting these phenomena.

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On the anatomy and affinities of *Paludestrina ventrosa*, Montague.

By

Guy C. Robson, B.A.

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With 12 Text-figures.

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1. INTRODUCTION.

THE Prosobranch mollusc which is described in this paper is a small insignificant animal found upon plants or bottom debris in the brackish water of creeks and tidal ditches in various parts of England, Wales, and Ireland. It is also found in the upper waters of estuaries. Jeffreys (9) records it from 'the sea coasts of Sweden, France, and Portugal, as well as of Algeria', though such cases are open to a great deal of doubt.

It has been selected for study for several reasons. In the first instance there is very urgent need for more information about the Taenioglossate Prosobranchs to which group the Paludestrinidae are referred. In the second place, though some substantial knowledge is available upon the classification and structure of the Paludestrinidae, the euryhaline habits of

some species of the genus and the general tendency in the group to show a transition from a marine to a fresh-water habit render them a peculiarly interesting group and worth an intensive study. In the last place the recent discovery of Parthenogenesis in *Paludestrina jenkinsi* (Boycott, 4) makes a closer study of the kindred species necessary.

Our knowledge of the European Paludestrinidae includes good accounts of the anatomy and histology of *Bythinella dunkeri* (Bregenzer, 6) and *Vitrella quenstedtii* (Seibold, 20), and more incomplete descriptions of part of the anatomy of *P. ulvae* (Henking, 10) and *P. jenkinsi* (Robson, 16). In spite of this amount of work a good deal remains to be cleared up as to the structure of these animals.

The material employed was obtained from tidal ditches at Leigh-on-Sea, Essex. It was fixed in Bouin's solution after the shell had been carefully cracked away so as to expose the columella. Reconstruction models of various organs were made. A rapid method, which may be capable of improvement, was devised, in which the usual plates were made up of modelling-clay mixed with varying proportions of glycerine and water and rolled out on pieces of thin paper. This mixture can be graded to give a harder medium than Plasticine, and is therefore more suited to making models of such parts as contain delicate ducts, nerves, &c. The paper, if cut larger than the plate, allows of rapid handling and can be cut away after the plate is in position. The surfaces and edges of the plates can be easily painted with water-colours.

The author is indebted to the late Dr. W. G. Ridewood for suggestions as to reconstruction models, and to Professor Paul Pelseneer for information upon the general morphology of the *Taenioglossa*.

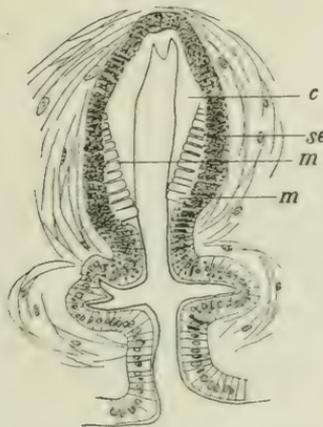
2. STRUCTURE.

1. The Alimentary System.

The oral cavity (Text-fig. 1) is usually deep and narrow. Ventrally it exhibits a pair of lateral diverticula which are sometimes forked. In general it agrees with that of other

members of the genus. It is lined by a cuticle which is fairly thin ventrally, but becomes thicker dorsally. This cuticle is secreted by a columnar epithelium which is continuous with that of the lips and adjacent parts. Each cell of this epithelium contains an elongate, deeply-staining mass of secretion which occupies the major portion of the cell and usually obscures the nucleus. These secretion-masses are especially well developed where the cuticle is deepest; and in these areas the

TEXT-FIG. 1.



Transverse section through the mouth. *c*, cuticle; *m*, mandibles
se, secretory epithelium.

whole epithelium is characterized by a mass of extra-cellular pigment in the shape of very small, subcircular granules.

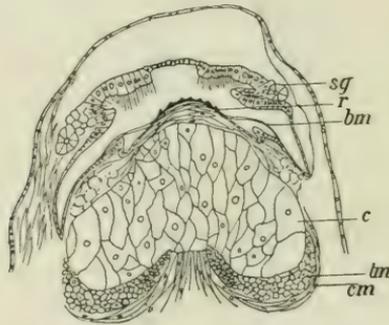
In the upper portion of the mouth is found a pair of mandibles. These consist of a number (minimum 13, maximum 20) of columnar pieces of specialized cuticle, each secreted by a single cell of the basement epithelium, as Seibold found in *Vitrella* (20). That the secretion-masses are intimately concerned in the formation of these is shown by the fact that plates are often continuous with the former. The mandibular plates stain very sharply with eosin, the rest of the cuticle being more or less unaffected by the stain. There is sometimes present, in addition to the mandibles, a specialized piece of

cuticle just below the mandibles on each side. There is also usually a median dorsal projection, dagger-shaped in transverse section. This also stains sharply with eosin, but less intensely than the mandibles.

Behind this projection are to be found at about the same level in the mouth on either side two glandular patches of unknown function which run backwards to the origin of the salivary ducts.

Posteriorly to the mandibles the mouth expands laterally and is flattened dorso-ventrally over the lingual cartilages.

TEXT-FIG. 2.



P. ventrosa. Transverse section through buccal bulb. *c*, oral cartilage; *cm*, circular muscles; *lm*, longitudinal muscles; *bm*, basal membrane of radula; *r*, radula; *sg*, salivary gland.

In this area it shows in transverse section three main divisions—a median, unpaired cavity with a thin roof, dorso-lateral expansions with ciliated and glandular walls into which the salivary ducts open, and ventro-lateral expansions which dip down beside the cartilages. These have a cuticular lining. The cilia of the dorso-lateral cavities no doubt serve to circulate the saliva. In *P. ulvae* Henking considers their function consists in driving the food particles backwards. In *Vitrella* and *Bythinella* the cilia are continued on to the roof of the median portion.

The lingual cartilages (Text-fig. 2) correspond to those found in other genera of the family, and in general to the

excellent description given by Bregenzer for *Bythinella*. They are two somewhat piriform bodies, united dorsally and in the median line. They are rather flattened dorso-ventrally. Posteriorly they diverge somewhat. Bregenzer states that in *Bythinella* they are each divided into a 'Haupt-Knorpel' and a 'Knorpelspange', and regards the difference between *Paludestrina* and *Bythinella* in this respect as of taxonomic value. Certainly no such division is apparent in *Paludestrina ulvae* and *ventrosa*. Lateral expansions ('Flügel') observed in *Bythinella* occur in *P. ventrosa* as well.

The tissue of the cartilages is composed of irregular polygonal cells with relatively small nuclei and a large amount of granular pigment.

It should be noted that Rougemont (18) states that in *Hydrobia* sp. the cartilages are capable of movement upon each other, if pressed downwards. In *Paludestrina*, *Vitrella*, and *Bythinella*, this is, of course, quite possible; as they are no doubt very elastic and have a certain amount of 'play' on each other.

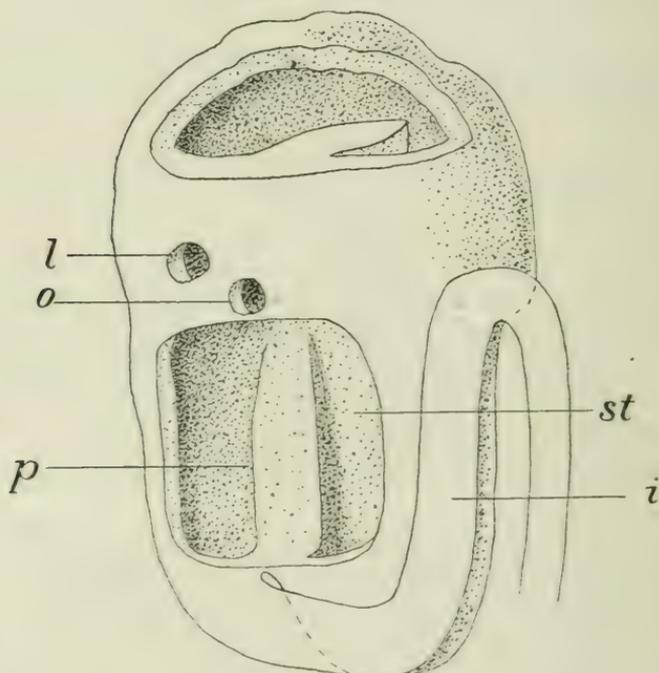
The *radula* has been described and figured by Woodward (24).

The salivary glands are two in number. In *P. ulvae* Henking describes two pairs with separate openings. Those of *P. ventrosa* appear to correspond with Henking's first (larger) pair. They run as far back as do the second pair of *P. ulvae* and sometimes cross over in a similar fashion.

The lateral diverticula of the pharynx disappear posteriorly, and the oesophagus develops a fresh series of diverticula in the form of deep longitudinal furrows. Behind the cerebral commissure the oesophagus in most cases shows a tripartite arrangement as in *Bythinella*. It is ciliated almost up to its distal extremity. The stomach (Text-fig. 3) is an irregularly-shaped organ with a forked appearance exteriorly due to the fact that the intestine and style-sac leave the stomach parallel to each other from its anterior end. The oesophagus and

hepatic duct open into the stomach at its upper (posterior) end. The pyloric part of the intestine and the style sac are in open communication with each other, as in *P. jenkinsi* (Robson, 16), by means of a longitudinal slit for a considerable way. Brezenzer does not refer to this as occurring in

TEXT-FIG. 3.



Diagrammatic reconstruction of stomach to show relationship of crystalline style sac. *p*, pylorus; *i*, intestine; *st*, style sac; *l*, hepatic duct; *o*, oesophagus.

Bythinella. No mention is made by Seibold of a style sac in *Vitrella*, and, as his account is painstaking and thorough, we have to assume that the sac is absent. This is a very curious fact and one to which we shall return later. The oesophagus and hepatic duct open into the stomach fairly closely together. In this region the stomach epithelium is ciliated, the ciliated area being continued downwards and

forwards into the pylorus. On the side of the stomach opposite to the oesophageal and hepatic apertures the epithelium gives rise to a dense cuticle which occupies the major part of the posterior part of the stomach but diminishes anteriorly.

Vitrella and *Bythinella* apparently differ conspicuously in the lining of the stomach. In the latter form only a small part of the stomach is ciliated, while the contrary is true of *Vitrella*. *P. ventrosa* is more or less intermediate between the two in this respect.

The base of the epithelium which secretes the cuticle is, as in the case of the oral cuticle, rendered conspicuous by a layer of densely-staining granules. The stomach is crossed by numerous ridges of which the most constant and most conspicuous is a large and strongly-developed one lying transversely in the cavity above the hepatic duct. Grooves with specialized cuticle are found in the neighbourhood of the latter.

The *style sac* is blunt externally and rather thimble-shaped. In transverse section it is circular and exhibits on the side towards the pylorus a groove of characteristic structure. The latter corresponds in its histological features to the similar structure in *Bythinella*. Bregenzer has offered no explanation of the function of this groove. Unfortunately the structure and relationship of the style itself cannot be demonstrated in fixed material. I am under the impression, however, that, in the living animal, the style is not loose in its sac but attached. If that is the case it may be secreted in the groove.

The rest of the sac is simple, being composed of a thick ciliated epithelium. The cilia are very dense and much longer than the cells.

No one familiar with the recent work on the style sac in Lamellibranchia can fail to be struck with the similarity between the structure here described and that figured by Nelson (14) for *Lampsilis anodontoides*. In both forms the pyloric part of the intestine communicates by a narrow slit with the style sac, the walls of which are composed of a single layer of columnar ciliated cells. In *Lampsilis* the resemblance to *Paludestrina* is still more emphasized by the

presence of a large mass of deeply-staining cells near the slit. In several figures given by Nelson and Edmondson (7) we find a lateral groove like that of *Bythinella* and *Paludestrina*. The similarity between the crystalline style of Gastropods and Lamellibranchs has been commented upon by various authors, and a short discussion may be found in Moore (12).

The pylorus is ciliated and passes gradually into the intestine proper. This follows the usual course. It is ciliated almost to its extremity; a well-marked typhlosole is found extending for some distance down the intestine. This is apparently absent in *Vitrella* and *Bythinella*.

The hepato-pancreas occupies the apical whorls as usual, and consists of branching finger-like processes. The duct is very short and fairly wide. No definite differentiation of the liver-cells into granular- and ferment-cells with different staining and contents could be made out. Vacuoles with inclusions are seen in the plasma of the liver-cells.

The rectum, when viewed transversely, exhibits a number of longitudinal folds. It runs forward in the roof of the pallial cavity projecting from the latter and ultimately becomes free for a short distance.

2. The Nervous System (Text-fig. 4).

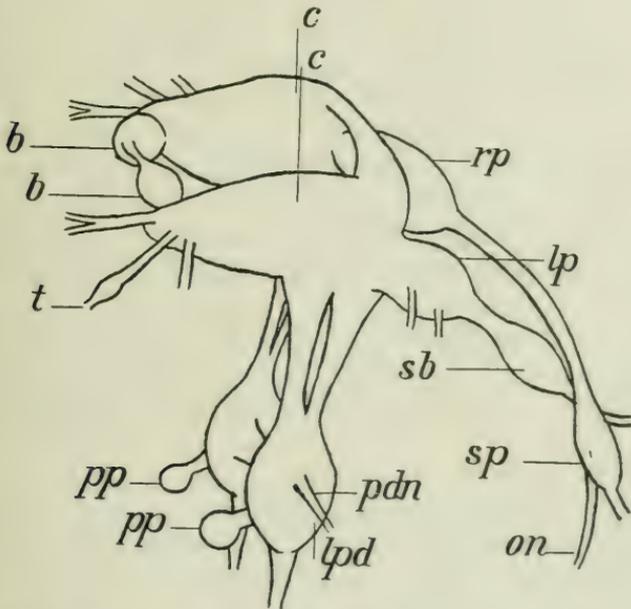
The only description of the nervous system of *Paludestrina* is that of Henking, which is insufficient and leaves a good deal to be desired.

The cerebral ganglia of *P. ventrosa* are long and rather pointed anteriorly. The cerebro-pedal commissure is normal though very short. The buccal commissure calls for some comment as it is extremely short and thus unlike the elongate form found in this and allied families. The buccal ganglia are closely applied to the anterior end of the cerebral ganglia. In one or two cases very short connectives were found; but such instances are rare. Henking does not refer to the buccal commissure as such in *P. ulvae*; but from his description and figure the connectives would appear to be as short as in *P. ventrosa*.

As in allied forms the cerebro-pleural connectives are absent, the ganglia being practically contiguous on each side. The right pleural ganglion is larger than the left. The pleuro-pedal connectives are short and closely applied to the cerebro-pedals.

The pedal ganglia are rather triangular in transverse section. Posteriorly they bear a pair of otcysts.

TEXT-FIG. 4.



Central nervous system (diagrammatic). *b*, buccal ganglion; *c*, cerebral ganglion; *lp*, *rp*, pleural ganglion; *lpd*, pedal ganglion (*l*); *pp*, propodial ganglia; *sb*, subintestinal ganglion; *sp*, supra-intestinal ganglion; *on*, osphradial nerve; *t*, tentacular nerve and ganglion; *pdn*, parapodial nerve.

The visceral commissure is of the shortened type and resembles that seen in *Vitrella* and *Bythinella*, though it agrees with the former in the amount of abbreviation, the supra-oesophageal pleural connective not being so shortened as in the latter. The supra-oesophageal portion follows the oesophagus more or less closely to the abdominal ganglion

which is situated in the columella region between the kidney and the reproductive organs. The subintestinal portion passes over the surface of the columella muscle to the abdominal ganglion.

Three main nerves are given off from the anterior end of the cerebral ganglia—a large tentacular nerve with a tentacular ganglion and two oral and labial nerves. No separate optic nerve was found, the innervation of the eye being achieved by optic fibres of the tentacular nerve. It may be recalled that Vayssière found that in *Truncatella* (23) the tentacular nerve is apparently responsible for the oculo-motor innervation.

From the left pleural ganglion are given off two nerves of pallial distribution. Each pedal ganglion gives off three main roots—anterior, ventral, and postero-lateral. The first-named always bear at a short distance large and well-defined propodial ganglia. The ventral pair is very stout and sometimes bears ganglia. The postero-lateral pair is very slender and, as in *Bythinella*, sometimes bears a diminutive ganglion. No certain trace of a metapodial commissure was found. It is absent in *P. ulvae* and present both in *Vitrella* and *Bythinella*.

From the supra-intestinal ganglion a nerve passes upwards over the roof of the pallial cavity to the osphradium.

Henking's description of the visceral commissure and its prolongations in *P. ulvae* is in need of correction. Bregenzer, who admits this, is inclined to make taxonomic capital out of his statement that two connectives from the pedal ganglion are found joining the 'Oberschlundganglion', and infers from this that the pleural and cerebral ganglia are completely fused. If this is correct it is difficult to account for Henking's 'accessorische' and 'unpaares' ganglia unless we call these respectively supra-intestinal, subintestinal and abdominal (!) It seems far more sensible to assume that Henking made a mistake about the point of insertion of the pedal connectives, to call his 'I. accessorische Ganglion' the left pleural and his 'unpaares Ganglion' the subintestinal.

3. Sense Organs.

The statocysts are in close proximity to the posterior surface of the pedal ganglia. The auditory nerve is very difficult to trace, and in a large number of preparations I have only succeeded in finding one in which it is clearly seen. It runs backward from the statocyst in close proximity to the cerebro-pedal connective and ultimately becomes indistinguishable from the latter. The cysts contain each a single moderately-sized otolith.

In certain other Taenioglossa, e.g. *Valvata* (Bernard, 1), *Melania* and *Paludina* (Pelseneer, 15), numerous small otoconia replace the single otolith of *Paludestrina*, *Vitrella*, and *Bythinella*. This diversity, which contrasts with the remarkable constancy in the number of otoliths found in Teleostean fishes, might well supply a subject for independent study both from the taxonomic and the physiological point of view.

The cysts are formed of an external layer of very thin epithelium covering an internal layer of irregularly-shaped cells. These are flattened and resemble rather those figured by Bernard (1) for *Valvata*.

In *Valvata* Bernard found no cilia, while Garnault (8) observed that in *Cyclostoma* they are very sparsely developed. In *Bythinella* and *Vitrella* they are not referred to. In *P. ventrosa* it is almost certain they are absent. There is quite definitely no ordinary ciliated layer and nothing more on the interior surface than occasional vague clumps of unrecognizable tissue. How the otoliths in this case stimulate the sensory layer is therefore uncertain unless the latter has a general tactile sensibility. In *Valvata* Bernard (loc. cit.) observed a sort of network formed of prolongations from the membrane of the lining cells.

The eyes do not call for special attention. They resemble those figured by Henking for *P. ulvae* in general form, though they differ in the closer approximation of the inner and outer cornea. In *Bythinella* the space between the two corneas is considerable and filled with connective tissue. In

the same form Bregenzer notes no special differentiation of the outer cornea from the adjacent epithelium of the tentacle base. This is not the case in *P. ventrosa*, in which the external cornea is always noticeably thinner.

The osphradium, as in *Vitrella* and *Bythinella*, is a simple ridge-like elevation on the left-hand side of the gill close to the junction of the roof of the pallial cavity with its floor. There are no foliations such as occur in some other *Taenioglossa*. The laterally-disposed pigment cells contain brownish pigment granules. The ciliated and sensory layer overlies a large and elongate osphradial ganglion.

4. The Respiratory and Circulatory System.

(1) Pericardium and Heart (Text-fig. 5).

The pericardium lies on the posterior side of the body-whorl in a superficial position covered only by the body-epithelium. It is placed at the posterior end of the pallial cavity on the left-hand side, and is roughly bounded by the kidney and the extremity of the style sac.

No trace of a reno-pericardial orifice could be found. Seibold was unable to find one in *Vitrella*; nor is it referred to by Bregenzer for *Bythinella*. It occurs in both *Cyclostoma* (8) and *Valvata* (1). The auricle was never found in an expanded condition, so that its general structure cannot be defined. The ventricle is, as usual, thick-walled and muscular, though a thinner-walled portion of varying extent is invariably to be seen.

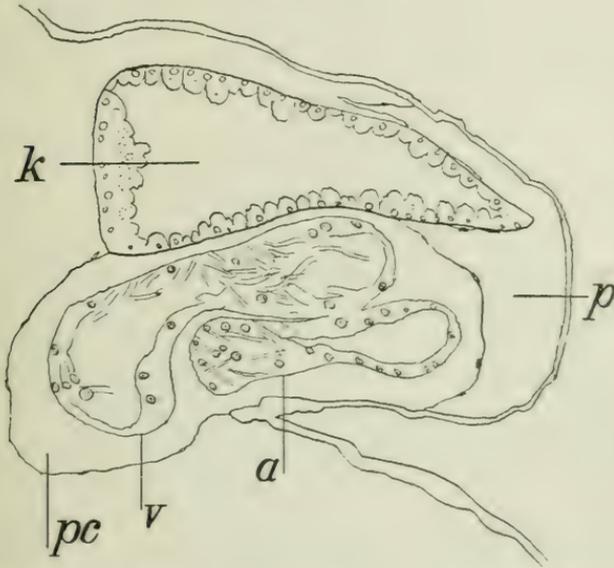
(2) Vascular System.

The descriptions of this system in other *Taenioglossa* are very unsatisfactory. The accounts given are usually incomplete, and frequently omit some portion from consideration. It may also be pointed out that on one point at least two of the most up-to-date treatises on the Mollusca are at variance. Lang (11, p. 322) says, ' Bald öffnen sich die Arterien . . . in arterielle Sinusse. Unter diesen verdient besonders der grosse Kopfsinus . . .' Pelseneer (15), who alludes to the

cavities in which the arteries terminate as 'interorganic lacunae', says (p. 100), 'the venous blood is collected into an anterior or cephalopedal sinus', &c. I share Professor Pelseener's view that the large cephalopedal cavity is venous.

The following outline, which is by no means complete and owing to the size and contractility of the animal is not founded

TEXT-FIG. 5.



Heart, pericardium, and kidney in section. *a*, auricle; *v*, ventricle; *k*, kidney; *p*, portal vein; *pc*, pericardium.

upon injections, may serve to enlarge our knowledge of this system in the *Taenioglossa*.

There appears to be a large general venous sinus of which the chief components (cephalic, pedal, and visceral) are in communication with each other. The two first-named are in open communication posteriorly, but are separated anteriorly by a horizontal septum. Both Henking and Bregenzer speak as though the latter completely separated the two cavities in *P. ulvae* and *Bythinella*. In none of my series, however, is this the case.

From the general venous sinus the blood passes (*a*) to the renal portal system by more or less clearly-defined vessels, (*b*) to the gills through the rectal sinus. From the kidney the blood passes into the portal vein (q.v.). The complete course of the latter is not easy to trace. In one or two cases it was found entering the auricle close to the root of the pulmonary vein, though in other cases its junction is not so clearly seen and it might even open into the pulmonary vein itself. It is possible, however, that some of the blood in the kidney may find its way into the gill directly, as the rectal sinus was adjacent to the kidney on part of its course. The rectal sinus proper appears to be cut off from the other venous sinuses, but to be in communication with them by means of a loose lacunar system.

From the rectal sinus afferent vessels run to the gill and pass along the base of the gill-lamellae (q.v.). The arterialized blood is carried from the gill by the pulmonary vein. It leaves the ventricle by the aorta, which divides into anterior and posterior branches. The first-named runs forward along the wall of the pericardium sending out branches in its course. The posterior branch passes between the stomach and intestine and probably enters a lacuna in this position. From the lacuna is given off among other vessels a clearly-marked genital artery which can be traced backwards to finer branches distributed to the various processes of the gonad.

(3) Respiratory System.

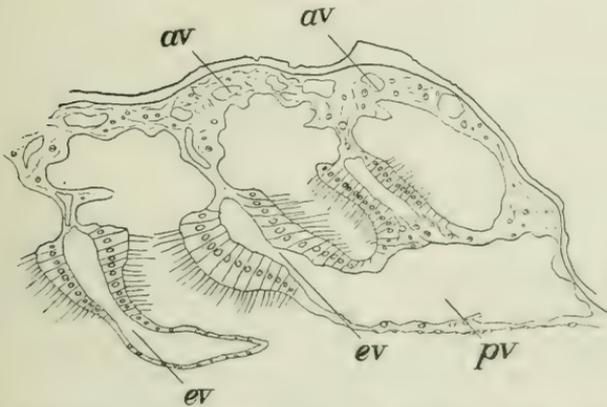
The gill (Text-fig. 6) is monopectinate and composed of broadly-triangular plates hanging freely in the pallial cavity. On the rectal side the extremity of each plate is free for a short distance.

The histology and structure correspond in general with that of *Bythinella*, but Bregenzer does not indicate the relation of the gill to the adjacent parts of the circulatory system.

On the whole it would seem that in *P. ventrosa* the apical portion of the lamellae is more elongate and triangular in transverse section than in other forms. It should be

noted that in this form and *Bythinella* the cilia are not distributed all over the lamellae as in *Vitrella* but are concentrated at the apex. Each plate shows longitudinal folds towards its basal part, becoming flatter where they join up with the efferent vessel. In *Bythinella*, *Vitrella*, and *Paludestrina jenkinsi* the lamellae are flat and unfolded. This folding seems rather difficult to explain. Were it not for the fact that similar folding occurs in *P. ulvae* (Henking) I would be inclined to think

TEXT-FIG. 6.



Gills in section. *av*, afferent vessel; *ev*, efferent vessel; *pv*, pulmonary vein.

that it might be due to shrinkage arising from excessive contraction of the transverse muscles in each filament. But in addition to the occurrence of similar folds in *P. ulvae* there is the fact that, if it were due to shrinkage, one would expect such contraction to take effect over all the lamella, which is not the case. If this ultimately proves to be an invariable character of these two species of *Paludestrina* it may very well be correlated with their brackish-water and marine habitat. It should also be pointed out that unless the text-figures G and H in Bregenzler's paper are diagrammatic the afferent vessels and blood-spaces are much smaller in *P. ventrosa* than in *Bythinella*.

From the rectal sinus blood passes by rather irregular and inconstant lacunar spaces to the roof of the pallial cavity, and finally into more definite and constant lacunae at the base of each gill-lamella. Thence it passes to the afferent vessels, and from these through the blood-spaces in the lamellae to the efferent vessels at the apex of the plates. The efferent vessels of all the lamellae are united on the left-hand margin of the gill by the pulmonary vein. On the left-hand side the afferent vessels apparently lose themselves in lacunae. On the rectal side the efferent vessels come to an end in the free portion of each lamella.

It is probable that blood is brought from the left-hand side of the mantle cavity in a wide irregular lacunar system which ultimately *de-bouches* into the same sub-lamellar spaces as the blood from the rectal sinus.

4. Renal System (Text-figs. 5 and 7).

The kidney lies between the body-wall, the pallial cavity, and the pericardium, sending ramifications among some of the other organs as in *P. jenkinsi*. It communicates with the pallial cavity at its own anterior end by a ciliated aperture furnished with sphincter and dilator muscles. In general the kidney is a thin-walled cavity with a lining of special secretory vacuolated cells as figured and described by Bregenzer (6, p. 248). Anteriorly, however, there is a special area between the body-wall and the pericardium characterized by a compact stroma of connective tissue with blood cavities communicating with the portal vein. This is the 'blood-gland' of various authors (cf. Sinroth, 21, p. 564). It is present in *Bythinella* but absent in *Paludina*, *Cyclostoma*, and other forms (Sinroth, *loc. cit.*). It is not specifically described in *Vitrella*, and it is not clear from Seibold's description if it actually occurs.

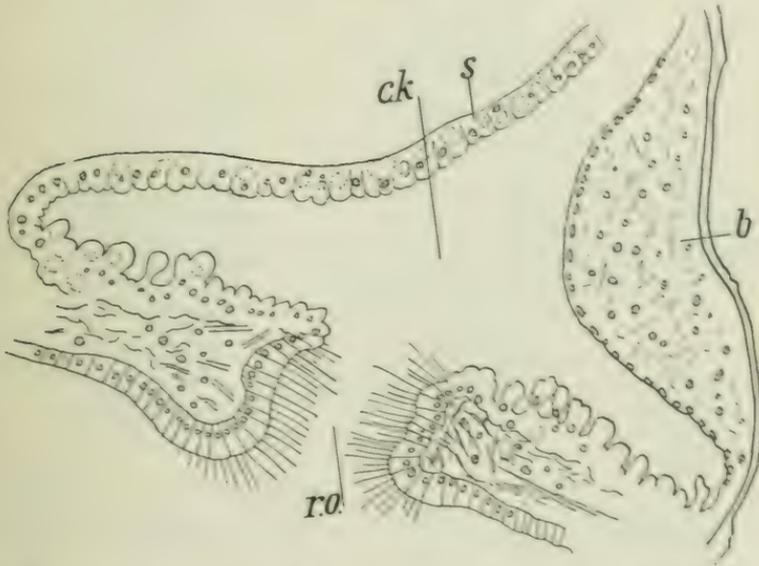
With regard to the epithelium covering this gland on the renal side I do not find the condition described by Bregenzer. The lining is usually a flat epithelium with flattened or roundish nuclei (fig. 7). I have never found the peculiar epithelium figured by Bregenzer (*loc. cit.*, Pl. xvi, fig. 15).

5. Reproductive System.

(1) Female Organs (Text-figs. 8, 9, 10).

The ovary lies as usual between the liver and columella muscle. It consists of branched tubular follicles. The material employed for this study was all collected and preserved during May and June when apparently the oögonia were approaching maturity, but were not being shed in any number. A few

TEXT-FIG. 7.



Renal aperture and 'blood-gland' in section. *ck*, cavity of kidney; *b*, blood-gland; *r.o.*, renal orifice; *s*, secretory epithelium.

were found in the oviduct, and in a small number of cases spermatozoa were found in the receptaculum seminis. It may be therefore considered that, speaking generally, the material examined represented a stage coincident with the beginning of the breeding season.

A great diversity of cellular elements was found in the ovary. The following types were invariably distinguished (Text-fig. 12):

(a) Ripe oögonia distinguished by their large size, large

- yolk content, usually with a clear slightly-granular nucleus and a deeply-staining nucleolus.
- (b) Ovarian cells only distinguished from (a) by the less intense staining of the yolk and their smaller size.
- (c) Small cells of various sizes, free or attached to the epithelium of the follicles with darkly-staining cytoplasm, clear nucleus, and dark nucleolus.

TEXT-FIGS. 8-9.

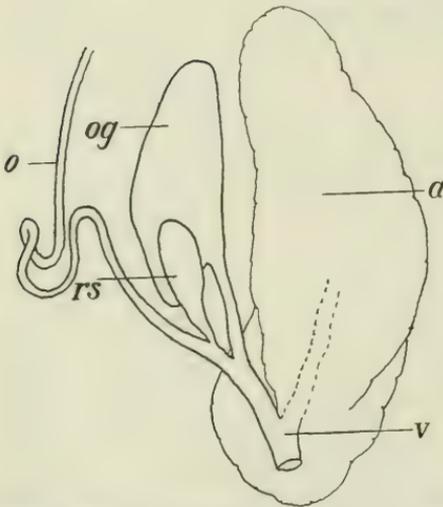


Fig. 8.—Female genitalia. *a*, accessory gland; *o*, oviduct; *og*, oviducal gland; *rs*, receptaculum seminis; *v*, vagina.

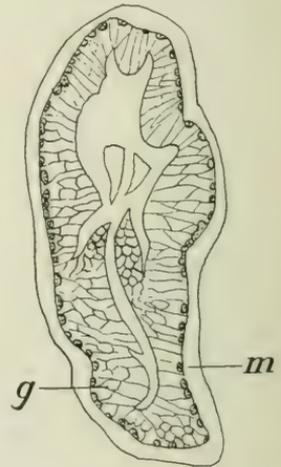


Fig. 9.—Section of oviducal gland. *m*, outer muscular layer; *g*, gland.

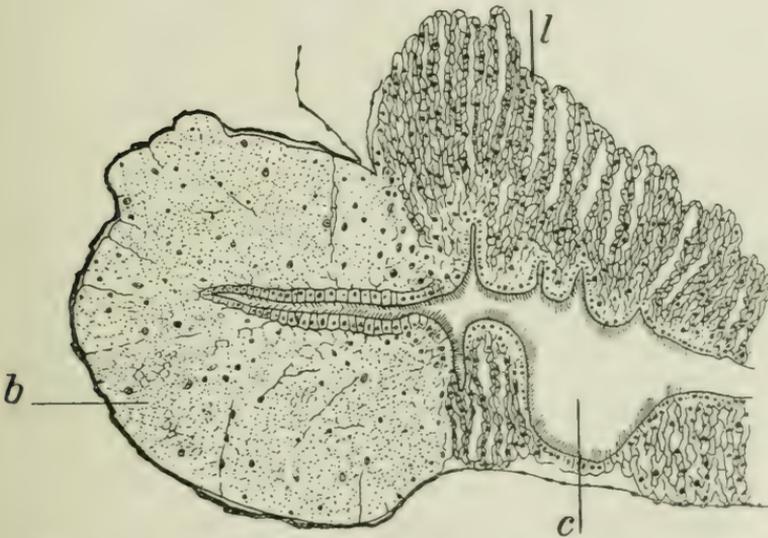
(d) Cells of the germinal epithelium in various stages, either very small and irregular or enlarged and approximating to (c).

The germinal epithelium was never found in the regular columnar condition seen in Bregenzner's figure U; and it is sometimes very difficult to interpret, being full of masses of deeply-staining material of irregular disposition and uncertain nature and often flattened out by the pressure of the ripening oögonia. The various types of ovarian cell with all the intermediate stages are frequently met with in one and the same follicle, and the gradual transition seems to indicate the

development of one main type of cell from the germinal epithelium, viz. oögonia. No traces of nurse-cells could be found.

The oviduct follows the usual course down the columellar region accompanied by the genital artery. It ultimately becomes thick-walled and convoluted. It gives off in succession a receptaculum seminis and an oviducal gland,

TEXT-FIG. 10.



Portion of accessory ♀ glands in section. *b*, darkly-staining area ; *l*, purple-staining area ; *c*, cavity continuous with vagina.

and opens into the vagina close to the entrance of the accessory glands of the latter. In its upper course its walls consist of a single layer of flattish epithelial cells. In the neighbourhood of the kidney its walls are formed of deeper and more columnar cells which contain at their apices (i.e. towards the lumen) a darkish secretion. They are ciliated and covered by an external layer.

The receptaculum seminis is rather club-shaped and has a short duct. It is surrounded by a muscular layer. The cells are columnar with basal nucleus and their structure

seems to indicate a glandular nature. Very occasionally spermatozoa were found in the receptaculum aggregated into small subcircular clumps.

(2) The Óviducal and Accessory Glands.

Some excuse is perhaps required for cumbering nomenclature with an additional obscurity. The appendage (Text-fig. 9) borne upon the oviduct just below the receptaculum seminis is called by Seibold the 'Anhangsdrüse des Receptaculum seminis' and by Bregenzer the 'Eiweissdrüse'. The latter's figures are not a sufficient indication whether structurally the organs are similar in *Bythinella* and *P. ventrosa*. Devoting our attention to the latter we find the 'oviducal gland', as I prefer to call it, to be covered by a strongly-developed muscular sheath with circular muscle fibres. In general form it is an irregular-shaped gland with a short duct. Internally it is very much folded. The cells of its inner layer when not loaded with secretion are tall and narrow. The nuclei are basal, and, when the cells are full of secretion, they become driven close up against the basal membrane. There are not very many accounts of the albumen gland in Gastropoda. But from those available we can safely assume that we are hardly warranted in calling this structure in *Paludestrina* by that name. In *Valvata* (1) on the one hand and *Physa* (22) on the other we see radically different types of 'albumen gland', and we can identify this form with neither.¹ Until more is known of this structure in Gastropoda, and particularly in Prosobranchia, it is perhaps better to avoid a too positive terminology.

The vagina is a narrow slit-like cavity surrounded by a large accessory glandular mass. It is thin-walled and ciliated internally. The glandular mass is very interesting but difficult to interpret. Previous authors of recent work upon *Taenioglossa* do not discuss it at any length, though Seibold pointed out that differences of staining could be observed in it. Subject to certain qualifications, we may state that this mass is divisible most

¹ Cf. Slugoeka's Pl. iv, fig. 20.

frequently into two parts which occupy more or less opposite sides of the vagina and, where they meet, show a certain amount of transition in their structure.

One portion is usually stained in haematoxylin and eosin a vivid light purple in which the pink tinge predominates. It consists of two kinds of cells. A layer of ciliated, cubical cells lines the cavity of the gland. Some of them are drawn out into irregular, elongate extensions with which are associated other rather elongate cells. These form irregular digitiform glandular masses. A distinct lumen is seen in these masses (Text-fig. 10). It is uncertain how they pass their secretion to the exterior, as I have never observed a communication between the lumen and the exterior. The second area usually stains a deep purplish blue with the same stain. Seen in its most characteristic form it is composed of the same columnar ciliated cells and an inner glandular mass. The latter is more compact, the nuclei of the constituent cells are fewer and often arranged at the periphery of rudely quadrate masses. One is tempted to conclude that this second portion only represents another stage of the condition observed in the first described part, and that in the one the cells are full of secretion and tend to obscure a structure like that described in the first case. In the compact portion it is very hard to make out cell outlines, and certainly nothing like the digitiform glandular processes can be seen. It is, on the other hand, very certain that in certain areas transitional masses are to be found.

I am inclined on the whole to consider that there are two functionally distinct portions of this gland mass, though intermediate stages are found. A comparison may be made with the rather similar structure of the accessory glands (oötype, shell-gland, &c.) of *Neritacea* which have been described by Bourne (3).

The cavity of the vagina is continuous with those of the glands.

(3) Male Organs (Text-fig. 11).

The testis consists of a number of branching follicular tubes and in general plan resembles the ovary. Only one kind of

spermatozoon was found, viz. the 'typical'. The definitive stage of the latter, which is found in the vas deferens and the receptaculum seminis of the female, exhibits an elongate conical 'head', a usually well-developed acrosome, an acute apical portion, no discernible middle-piece, and an elongate tail. The precise length of the latter could not be very satisfactorily ascertained, but it is apparently very much longer than that of *Bythinella*, in which the tail is between twice and thrice as large as the head. In *P. ulvae* and *P. taylori* (Robson MS.) the tail is relatively enormous. One of the constant features of spermatogenesis is the occur-

TEXT-FIGS. 11-12.

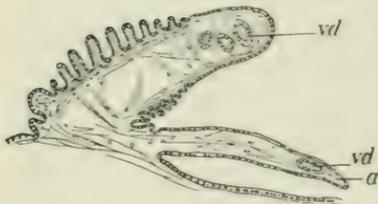


Fig. 11.—Section of penis. *a*, free portion of 'appendage'; *vd*, vas deferens.



Fig. 12.—Transverse section through ovarian follicle.

rence in the spermatids of an arrangement of the chromatin of the nucleus in bent rods or half-hoops at the periphery of the nucleus. My friend Dr. J. B. Gatenby has pointed out to me the rather similar concentration of chromatin at the posterior part of the nucleus in the spermatid of *Murex trunculus* recorded by Schitz (19). I am also indebted to Dr. Gatenby for pointing out to me the frequent occurrence of abnormal stages of spermatocytes, though of course, as has been stated above, the spermatozoa are monotypic.

The vas deferens is thin walled during the first part of its course. It passes down the columellar region and in the neighbourhood of the kidney gives rise to a large glandular swelling, the prostate. The latter has plicate walls in-

teriorly, lined with columnar ciliated cells with more or less basal nuclei. The rest of the structure of this gland, which stains violet with haematoxylin and eosin, is not unlike that of the lighter-staining portion of the accessory gland of the female.

Below the prostate the vas deferens becomes smaller, thick-walled, and ciliated. It eventually runs just below the epidermis in the floor of the pallial cavity to the penis, which it traverses up to its apex. The penis is single in contrast with the remarkable complexity of *Bythinella* and *Bythinia* (Moquin Tandon, 13), in which a flagellum and a second branch occurs. It therefore exhibits the condition seen in *Cyclostoma* (Garnault) and *Vitrella*. In *P. ulvae* the penis is quite simple according to Henking, while drawings made from the living animal by my friend Dr. H. Quick also show no accessory structures upon the male organ. The intromittent portion in *P. ventrosa* is long and pointed.

3. HABITS, ETC.

A preliminary attempt has been made (Robson, 17) to analyse the ecological conditions under which *P. ventrosa* is found. But a great deal remains to be done upon this subject as well as upon the distribution and ecology of the plants associated with it and upon which it may be presumed to depend. Though a more definitely brackish-water form than *P. ulvae*, the case worked out at Leigh-on-Sea demonstrated a greater adaptability and tolerance on the part of *P. ventrosa*. If, as we may rightly assume, the British Paludetrinidae show a progressive tendency to become adapted to fresh-water, *P. ventrosa* represents an intermediate stage of adaptation, but exhibits the tendency in its initial rather than its later stages. Little can be said upon the more intimate habits of this animal. It is usually found upon some water-plant, but quite frequently upon mud or bottom debris. In several examples from Leigh, Wakering Wick, and elsewhere, the stomach contained a variety of diatoms and a few foraminifera.

fera. The rest of the contents were usually too much digested to enable their nature to be made out. No remains of plant fibre, &c., was ever found. I am inclined to think that it browses upon the microfauna and microflora of the plants upon which it lives, and that it does not actually chew the leaves of the latter.

4. AFFINITIES.

(a) I cannot agree with Bregenzer's verdict upon the immediate relationships of *Paludestrina* (6, p. 276). According to her, the latter genus is separated into a group distinct from *Bythinella* and *Vitrella* upon the following characters :

- (1) Fusion of the cerebral and pleural ganglia.
- (2) The possession of two pairs of salivary glands.
- (3) Reduction of the 'Knorpelspange' of the lingual cartilages.
- (4) Brackish-water habitat.

Of these characters the first is open to question. In *P. ventrosa* there is no more fusion of the ganglia in question than in *Bythinella*, while in *P. ulvae* we have seen (p. 168) that Henking's statements are open to question. In the second place, only one pair of salivary glands is found in *P. ventrosa*. As to the third character it is scarcely worth anything as the 'Knorpelspange' is absent in *Vitrella*! Lastly, *Paludestrina* is not restricted to brackish water, at least as far as England is concerned. As the result of a scrutiny of the characters available for taxonomic purposes we might with equal justification select the simple penis and longer super-intestinal part of the visceral commissure in order to unite *Paludestrina* with *Vitrella* as against *Bythinella*, or the crystalline style and certain features of the radula to unite *Paludestrina* and *Bythinella* against *Vitrella*. In any case I venture to think that an animal's taxonomic position cannot be summarily decided in this fashion. Until we have objective evidence as to the taxonomic value of the various characters such groupings as those discussed above are of little value. On the whole we can safely consider these

genera as referable to the same family ; but I feel that we require another technique for deciding their closer affinities than a mere inspection and assorting of characters. A character such as the absence of the crystalline style in *Vitrella* would appear in the first instance to be profoundly important. But we do not know the precise significance of its absence.

As an alternative to a close study of genetics, evolution, habits, and ecology in relation to structure which alone can give us a sound taxonomic method, the only procedure that could be suggested would be a complete enumeration of characters and a grouping based upon agreement or disagreement in a large number of structures. This method would be crude, but it would be better than an arbitrary selection of a few characters. In the present case I have distinguished a total of twenty-one important characters. The agreement or disagreement of the three genera in question is indicated as follows :

Paludestrina=	Bythinella alone in	5
„	=Vitrella alone in	2
„	=Bythinella and Vitrella in	4 21
„	=neither in	8
	relationship uncertain in	2

(b) Though it would be beyond the scope of this paper to offer a criticism of the present arrangement of the Taenioglossa, we may nevertheless attempt to define the position of the Paludestrinidae with regard to some of the main tendencies of Prosobranch morphology.

The Paludestrinidae represent a stage in the abbreviation of the nervous system which involves the pleural-intestinal portions, and is seen in its extreme condition in *Bithynia* and *Valvata* in which the sub- and supra-intestinal ganglia are either fused or closely approximated to the pleural ganglia. In *Melania* and *Cerithium* this condition of close approximation is seen on one side only, the ganglia being separated on the other side. In *Paludestrina* and *Bythinella* they are slightly separated on both sides, while in *Littorina* and *Paludina* they are widely separated.

Paludestrina agrees with *Pterocera*, *Tiphobia*, *Lithoglyphus*, and a few others in possessing a crystalline style. We may assume, however, that this is without phylogenetic significance within the group.

Another interesting tendency which may not be of phylogenetic importance is the possession of a single otolith in the *Paludestrinidae*. It shares this character with *Littorina*, *Truncatella*, some *Melantias*, and *Natica*. On the other hand, *Paludina*, *Ampullaria*, *Valvata*, *Cyclophorus*, and others have multiple otoconia. A blood-gland is absent from the kidney of *Paludina*, *Valvata*, *Cerithium*, &c., and is found in *Littorina* and in the *Paludestrinidae*.

Finally, while possessing a simple osphradium, *Paludestrina* exhibits a definite osphradial ganglion—a stage apparently more advanced than such forms as *Littorina* and *Bithynia*, in which (Bernard, 2) no osphradial ganglion is found.

5. SUMMARY.

1. *Paludestrina ventrosa* possesses the general *Taenioglossate* organization.
2. It represents a genus of *Paludestrinidae* equivalent to *Bythinella* and *Vitrella*.
3. It is peculiar within the family as possessing:
 - i. Folded gills;
 - ii. A slit connecting the style sac through nearly all its length with the intestine;
 - iii. A typhlosole;
 - iv. A non-ciliated roof to the median part of the pharynx.

It represents an intermediate stage in the acquirement of the fresh-water mode of life, being essentially a brackish-water form with a fairly well-marked euryhaline tendency.

4. Several structures not fully described by previous authors are discussed in this paper (e.g. the accessory female and circulatory organs), and it is not certain in what form these structures occur in other *Taenioglossa*.

5. Within the Order Taenioglossa, Paludestrina is referable to the group which possesses :

- (1) a brevicommissurate visceral commissure ;
- (2) a single otolith ;
- (3) an osphradium with basal ganglion ;
- (4) a renal-portal system and blood-gland ;
- (5) an ' oviducal ' gland immediately adjacent to the receptaculum seminis.

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The Gastric Mucosa.

By

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With Plate 8 and 1 Text-figure.

INTRODUCTION

THE gastric mucous membrane is described as being disposed in three regions, known as the cardiac, fundic, and pyloric. These regions, although distinguished from one another by definite microscopic characters, yet merge gradually the one into the other, so as to present no well-defined lines of demarcation. The actual extent of each region varies in different animals. It has not been sufficiently recognized, however, that the cardiac and pyloric areas are very small, especially in the carnivora. In the cat, the (microscopic) pyloric region is a narrow zone, extending for not more than 35 mm. from the junction of pylorus and duodenum; it may not even correspond in extent with the so-called pyloric antrum. In view of this fact it is possible to doubt the exactness with which pure pyloric pouches can be isolated either by the Heidenhain (13) or Pavlov (21) technique.

What is known regarding the functions of the different regions of the stomach is not compatible with their differences in structure. And current descriptions of the cells forming the gastric glands are by no means uniform, much confusion being due to the fact that the histological descriptions vary according to the method of fixation and staining employed and the species of animal investigated.

The following description of the histology of the gastric glands is divided into three parts. In Part I only the gastric mucosa of the cat will be described, since the observations

I have made upon its stomach are more complete than in the other cases. Other animals, both adult and foetal, have, however, also been investigated, and the special features of some of the cells of their glands are described in Parts II and III.

PART I. THE GASTRIC MUCOSA OF THE CAT.

The cats were killed both while fasting and at various intervals after a meal. They were usually fed on boiled fish, milk, and bread, but some were put on a meat and milk diet. In all twenty-five animals have been examined.

HISTOLOGICAL TECHNIQUE.

For microscopical purposes the animals were killed either by carbon monoxide or chloroform. The stomach was then examined fresh or was prepared for sections.

For the fresh preparations a piece of the mucous membrane was either scraped off and teased in Ringer or serum, or the fresh tissue was frozen in a little serum and cut up with a microtome. The fresh sections, however, gave no more information than those obtained after fixation, so that this method was discontinued.

For permanent preparations the fixatives used were Zenker, Altmann's fluid, osmic acid 1 per cent. and formol (either neutral 20 per cent. or acid 10 per cent.). When Zenker or formol was employed the stomach was slightly distended with the fixative and suspended in the same solution for the period necessary for penetration. It was then cut into suitable pieces, which were either placed in gum or carried through in the usual way into paraffin. For some preparations pieces of fresh stomach were pinned out on a cork and immersed in the fixing reagent; this was the chief method when using osmic acid solutions, but a few pieces were fixed in osmic without stretching.

The stains employed were alcoholic eosin and methylene blue (16), haematoxylin and eosin, van Gieson, iron haema-

toxylin (Heidenhain), Mallory (24), or polychrome methylene blue. It has not been thought necessary to give details of the application of the above stains ; they may be found in the references indicated. In the description which follows acid formol fixation is implied, although the observations recorded have been corroborated by other methods. Where a notable difference occurs the special fixative concerned is mentioned.

THE MUCOUS MEMBRANE AS A WHOLE.

It is not intended to describe the naked-eye appearances. Suffice it to say that with a lens (Sprott Boyd (28)) differences may be noted between the duct orifices of the pyloric region and those of the remainder of the stomach. In the former the mucous membrane is thicker and the ducts wider, longer, and more funnel-shaped than in the latter.

The gland-tubes are simple, but may branch slightly towards their blind ends. Several gland-tubes are usually served by a common duct. Only in the part of the pyloric canal close to the duodenum do the glands become markedly racemose, but the glands adjacent to the oesophagus may also take on a racemose character.

A gastric gland-tube may be described as consisting, besides the duct, of a superficial part, which is the portion of the gland-tube immediately below the duct, and a deep part composed of the remaining portion of the gland.

THE CONNECTIVE TISSUE.

Between the glands lies the supporting connective tissue (interglandular tissue) which contains plain muscle-fibres arranged vertically, blood-vessels, lymphatics, and nerves. In addition to these there are three kinds of cells in the tissue :

(1) **Finely Granular Branched Connective-tissue Cells.**—These stain a deep magenta with polychrome methylene blue and a purplish blue with alcoholic eosin and methylene blue. They form by far the most numerous variety and are more numerous in the stomach than in other

portions of the digestive tract. This has already been noted by Cade (5).

(2) *Finely Granular Oxyphil Leucocytes*.—These are sometimes massed together in groups: more generally they are scattered throughout the mucosa.

(3) *Coarsely Granular Eosinophil Cells* (Pl. 8, fig. 5, *g*). These are present in least numbers. They occur mainly near the surface, and may be found between the cells lining the duct of the gland as well as in the interglandular tissue. The eosinophil granules or globules vary considerably both in number and size, some being as much as $2-3\mu$ in diameter. They stain with iron haematoxylin, which does not colour the oxyphil granules of leucocytes; they are thus not unlike the cells of Paneth of the small intestine.

All three types may be found in the interglandular tissue of other animals, e. g. dog, pig, and rabbit.

The interglandular tissue is more abundant at the cardiac and pyloric ends of the stomach than in the middle of the fundic region. Here the connective tissue is more plentiful immediately under the surface epithelium.

The mucosa rests on a thick condensation (membrane of Zeissl, *stratum compactum* of Oppel (Text-fig. 1, *a. sc*)) of white fibrous tissue, immediately underneath which lies the muscularis mucosae. This membrane-like condensation is of interest as it is not common to all animals, e. g. it is absent in man, pig, and rabbit, but is present in cat and rat. Further, it is non-elastic and separates the muscle-fibres within the interglandular tissue from the muscularis mucosae. It is perforated by vessels, and the plain muscle-fibres reach the mucosa by the same communications.

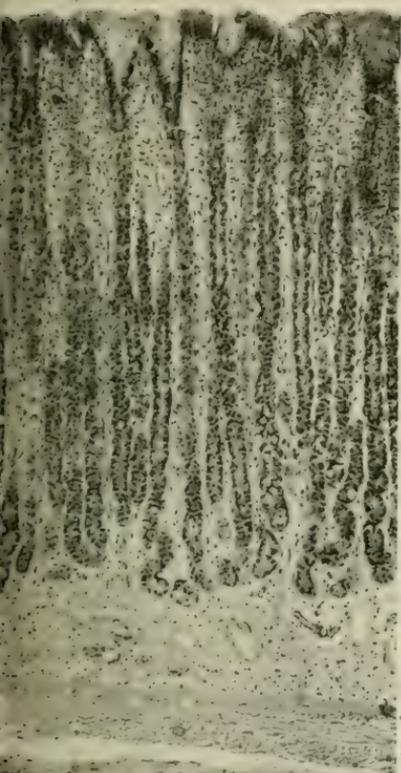
THE SURFACE EPITHELIUM.

This epithelium includes the cells covering the surface and those lining the ducts. These cells are essentially of one type. Those on the surface are columnar, becoming shorter and more cubical as they are traced into the ducts. A corresponding change may also be noted in the nucleus, which is elongated

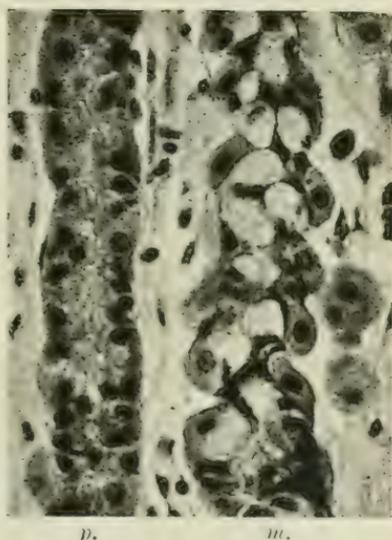
on the surface but almost spherical within the ducts (see Pl. 8, fig. 5).

The cytoplasm is finely granular in the fresh and certain fixed (neutral formol, osmic) specimens, and may be differen-

TEXT-FIG. 1.



Low power ($\times 75$). *sc*, stratum compactum; *mm*, muscularis mucosae.



B. High power ($\times 400$). *p*, gland lined with peptic cells; *m*, gland lined with mucoid cells; oxyntic cells occur in the parietal parts of both glands.

Glands from the middle of the anterior surface of the stomach. Cat 10: 24 hrs.; acid formol; haematoxylin and eosin. (Photograph.)

tiated into two parts (Ellenberger and Scheunert (11) by staining methods, viz. an outer goblet-shaped part, which is clear but tinted red in haematoxylin and eosin preparations, stained blue by Mallory and a pale blue by polychrome methylene blue, and an inner part consisting of the remainder

of the cell, in which the nucleus is situated, and which is stained of a reddish colour by Mallory.

The surface cells show a larger goblet part than the duct-cells (see Pl. 8, figs. 3 and 5). During active digestion this part diminishes in size, but in both fasting and feeding animals cells in which the goblet part is defined but not stained may be seen. This presumably indicates that the cells in question have discharged their contents and have not had time to supply the part with new material (granules).

With regard to the mode of attachment of the cells to one another I have sometimes observed the intercellular bridges described by Carlier (5 *a*). These, however, are only apparent when the cells appear unduly vacuolated. In sections tangential to the surface I have seen no indications of bridges.

The surface epithelium is continuous with the epithelium of the gland-tubes, the transitional cells losing their goblet portions and staining a uniform bluish colour with Mallory. The transition is short (see Pl. 8, fig. 5, *t*).

THE CARDIAC REGION.

The junction of the oesophagus and the stomach is well defined in the cat, the stratified epithelium of the former stopping abruptly and being replaced by the columnar epithelium of the latter. At this junction a lymph follicle may sometimes be seen, but there is more frequently a large vesicle or cavity lined by one or two layers of cubical cells.

The cardiac region (when present) is extremely narrow, measuring about 2-3 mm. from the cardio-oesophageal junction to the nearest group of parietal or oxyntic cells. It includes only cells of one type (cardiac cells) unmingled with others. Beyond this there is a boundary zone extending for another 3 mm., which contains both oxyntic and cardiac cells. Frequently there is no definable cardiac area; oxyntic cells are found at the junction itself and only the 'boundary zone' is present. Beyond the boundary zone another type of cell, characteristic of the fundus, is met with; this may be regarded as the cardiac limit of the fundic region.

The glands of the cardiac region consist of relatively simple tubes, with short ducts and somewhat wide lumina. In most animals they are fairly numerous, in others only a few such glands are to be found near the oesophagus. They are lined by a single layer of columnar or cubical epithelium, which appears granular in the fresh condition. In sections, however, granules are absent and a fine reticulum is seen in its place. The reticulum is irregularly distributed throughout the cell, and is stained blue by alcoholic eosin and methylene blue, pale magenta by polychrome methylene blue, and blue with Mallory (Pl. 8, fig. 1). In some cases a reticulum which stains reddish with Mallory is present in addition to the above finer reticulum which stains blue. Haematoxylin hardly stains the 'blue' reticulum at all, nor does it tint the spaces between the reticulum. In the case of the other stains just mentioned the spaces are coloured in the same way as the reticulum, but more faintly.

The nucleus is irregularly rounded or ovoid and is invariably situated towards the base of the cell. In a fasting animal the cell is more columnar and the nucleus less flattened than in an animal which has been fed. On the whole, however, there is little change to be noted in these cells.

No compound tubular glands such as have been described by Ellenberger (10), Edelmann (8), Schaffer (27), and others in various animals are present in the cat, nor are any structures resembling crypts of Lieberkühn met with: this also applies to other regions of the cat's stomach.

The simple tubular glands of the cardiac region were first described by Schafer and Williams (26) in the kangaroo, and with their description those of the cardiac region agree. It will be shown later that the cardiac cells do not constitute a special type, but form a variety of mucoid cells, a term which is explained elsewhere.

THE PYLORIC REGION.

The pyloric region is considerably larger than the cardiac in area, although smaller than is generally supposed. It extends

for about 15 mm. from the pyloro-duodenal junction along the greater curvature and about 12–15 mm. along the lesser curvature. Beyond these limits small oxyntic cells make their appearance, and about 20 mm. further full-sized oxyntic and peptic cells are met with in large numbers. Here lies the pyloric limit of the fundic region.

With regard to the general features of the pyloric glands, they have long and wide ducts and become more racemose and exhibit more interglandular tissue near the intestine. Lymph-follicles are numerous in this region of the stomach, several being invariably present at the pylorus itself. At the pyloro-duodenal junction the pyloric glands pass through the muscularis mucosae, which is here incomplete, and become Brunner's glands of the duodenum. The lumen of the glands is large, and this, along with their racemose character, serves to distinguish the pyloric glands from those of the cardia which they otherwise resemble.

The glands of the pyloric region are lined by a single layer of cells, which are columnar or cubical in shape and irregularly reticulated (Pl. 8, fig. 4). They are stained in the same way as the cardiac cell, the whole cytoplasm appearing blue with methylene blue combinations and with Mallory, pale magenta with polychrome methylene blue, and colourless with haematoxylin. As is the case with the cardiac cell, the basal portion of the cell may in some animals be occupied by a second reticulum which stains red with Mallory. This may be seen in fasting and fed animals, but more often in the latter condition. The nuclei are irregularly rounded and situated basally. During activity the cell becomes shorter, indicating a discharge of its contents, and the nucleus appears more spherical, i. e. less compressed.

The similarity between the cardiac and pyloric glands has been noted by many observers (Cobelli (6), Ebstein (7), Schaffer, Stohr (29), and others). Bensley (2, 3) compares the pyloric cells with the cells lining the 'neck' of the fundic gland as well as with the cardiac cells. On the other hand, Heidenhain (13), Langley and Sewall (15), Kranenberg (23),

and all later writers believe that they are fundamentally the same as the 'chief' cells of the fundus. It will be shown later that there can be no doubt regarding their difference from the 'chief' cells, and their resemblance to the cardiac gland-cell is too close not to regard them as identical in structure if not in function.

THE GLANDS OF THE FUNDUS.

Histologically, the portion of the stomach between the cardiac and pyloric regions just described has a uniform structure. The glands of this intermediate area are generally known as the glands of the fundus, though they might be more appropriately termed the glands of the body of the stomach. The general form and arrangement of the fundic glands have already been noted. They are simple tubes with short ducts, and as the glands are closely packed together there is little interglandular tissue.

Three kinds of cell occur in the glands of this region, although hitherto, with the exception of Bensley (1) and Cade (5), histologists have recognized only two, namely 'central' or 'chief' and 'parietal' or 'superadded' cells.

(1) Peptic Cells.—These are usually known as 'chief' cells; they are quite distinct from a second type of central cell which are intermingled with them, and are described later as mucoid cells. Peptic cells occur throughout the lower or deep half of the gland-tube, although it is comparatively uncommon to find this part of the tube lined wholly by such cells. They look somewhat columnar in shape in section, but when isolated are polyhedral.

The cytoplasm contains granules in the fresh state (Langley and Sewall); these are irregular in size. On examination in saline, weak acids, or alcohol, the granules tend to increase in size and become less distinct. Finally they disappear, apparently by passing into solution. A few granules always remain unaffected.

In fixed preparations, whether formol, Zenker, or osmic, the granules are replaced by a coarse but regular reticulum (Pl. 8.

figs. 2 and 3, *p.* XXI). Nevertheless, with both formol and osmic, a few granules may be preserved; this is especially the case after osmic fixation (Pl. 8, fig. 3, *p.* XX, III). The regularity of the reticulum suggests that the extra-granular cytoplasm is coagulated before the granules are dissolved out. The reticulum may therefore be taken as a rough index of the amount and size of the granules contained in the cell.

With regard to their reaction to various dyes, both the reticulum and the granules become intensely stained blue with alcoholic eosin and methylene blue, deep purplish blue with polychrome methylene blue, and brownish violet with Mallory. They are only lightly stained by haematoxylin, but more strongly so by the iron haematoxylin method. The nucleus is irregularly ovoid or rounded; it varies in shape and position according to the activity of the cell.

Functional changes are easily noted in these cells. In the fasting condition the nucleus is found towards the base of the cell and the cytoplasm is reticulated throughout. After a period of activity, i.e. during digestion, the cell gradually shrinks, and the nucleus becomes larger and occupies a more central position. Teased preparations seem to show that the granules are on the whole larger in the fresh condition, while in fixed specimens the meshes of the reticulum are wider. Ergastoplasmic fibres occur at the base of the cell, while the reticulations (granules) diminish near the lumen of the gland. In some cases (five to six hours after a large meal) half of the cell may be occupied by ergastoplasmic fibres. These fibres stain in the same way as the reticulum, although more definitely than it (Pl. 8, fig. 3, *p.* III, and fig. 5, *p.*). Langley was the first to demonstrate the diminution of granules during activity; he also stated that the cells become clearer at their bases. Later Bensley, Zimmermann (30), and Theohari (23) showed that the basal clear zone is occupied by ergastoplasmic fibres (prozymogen of Macallum (19)). These observations I can confirm in the cat. The swelling of the granules during digestion appears to be a stage in the conversion of zymogen into soluble ferment and occurs more rapidly than the formation

of new granules. Hence the diminished reticulated area, and the absence of any increase in the size of the cell, contrary to Heidenhain's observation.

(2) *Mucoid Cells*.—This other type of central cell has somewhat finer granules, and when fixed they are replaced by a fine reticulum (perhaps a precipitate) (Pl. 8, fig. 3, *m*, xx, xxi *b*). No granules ever remain intact after fixation. In the fresh condition these granules are more rapidly dissolved by reagents than those of the peptic cells; this, perhaps, partly explains the entire absence of granules after fixation. Mucoid cells occur mainly in the superficial half of the gland-tube, but are interspaced among the coarser reticulated peptic cells towards the deeper part, and may be found throughout the whole gland-tube. In places a portion of a gland may be lined entirely by these cells. In form they are roughly globular, but variations in shape occur according to their position and fit in the tubule (Pl. 8, fig. 5, *m*).

Their staining reactions render them distinctive. They are coloured a pale blue by alcoholic eosin and methylene blue, a pale magenta by polychrome methylene blue, and a deep blue by Mallory; as is the case with the fasting peptic cells, they are unaffected by haematoxylin. When a definite reticulum is present it stains blue with Mallory, but in some of the cells the basal portion takes on a brownish or even reddish tinge. When there is no reticulum the precipitate-like material invariably stains blue.

The nucleus is small and compressed against the base of the cell: it is generally deeply stained. Changes during digestion consist in the cell becoming first larger and later smaller and staining less heavily with Mallory, while the nucleus appears to be a little more prominent. Mucoid cells are most marked in the boundary zones, where they are continuous with the cardiac cells on the one side and the pyloric cells on the other.

(3) *Oxyntic Cells*.—In the cat these cells are mostly found wedged in between the central cells with a corner abutting on the lumen; nevertheless, they lie sufficiently far outwards to be termed parietal cells. They are most numerous

in the superficial half of the gland, and may form the sole lining of a portion of the gland-tube. They may be found even between the columnar cells of the gland-ducts. In shape (judging from vertical and transverse sections) they are roughly pyramidal, but there are many variations from ovoid to crescentic. Unlike the peptic and mucoid cells the granules of the oxyntic cells are very fine, and are not readily attacked by reagents. They are fixed by all the methods employed; with osmic acid those situated immediately underneath the membrane of the cell may be demonstrated to be lipid in character. Similar observations have been made by Böhm and Davidoff (4) in the rat. The staining reactions of the oxyntic cell-granules are as follows: red with alcoholic eosin and methylene blue, haematoxylin and eosin and Mallory; pale blue with polychrome methylene blue; and dark brown with osmic.

The nucleus is spherical and usually central. Occasionally it is excentric or there may be two nuclei within the same cell.

A number of the cats examined showed the presence of parasitic spirochaetes (Lim (18)). These organisms were sometimes found within oxyntic cells in what appeared to be a single dilated canaliculus, continuity with the lumen of the gland being demonstrated. Otherwise there was no histological disturbance. Vacuoles may often be seen within the oxyntic cells of all animals.

With regard to functional changes, oxyntic cells appear on the whole to become larger (Heidenhain) during digestion and their granules more easily distinguished, being less closely packed together and probably fewer in number. The difference, however, is not marked, and may be partly due to shrinkage of the central cells.

It ought to be noted that oxyntic cells occur throughout the whole stomach, being absent only some 3 mm. from the oesophagus and about 15 mm. from the pyloro-duodenal junction. The oxyntic cells of the pyloric boundary zone are somewhat small in size and are situated mainly in the superficial portion of the gland; they are probably primitive in

character. These have already been described in other animals (Stöhr (29), Trinkler (23), Nussbaum (20)). Nussbaum, however, does not consider these smaller cells to be the same as oxyntic cells.

GENERAL CONSIDERATIONS.

These observations show firstly that the term 'chief' or 'central' cells is inadequate, since there are two types differing widely from each other. Secondly that the cells of the cardiac and pyloric regions are similar in structure and of the same characteristics as the mucoid cells of the fundus. Thirdly that the fundus is the all-important region of the stomach from the point of view of the secretion of gastric juice, the other two regions being small by comparison and containing no recognizable zymogen-secreting cells.

Let us first consider the characters of the two types of central cells. We have seen that the peptic cell is granular (or reticulated) and that after a period of activity the granules diminish in number and are replaced at the base of the cell by ergastoplasmic fibres. In the case of the mucoid cell the cytoplasm is also granular (when fresh), but functional changes do not cause any alteration in its architecture. The nucleus of the peptic cell at rest is irregularly rounded or ovoid, and is applied against the basement membrane, but during digestion is more regular in outline and frees itself from the base so far as to occupy a more central position. The mucoid cell-nucleus, on the other hand, is not markedly changed either in shape or position. There are also the differences in staining reactions. The peptic cell is coloured in an entirely different manner from that of the mucoid cell (compare *m* and *p*, Pl. 8, fig. 3). This difference is manifested not with one staining method alone but with several, although Mallory's is the best for the purpose. Both types of central cell may be seen in man, dog, and rabbit (and also in the frog); they are probably common to all mammals.

There can thus be no doubt regarding the separate existence of these two types of cells. Edinger's theory that all the

varieties of cells found in the stomach are functional modifications of one type is untenable. It is impossible to reconcile this view with the differences in structure and reactions in both fasting and feeding animals.

Heidenhain (13) long ago observed that some chief cells stain more readily with aniline blue than others—and referred this to functional changes. This was later confirmed by Greenwood (12) in the pig's stomach; she suggested that the 'clear' cells might be mucus cells, thus anticipating the results of two subsequent observers. Both Bensley and Cade have distinguished two types of central cells (older observers from Edinger (9) and Pilliet (22) downwards have found various modifications of the central cells but not separate types), which appear to be similar to the peptic and mucoid varieties described here. Bensley was the first to note that the cells of the 'neck' region of the fundic glands stain in the same manner as mucus-secreting cells; these cells he termed 'indulinophilous mucous cells'. Cade confirmed Bensley's finding with indulin and called them 'cellules principales du col'. In the cat the neck region is lined by oxyntic and transitional cells, i.e. cells which have almost lost the division of the cytoplasm into two zones so characteristic of the surface mucous cells (see Pl. 8, fig. 5, *t*). It is the portion of the gland below the neck, therefore, that is lined chiefly by mucoid cells (see Pl. 8, fig. 5). Bensley (2) does state, however, that an occasional 'indulinophilous cell' may be found among the central (peptic) cells of the deeper part of the gland, and from an examination of his figures (Pl. 8, fig. 6) it is clear that the neck region he describes includes the superficial portion of the gland. To him credit is due for their discovery, although a more definite description and wider distribution of the mucoid cells must now be recognized.

'Mucoid' cells are described in only two text-books in English, Schafer's 'Essentials of Histology' (25), and the American edition of Böhm and Davidoff, translated by Huber (4). Of continental works I can only find a mention in Prenant, Bouin, and Maillard (23), who have an excellent diagram in

their text-book of Histology showing typical mucoid cells—which they hesitatingly label ‘cellules principales muqueuses?’—to illustrate the mucus cells of Bensley. It is evident, therefrom, that hitherto the distribution and even the existence of mucoid cells have scarcely been recognized.

The names ‘peptic’ and ‘mucoid’ have been chosen for obvious reasons. The structure of the peptic cell is characteristically that of a zymogen-secreting cell, and by the term ‘chief’ or ‘central’ this cell was meant, so that there is no need to dispute its function. The term mucoid is applied because the cell resembles other mucus-secreting cells, but it is not identical either with the mucus-secreting cells lining the surface or with the goblet cells of the intestine (compare cells *m* and *s* in Pl. 8, fig. 3; also see Lim (17)).

We may next consider the relation between the cardiac, pyloric, and mucoid cells. We have seen that there is little or no difference structurally between the two former (cardiac and pyloric) cells, and that the mucoid cells resemble them in most respects except position. They are stained in the same way, and their structural characters are very similar both during rest and activity. The cardiac and pyloric cells show in some animals a reddish basal reticulum; this may or may not constitute a difference, although it is to be noted that the reticulum is more frequently absent than present. Lastly, they are continuous with each other, for cardiac cells can be traced into the fundus in the form of mucoid cells; the same applies to pyloric cells. The close resemblance which thus exists between these three types (they are all obviously mucoid) presumes a similarity in their functions.

The striking differences in structure between the peptic and pyloric cells have been quite missed by all the workers on pyloric pouches, and it is possible that their histological examination was inadequate to ensure the purity of the pouches which they made. But apart from this the suggestion that pepsin is secreted by cells which are not typical of the zymogen-secreting type calls for a closer investigation into the origin of the secretion of the pyloric pouches.

SUMMARY.

The gastric mucous membrane is principally formed by relatively simple tubular glands which become more complex near the orifices of the viscus, especially near the pylorus. The glands are lined by one or more kinds of cells; the following types may be recognized:

1. Surface mucus-secreting cells, which include the cells lining the surface and the gland-ducts leading therefrom.

2. Mucoid cells, of which there are two closely allied groups, viz.:

(a) The cardiac and pyloric cells which form the sole lining of the glands within about 0-2 mm. and 15 mm. of the oesophageal orifices respectively.

(b) The mucoid cells proper, which occur in the large intervening region (fundus) where they are intermingled with the peptic and oxyntic cells; they chiefly occupy the superficial or upper part of the gland-tube.

3. Peptic cells, which are found (often in conjunction with mucoid cells) within the deep part of the gland; both peptic and mucoid cells were formerly described as 'chief' or 'central' cells.

4. Oxyntic cells, which chiefly occupy the upper portion of the gland where they are found between the mucoid cells; in the deeper portion of the gland they take up a parietal position.

The interglandular tissue contains basiphil connective-tissue cells, oxyphil leucocytes, and a few cells with large eosinophil globules.

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PART II. THE GASTRIC MUCOID CELLS OF FOETAL AND NEW-BORN ANIMALS.

The stomachs of two litters of new-born and of one foetal cat have been examined, and in addition those of three still-born children and one four months' human foetus. The method employed was acid formol fixation; the staining was effected with either Mallory's stain or Heidenhain's iron haematoxylin.

CAT.

In a foetus of about six weeks the stomach exhibits a simple lining of columnar epithelium, which is entirely

devoid of a superficial mucous portion. The cytoplasm stains reddish with Mallory. Only a few invaginations represent the primitive gland-tubes.

At birth short simple gland-tubes are present. They are lined by oxyntic and mucoid cells. Some of the latter are wholly, others are only partially, mucoid, having a portion of non-mucoid (red-staining with Mallory) cytoplasm within the basal half of the cell. The surface cells are similar to those of the adult.

One week after birth the glands are larger and the oxyntic cells more prominent. Mucoid cells are present in large numbers; a few developing peptic (?) cells are visible. These show no mucoid reaction; they are coloured principally by the red and brown dyes in Mallory's mixture. The pylorus is now becoming defined; it contains only mucoid cells.

Three weeks after birth the peptic, mucoid, and oxyntic cells are all plainly evident; the appearance of the mucous membrane now approximates that of the adult.

HUMAN.

In a foetus of about four months the stomach is lined by a mucous membrane of the simple type, bearing only short gland-tubes. These are formed partly by mucoid and partly by red-staining non-mucoid cells; oxyntic cells are as yet absent. The junction between the stomach and the duodenum is sharply marked off by the pyloric sphincter, but the mucous membrane does not show a corresponding division. The pyloric portion of the stomach for some distance from the actual muscular junction contains both goblet and columnar cells with striated borders. The glands are wholly mucoid.

At birth peptic and oxyntic cells are fully developed; the glands are much longer than at four months and altogether more like the adult.

CONCLUSIONS.

It is quite clear that the gastric glands are in the first instance formed of non-mucoid, red-staining cells. Later these

cells become mucoid in character throughout the whole stomach. The next type to differentiate is the oxyntic, and at a later stage still comes the peptic.

Peptic cells are present in the human foetus at birth, but in the cat do not appear until between the second and third week after birth. This difference may give an important clue to the function of the fundic mucoid cells, for it has been observed that the new-born human stomach contains pepsin while the stomach of the new-born cat contains none, and does not exhibit a ferment until the third week after birth ((Hammarsten 1874, Zweifel 1874, Morrigia 1876) quoted by Moore (2), Sewall (3)). Obviously pepsin is not secreted by the mucoid cells.

These cells are essentially primitive, or at least less specialized than either the peptic or oxyntic. Cade arrives at a parallel conclusion from an entirely different point of view (1). He found that oxyntic cells disappear and peptic cells lose their granules in the vicinity of gastero-enterostomy openings, and all the cells appear mucoid in character. He thus inferred that the altered conditions had caused the specialized cells to revert to the more primitive mucoid cells. In cats I have been able to confirm Cade's observation completely.

Thus while the mucoid cells are undoubtedly a definite variety of the gastric gland-cells they are closely allied to the peptic cells to which they give rise in early and perhaps in later life.

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PART III. THE GASTRIC MUCOID CELLS IN MAN, DOG, RABBIT, AND FROG.

The gastric mucous membrane of several species of animal has been examined in order to compare the histological features and the distribution of the mucoid-reacting cells in each

species, and to determine the general relationship which exists between the mucoid group and the peptic cells of the fundus. The technique employed is similar to that referred to in Part I. The material was invariably obtained from the newly-killed or from the living anaesthetized animal. Human material came partly from the operation table, partly from the post-mortem. Acid formol fixation and Mallory's and Heidenhain's methods of staining were the routine procedures.

THE MUCOID CELLS OF THE FUNDUS.

Human.—In man mucoid cells are abundantly present. They have the same characteristics as those of the cat except that their cytoplasm is more homogeneous and stains a lighter blue with Mallory. Their distribution is somewhat different: they form the entire central lining of rather less than the superficial two-thirds of the secreting tubule—hence their regular cubical outline. This portion of the tubule is thinner than the deeper portion which (with rare exceptions) contains typical peptic and oxyntic cells. A few tubules are lined throughout their whole extent by mucoid cells. There is not the same amount of intermingling between the mucoid and peptic cells as in the cat, and thus the mucoid portion of the tubule is more easily defined, especially since it is narrower than the peptic portion.

Dog.—The mucoid cells of the dog are intermediate in appearance between those of man and the cat. In some individuals the cytoplasm is almost homogeneous and stains lightly with Mallory; in others it is more reticular and stains heavily as in the cat. This may be due to functional changes. The distribution of the cells, however, shows fewer mucoid cells in each tubule, i.e. they line less than the superficial half; nor do the mucoid and peptic cells intermingle to any great extent. The widening of the calibre of the deep portion of the tubule occurs gradually as in the cat, but nevertheless the mucoid and peptic portions are sharply marked off from each other.

Rabbit.—The mucoid cells of the rabbit stain faintly blue with Mallory and are nearly homogeneous; they appear like those of man. They are not easily made out since they are

hidden by the numerous overlapping oxyntic cells. This seems to be a very characteristic feature in the rabbit and accounts for the shape of the cells being very irregular. These cells occupy the superficial three-fourths of the tubule, but there is a good deal of intermingling with peptic cells. The deep portion of the tubule rarely shows mucoid cells. This is best shown in iron-haematoxylin-stained sections of the actively secreting stomach, the presence of the overlapping oxyntic cells making it difficult to examine the more centrally situated cells. In the above preparations the peptic cells alone are clearly stained on account of the marked development in them of ergastoplasmic fibres. The mucoid cells are left unstained by iron haematoxylin. The proportion of mucoid to peptic elements in each tubule varies in different parts of the fundus; from two-thirds to four-fifths of the whole tubule may be mainly mucoid.

Frog (*Rana temporaria*).—In the frog's stomach only oxyntic and mucoid cells are to be seen. The latter have a clear cytoplasm which stains a faint blue with Mallory. They are found in the superficial third of the gland-tube and rarely extend to the deeper parts.

THE CARDIAC AND PYLORIC MUCOID CELLS.

The cells forming the cardiac and pyloric glands are so similar in appearance and staining reactions that they may be grouped together for consideration. They differ from the mucoid cells of the fundus in their regular shape and in sometimes exhibiting a red-staining reticulum with Mallory. The extent of the cardiac and pyloric zones along the two curvatures of the stomach have been measured and are set forth below.

<i>Animal.</i>	<i>Cardiac Cells.</i>	<i>Cardiac and Oxyntic Cells.</i>	<i>Pyloric Cells.</i>	<i>Pyloric and Oxyntic Cells.</i>	<i>Curvatures.</i>
Cat	0.4 mm.	3 mm.	15 mm.	20 mm.	Greater
	0.3 mm.	3 mm.	12-15 mm.	20-5 mm.	Lesser
Dog	—	2 mm.	20 mm.	40 mm. ¹	Greater
	—	3 mm.	25 mm.	45 mm. ¹	Lesser
Rabbit	0.1 mm.	2 mm.	35 mm.	2 mm.	Greater
	0.2 mm.	2-3 mm.	40 mm.	2-3 mm.	Lesser

¹ Oxyntic cells small and primitive.

Human.—The pyloric cells of man resemble those of the cat in every respect except that they are longer and stain more lightly. Sufficient material was not available from which measurements of the cardiac and pyloric regions could be made.

Dog.—There are no pure cardiac glands in the dog. Oxyntic cells may be found at the cardio-oesophageal junction along both curvatures, while peptic cells are present within 2–3 mm. of the junction. In this small zone the cells are longer but otherwise show the same features as those of the cat. Racemose glands are very constantly present; they extend from the oesophagus into the cardia under the muscularis mucosae. Their acini are mucous with a few serous crescents here and there. They are thus not to be considered as cardiac glands, but as part of the salivary apparatus which occurs abundantly in the mucosa of the oesophagus.

The pyloric region extends for about 40 mm. along the greater curvature and 45 mm. along the lesser. The boundary zone bearing full-sized oxyntic cells and pyloric cells occupies only about 2 mm., but small (primitive) oxyntic cells may be observed especially at the neck of the glands within 20–5 mm. of the pylorus. The cells, like the cardiac group, resemble those of the cat—the red-staining reticulum being more constantly present; this is best seen in those near the duodenum.

Rabbit.—There are few cardiac glands corresponding to those seen in the cat. These usually occur along the lesser curvature, occupying a small zone of about 2 mm. distal to the oesophagus. Along the greater curvature and sometimes along both curvatures oxyntic cells may be found right up to the cardio-oesophageal junction. When the cardiac glands are present the cells which form them are not typical. They only show a faint mucoid reaction near the surface; elsewhere the cytoplasm is both granular and reticular, and stains reddish with Mallory. The condition appears to be an exaggeration of the 'red reticulum' seen in the cat and other animals. In addition to this peculiarity glands of the racemose type are also met with under the muscularis mucosae. They extend

(along the lesser curvature) for only a very short distance (about 3 mm.). The acini are mainly serous, a few being mucous; the cells lining the terminal ducts have granules in striae and have centrally-placed nuclei. True mucoid and peptic elements are present beyond the cardiac area described above, the former forming a boundary zone of about 3-4 mm. with the oxyntic cells before the latter are met with.

The pyloric region is somewhat larger than that of the cat, since oxyntic cells are only seen about 35-40 mm. from the duodenum (see table, p. 207). There is almost no boundary zone: the peptic cells appearing a few millimetres beyond the oxyntic. The gland-cells are more mucoid than those of the cardia, but like these show a well-marked non-mucoid basal area.

Langley (5) described the cells of the rabbit's fundus along the greater curvature as being finely granular and similar in appearance to the pyloric cells, while the cells of the remainder of the fundus are coarsely granular. I have not been able to make out this distinction, but perhaps Langley took the superficial mucoid cells to be the only kind of central cell and failed to see the peptic (coarsely granular) cells in the deepest part of the mucous membrane.

Frog.—There are no true cardiac glands in the frog; the peptic cells merely stop short at the end of the oesophagus while mucoid and oxyntic cells make their appearance. The pyloric region extends about 3-4 mm. from the duodenum; its gland-cells are not different from the mucoid cells of the fundus.

GENERAL CONCLUSIONS.

The results of this investigation confirm those of Bensley (1) and more especially those of Cude (2), who has examined all the species dealt with here. They show that the fundic mucoid cells vary slightly in appearance in different animals, and that their distribution in the tubule is roughly about the superficial half. From the study of new-born cats it is found that the peptic cell arises from cells of the mucoid type. This

is also probably true for animals other than the cat, since the peptic cells are invariably found in the deep or blind end of the tubule, which may be considered to have developed last. This encourages the view that the mucoid cell gives rise to the peptic cell, without suggesting that the latter is merely a functional phase of the former. Mucoid and peptic cells are undoubtedly different functionally and structurally. In this connexion it is noteworthy that mitoses have never been observed in peptic cells, while they have been seen in mucoid and more frequently in oxyntic cells. In short the mucoid cell is a stage in the genesis of the peptic cell. Transitions from the one state to the other are difficult to demonstrate, but considering the differences, slight though they may be, which occur in the mucoid cells of the same and of different animals, and especially the occurrence of the basal 'red-staining', the gap in the genesis of the peptic cell is perhaps partially filled. Looked at in this light, the observation of Cade on the retrogression of the peptic cells in the vicinity of gastero-enterostomy openings (see Cade (2) and Part II of this paper) may be translated as the inhibition of peptic cell-formation and the arrest of its genesis in the mucoid stage.

Utilizing the above hypothesis, the cells of the cardiac and pyloric glands may be regarded as cells which have been prevented from attaining full development by the conditions existing at the orifices of the stomach.

The relationship between the various gastric cells may therefore be classified as follows. The mucoid cell of the fundus forms the lowest functional type, for it apparently does not secrete pepsin. The cardiac and pyloric cells are a little more advanced, since Klemensiewicz (4) and Heidenhain (3) have shown that the pyloric region secretes a proteolytic ferment. Structurally these cells show the basal 'red-staining' more constantly (especially in the rabbit) than the mucoid cell, and this may be taken as indicating a certain degree of zymogen formation. The cardiac cells may not function exactly as the pyloric cells do, but they are at least cells of the same developmental order, and they constitute such a small element in the

animals under consideration that they probably have no physiological significance. The peptic and oxyntic cells are the most highly specialized.

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3. Heidenhain.—'Pflüger's Arch.', 1878, xviii. 169.
4. Klemensiewicz.—'Jahresb. ü. d. Fort. d. Tierchem.', 1875, v. 162.
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EXPLANATION OF PLATE 8.

(All are from the cat.)

Fig. 1.—A cross section of a gland-tube from the cardiac end of the stomach along the lesser curvature, about 1 mm. from the oesophagus. Animal killed fourteen hours after last meal. Acid formol fixation; stained with Mallory.

Fig. 2.—A cross section of a gland-tube from the cardiac end of the stomach along the lesser curvature, about 6 mm. from the oesophagus. From the same preparation as fig. 1. *m*, mucoid; *p*, peptic; *o*, oxyntic. These cells are in the resting condition.

Fig. 3.—Cells from the glands of the middle region of the stomach. *s*, surface mucus-secreting cells; *m*, *p*, as in fig. 2.

xx, Cat 20; 14 hrs.; Altmann's fluid; Mallory. The peptic cell on the left is somewhat homogeneous (granules intact), while the cell on the right shows the more usual reticulated appearance. Note the cytoplasm of the mucoid cell.

i, Cat 1; 1 hr.; acid formol; alcoholic eosin and methylene blue. The granules in the peptic cell are imperfectly preserved; the cytoplasm stains intensely in a blotchy manner. Note the almost homogeneous appearance of the mucoid cell.

iii, Cat 3; 6 hrs.; acid formol; very dilute polychrome methylene blue. The peptic cell here shows well-marked ergastoplasmic fibres and zymogen granules, and is in striking contrast with the mucoid cell.

xxi a, Cat 21; 24 hrs.; acid formol; iron haematoxylin.

XXI *b*, same tissue; Mallory. The peptic cells show a well-marked reticulated appearance. Note that the mucoid cells also show a reticulum.

Fig. 4.—Cross section of a pyloric gland from the lesser curvature about 19 mm. from the pyloro-duodenal junction. Cat. 21; 24 hrs.; acid formol; iron haematoxylin. The sparse reticulum which can be seen here stains red with Mallory.

Fig. 5.—A longitudinal section of a gland-tube from about the middle of the greater curvature. Cat 3; 6 hrs.; acid formol; Mallory. *s*, *m*, *p*, *o*, as in figs. 2 and 3; *t*, transitional cells; *g*, cell containing large eosinophil globules. This drawing gives an idea of the distribution of the various cells which compose a gastric gland-tube in the fundic region. The cells are in an exhausted condition. Compare the mucoid cells with the peptic, and also this figure with fig. 2.

FIG. 1



FIG. 2

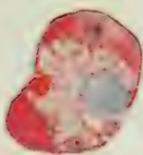


FIG. 4

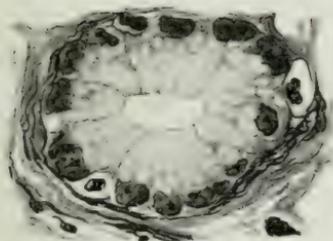


FIG. 5



FIG. 3



On the Labral Glands of a Cladoceran (*Simocephalus vetulus*), with a description of its mode of feeding.

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With Plates 9, 10 and 2 Text-figures.

LEYDIG (12) in 1860 was the first worker to point out that the possession of labral glands is common to all the Cladocera. In 1846, however, Schödler (16) had observed that in the labrum of *Acanthocercus* there exist paired glandular bodies; he states, 'Im sog. Labrum (des *Acanthocercus*) glauben wir ein paar rundliche, fast niereenförmige Conglomerate als drüsige Körper (vielleicht als Speicheldrüsen, glandulae salivales) ansprechen zu müssen'. Claus (3) in 1876 mentioned these glands in his work on the anatomy of Daphnids, and later Cunnington (5) in 1903 described them in *Simocephalus sima* (*Simocephalus vetulus*).

Among the other Phyllopoda, Claus (4) in 1886 mentions and figures the glands in *Branchipus* and *Artemia*. Referring to the labrum he states: 'endlich in dem terminalen Theil die grossen als Speicheldrüsen gedeuteten Drüsenzellen, deren Ausführgangsöffnung und Drüsenstructur auf Querschnitten leicht zu constatieren sind'. Sars (15) states that these glands exist in *Limnadia* and *Limnetis*, and in his figures of other Phyllopoda large cells are indicated in the interior of the labrum.

With regard to the anatomy Claus (3) was the first to give a description in any detail, but apart from this the only description at all complete is due to Cunnington in his description of the glands in *Simocephalus sima*. Claus considered that

the glands could be separated into two groups, the first group lying under the brain and over the oesophagus and the second group consisting of very large cells lying nearer the tip of the labrum. The first group sent out a long thin efferent duct which, after making many twists, allowed the exit of the secretion in front of the mouth. Cunningham's description differs essentially from this in that he could not observe a duct from the first group but did observe an efferent duct from the second group. Cunningham also distinguishes two groups of cells—a proximal group of several small cells and a distal group of large cells. The proximal group, he states, lie close against the chitinous cuticle and are obviously modified epidermal cells and possibly act as replacement cells, taking the place of cells in the distal group when these lose their secretory power. The latter group usually consists of four cells only and these are placed one behind the other, the most extreme possessing a duct opening on the inner side of the labrum. They have characteristic nuclei, which are shaped like a hollow bowl and thus appear circular or semi-circular in section. The secretion is formed in the neighbourhood of the nuclei in the form of little drops which fuse to larger drops or rods or bands and pass to the exterior. Cunningham suggests that the duct of the extreme cell of the distal group acts as a common duct for the whole group.

METHODS.

For *Simocephalus vetulus* the best fixative was found to be cold saturated sublimate in distilled water. This gave excellent fixation and did not produce distortion as did most other fixatives. Good results were also obtained with a mixture of equal parts of saturated sublimate in distilled water and 1 per cent. osmic acid. This mixture, a modification of Mann's fixative, was allowed to act for about an hour. In comparing *Simocephalus* with other Cladocera, it was found that for *Daphnia* the best results were obtained with sublimate acetic acid, while for *Graptolebris* and *Campitocercus* Carnoy gave the best fixation. A young *Chiro-*

cephalus metanauplius was fixed in cold saturated sublimate and was found to be very well fixed.

Ehrlich's haematoxylin was used considerably for staining. Iron haematoxylin gave too intense a stain for the gland-cells. The best differential stain, however, was obtained by using Mallory's triple method for connective tissue.

The fixed material was embedded direct into paraffin and cut 8μ .

ON THE ANATOMY OF THE LABRAL GLAND.

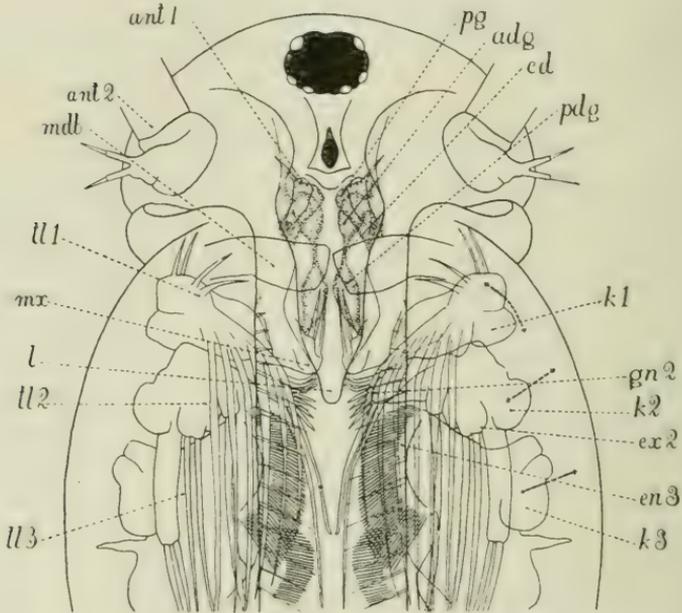
The two groups of gland-cells, as described by Cunningham, were found to be very distinct and will be described separately, but before doing so the extent and position of the labrum must be stated. The labrum, or upper lip, is an immediate prolongation backwards of the ventro-posterior part of the head, passing ventrally to the two laterally-working mandibles and ending under the maxillae which are immediately behind the mandibles. When viewed from the ventral side it may be described as dagger shaped, but its contour is peculiar and reference must be made to Text-fig. 1, which is a ventral view of the animal as it is seen resting normally in a watch-glass, and to Pl. 10, fig. 13 which is a diagrammatic lateral view of the animal. Anteriorly the labrum is marked off from the dorsal part of the head by a groove on each side (Pl. 9, fig. 3) which extends forward to the level of the nauplius eye and then expands dorsally into the bay from which arises the second antenna.

In the living animal the labral glands can be seen indistinctly in the anterior part of the labrum and are of a pale-yellow colour, as was observed by Leydig (12).

Proximal Group.—This consists of two laterally placed groups of epidermal cells which almost meet in the mid-ventral line between the first antennae. Each group commences just in front and close to the ganglion of the nerve to the first antenna (Pl. 10, fig. 13), and extends postero-dorsally over a lozenge-shaped area lining the lateral cuticle of the labrum as far back as the labral nerve (Pls. 9 and 10, figs. 1-4 and 13). Each group consists of about twenty cells, and the nuclei of these

vary in size, being smallest at the base of the first antennae and largest about the centre of the group. The nuclei are usually about 20μ long, but the smallest are never less than half this

TEXT-FIG. 1.



Semi-diagrammatic ventral view of *Simocephalus vetulus*. The thick dotted lines ending in arrow heads on the animal's left side indicate the direction and extent of the normal movement of the appendages figured on that side.) *adg*, anterior pair of distal gland-cells; *ant 1*, first antenna; *ant 2*, second antenna; *cd*, connexion between anterior and posterior pairs of distal gland-cells; *en 3*, proximal endite of third trunk-limb; *ex 2*, exopodite of second trunk-limb; *gn 2*, gnatho-base of second trunk-limb; *k 1*, *k 2*, *k 3*, branchiae of first, second, and third trunk-limbs respectively; *l*, labrum; *mdb*, mandible; *mx*, maxilla; *pdg*, posterior pair of distal gland-cells; *pg*, proximal gland; *ll 1*, *ll 2*, *ll 3*, first, second, and third trunk-limbs respectively.

length. For comparison it may be stated that the length of the nuclei of nerve-cells or of muscle-cells, which are of very uniform size and oval shape, is 4μ . Thus the volume of these large gland-cell nuclei must be many times, at least twenty

times, that of the nucleus of a nerve- or muscle-cell. The chromatin in these nuclei is distributed fairly evenly in small clumps (Pl. 10, fig. 9), and there is a conspicuous oval nucleolus which stains red with Mallory's stain. The cell outlines are not distinct, but where one would expect the cell boundaries to be there are accumulations of large clear vacuoles (Pl. 10, fig. 9), undoubtedly the secretory product of these cells. In the peripheral cells of this group the cytoplasm is not very vacuolated, the vacuoles being very markedly intercellular; but more centrally and towards the anterior end the whole of the cytoplasm of the cells is full of small vacuoles while the larger vacuoles lie in between the cells. In this region the proximal group is seen to be attached to the distal group of gland-cells (Pls. 9 and 10, figs. 3 and 9).

The proximal group is supplied by a small branch of the nerve to antenna 1 which comes off very near to the brain. There is no efferent duct from the proximal group as described by Claus.

The Distal Group.—The distal glands (Text-fig. 1) on each side consist of five cells, four gland-cells and a duct-cell. The gland-cells are arranged in two pairs situated anteriorly and posteriorly, connected with each other—the hinder pair embracing the duct-cell.

The anterior pair of cells are in direct connexion with the posterior side of the nerve to the first antenna at a point a little further from the brain than the branch to the proximal group (Pls. 9 and 10, figs. 2 and 13), and there is a conspicuous group of nerve-cells in the nerve in this region (Pl. 9, fig. 2). Laterally, as stated above, these cells are connected with the proximal group, and at this point the vacuolated cytoplasm of the proximal gland-cells is seen to be continuous with that of the distal gland-cells, the vacuoles passing freely from one group to the other (Pl. 10, fig. 9). The peripheral cytoplasm, except at this point of juncture, is denser than that in the interior of the cells, and is free from vacuoles of secretion (Pl. 10, fig. 9). There is no distinct division between these two cells, but in between the two nuclei there is a confused mass of vacuoles.

Centrally these vacuoles coalesce and form an irregularly flat, ill-defined reservoir (Pl. 10, fig. 9). The vacuoles are not very transparent, and in passing from the proximal glands to these two cells of the distal glands one can see the vacuoles becoming more opaque.

The nuclei are not cup-shaped as Cumington (5) stated to be the case generally with the nuclei of the distal glands, but are roughly spheroidal (Pl. 10, fig. 9). Their diameter is not usually so great as the length of the largest nuclei in the proximal group, but there is probably not much difference between the volumes of these nuclei. There are larger clumps of chromatin in the nuclei than in those of the proximal group, and also the nucleoli, which stain red with Mallory's stain, are about twice as large. But there is also a diffuse scattering of chromatin all through the nucleus which gives it a much darker appearance in a stained preparation.

These two anterior cells of the distal group are connected by an attenuated process with the two posterior gland-cells (Text-fig. 1; Pl. 10, fig. 13). The reservoir in the anterior pair is not continuous as a duct through this drawn-out connexion, but vacuoles are to be seen here, so that presumably the secretion can pass from the anterior to the posterior pair of cells. This connexion is always attached to the dilatores oesophagi (Pl. 9, fig. 4), and its middle point is a little posterior to the labral nerve loop (Pls. 9 and 10, figs. 4 and 13).

The nuclei of the posterior pair of gland-cells are cup-shaped, as Cumington states. Most of the nucleus forms a thin lamella but there is usually a swelling in the region of the nucleolus (Pl. 10, fig. 12). This is large and usually flat and shows the same staining reactions as the nucleoli of the other gland-cells. The chromatin is gathered together in clumps as shown in Pl. 10, fig. 10, but a more irregular clumping as shown in Pl. 10, fig. 12, is more characteristic.

The cytoplasm is pervaded with vacuoles of secretion which are opaque to varying degrees, and these are very conspicuous in the hemispherical recesses formed by the nuclei. As before,

there is no distinct division between these two cells, but the nuclei are placed with their concave sides facing towards each other and in between the two is a very conspicuous and clearly-defined reservoir (Pl. 10, figs. 10 and 12). This is apparently formed of a flat plate of transparent coalescing vacuoles of the secretion produced by the gland-cells.

Neither of these cells possesses an efferent duct as figured by Cunningham, but posteriorly they embrace a separate duct-cell (Pls. 9 and 10, figs. 6, 11, and 12). This cell has the form of a tube opening to the exterior at its posterior end and anteriorly opening into the reservoir of secretion. The lumen of this tube is often flat (Pl. 10, fig. 11) especially at its posterior end. The nucleus of this duct-cell stains very lightly and is small compared with that of a gland-cell, although it is slightly larger than that of a nerve- or muscle-cell. The cytoplasm stains very lightly and is not vacuolated.

In sublimate material there is in the secretion reservoir a granular coagulum which stains faintly blue with Mallory's stain, while in the lumen of the duct-cell it stains red. Presumably the cytoplasm of the duct-cell alters the constitution of the secretion in some way, so that its staining reaction when fixed is changed. A section through the duct-cell at its anterior part shows the secretion in contact with the walls of the tube staining red, while that more centrally placed, which has not yet been acted upon by the duct-cell, still stains blue. The external apertures of the duct-cells form two small slits on the side of the labrum near its tip (Pl. 9, fig. 7) where the latter is compressed laterally. They are situated a little towards the dorsal surface of the labrum and are ventral to about mid-way between the mandibles and maxillae.

In other Daphnids studied it was not found possible to obtain preparations sufficiently well fixed on which to base critical considerations, but it is evident that the same ground-plan underlay all the cases studied. In *Chirocephalus*, however, the results obtained are very good and agreed comparatively well with Claus's (4) figure for *Branchipus*. The proximal group is very scattered and ill defined. Its cells do not all line

the chitinous cuticle, but, however, they are connected with the gland-cells of the distal group and loosely fill the anterior part of the large labrum. The distal group is represented by three pairs of gland-cells—two placed laterally and one medially—slightly nearer the tip of the labrum. The nuclei of these cells are very large but not cup-shaped. In each pair of cells is a secretion reservoir which opens into the lumen of a very conspicuous duct-cell just as in *Simocephalus vetulus*.

ON THE MANNER OF FEEDING.

Simocephalus vetulus feeds on small particles and planktonic organisms contained in a current of water which it maintains over its mouth appendages. In observing the animal it is usually on its back as figured in Text-fig. 1, but in describing the method of feeding, to avoid confusion, the animal will be assumed to be dorsal side uppermost.

The valves of the carapace form an incomplete tube about the posterior part of the animal, this tube being effectively completed by the hairs along the ventral edges of the carapace (Text-fig. 1). Posteriorly the tube is open to the exterior and anteriorly it expands at each side of the labrum into the bays from which arise the second antennae. Further, this tube is incompletely divided into a dorso-lateral chamber, which includes the brood-pouch and in which are the branchiae, and a median ventral food passage. The latter is bounded dorsally by a well-marked food groove (Pl. 9, figs. 6, 7, and 8) which runs along the ventral side of the trunk. Ventral to it are the hairs along the edges of the carapace while laterally are the trunk limbs. The current of water carrying the food passes in at the bases of the second antenna, and so passes close to the first antenna on which are situated, according to Scourfield (17), the supposed olfactory organs, and passes out at the postero-ventral angle of the carapace in the neighbourhood of the anus.

The appendages chiefly responsible for maintaining the food-stream are the first, third, and fourth trunk-limbs. Calman (2) states that the third and fourth pairs of trunk-limbs 'are

characterized by the development of the proximal endite with its comb-like row of setae'. These endites are placed almost vertically with their setae pointing upwards into the food groove. They diverge slightly from behind forwards and in passing upwards towards the trunk they slope inwards. They move in and out laterally. From the fact that they are nearest together at their posterior end the outward movement sucks in the water from before backwards. Since also they are not placed vertically but are slightly further apart at their proximal end than they are at the end of the comb of setae in the food groove, the outward movement, in all probability, causes a small backwash in a forward direction in the food groove.

Although the food current is produced mainly by the third and fourth trunk-limbs the first also plays an important part. The shape and arrangement of the first trunk-limbs can best be seen from Text-fig. 1. Its setae form a curved shield over; that is, ventral to, the second trunk-limb. In its normal movement it synchronizes with the other trunk-limbs but is not in the same phase. It commences its backward stroke just after the other limbs begin to beat outwards. The outer part of the limb moves in an arc of a circle with the tip of the labrum as centre (Text-fig. 1) so that those setae which lie against the side of the labrum scarcely move at all. The two limbs together thus form a funnel-like entrance to the food passage down the centre of which projects the labrum. The reason for the retarded lateral movement of this pair of limbs is not at all certain, but in all probability it is to secure a passage of water over the branchiae.

The second trunk-limbs are peculiar in possessing a large and specialized *processus maxillaris* or *gnathobase*. Their exopodites or outer branches lie over, that is ventral to, the succeeding limbs, and their function is probably merely to assist by their oar-like movements in maintaining the food-stream. The *gnathobases* point inwards and are beset with setae which point inwards and almost meet in the middle line. On each *gnathobase* there are ten setae. The posterior seven point backwards while the anterior three point forwards. They

are numbered in Text-fig. 2 from behind forwards. No. 1 is very long, reaching back to the hind end of the body, and is beset with long hairs. No. 2 is much shorter and ends in a small hook, and possesses a comb-like row of minute closely-set hairs over a little more than half its length on one side. Nos. 3, 5, 6, and 7 really form a series. They are short stout setae ending in a brush-like tuft of hairs. No. 4 differs slightly from them in being shorter but terminating in a long thick

TEXT-FIG. 2.



Gnathobase of second trunk-limb of *Simocephalus vetulus*
(for explanation see text).

hair projecting beyond the rest. Lilljeborg (13) does not figure this difference. Nos. 8, 9, and 10 have the form of forwardly projecting combs. No. 8 is usually bent at an angle at about its middle point, while Nos. 9 and 10 are curved. The hairs on No. 8, which only occur on its distal half, are very fine and regular, and are about twice as closely set as those on Nos. 9 and 10, which occur along the whole length of the setae. During the movement of the second trunk-limb outwards and forward the gnathobase also moves upwards so that its three anterior setae comb the side of the tip of the labrum. When the limb is in its most forward position these three setae have passed across the labrum on to the maxillae.

Each maxilla is armed with three setae which do not point dorsally as figured by Cumington (5), but point forwards along

the food groove (Pl. 10, fig. 13, and Pl. 9 figs. 5 to 8). Each seta is beset with a double row of hairs on its inner side. During the movement of the maxillae the setae move backwards and forwards and in their forward movement move inwards, so that the hairs of the opposite setae meet in the food groove.

At the hinder margins of the biting surfaces of the mandibles there are blunt spines (Pls. 9 and 10, figs. 6 and 13), while the anterior parts are scored with vertical serrated ridges. The two mandibles, which are never symmetrically apposed to one another, appear to work like two cog-wheels fitting into one another and thus crush the food and at the same time force it forwards into the beginning of the oesophagus, up which it rapidly passes by peristalsis.

The mechanism of the method of feeding is as follows: food particles in the food-stream, drawn in by the action of the united movements of the trunk-limbs, are diverted towards the median groove along the side of the labrum, by the first trunk-limbs. At the tip of the labrum they are caught by the anterior setae of the gnathobases of the second trunk-limbs and brushed dorsally into the food groove above the tip of the labrum and between the maxillae. The brush-like setae of the gnathobase are in all probability the main agents in bringing this about. The more anteriorly-placed comb-like setae which brush the side of the labrum also assist in collecting the food on to the maxillae, but their chief function seems to be to brush the secretion of the labral glands on to the food as it collects between the maxillae. Hardy and MacDougall (8) state that when the food is swallowed it consists of particles—'which are glued together by some sticky substance'. It is suggested that this sticky substance is the secretion of the labral glands. The food which collects as a bolus between the two maxillae is now and again pushed forwards by the movements of the appendages on to the mandibles. Pl. 10, fig. 13, shows how the hairs on the setae of the maxillae point forwards, and Pl. 9, figs. 6, 7, and 8, show how the hairs of the adjacent setae fit together and so make an admirable broom for sweeping a bolus forwards on to the mandibles. A movement of the maxillae

is always followed immediately by a movement of the mandibles, but the latter rotate many times without any movement of the maxillae, so that probably the maxillae push forwards a large bolus on to the mandibles and these gradually pass it into the oesophagus.

Hardy and MacDougall (8), referring to *Daphnia*, which is no doubt essentially similar in its feeding to *Simocephalus vetulus*, state that food particles are carried over the mouth by a current of water and 'many of them adhere to the sticky surfaces of the mouth appendages', and that these adherent particles are formed into a bolus by the movements of the appendages. To observe the method of feeding these workers fed the Daphnids on milk, yolk of egg, and carmine. When the animals are fed on any of these substances they always become dirty, the particles adhering all over their bodies. With the former two substances they become greasy and break through and adhere to the surface of the water. It is thought that this is merely due to the presence of an abnormally large quantity of food. In the normal animal, feeding on its normal food, no particles are to be seen adherent to the appendages. If the animal is at all moribund it soon becomes covered with adherent particles.

If the animal be fed on milk—a drop of milk is carefully placed at the bottom of a watch-glass containing the water in which the water-fleas are swimming—the regular movement of the appendages is often stopped while the setae of the first trunk-limb are combed over the lateral surface of the labrum to remove any milk adhering to it. Also by this method of feeding a large amount of fatty drops collect in the food groove posterior to the maxillae. These are in all probability drawn there by the backwash previously mentioned that must pass out along this groove. When this accumulation of food becomes too great the labrum is raised by its levator muscle—which runs from the base of the labrum to the covering of the brain—the trunk is flexed forwards, and, with the caudal furca, the accumulation is lifted out of the food groove and, by the extension of the body, removed to the exterior.

ON THE FUNCTION OF THE LABRAL GLANDS.

Some preliminary experiments of staining *Simocephalus* *intra vitam* suggested that further investigations might elucidate the functions of the labral glands. The experiments which were accordingly carried out did not prove of much use in the direction expected, but were interesting and will be described here.

Fischel (6) describes experiments on *intra vitam* staining using, among other stains, alizarin, neutral red, Bismarck brown, Nile blue sulphate and hydrochloride. In repeating his experiments using the stains named, the only stains with which successful results were obtained were neutral red and Bismarck brown. It may be mentioned that these two stains were Grüber's chemicals while the others were not.

In Fischel's figure of *Daphnia magna* stained *intra vitam* with neutral red, there are figured two large red patches in that region where the labrum should be drawn which probably represent the labral glands. He states that these glands are always to be found faintly stained in animals stained *intra vitam* with neutral red. In adult *Simocephalus vetulus* the most conspicuously-stained organs in such animals are the labral glands and the body which Fischel describes as a gland of unknown nature, which has since been shown by Langhans (10) to be the end-sac of the shell gland, and both these stain intensely. In the labral glands both proximal and distal groups stain, but the duct-cell remains unstained. The connexion between the anterior and posterior pairs of cells of the distal group appears very distinctly, and was at first thought to be a distinct duct. In the gland-cells there appear accumulations of an intensely staining material—these accumulations being often as large as the nuclei of the cells. The reservoir of secretion which can be seen in the living animal remains unstained.

Fischel maintains that the staining with neutral red is not due to the staining of passive metabolic products but to the staining of preformed elements in the protoplasm. In support

of this he states: 'ist einmal die Granularfärbung eingetreten, so bleibt sie auch konstant, das Bild derselben ändert sich in keiner Weise, wie lange auch die Tiere beobachtet werden mögen. Und was ebenso wichtig ist, färbt man eine grössere Anzahl von Tieren, so weisen Zellen der gleichen Art stets auch die gleiche Granulierungsart auf'. In the experiments on *Simocephalus vetulus* no such constancy was observed in the labral glands. While these remained stained they did not continually present the same appearance; moreover, not only did the glands of different individuals stain differently, but the glands of the different sides of the same individual stained differently, which is what one would expect from the mobile, vacuolated nature of the protoplasm constituting the labral glands. However, quite apart from this case, this constancy in the appearance of a cell stained *intra vitam* with neutral red does not agree with the fact that by such staining methods the mitochondria are stained (Gatenby (7)). Lewis and Lewis (11) have shown that not only do mitochondria continually change their shape but also are continually shifting their position.

If specimens are fixed in sublimate after staining *intra vitam* with neutral red and dehydrated rapidly some of the stain remains in the specimen. If they are now embedded and sectioned, on mounting the ribbon the stain can be seen in patches in the labral gland, and the position and shape of these can be drawn with reference to the contour of the glands. If now the wax is removed and the sections brought down to water the remaining stain is washed out. Staining now with an aqueous solution of thionin there appear dark bodies in the section staining an intense violet, almost black, and these patches agree with those stained by the neutral red. In sections of the animals which have not been stained with neutral red but which had been similarly fixed and stained in thionin, these very conspicuous dark bodies do not occur, and it seems safe to assume that they are formed by the action of the neutral red on the animal.

Weak solutions of neutral red apparently always have a harm-

ful effect on *Simocephalus vetulus*. No individual of *Simocephalus exspinosus* was found to survive a weak solution longer than twelve hours. In *Simocephalus vetulus* the movements of the limbs is always retarded when the animals have been in such a solution for about twelve hours. Advantage was taken of this fact to study the movements of the limbs during feeding. Usually, even if the stained individuals are removed to pure water, they survive only a few days. Sometimes, however, with young individuals they survive and completely lose all effects of the stain. No adults have been obtained to survive long the effects of the stain, but among these adults the stain often shows signs of disappearing and yet the labral glands always remain as conspicuously stained as at first. It was thought from these results that the labral glands might be partly the agents causing the disappearance of the neutral red. However, in the well-known experiment of feeding Daphnids on carmine, while the end-sac of the shell gland is stained by the carmine there is never any trace in the labral glands. This experiment was also repeated with neutral red, Bismarck brown, Nile blue sulphate and hydrochloride, using a filtered mixture of the stain with milk to feed, but there was no indication as to where the stain was excreted.

Apparently with neutral red and Bismarck brown the staining effect is not produced through the gut but the stain acts directly through the cuticle. Thus young embryos in the brood-pouch stain just as markedly as their parent. Both these stains show a great affinity for yolk. Individuals with nearly fully-developed embryos in the brood-pouch were stained in neutral red for twenty-four hours. Those individuals were then selected which had given birth to their brood, but had not yet laid their next batch of eggs, and these had deeply-stained ovaries. These were returned to fresh water. The eggs which were subsequently laid were stained deep red. As these developed the stain was seen to be confined chiefly to the yolk. In most cases the adults died before giving birth to the young but in a few cases the young were born, but the adults never

survived the succeeding ecdysis. The young in these cases showed stain chiefly in the tips of the first and second antennae and in the 'Haftorgan'. By the third instar all traces of the stain had disappeared.

These experiments show a similarity to those of Sitowski (18) on *Tineola biselliella*. This worker fed these caterpillars on food stained with Sudan red, and their fat became stained red, giving them a red appearance. The eggs laid were also stained red while the animals hatching from them showed signs of a slight red coloration.

They are also most probably similar to a certain experiment of Agar (1) on *Simocephalus vetulus*. In Agar's experiment he fed the Daphnids on a food which produced in them a curious abnormality, which consisted in a change from the normal, of the curvature of the valves of the carapace. On removing the abnormal individuals to normal conditions the abnormality disappeared in a few generations, and up to this point the result is analogous to Sitowski's results and to the experiment recorded here. However, Agar states that not only did the abnormality disappear, but in the third generation of the offspring there was a 'very decided reaction'—the valves of the carapace not only came back to their normal position but overshot the mark and became more curved in the opposite direction. This is stated to be due to the overproduction of an anti-body antagonistic in its effects to the substance causing the abnormality. This occurrence of a reaction was supported by a table of ratios representing the transmission of the abnormality, and about this table Agar says that, by itself, 'it cannot be said to give unequivocal evidence, especially when the high degree of inaccuracy in the original measurements is considered', but that this table bore a 'striking resemblance' to a second table representing the transmission of another abnormality which was based on much more accurate measurements and on a much greater number of individuals. But, even supposing that this latter table accurately represents the course of the second experiment, the value of the resemblance

between the two tables as a basis on which to postulate similarity between the two sets of experiments, of which the tables are representative, depends solely on the accuracy of the first table. While it is admitted that the second table is no doubt comparatively accurate, it must be emphasized that in the first table referring to the abnormal curvature of the carapace, the presence of a reaction in F₃ generation was indicated by an increase among only forty-seven individuals, of 6 per cent. over a normal ratio—and in measuring this ratio an error could be made of as much as 20 per cent.—the average error according to a table quoted by Agar to show this inaccuracy is roughly 10 per cent. It appears very uncertain, on such data, to make the definite statement that there was a 'very decided reaction'. The repetition of these results of Agar was abandoned because, in the individuals used, the inaccuracy of the measurement of the ratio which indicated the extent of the abnormality was even more marked than in those used by Agar.

It may be mentioned here that Agar merely stated that the food producing the abnormality was a 'culture of protophyta grown in a mixture of cowdung, soot, and water'. It was found that the abnormality can be produced by feeding a culture containing no other protozoon than a species of *Chlamydomonas*. Also, contrary to Agar's finding, it was not found possible to produce the abnormality in *Simocephalus exspinosus*.

In the experiments recorded here there seems no evidence as to how the neutral red disappeared. As Agar suggests for cases of parallel induction, it may have disappeared by mere dilution caused by the increase in the bulk of the protoplasm without a corresponding increase in the amount of the stain. Partly the stained matter may be oxidized or changed in some way into a colourless material which may or may not be excreted ultimately.

These experiments on *intra vitam* staining were carried out before the mechanism of feeding was closely studied. The latter investigation made it obvious that, as already stated,

the food is entangled in some substance before it reaches the mouth, and from the disposition of the appendages and their method of working it seems most probable that this substance is produced by the labral glands. This brings *Simocephalus vetulus* into the same category of feeders as those *Gastropoda*, *Pelecypoda*, *Protochordata*, and *Branchiopoda* whose method of feeding is described by Orton (14), in which the prehension of the food is brought about by the secretion of some food-entangling substance. The nature of this substance in *Simocephalus* is, however, peculiar. Sections of the glands were stained according to the method recently described by Keilin (9) using thionin as a metachromatic stain for mucin. The nuclei of the gland-cells stained blue while the cytoplasm was purplish, as would occur in a mucous gland, but the secretion filling the reservoir not only did not stain red, as it would do if it contained mucin, but showed a pale-blue tint. Bismarck brown also left the contents of the reservoir unstained. If, then, the metachromasy of thionin is used as a definite method for the detection of mucin, the labral glands of *Simocephalus vetulus* must not be described as mucous glands.

From the quotation from Schödler's work on *Acanthocercus* at the beginning of this paper it will be seen that he suggested that the labral glands were possibly salivary glands. Claus does not discuss their function but merely states: 'Die grossen Zellen der Oberlippe . . . betrachte ich als Lippendrüsen'. Cumington, discussing the physiological significance of the secretion from the labral glands, states that the fact that the secretion flows out in front of the mouth suggests that the gland is a salivary gland.

The term 'salivary gland', derived as it is from vertebrate and more especially human anatomy, is now unfortunately used in a variety of senses in the different groups of animals. In some groups a certain physiological sense is implied, while in others the term is used only in a topographical sense. Among the *Arthropoda*, it is not possible to find, from the physiological sense, a character common to all the secretions of their salivary glands, while from a morphological standpoint,

owing to the very uncertain homologies of the various mouth parts in the different classes, it is not advisable to base a definition of salivary gland in this group on such considerations. Hence the term salivary gland should not be extended still further to include the labral glands of *Simocephalus vetulus*.

SOUTH KENSINGTON.

August 1921.

SUMMARY.

1. The labral glands consist of a proximal and a distal group of gland-cells.

2. The proximal group consists on each side of about twenty cells. The cells possess large flat nuclei and their secretion collects as intercellular vacuoles.

3. The distal glands which are in connexion with the proximal groups consist on each side of five cells—four gland-cells and a duct-cell. The anterior pair of gland-cells possess large spheroidal nuclei between which is an ill-defined reservoir of secretion. The posterior pair have cup-shaped nuclei between which is a very definite reservoir of secretion.

4. The duct-cell is in the form of a hollow tube, one end opening to the exterior near the tip of the labrum and the other end opening into the reservoir of secretion between the nuclei of the posterior pair of distal gland-cells. The duct-cells act as ducts for the whole of the labral glands, the secretion passing as vacuoles from cell to cell.

5. The duct-cell alters the reaction of the secretion before passing it to the exterior.

6. Food particles carried in the stream which is maintained by the trunk-limbs through the carapace are abstracted by the gnathobases of the second trunk-limbs.

7. There are ten setae on the gnathobase of the second trunk-limb, the anterior three of which are comb-like and brush the secretion of the labral glands on to the food particles as they collect between the maxillae.

8. The setae of the maxillae are directed anteriorly, and by their action pass the food on to the mandibles at the entrance of the oesophagus.

9. The labral glands stain very markedly *intra vitam* with neutral red and Bismarck brown. There is no evidence that this effect is due to the staining of the preformed structures in the protoplasm.

10. Females stained *intra vitam* with neutral red, when removed to fresh water will lay red eggs from which young will hatch which are also stained. The stain disappears from these during growth.

11. Agar's experiments on the transmission of an abnormality produced by a certain food are criticized. This abnormality can be produced by feeding *Simocephalus vetulus* with *Chlamydomonas*.

12. The secretion of the gland contains no mucin.

EXPLANATION OF PLATES 9 AND 10.

DESCRIPTION OF FIGURES.

All figures are from Camera lucida drawings. Figs. 1-8 were drawn using a Zeiss D. objective and are at a magnification of 222 diameters. Figs. 9-12 were made with a Zeiss apochromatic N.A. 1.4, 2 mm. objective and compensating ocular 8. The magnification is about 860.

LIST OF ABBREVIATIONS.

a.l.g. external opening of duct of labral gland; *br*, brain; *c.c.* circum-oesophageal commissure; *d.c.* duct-cell; *d*, duct of labral gland; *d.o.* dilatores oesophagi; *e.c.* epidermal cell; *f.g.* food groove; *ga 1*, ganglion of Antennarius 1; *g.pg.* group of nerve-cells in Antennarius 1 at root of branch to proximal group; *l*, labrum; *l.d.c.* lumen of duct-cell; *l.g.* lateral groove dividing labrum from dorsal part of head; *ll.* labral nerve-loop; *md*, mandible; *mg*, mid-gut; *mx*, maxillae; *n.a. 1*, Antennarius 1; *n.a. 2'*, Antennarius 2 major; *n.a. 2''*, Antennarius 2 minor; *n.md.* mandible nerve; *n.mx.* nerve to maxilla; *n.n.e.* nerve to nauplius eye; *n.pg.* nerve to proximal group of gland-cells; *nl*, nucleolus; *nu.p.g.* nucleus of proximal

gland-cell; *nu.a.d.* nucleus of cell of anterior pair of distal gland-cells; *nu.p.d.* nucleus of cell of posterior pair of distal gland-cells; *nu.d.c.* nucleus of duct-cell; *oe.* oesophagus; *o.g.* olfactory ganglion; *p.c.* peripheral layer of cytoplasm of anterior pair of distal gland-cells; *r.* reservoir of secretion; *s.m.* setae of maxilla; *v.* vacuoles of secretion.

Figs. 1-8 form a series of transverse sections through the labrum and adjacent parts of an adult specimen of *Simocephalus vetulus*. The position of these sections is indicated in fig. 13 by the series of vertical lines numbered 1-8 at their upper ends. Figs. 9, 10, and 11, are drawings at a higher magnification of parts of the same sections that are represented in figs. 3, 5, and 6 respectively. The orientation of figs. 9-11 with respect to the plate is the same as it is in figs. 3, 5, and 6. In figs. 1-8 and in fig. 13 the proximal glands are shaded thus */// //////////////* and the distal glands are shaded thus *////////////////*.

Fig. 1.—Section cutting the most anterior part of the proximal gland at the level of the nerve to the first antenna.

Fig. 2.—Section through anterior end of the distal gland. This section passes through the nerve to the proximal gland.

Fig. 3.—Section through the connexion between the proximal and distal groups of gland-cells.

Fig. 4.—Section through the attenuated connexion between the anterior and posterior pairs of gland-cells of the distal gland. This section passes through the commencement of one of the mandibles and includes the labral nerve-loop.

Fig. 5.—Section through the posterior pair of cells of the distal gland. This section includes the tips of the hairs on the setae of the maxillae.

Fig. 6.—Section through the duct-cell.

Fig. 7.—Section through the external opening of the duct of the labral gland.

Fig. 8.—Section through the maxillae and the tip of the labrum.

Fig. 9.—See fig. 3.

Fig. 10.—See fig. 5.

Fig. 11.—See fig. 6.

Fig. 12.—Horizontal section, the position of which is indicated in fig. 13. It passes through the aperture of the duct of the labral gland.

Fig. 13.—Somewhat diagrammatic figure of a side view of *Simocephalus vetulus*. Of the appendages behind the antennae only the right mandible and maxilla are represented.

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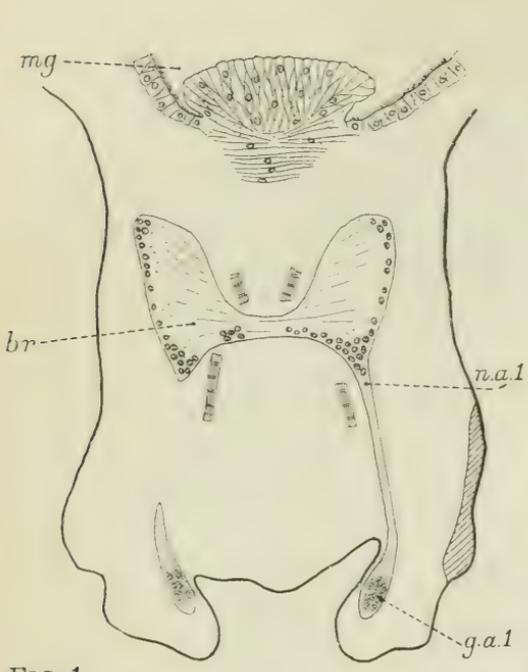


FIG. 1

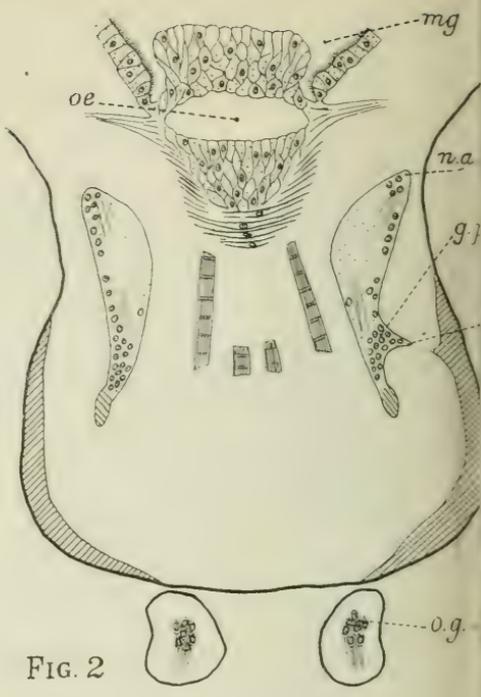


FIG. 2

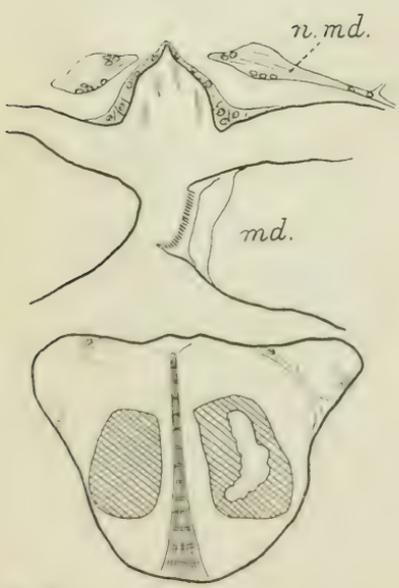


FIG. 5

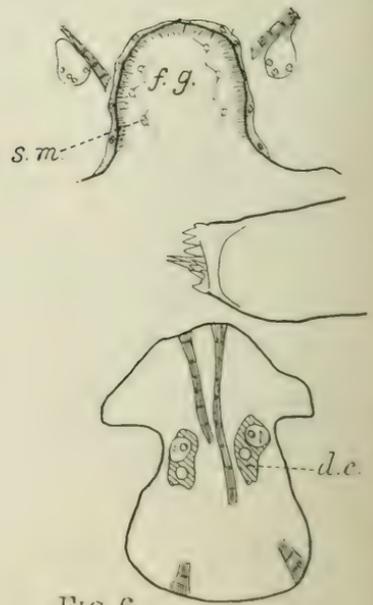
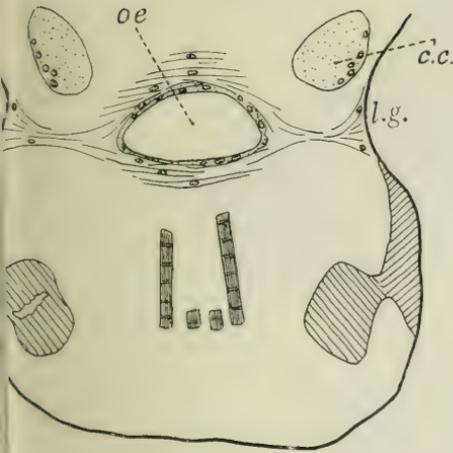


FIG. 6

H. G. Cannon, del.



G. 3

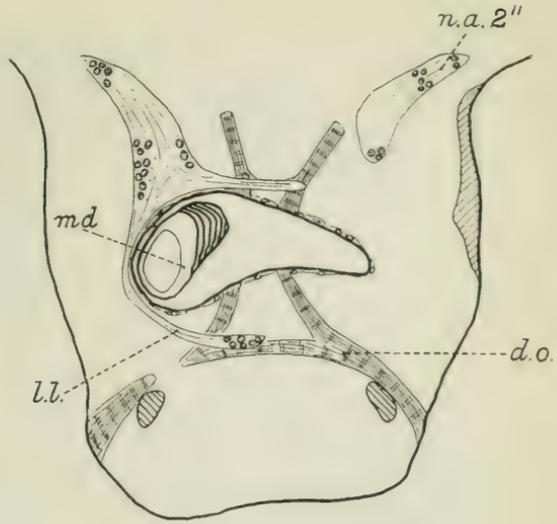


FIG. 4

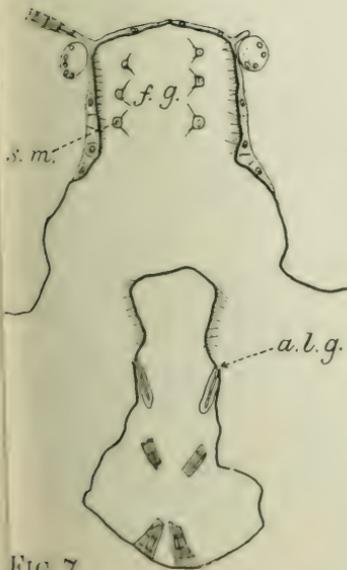


FIG. 7

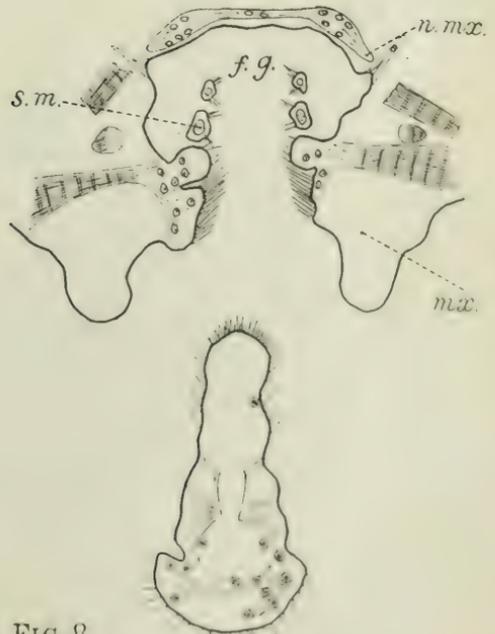


FIG. 8

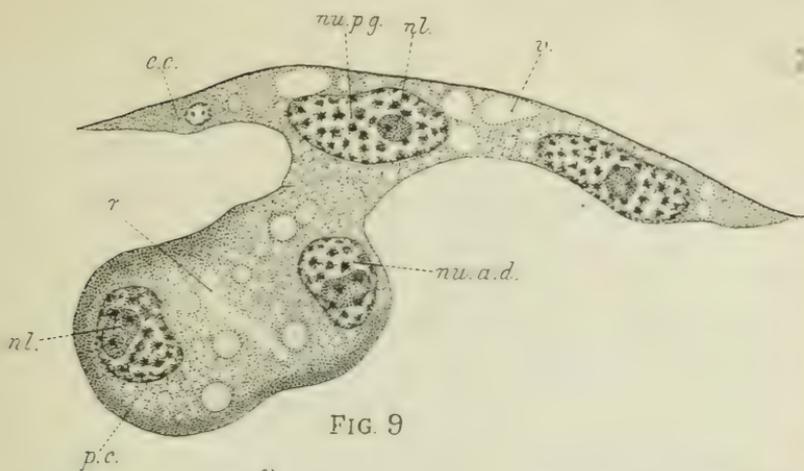


FIG. 9

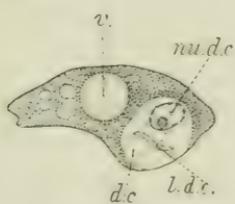


FIG. 11

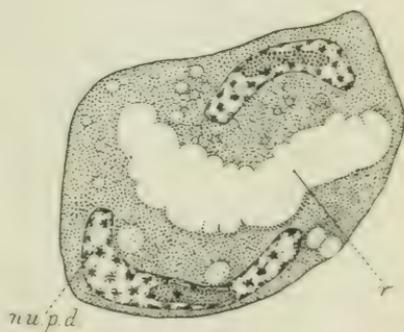


FIG. 10



FIG. 12

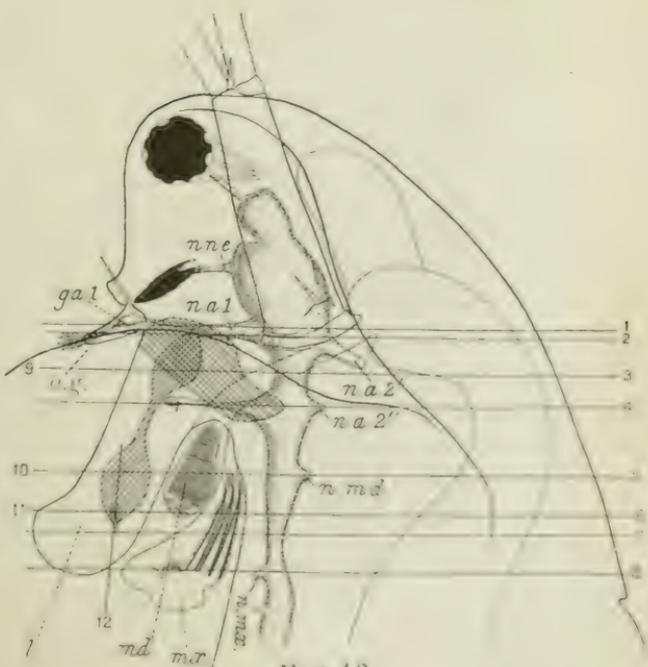


FIG. 13

Surface Tension and Cell-Division.

By

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With 9 Text-figures.

THE series of changes which a dividing cell exhibits has long suggested to biologists that surface tension plays a dominating rôle in the process of cleavage. Without exception, theories based on this suggestion have postulated regions of differential tension on the cell-surface; the surface tension at the equator of the cell has been held to be either higher or lower than that at the polar regions of the cell. Such theories have proved of but little value as a means of further investigation, since there is no apparent means of determining how such a state of affairs could arise, nor is there any apparent differentiation in the microscopical structure near the equator of the cell-surface.

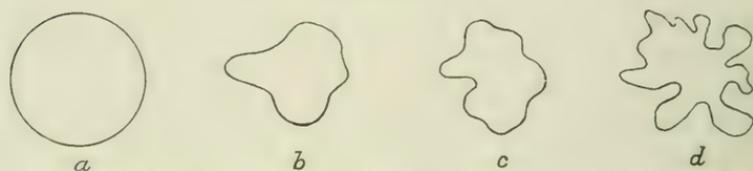
The evidence here presented suggests that regions of differential surface tension are unnecessary assumptions, and that cell-division does not take place owing to a change in surface tension at the cell-surface, but owing to a force inside the cell which operates against the surface tension. It is the equilibrium between this force and the normal surface tension which determines the shape of the dividing cell.

The fertilized eggs of *Echinus miliaris* form very satisfactory material for a study of cell-division, since the protoplasmic surface of the egg is in direct contact with an aqueous medium and because the actual process of cell-division can readily be followed under the low powers of the microscope. The normal egg is spherical, and if it be crushed or broken the resultant portions show no tendency to mix with the water, but rapidly acquire a more or less spherical shape. From this we may infer that the protoplasm of the egg resembles that

of many other cells, in that it is essentially a liquid which is immiscible with water. Further, an overwhelming body of evidence is available to show that the protoplasmic surface contains a lipoid or oily phase. To what extent is the form of the egg dependent upon those conditions which determine the form of inert drops of oil surrounded by water?

Consider the simple case of oil-drops in water. The drops are all spherical owing to the existence of a force—usually called surface tension—acting at the oil/water interface. In any such system the amount of free energy will tend to reach a minimum, and since the volume of oil presents a minimum amount of surface when the drop is spherical it is obvious that

TEXT-FIG. 1.



Form of oil-drops in (a) acid water (b-d) increasing amounts of alkali in water.

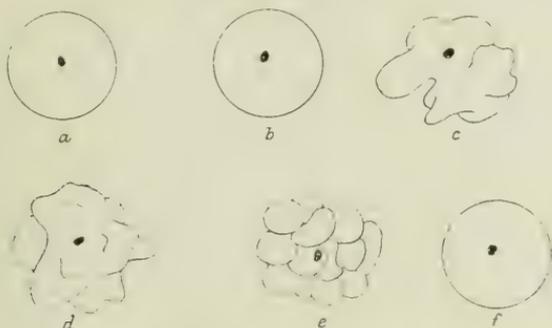
the position of stability is reached when the drops are round. The higher the surface tension the more rapidly is the spherical form assumed, and the more resistant is the form of the drop to external or internal disturbance. Now, it has been shown that the surface tension at an oil water interface is materially affected by the hydrogen-ion concentration of the water. Hydrogen ions raise the surface tension; hydroxyl ions lower it. The effect of such changes is a marked alteration in the form of the oil.

In an acid solution the oil-drop remains perfectly spherical and is not readily deformed by external forces. In an alkaline solution, however, the drop becomes very irregular in shape and is readily deformed. In highly alkaline solutions the surface tension may actually become 'negative', and the condition of stability is reached by the splitting up of the drop to form an emulsion.

To what extent the drop of protoplasm responds to similar changes in its environment is seen from the following figures.

It will be seen that in an alkaline medium the capacity of the egg (like that of an oil-drop), to retain a spherical form is lost. The outline of the egg becomes distorted by the production of numerous blunt irregular processes, just as is the surface of the oil. On returning such eggs to normal sea-water the spherical form is gradually reformed; in acid sea-water the spherical form is quickly regained. In some cases the recovery

TEXT-FIG. 2.



a, Egg in normal sea-water, P_{H} 7.9; *b*, egg in acid sea-water, P_{H} 6.0; *c-e*, successive stages in alkaline sea-water, P_{H} 9.6; *f*, same egg transferred from alkaline to acid water.

of the spherical form causes the protoplasm to divide completely into two or more parts. These parts are always spherical or elliptical. The nucleated fragment alone divides to form normal blastomeres. Saponin possesses the power of lowering the surface tension at an oil/water interface, and produces similar changes in the form of the egg to those produced by alkalis.

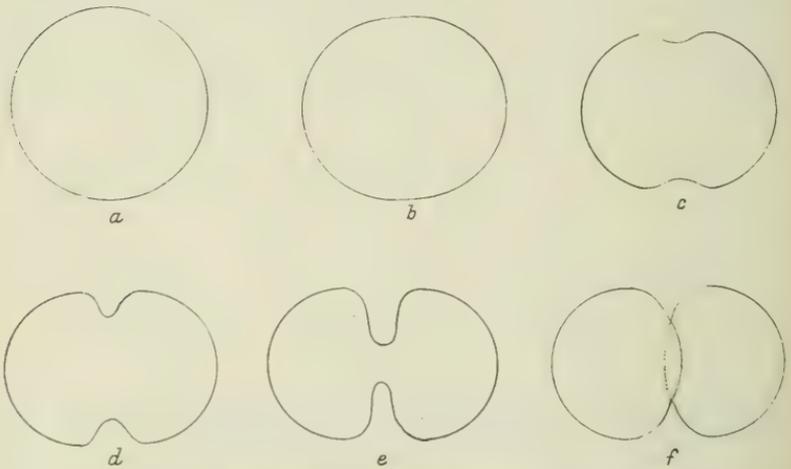
It seems reasonable to conclude that the form of the undivided egg is determined, at least in part, by the surface tension at the egg-surface.

Before proceeding to consider the part played by surface tension during the process of division, it is necessary to draw attention to certain facts in connexion with normal cleavage. The first division in the egg of *Echinus miliaris* takes

place (at 15° C.) about fifty minutes after fertilization, and takes roughly three minutes. The process of cleavage is shown diagrammatically below.

It is important to note that during cleavage there is a progressive increase in the length of the main axis of the egg; this is just as distinctive as the production of the shorter axis which is brought about by the development of the cleavage furrow.

TEXT-FIG. 3.



Stages in normal cell-division of egg of *Echinus miliaris*.

As the egg elongates so the polar regions become more and more convex, while the equatorial region becomes more and more concave.

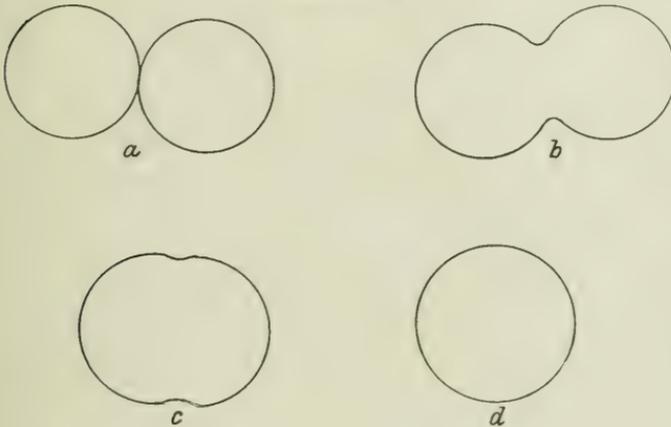
In making a comparison between the dividing egg and an oil/water system it is convenient to consider the fusion of two oil-drops rather than the division of a single drop into two equal parts. When two oil-drops fuse it is obvious (Text-fig. 4) that a reversal of the process would approximate very closely to the process of cell-division.

Now, in fusing together the amount of free energy at the surface of the oil is reduced in the ratio of 5 : 4, so that when the single drop is mechanically shaken into two equal parts,

or when the egg divides into two equal blastomeres, it is necessary to provide the surface of the two systems with free energy. Hence, during cell-division the egg must do work in order to provide free surface energy.

Before proceeding further with this argument, let us consider the effect of altering the surface tension at the protoplasmic/water interface during the actual process of cleavage. In order to do this, eggs at different stages of cleavage are transferred

TEXT-FIG. 4.



Stages in the fusion of two oil-drops.

to acid and alkaline sea-water. In the latter case division occurs quite normally; in the former case striking effects are produced.

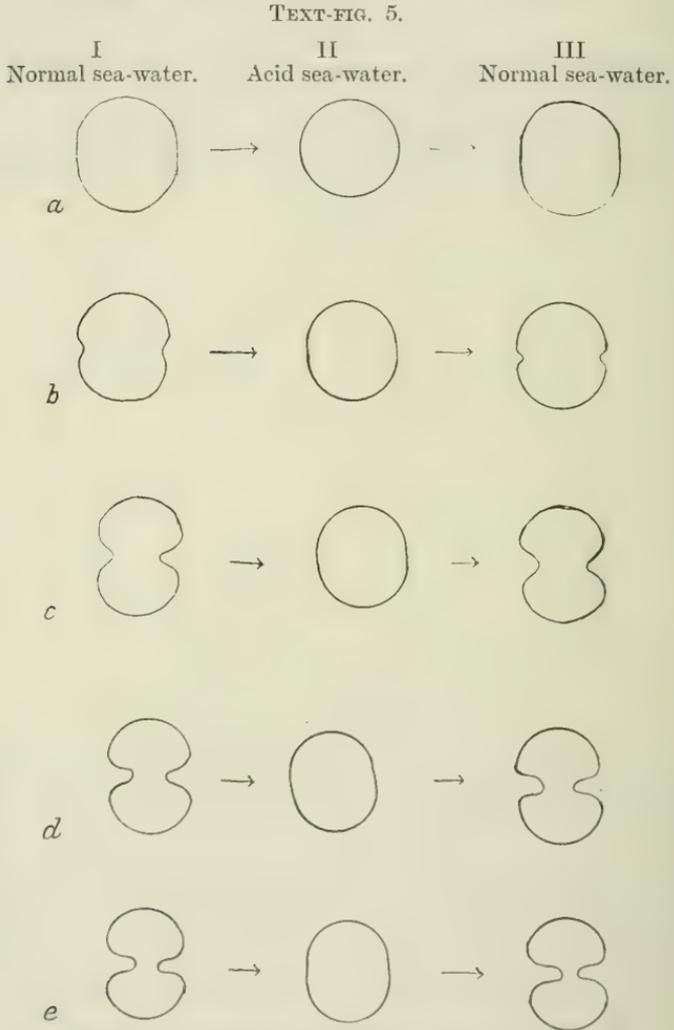
The effects of acid sea-water may be summarized as follows :

(i) The cleavage furrow is entirely lost, and in the early stages of cleavage the egg tends to become spherical in form.

(ii) In the total abolition of the cleavage furrow there is distinct evidence that the egg is elongated along the main axis of the astral figure, so that in the latter stages of cleavage the form of the egg is that of a well-marked cylinder with hemispherical ends.

(iii) The effect of acid sea-water is entirely reversible. If an egg which has been taken from normal to acid sea-water be

replaced in normal sea-water, it very rapidly returns to the stage of cleavage which it had reached prior to being placed in



Effect of acid sea-water on the form of a dividing egg.

acid sea-water: it then proceeds to complete the division at the normal—relatively slow—rate. The distinction between the two phases of recovery from the acid is most marked.

There can be but little doubt that the explanation of these facts is as follows: the acid sea-water raises the surface tension at the egg-surface, and tends to make the egg regain its spherical shape. Owing, however, to some force, which elongates the main axis of the egg, equilibrium is reached (during the latter stages of cleavage) when the egg is cylindrical and not spherical.¹ It will be noted that the increase in length of the main axis, which was noticed in normal cleavage, is much more obvious in acid sea-water owing to the abolition of the cleavage furrow.

It has been shown that when the egg is removed from acid to normal sea-water the cleavage furrow reappears at once. The amount to which the cleavage furrow develops depends entirely on the equilibrium between (a) the surface tension at the egg-surface and (b) some other force within the egg. In acid sea-water the surface tension is high and equilibrium is reached when the egg is a cylinder with hemispherical ends: in normal sea-water equilibrium is reached with a well-marked cleavage furrow. Whereas the effect of a change in surface tension is very rapidly reflected in the form of the cleavage furrow, it is also clear that the elongation of the main axis is the active process whereby free energy is supplied to the egg-surface and allows the furrow to form under normal conditions. This process is stopped in an acid solution (like so many other physiological processes) and is resumed on return to normal sea-water. The rate at which this force acts is entirely independent of the experimental rate of change of the surface tension of the egg-surface.

That the elongation of the egg axis is the active process involved is shown from the experiment of Plateau.²

If a drop of oil be placed between two metal rings A and B so as to form a complete cylinder (Text-fig 6, *a*), and if the rings be now moved apart, then when the distance of A to B becomes

¹ The relative surfaces which enclose an equal volume of protoplasm are

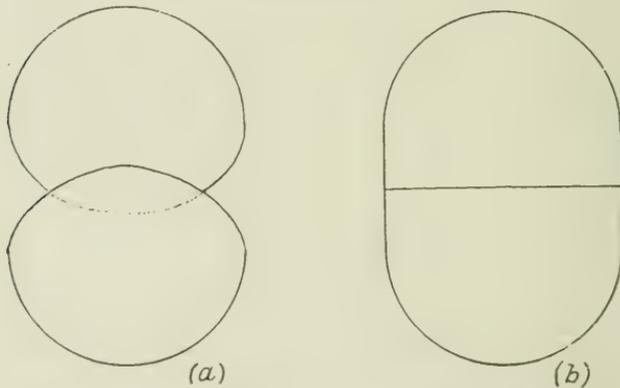
(i) Sphere	100
(ii) Cylinder with hemispherical ends	105
(iii) Two spheres (each half vol. of i)	126

² 'Statique des liquides', vol. ii.

greater than $\frac{2}{3}$ of the diameter of the rings (Text-fig 6, *b*), the form of the drop changes in the same way as that of a dividing egg. The further A is moved away from B the more convex do the ends of the cylinder become, and the more marked is the development of the 'cleavage' furrow; finally the drop is resolved into two separate parts.

There is, however, one respect in which the protoplasmic system differs from that of an oil-drop. When a drop of oil is divided into two—as in Plateau's experiment—it is a simple matter to reverse the process and reform a single drop of oil.

TEXT-FIG. 6.



Form of completely divided egg in (*a*) normal sea-water. (*b*) Acid sea-water.

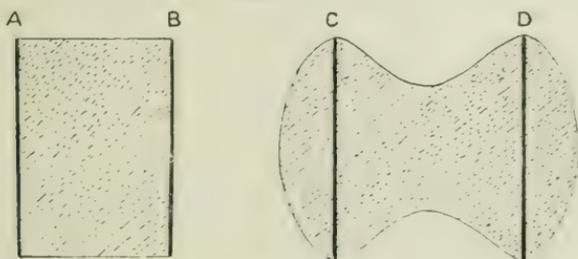
In the case of the living cell this does not occur. It would seem that this is due to the existence of a surface layer (Traube membrane) which is automatically formed when protoplasm comes into contact with water, and that the blastomeres fail to fuse with each other just as oil-drops fail to fuse together if they are shaken in smaller drops in the presence of a soap or any similar substance which can form a condensation layer at the surface of the oil.

The conclusion reached is that division of the cell is brought about by the elongation of one axis of the cell, and that the cleavage furrow results as an equilibrium between this process and the normal surface tension at the cell-surface. It need

hardly be mentioned that the existence of any form of mechanical membrane, or the presence of elements (e.g. other cells) capable of exerting a pressure in any particular direction will materially alter the system which is under discussion.

It can hardly be doubted that the elongation of the cell-axis is associated in some way with the elongation of the astral

TEXT-FIG. 7.



TEXT-FIG. 8.

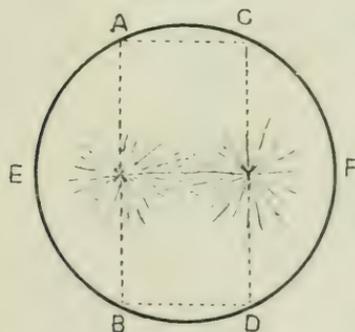


figure. Since the latter process goes on continuously during mitosis and does not begin with the elongation of the whole cell, it follows that the elongation of the cell is probably the result and not the cause of the elongation of the astral figure.

If this conclusion is correct the cell can be divided into three parts; a central cylindrical portion $ABDC$ (Text-fig. 8) which is tending to increase the length of its shorter axis, and two convex ends to this cylinder AEB and CFD . Until the ratio $\frac{AC}{AB}$ approaches $\frac{2}{3}$ the form of the cell will not change, but as

this figure is approached the sides AC and BD will begin to flatten; as soon as $\frac{AC}{AB}$ is $> \frac{2}{3}$, a definite cleavage furrow will result between A and C and between B and D. At the same time the convexity of the surfaces AEB and CFD will increase.

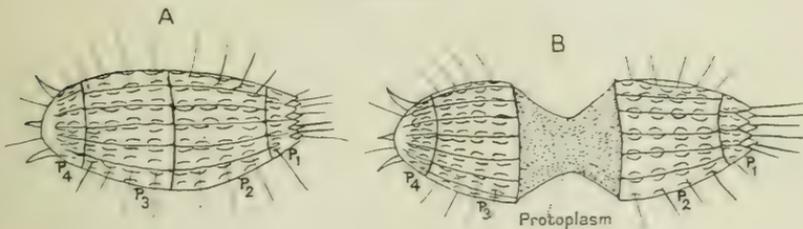
If the change in length of the axis AC is dependent upon a change in the distance of one centrosome from the other, then one would expect to find a similar relationship between this latter distance and the sector AB. The following measurements appear to show that in a variety of cells the cell begins to be deformed in appearance at the equatorial region when the distance between the asters is about .67 of the sector of that part of the cell lying between the asters, and that the cleavage furrow becomes well marked when the ratio has reached the value .8 or .9.

Type.	Authority.	Stage of Division.	Length of Axes.		Ratio $\frac{XY}{AB}$	
			XY	AB		
Multicilia lacustris	Lauterborn	Stage immediately before development of concave cleavage furrow.	1.15	2.0	.58	Average $\frac{XY}{AB} = .65$
Diplogaster longicauda (2 celled)	Ziegler		1.15	1.9	.60	
Diplogaster longicauda (4 celled)	Ziegler		1.7	2.65	.64	
Acanthocystis	Schaudinn		1.45	2.10	.69	
Ascaris	Boveri		2.2	2.9	.76	
Multicilia lacustris	Lauterborn	Definite concave cleavage furrow.	1.85	2.15	.86	Average $\frac{XY}{AB} = .8$
Diplogaster longicauda	Ziegler		1.27	1.64	.77	
Diplogaster longicauda	Ziegler		1.35	1.70	.80	
Diplogaster longicauda (4 celled)	Ziegler		1.15	1.38	.83	
Acanthocystis	Schaudinn		1.80	2.00	.90	
Ascaris	Boveri	2.65	2.9	.91		

Prior to any visible change in the appearance of the cell the ratio $\frac{XY}{AB}$ is progressively increasing from the beginning of the formation of the astral figure: it then passes through the critical value at which the furrow appears and continues to increase until complete cell-division is effected.

It is somewhat rash, perhaps, to press too far the analogy of the astral figure to the rings in Plateau's experiment. It is obvious that the mechanical model is a limited one, and that subsequent work may show that regions of differential viscosity such as are suggested by Chambers's¹ work may be found to be involved. Yet in the particular case of a cell which possesses a structure curiously fitted to play the part of Plateau's rings, division is found to proceed on just those lines demanded by the above analysis. Text-fig. 9 shows the division

TEXT-FIG. 9.



Division of *Coleps hirtus* (after Doflein). The undivided form A gives rise to two daughter individuals by passing through stage B.

of the protozoon *Coleps hirtus*. The body is covered by four hard skeletal plates P_1 - P_4 . When division occurs the distal ends of the cell remain fixed in form owing to the existing plates, and between plates P_3 and P_4 the cell becomes elongated in exactly the form demanded by the hypothesis put forward. In the stage of division illustrated a well-marked cleavage furrow has formed and the 'Plateau' ratio is about .8.

SUMMARY.

Cell-division may be accounted for by the movement of the two asters away from each other. The appearance of the cleavage furrow is due to an equilibrium between the effect of this movement on the protoplasm and the surface tension at the cell-surface. The behaviour of the cell under these forces is precisely similar to a drop of oil subjected to similar conditions. There is no necessity to postulate regions of differential surface tension at the poles or equator of the cell.

¹ 'Journ. Exp. Zool.', vol. 23, p. 483, 1917.

On the Classification of Actiniaria.

Part III.—Definitions connected with the forms dealt with in
Part II.

By

T. A. Stephenson, M.Sc.

University College of Wales, Aberystwyth.

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1. DEFINITIONS.

N.B.—In the following pages only a necessary minimum of synonymy is given ; species-lists are not necessarily exhaustive ; and dubious forms are often omitted. Except where stated explicitly, all reasons for the classification here used will be found in Parts I and II.

Sub-class ZOANTHACTINIARIA. Van Bened.

(As used by Bourne, 1916, pp. 514-15 = DODECACORALLIA.
Carlgr., Bronn's 'Thierreich', 1908.)

Order DODECACTINIARIA.

(As used by Bourne, 1916, p. 515.)

Sub-order ACTINIARIA.

Tribe 1. PROTANTHEAE, Carlgr.

Founded by Carlgren in 1891 : used here in its original and narrow sense, as covering Gonactinia and Protanthea

(and probably *Oractis*) only, not in the wider sense of Carlgrén's later work.

Actiniaria with or without a definite base, but without basilar muscles. The body is smooth. There is a sheet of longitudinal muscle-fibres in the ectoderm of body-wall and actinopharynx as well as of disc and tentacles, and the body-wall ectoderm at least has also spirocysts. Sphincter absent or weak diffuse endodermal. Tentacles few or more numerous, simple. In normal animals only eight mesenteries are perfect, and these are the analogues of the eight macrocnemes of *Edwardsia*. The mesenterial muscle is weak, often hardly more developed than the body-wall muscle, and not forming a very definite retractor usually. The number of mesenteries present beyond the eight protoenemes varies, but the eight are not sharply marked off from the others as macrocnemes, although they have a certain predominance, especially in *Oractis*. Four imperfect mesenteries pair with the lateral protoenemes, and the rest form a secondary cycle or cycles. The distribution of gonad and filament may affect the protoenemes only (*Oractis*), or the protoenemes and their lateral partners (*Gonactinia*), or the whole of cycle 2 as well (*Protanthea*). The filaments have no ciliated tracts. There are no well-marked siphonoglyphes.

Family 1. GONACTINIIDAE, Carlgr., 1893.

Used here *sensu stricto* for *Gonactinia*, *Protanthea*, and *Oractis* only.

With the characters of the tribe *Protantheae*.

GONACTINIA, Sars, 1851, p. 142.

Gonactiniidae with the gonads confined to the lateral protoenemes normally, whereas the filaments are found not only on these but also on their partners and on the directives. There is a definite base. Reproduction often asexual.

Species :

G. prolifera, Sars, 1835, p. 3. (See Carlgrén, 1893, p. 31.)

PROTANTHEA, Carlgr., 1891.

Gonactiniidae with gonads and filaments on the mesenteries of cycles 1 and 2, and beyond these cycles small mesenteries devoid of appendages and confined to the uppermost parts of the body. There is a definite base.

Species :

P. simplex, Carlgr., 1891, p. 81; 1893 p. 24.

ORACTIS, McM., 1893, p. 138.

Gonactiniidae (?) in which only the eight protoconemes are fertile and filamented. There is no definite base, and there are only ten tentacles.

Species :

O. diomedae, McM., 1893, p. 138.

This genus is not yet very fully known, but is probably referable to this family.

Tribe 2. PTYCHODACTEAE, mihi.

Containing the family Ptychodactidae only.

Actiniaria with a definite base, which may rather merge into the column, but without basilar muscles. Body-wall smooth or with vertical rows of hollow outgrowths; in structure, however, similar to the tentacles, with ectodermal muscle-sheet and at least usually spirocysts. Sphincter absent or weak diffuse endodermal. Tentacles few or more numerous, simple. Actinopharynx either quite rudimentary and reduced to a narrow band, or else quite well developed and provided with siphonoglyphes and ectodermal muscle-fibres. Six to twelve or more pairs of mesenteries perfect. Musculature of mesenteries weak, hardly forming retractors. The free borders of the mesenteries (or their representatives if the mesenteries fuse below) are occupied by filament above and gonad below, not both together. The filaments have no ciliated tracts, but those of the imperfect mesenteries end up above in a curious structure like a bisected funnel, unusual in form and make-up.

Family 1. PTYCHODACTIDAE, Appellöf, 1893.

See also Carlgren, 1911, p. 12, &c.

With the characters of the tribe Ptychodacteae.

Genera : *Ptychodactis*, *Dactylanthus*

PTYCHODACTIS, Appellöf, 1893, p. 4.

Ptychodactidae with about 100 tentacles or more. Smooth body. No sphincter. Actinopharynx rudimentary, reduced to a narrow band just inside the lip, which is produced at certain points into lappets for the attachment of larger mesenteries. Mesenteries irregularly arranged: primaries and usually secondaries perfect.

Species :

P. patula, Appellöf, 1893, p. 4. (See also Carlgren, 1911.)

DACTYLANTHUS, Carlgr., 1911, p. 2.

Ptychodactidae with twenty-four tentacles. Body with twenty-four vertical rows of hollow outgrowths or vesicles, corresponding to the twenty-four regular endocoels and exocoels. Sphincter very weak diffuse. Actinopharynx quite well developed, with two siphonoglyphes and with curious pockets between the insertions of some of the mesenteries. Twelve pairs of mesenteries, six pairs or all of them perfect, all fusing together down below in the gonad region, in such a way that the gonads occupy no longer the now non-existent free edge of the mesentery, but the region nearest to the point of fusion.

Species :

D. antarcticus, Clubb, 1908, p. 5. (See Carlgren, 1911.)

Tribe 3. NYNANTHEAE, Carlgr.

Used here in a different sense than that of Carlgren, so that it excludes Edwardsiaria, Corallimorphidae, and Discosomidae, but includes Boloceroides and the Endocoelactids.

Actiniaria with or without a definite base, with or without basilar muscles. Body-wall smooth or with verrucae or outgrowths of one sort or another. The presence of a sheet of ectodermal muscle in body-wall or actinopharynx is exceptional, occurring sporadically, and sometimes reduced to a vestige such as ectodermal muscle in the siphonoglyphes. Spirocysts in body-wall ectoderm are also exceptional save in Endocoelactaria. A sphincter may or may not be present, and if present may be weak or strong, endodermal or mesogloal. Tentacles few or many, simple or complex, their longitudinal musculature ectodermal or mesogloal. Siphonoglyphes are typically present. The mesenterial filaments have ciliated tracts. Pairs of perfect mesenteries are present save in abnormal cases, and usually at least six pairs, often more. Six is a fundamental number for arrangement of parts, but there are a good many deviations. Mesenterial musculature does not often exhibit so low a grade as in Gonactiniidae, Ptychodactidae, and many Madreporaria—often it is highly developed, very definitely marked off retractors being formed—cases of weakness are usually sporadic and secondary rather than universal and inherent.

Sub-tribe 1. ATHENARIA, Carlgr.

Used here as covering Halecampids and Nynanthids but not Edwardsians.

Nynantheae representing those forms which being the outcome of a Haleampa-like ancestor have retained more similarity to that

ancestor than most forms, and live a more or less burrowing life. Size variable, predominating shape vermiform, this being attained in greater or less degree in different cases; the diameter of the body in some forms, or at least in some states, bearing a fair proportion to its length. There is no adherent base, the aboral end being a physa, which does sometimes adhere to small objects. There is little or no sphincter, but if present it may be endodermal or mesogloal. Cinclides often present. Number of tentacles usually small, even at greatest not passing about forty; not more than one communicates with each endocoel and exocoel. Number of mesenteries similarly limited, and they are either all macrocnemes or else a division into macro- and microcnemes is to be found—with an intermediate condition in the case of *Peachia*. Secondary mesenteries develop in the exocoels of the primaries. Sometimes the larvae seem to be parasitic on medusae.

Family 1. HALCAMPIDAE, Andres.

Used here in the general sense of Andres, 1883, p. 312.

Hyanthidae as used by Gosse, *pro parte*.

Including *Halcampoidinae*, Appellöf, 1896, p. 13; *Halcampomorphae*, Carlgr., 1893, p. 38; *Halcampinae*, Carlgr., 1893, p. 38; *Monaulidae*, Hertw., 1882, p. 104; *Halianthinae*, Kwiet., 1896; and 'Fenja' and 'Aegir'.

Athenaria of more or less vermiform shape, with or without suckers or papillae on the body, with or without cuticle or incrustation. Cinclides may occur in the physa. Tentacles eight to twelve, fourteen, twenty, or more, and with other variations, their longitudinal musculature ectodermal. Sphincter absent, weak mesogloal, or weak endodermal. The mesenteries have as their main feature six pairs of macrocnemes, but there are variations; the full six pairs may not be developed, or there may be one or two unpaired macrocnemes in addition to them. Microcnemes are sometimes present, their number varying.

Genera: *Halcampa*, *Halcampoides*, *Pentactinia*, *Scytophorus*.

*HALCAMP*A, Gosse, 1858.

See Carlgren, 1893, pp. 37-8; Kwietniewski, 1896, p. 585; Haddon, 1889, p. 335; Carlgren, 1900 (on *Pentactinia*), p. 1170, &c.; and Stephenson, 1918 A, pp. 8-10. *Halianthus*, Kwiet. *Halianthella*, Kwiet. ? *Halcampella*, Andres.

Halcampidae typically worm-like, more or less, but very changeable in form (see Part II, Text-fig. 7, c, d). There is a physa which may or may not be retractile and which has cinclides in it (always?). The main part of the body, or scapus, may be without suckers, or it may have suckers to which sand adheres, so as to make a more or less dense covering. A clear external separation into capitulum, scapus, and physa is not necessarily present. Some species have no sheath. Sometimes the scapus has solid papillae. Tentacles retractile, eight to twelve or more (e. g. thirty-two), their longitudinal muscle ectodermal. Sphincter weak mesogloal (see Part I, Text-fig. 1, and Pl. 22, fig. 7). Mesenteries either all macrocnemes, or else divided into macro- and microcnemes. Macrocnemes six pairs (rarely one or two additional unpaired ones) or fewer; microcnemes if present variable in number (see Part II, Text-fig. 8).

Species:

- Genotype, *H. chrysanthellum*, Peach, Johnst., 1847, p. 220.
 (See also Gosse, 1860, p. 247; Haddon, 1889, p. 335; Walton and Rees, 1913, p. 65; Haddon, 1886, p. 1; Faurot, 1895, p. 127; Stephenson, 1918 A, p. 9; 1920 A, p. 440.)
- H. duodecimcirrata*, Sars, 1851, p. 142; Carlgr., 1893, p. 38.
- H. arctica*, Carlgr., 1893, p. 45.
- H. limnicola*, Annan., 1915, p. 89.
- H. aspera*, Steph., 1918 A, p. 10.
- H. chilensis*, McM., 1904, p. 223.
- H. kerguelensis*, Studer, 1878, p. 546. (See Kwietniewski, 1896.)
- H. arenaria*, Haddon, 1886, p. 616; 1889, p. 335. (See also Walton and Rees, 1913, p. 66.) And probably others.

I have been obliged to transfer *H. aspera* from *Halcampoides* to *Halcompa*, because on re-examination of some sections of it I find appearances which I take to indicate a mesogloal sphincter. The reasons for my overlooking it in my original investigation were that there is not much of it, and it was not until I had subsequently examined several other species with insignificant sphincters that I found out exactly where one must look for it in a deeply introverted and somewhat twisted specimen such as mine was. I had only transverse, and not the more serviceable longitudinal sections of the region where it lies, being further misled by mistaking certain parts of the endodermal circular muscle for a slight endodermal sphincter. There is also perhaps a fourteenth

perfect mesentery, but if so, whether it has a retractor is uncertain, and it is probably asymmetrical, not placed as in *Scytophorus*. But only more and better material can clear it up. *H. arenaria* I have left in the genus on the assumption that it has a mesogloal sphincter, but that remains to be proved.

HALCAMPOIDES, Dad., 1887.

See Appellöf, 1896, p. 3, and the references given under *Halcampa*. Fenja, Dan. Aegir, Dan. *Halcampomorpha*, Carlgr. *Halcampa* as used by Kwietniewski. *Halcampella* as used by Hertwig. ? *Halcampella*, Andres.

Halcampidae typically more or less worm-like, not necessarily with a clear distinction into scapus, capitulum, and physa. *Cinclides* may occur in the physa. Naked or incrusted. Tentacles twelve or more, can be retractile, and with the tentacular longitudinal muscle ectodermal. No mesogloal sphincter, but there may be a slight endodermal one. Six pairs of macrocnemes. Microcnemes present or not.

Species:

H. abyssorum, Dan., 1887.

H. clavus, Q. and G., 1833, p. 150. (See Hertwig, 1882, p. 92; Pax, 1912, p. 310; Appellöf, 1896, pp. 3, 13, &c.; and Haddon, 1889, p. 336.)

H. maxima, Hertw., 1888, p. 29. (See Wassilieff, 1908.)

H. kerguelensis, Hertw., 1888, p. 28.

H. purpurea, Studer., 1878, p. 545. (See Kwietniewski, 1896.)
And probably others.

H. minuta, Wass., seems to be more like a *Haloclava* than a *Halcampid*. It is possible that of the species listed some may be synonyms of others—it has been suggested that *H. clavus* is the same as *H. purpurea* and *H. abyssorum*. *Halcampella endromitata*, Andres, and others, cannot yet be definitely allocated. In Wassilieff's description of *H. maxima* there are some remarks which suggest that he had some form other than a *Halcampoides* to deal with; but they may be due to a couple of critical misprints.

PENTACTINIA, Carlgr., 1900, p. 1166.

Halcampidae with body which may be long, and physa. Scapus with papillae to which fragments may adhere. Tentacles typically twenty, their longitudinal musculature ectodermal. No sphincter. Ten macrocnemes present—the 'Edwardsia eight' + one couple pairing with the dorsolateral protocnemes. The sixth primary couple represented by two perfect but weak mesenteries. Four pairs of microcnemes, confined to distal part of body.

Species :

P. californica, Carlgr., 1900, p. 1166.

SCYTOPHORUS, Hertw., 1882, p. 104.

Halcampidae with body which may be long, with cuticle developed chiefly on the scapus. The physal end may attach itself. No verrucae. Tentacles fourteen, their longitudinal musculature ectodermal. Mesenteries fourteen, the usual primary six pairs + one couple, the individuals of the couple with their retractors facing one pair of directives. All these mesenteries are macrocnemes, but some may be without gonads. No sphincter. The ciliated tracts of the filaments may be peculiar.

Species :

S. striatus, Hertw., 1882, p. 104.

S. antarcticus, Pfeff., 1889, p. 11. (See Carlgren, 1899, p. 7.)

Family 2. ILYANTHIDAE, Gosse.

Ilyanthidae as used by Gosse, 1860, *pro parte*, i.e. excluding Ceriantharia, Edwardsiaria, and Halcampids.

Athenaria often attaining a fair size, frequently with stout bodies which are often capable of becoming vermiform. Suckers present or absent. Cinclides may occur in the physa or on the scapus. Patches of cuticle are sometimes present. Tentacles simple or capitate, eight, twelve, twenty, or more, up to about forty. Sphincter absent or slight and endodermal. Never fewer than ten pairs of mesenteries in adult animals, the number varying up to about eighteen pairs. They are usually all macrocnemes, even if there is some distinction among them, though in *Peachia* four at least of the ten pairs are imperfect and without gonad or filament, but they have strong retractors and cannot be called microcnemes. There is often only one siphonoglyphe, and this may bear a specialized upper end or conchula.

Genera: *Ilyanthus*, *Eloactis*, *Haloelava*,
Harenactis, *Peachia*.

Polyopis striata, Hertw., may just possibly have been a battered member of this family.

ILYANTHUS, Forbes, 1840.

Ilyanthidae with a physa. Body-form may be thickish, without suckers, but there may be patches of cuticle. Margin of scapus forms a collar with a narrow capitulum above it. Tentacles simple, in 3 cycles, from about 28 to 36. No conchula. Mesenteries the same in number as the tentacles, all macrocnemes (perfect, with circumscribed retractor, and filament), but not all fertile. Seven tentacles form the primary cycle; these are held permanently over the mouth, and divide up those of the outer cycles into radiating groups. The arrangement is exactly bilateral, and not radial; *one directive tentacle is a primary, the other a secondary.* Tentacles of cycle 3 the longest.

Species :

I. mitchelli, Gosse, 1853, p. 128; 1860, p. 232.

? *I. scoticus*, Forbes, 'Ann. N. H.', I, v. 183.

The only species to be certainly referred to this genus is *I. mitchelli*, which I have been able to study alive and anatomically. It is a unique and extraordinary form, and further details about it will, I hope, be shortly forthcoming. It is clear that *I. parthenopeus*, Andres, is something quite different from *I. mitchelli*, and merits at least a distinct genus, and as it seems to me a distinct family; see Andresiidae for further detail.

ELOACTIS, Andres, 1883, p. 464.

Ilyanthidae with a physa which may adhere slightly. Column without verrucae, its upper margin well marked. Tentacles twenty, capitate, not fully retractile, the outer largest; tentacular longitudinal muscle ectodermal; tentacle-heads especially rich in sting-cells. No conchula. No sphincter. Mesenteries ten pairs, all macrocnemes (see Part II, Text-fig. 9), probably all fertile. One siphonoglyphic.

Species :

E. mazeli, Jourd., 1880, p. 41. (See Faurot, 1895, p. 152; and Rees, 1913, p. 70.) Perhaps others.

HALOCLAVA, Verr., 1899, p. 41.

Ilyanthidae with body which may be long. Base physa-like but capable of adherence to small objects. The body has rows of adhesive suckers above. There is some sort of sphincter. Tentacles twenty, usually clavate. No conchula. Mesenteries ten pairs, all perfect, very muscular, but six pairs are larger and form a primary cycle.

Species:

H. producta, Stimp., 1856, p. 110, is the genotype, and perhaps, others should also be included. (See Verrill, 1899.)

HARENACTIS, Torrey, 1902.

Hyanthidae with a physa which can flatten into a disc and stick to something. The body may attain great length; it is smooth, but has a vertical row of cinclides in the upper part of it, corresponding to each endocoel and exocoel. No conchula. No sphincter. Twenty-four simple tentacles. One siphonoglyphe. Longitudinal musculature of tentacles ectodermal. Mesenteries all macrocnemes, twelve pairs, only the six primary pairs usually fertile.

Species:

H. attenuata, Torrey, 1902.

PEACHIA, Gosse, 1855.

Siphonactinia, Dan. and Kor., 1856.

Hyanthidae with a physa, in which there are typically cinclides. Column can be thick, or can become vermiform by attenuation (see Part II, Text-fig. 7, A), without verrucae. A trifold conchula (which may have further subdivisions beyond the three main ones) is usually present in connexion with the opening of the one siphonoglyphe. No sphincter. Tentacles simple, eight or twelve. Mesenteries ten pairs (or six pairs in younger specimens); usually six pairs perfect, with strong diffuse or circumscribed-diffuse retractors, gonads, and filaments—more rarely only the eight protoenemes are perfect, but the other primary couples have their filaments and retractors. There are four secondary imperfect pairs without gonad and filament, but they have strong retractors and are not microcnemes.

Species:

P. hastata, Gosse, 1855, p. 294. (See also Gosse, 1860, p. 235; Faurot, 1895, p. 136; Haddon, 1889, p. 337; Stephenson, 1920 A, pp. 446, &c.; Haddon and Dixon, 1885.)

P. undata, Gosse, 1858, p. 418; 1860, p. 239.

P. triphylla, Gosse, 1860, p. 243. (See Hornell, 1894, p. 78.)

P. quinquecapitata, McMurrich, 1913.

P. koreni, McL., 1893, p. 144.

P. hilli, Wilsmore, 1911, p. 39.

And probably others.

I have cast the definition so that it covers the aberrant *P. hilli* as well as the typical forms, but it would not be surprising to find that that species merits separation from the

rest of the genus, on account of its seeming lack of a conchula and imperfection of some of the primary mesenteries. Certain species have been described under the generic names *Bicidium* and *Philomedusa*, but these are very likely larvae of various species of *Peachia* and *Halcampa*, parasitic on medusae.

Sub-tribe 2. ENDOCOELACTARIA, mihi.

Nynantheae with a definite base. Form variable, but the column without vesicles. Ectoderm of column nearly always with spirocysts, but probably without ectodermal muscle, at any rate usually. No sphincter. One or two siphonoglyphes. Body-wall may be thick and heavy, and the opal disc may be lobed—or both may be quite ordinary in appearance. Tentacles few or numerous, simple or with variously developed aboral swellings of mesogloea; either in two alternating marginal cycles or else arranged in a way unlike the usual anemone plan; but there is never more than one tentacle to each mesenterial space. Radial muscle of disc and tentacles ectodermal or with a mesogloea tendency. Mesenteries varying in detail, but with this in common, that after the first six couples are formed all subsequent pairs appear in the lateral endocoels, and have their longitudinal muscles oriented as in directives.

Family 1. HALCURIIDAE.

Halcuriidae as used by Carlgren, 1918, p. 24, pro parte, i. e. for Halcurias.

Endocoelactidae, Carlgr., 1897, p. 169.

Endocoelactaria without peculiarity of body-form such as lobing of disc and basal thickening of tentacles, and with a tendency to develop little groups of nematocysts in the body-wall ectoderm. One siphonoglyphe only. Tentacles simple, up to about seventy. Mesenteries rather well marked out into a few (six or ten) large perfect fertile filamented pairs with circumscribed retractors, and a number of further pairs without these appendages. Some of these smaller mesenteries are perfect, but they are all, beyond the first ten mesenterial pairs, confined to the uppermost part of the body and are virtually microcnemes.

Genera: Halcurias and Carlgrenia.

HALCURIAS, McM., 1893, p. 142.

Endocoelactis, Carlgr., 1897, p. 169.

Halcuriidae with definite but sometimes small base. The body is

sometimes elongate, and is smooth but for the presence, sometimes, of ectodermal batteries of nematocysts. Margin tentaculate or with a parapet, no distal lobing. Tentacles up to about seventy. Macrocnemes ten pairs, fertile, filamented, and with strong circumscribed to circumscribed-diffuse retractors. Microcnemes confined to upper part of body, either in regular cycles or irregularly placed, some of them usually perfect.

Species :

- H. pilatus*, McM., 1893, p. 142. (See also McMurrich, 1898 ; 1901, p. 155 ; Carlgren, 1918, p. 25.)
H. carlgreni, McM., 1901, p. 159. (See also Carlgren, 1897, p. 159, &c. ; 1914 ; and 1918, p. 26.)
H. endocoelactis, Steph., 1918 A, p. 14.

CARLGRANIA, Steph., 1918 B, p. 109.

Haleuriidae with definite base, slight parapet and fosse, and no distal lobing. Ectoderm of column, at least in upper part, with nematocyst batteries. Tentacles about forty in the specimens so far collected. Macrocnemes six pairs, fertile, filamented, strong circumscribed retractors. In the lateral endocoels are four pairs of perfect microcnemes which run down the whole length of the body (see Part II, Text-fig. 16, c) ; beyond these first ten pairs (six pairs macrocnemes and four pairs microcnemes) any additional microcnemes are confined to the upper part of the body.

Species: *C. desiderata*, Steph., 1918 B, p. 109.

Family 2. ACTINERNIDAE, n. fam.

Haleuriidae as used by Carlgren, 1918, p. 24, *pro parte*.

Endocoelactaria with definite base. Body cylindrical or, more usually, expanded above, and in this case often divided into lobes, which are typically four or eight in number. There may or may not be collections of nematocysts in the body-wall ectoderm. Tentacles may be numerous, simple, or with mesogloal swellings on the aboral sides of some or all of them. A thick body-wall is a frequent characteristic. Two siphonoglyphes. There are a good many mesenteries, and the older ones are not marked off from the others as macrocnemes ; many are perfect ; the later ones may develop cyclically or bilaterally, and the partners of a pair be equal or unequal. The mesenterial musculature is not strong, and at least all stronger mesenteries are fertile.

Genera: *Actinernus*, *Synactinernus*, *Isactinernus*, *Synhalcurias*.

ACTINERNUS, Verr., 1879.

This is only part of Verrill's genus. Other forms sometimes assigned by mistake to it will be found under *Actinoscyphia* and *Polysiphonia*.

Porponia, Hertw., 1882, p. 125.

See Carlgren, 1914; Carlgren, 1918, p. 31, &c.; Stephenson, 1918 B, p. 127; Stephenson, 1920 A, p. 540.

Not *Actinernus* as used for *A. plebeius*, *A. saginatus*, *A. aurelia*.

Actinernidae with thick body which expands more or less distally, and is often but not always lobed, the lobes usually eight in number. Tentacles (except the youngest and sometimes the inner ones) with aboral mesogloecal swellings or bridges of varying development in different species, which may run up the tentacles almost to their tips. The tentacles usually in two cycles, and largest at the apices of the lobes. Numerous mesenteries, the older ones developed as usual in *Endocoelactaria*; after a certain point, however, they continue to appear in definite zones in a bilateral way, from the outer side of the zone inwards, usually. These mesenteries have the partners of a pair unequal. Mesenterial muscle weak.

Species:

A. nobilis, Verr., 1879, p. 474. (See Carlgren, 1918, p. 32.)

A. elongatus, Hertw., 1882, p. 111. (See Carlgren, 1918, p. 33.)

A. robustus, Hertw., 1882, p. 113. (See Carlgren, 1918, p. 34.)

A. michaelisarsi, Carlgr., 1918, p. 33.

A. antarcticus, Carlgr., 1914, p. 50; 1918, p. 35.

SYNACTINERNUS, Carlgr., 1918, p. 30.

Actinernidae with the body expanded above into eight lobes, four larger and four smaller alternating. Tentacles in at least two cycles, without basal swellings, largest at apices of the lobes, numerous. Radial muscle of disc and tentacles chiefly ectodermal. At least half the numerous mesenteries perfect. Mesenterial muscle not forming projecting retractors. The perfect mesenteries are cyclic in arrangement, beyond these are others of unequal size in upper part of body.

Species:

S. flavus, Carlgr., 1918, p. 31.

ISACTINERNUS, Carlgr., 1918, p. 29.

Actinernidae with distal part of body four-lobed, the lobes able to bend in over the mouth. Wall with very little papillae forming nematocyst batteries. Tentacles numerous, in at least two cycles, largest at the apices of the disc-lobes, the inner with small aboral mesogloea basal swellings, the outer with the swellings slighter or absent. Radial musculature of disc and tentacles chiefly ectodermal. Actinopharynx with very thick mesogloea. Mesenteries numerous, almost regularly arranged, cyclic, the partners equal, many of them perfect. Weak retractors in lower parts of older mesenteries.

Species :

I. quadrilobatus, Carlgr., 1918, p. 29.

SYNHALCURIAS, Carlgr., 1914, p. 68.

Ilyanthopsis as used by Wassilieff, 1908, *pro parte*.

Actinernidae with the body not lobed above. There are little nematocyst batteries in the column ectoderm. Tentacles without basal swellings, up to over 100. Radial muscle of disc tending to become mesogloea, longitudinal muscle of tentacles ectodermal. Numerous mesenteries, all perfect in old specimens, their arrangement cyclic but not very regular, the two partners of a pair generally about equal. Mesenterial musculature weak, not forming projecting retractors.

Species:

S. elegans, Wass., 1908, p. 8. (See Carlgr., 1914, p. 68; and 1918, p. 27.)

Sub-tribe 3. **MESOMYARIA**, mihi.

Nynantheae usually with a definite base and basilar muscles, but sometimes with a physa or an intermediate grade of base; sometimes the base is reduced; or it may be hollowed out into a cup or elongated as a slit, &c. The form is variable, from a worm-like burrowing condition to a broad flat dish shape or a flattened wrap-like condition, but the typical anemone form (more or less cylindrical or vase shaped) is the usual thing. In the advanced forms there is often a thick body-wall, and some of these exhibit ornamental knobs or roughnesses, crests, and so on, and in this connexion the tentacles may have aboral basal swellings of mesogloea. In some forms the body is divided into scapus and capitulum, and the scapus may have cuticle on it. There are never any true vesicles or aerorhagi, but sometimes suckers or verrucae. Cinclides often occur. The tentacles are usually simple, but may have the above-mentioned swellings, or even, rarely, thickenings of other different kinds. Their longitudinal musculature is ectodermal to mesogloea,

the latter condition fairly frequent among advanced forms. Not more than one tentacle communicates with each exo- and endocoel. The sphincter, if present, is mesogloea. Acontia are often present, and are the typical special stinging organs of the group; sometimes they are rudimentary and hard to detect. Secondary mesenteries appear in exocoels, not endocoels. Ectodermal muscle in the body-wall is exceptional. Mesenterial musculature is typically well developed; at its best very strong.

The ten families of Mesomyaria and the contained genera are defined in Part I of this paper. The only modification required is, that the word 'Mesomyaria' be substituted for the word 'Actiniina' in the family definitions there given. 'Actiniina' was used provisionally, pending the working out and publication of the groups set up in Part II.

Sub-tribe 4. ENDOMYARIA, mihi.

Nynantheae with a definite base save in one case, but it may be reduced or rather physa-like, or slit-like, or converted into a float, &c. Usually there are basilar muscles. Form variable, body-wall either smooth or with verrucae or acorhagi or vesicles, which may become complex outgrowths—more than one of these things may be present in the same animal. There is not the same tendency to ponderous body-walls (though of course these are sometimes fairly thick) and knobs and crests of mesogloea as in some Mesomyaria. Ectodermal muscle in the body-wall is exceptional. Cinclides do occur, but their distribution is little known. The tentacles are simple or complicated in various ways (see Part II, Text-figs. 14 and 19), but do not have basal mesogloea swellings. Their longitudinal musculature is less often mesogloea than in Mesomyaria. At their best development, in forms of warm seas, they may form complex tufts and give a frill-like or seaweed-like effect (see Part II, Text-fig. 19, and Text-fig. 18 for vesicles). In some forms not more than one tentacle joins each endocoel and exocoel. In others there is more than one on some or all of the endocoels, but not more than one on each exocoel. In still others the exocoels also may have more than one—so that sometimes there are many per endo- and exocoel. Sometimes the tentacles are reduced to sessile vesicular structures. The sphincter, if present, is endodermal of some grade, may be very strong and circumscribed at its best though often weak or diffuse, &c. There are never any acontia. Secondary mesenteries develop in exocoels. Mesenterial musculature is typically well developed; at its best very strong.

Family 1. CONDYLANTHIDAE, n. fam.

Antheadae as used by Carlgren, 1899, p. 9, pro parte.

Actiniidae as used by Pax, 1907, pro parte, &c.

Endomyaria with definite base. Body-wall unspecialized, without verrucae, the body sometimes elongate and half vermiform. Tentacles retractile, in several cycles, simple, not more than one to each exo- and endocoel. No sphincter. Radial musculature of disc and tentacles ectodermal. Mesenteries divided into macro- and microcnemes, macrocnemes (bearing gonad, filament, and strong retractor) six pairs; microcnemes without those structures.

Genus: *Condylanthus*.

CONDYLANTHUS, Carlgr., 1899, p. 15.

Charisea, Torrey, 1902.

With the characters of the family.

Species:

C. magellanicus, Carlgr., 1899, p. 15.

C. saxicola, Torrey, 1902.

There seems to be no adequate way of separating off these two species into two genera, the differences being seemingly of specific value only. I have therefore listed *Charisea* as a synonym of *Condylanthus*, which has priority.

Family 2. MYONANTHIDAE, n. fam.

Antheadae as used by McMurrich, 1898, p. 146, pro parte.

Actiniidae as used by Pax, 1907, pro parte, and Haddon, 1898, p. 414, pro parte.

Gonactiniidae as used by Carlgren, 1900, p. 15, pro parte (*Boloceroides*).

Endomyaria with definite base, which may be slight, with or without basilar muscles. Body-wall smooth or with suckers. Ectodermal muscle and spirocysts in body-wall and actinopharynx present or absent. No vesicles. Tentacles simple, not more than one per exo- and endocoel; they may be deciduous or not; their longitudinal muscle is ectodermal. Sphincter absent or endodermal, not very strong endodermal, diffuse or circumscribed-diffuse. Siphonoglyphes present or absent. Mesenteries NOT divided into macro- and microcnemes. Perfect mesenteries six pairs. Fertility affects the older mesenteries, excepting sometimes the directives. Retractors strong or weak.

Genera: *Myonanthus*, *Macroactyla*, *Bolocerooides*, *Nevadne*.

MYONANTHUS, McM., 1893, p. 151.

Myonanthidae with smooth body and slight fosse within the margin. Tentacles retractile, without sphincters. Sphincter definite, diffuse, and may have a good deal of anastomosis between its processes. All mesenteries fertile save the directives and the youngest.

Species:

M. ambiguus, McM., 1893, p. 151.

MACROACTYLA, Haddon, 1898, p. 431.

Myonanthidae with a delicate skin covered with little adhesive suckers. Large suckers on upper part of body. Fragments may adhere to it. No capitular rim or acrorhagi. Tentacles may be long and large, and are without sphincters. Sphincter not strong, sessile, circumscribed diffuse. Retractors strong, circumscribed diffuse. All mesenteries fertile.

Species:

M. aspera, H. and S., 1893, p. 124; Haddon, 1898, p. 431.

There is a slight ambiguity in Haddon's definition of this genus—I take his meaning to be that there are six pairs of perfect mesenteries, but if by any chance it is otherwise the genus will have to go to Actiniidae.

Bolocerooides, Carlgr., 1899, p. 43.

Myonanthidae with smooth body and tentaculate margin. No sphincter, no true siphonoglyphes, no basilar muscles. Ectoderm of body-wall and actinopharynx has spirocysts and muscle fibres as well as that of disc and tentacles. Tentacles provided with sphincters, therefore deciduous. All stronger mesenteries fertile save directives.

Species:

B. McMurrichi, Kwiet., 1898, p. 394. (See also Carlgr., 1899, p. 43; 1900, p. 16.)

B. hermaphroditica, Carlgr., 1900, p. 18.

A discussion of the systematic position of this genus will be found in Part II, p. 506, &c.

NEVADNE, n. gen.

Gyrostoma as used by Annandale for *G. glaucum* (1915, p. 70).

Myonanthidae. The one known form has longish body and long

tentacles, living in brackish water in India. There is a slight base. Body smooth but for microscopic prominences containing nematocysts. The outer tentacles are the largest. Six pairs of perfect mesenteries. Gonads on the older imperfect mesenteries. No true retractors or sphincter. Tentacles without sphincters.

Species:

N. glauca, Annan., 1915, p. 70.

It has been necessary to erect a new genus for Annandale's *Gyrostoma glaucum*, which cannot come within the genus *Gyrostoma* or even into the Actiniidae, with its six pairs of sterile perfect mesenteries and their feeble musculature. It seems to be a distinct and interesting form, which fits into the Myonanthidae well.

Family 3. ANDRESIIDAE, n. fam.

Ilyanthidae as used by Andres, 1883, p. 457, pro parte.

Endomyaria. The only known genus has a body capable of attaining a great length, and devoid of a pedal disc, being adapted for burrowing. Body without verrucae, but with a notched parapet and a fosse at the margin. Tentacles long, retractile, in four regular cycles, graded in size from within outwards. Small circumscribed endodermal sphincter. Longitudinal musculature of tentacles ectodermal. Twenty-four pairs of mesenteries in three cycles, all perfect but in varying degrees, all fertile save sometimes the directives, and provided with diffuse retractors which are not confined to the larger mesenteries only.

The above is a short statement of the chief characteristics of the only species as yet referable to this family, *Andresia parthenopea*. This form was described by Andres (1883, p. 459) as *Ilyanthus parthenopeus*, and further dealt with by Faurot (1895, p. 154) and Simon (1892). It is a species which does not conform to one's idea of Athenarian structure at all well, and certainly cannot remain in the genus *Ilyanthus*, as represented by *I. mitchelli*, which I have been able to investigate anatomically. I here suggest the generic name *Andresia* for it, after Angelo Andres, author of the largest monograph on Actinaria yet attempted. I have proposed (Part II, p. 522) to have a separate family *Andresiidae* for it, because it cannot well be placed in any known

family without making that family rather heterogeneous. It might be included in Actiniidae on the same principle by which I included *Halcampactis*, a genus with no base, in the Phelliidae—but there it was a case where there was transition to be traced between forms with no base and those with a base; and it does not follow that the same course need be observed in the two cases. At any rate I put forward the Andresiidae tentatively, as being the most expedient plan at the moment.

Family 4. ACTINIIDAE, sens. strict.

Actiniidae, Gosse + Bunodidae, Gosse, as used by Haddon, 1898, p. 414, but excluding *Antheopsis*, *Macroactyla*, and *Myonanthus*, which he includes.

Including Actiniidae (or Antheadae) and Bunodidae of most authors, with certain genera removed. Bunodidae, Gosse = Cribrinidae, McM. = Bunodactidae, Verr.

Including Boloceridae, McM.; Glyphactininae, Roule; Tealidae, Hertw.; Isohexactiniae, Kwiet.; Antheomorphidae, Hertw.; Liponemidae, Hertw., pro parte; Holactiniae, Boveri, &c.

Endomyaria with a base which is sometimes rather reduced, usually well developed; or it may be elongate through attachment to a spine. Body-wall smooth or with verrucae, but without vesicles. Margin tentaculate or distinctly marked off as a parapet or collar; with or without acrorhagi. Tentacles simple or with paired lateral enlargements; provided with sphincters rarely, more usually not; their longitudinal musculature usually ectodermal, rarely mesogloal. Sphincter absent or endodermal; weak or strong; exhibiting various grades of development—diffuse, circumscribed, &c. Not more than one tentacle to each exo- and endocoel. Mesenteries not divided into macro- and microcnemes, number of perfect mesenteries usually considerable, always more than six pairs in adult animals. Retractors variable, but often strong. Usually fertility affects either all the mesenteries or most of the older ones, though in other cases these are sterile.

Genera: *Actinia*, *Anemonia*, *Gyrostoma*, *Condy-lactis*, *Parantheopsis*, *Bunodactis*, *Tealia*, *Epiactis*, *Isotealia*, *Pseudophellia*, *Bolocera*, *Leipsiceras*, *Boloceropsis*, *Dofleinia*, *Ixa-lactis*, *Glyphostylum*, and perhaps others; see below.

ACTINIA, Browne.

Diplactis, McM., 1889, p. 110.

Hormathia as used by Hertwig for *A. delicatula* (1888, p. 15).

Actiniidae with smooth body having a collar-like margin, with a fosse between itself and the tentacles, in which lie acrorhagi. The latter are simple or more or less compound, usually conspicuous (e.g. blue) in colour, and can be covered up by the margin in contraction. Tentacles simple and typically retractile, their longitudinal musculature mostly or wholly ectodermal. Sphincter absent to weak or strong diffuse, sometimes with a mesogloal tendency, but endodermal actually. Mesenteries may be all fertile save directives. Retractors diffuse, may be strong. (Sphincter of *A. equina*, Part II, Text-fig. 11, B.)

Species:

A. equina, L., 1766-8, p. 1088. (= *A. mesembryanthemum*, Ellis and Solander, 1786, p. 4 = *A. cari*, D. Ch., 1825, p. 233.

See Gosse, 1860, p. 175; Pax, 1907, p. 53; Clubb, 1898; Andres, 1883, pp. 397 and 402; and Simon, 1892.)

A. delicatula, Hertw., 1888, p. 15. (See Haddon, 1898, p. 459; Carlgr., 1900, pp. 31-3.)

A. bermudensis, McM., 1889, p. 111.

A. tenebrosa, Farquhar, 1898, p. 535; Stuckey, 1909, p. 380.

A. kraemeri, Pax, 1914, p. 413.

I have listed under *Actinia* those species which seem to definitely belong to the genus as here defined. Sometimes rather vague forms are allotted to it, and there seems to be a certain amount of confusion with regard to it, and to the allied genera. It is actually quite a distinct genus, and there seems to be no reason for mixing it up with *Anemonia* or *Gyrostoma*. *A. equina* is of course the commonest of our British anemones, and *A. tenebrosa* seems to be its southern representative. Verrill ('Trans. Connect. Acad.',

xi, p. 51) describes an *A. melanaster*, but its systematic position is uncertain.

ANEMONIA, Risso.

Isactinia, Carlgr., 1900, p. 33.

Actiniidae with smooth body having a parapet formed by acrorhagi, which cannot therefore be covered up by the margin; they may vary a good deal in extent of development, and in some cases are almost suppressed, next to invisible. The tentacles may be long, and are typically non-retractile; their longitudinal musculature ectodermal. Sphincter diffuse or more or less circumscribed or intermediate, not very strong. Retractors variable, may be strong. Gonads may appear from the first cycle onwards. Marked siphonoglyphes not always present, nor directives necessarily. (Sphincter of *A. sulcata*, Part II, Text-fig. 11, E.)

Species:

A. sulcata, Penn., 1766. (= *Anthea cereus*, Ellis and Solander, 1786. See Gosse, 1860, p. 160; Andres, 1883, p. 405; Pax, 1907, p. 62; and Simon, 1892.)

A. manjano, Carlgr., 1900, p. 41.

A. theloteria, Pax, 1907, p. 69.

A. badia, Carlgr., 1900, p. 33.

A. hemprichi, Klunz., 1877, p. 72. (See Pax, 1907, p. 57.)

A. carlgreni, Lager, 1911, p. 226.

A. citrina, H. and S., 1893, p. 125; Haddon, 1898, p. 416.

Possibly *A. erythraea*, H. and E., and others may come here. Probably *Comactis flagellifera*, Dana, is only *A. sulcata*. I have fused *Anemonia* and *Isactinia* and made one definition cover both, because I cannot feel convinced of any real distinction between them. The main point seems to be a slightly different grade of sphincter, but it is not enough for separation in a family like this.

GYROSTOMA, Kwiet., 1898, p. 424.

Paranemonia, Carlgr., 1900, p. 61.

Actiniidae with smooth body and a more or less well-marked margin and usually some sort of fosse. There are no acrorhagi, but sometimes the margin is notched. Tentacles simple, their longitudinal musculature ectodermal. Sphincter absent, or diffuse or circumscribed, but not very strong; sometimes with a mesogloal tendency though actually endodermal. Retractors weak or strong, diffuse to circumscribed diffuse.

Gonads may appear from cycle 1 onwards. Siphonoglyphes variable in number, there may be several—also directives; latter may be absent.

Species:

- G. hertwigi*, Kwiet., 1898, p. 424, is the genotype. (See Haddon, 1898, p. 420.)
- G. ramsayi*, H. and S., 1893, p. 124; Haddon, 1898, p. 420.
- G. kwoiam*, H. and S., 1893, p. 125; Haddon, 1898, p. 422.
- G. cinerea*, Cont., 1844, p. 183. (See Pax, 1907, p. 36.)
- G. tristis*, Carlgr., 1900, p. 36.
- G. dubia*, Carlgr., 1900, p. 38.
- G. Stuhlmanni*, Carlgr., 1900, p. 39.
- G. Sancti-thomae*, Pax, 1910, p. 177.
- G. incertum*, McM., 1904, p. 230.
- G. selkirkii*, McM., 1904, p. 227.
- G. dysanacritum*, Pax, 1907, p. 48; 1909.
- G. haddonii*, Lager, 1911, p. 229.
- G. sulcatum*, Lager, 1911, p. 230.

Other species which may be referred with a query to the genus are *G. tulcarensis*, Pax, 1909, p. 404; *G. inequale*, McM., 1893, p. 149; *G. adhaerens*, H. and E., 1832, p. 258 (Pax, 1907, p. 51); *G. dichogama*, Kirk and Stuckey, 1909, p. 384; *G. olivacea*, Hutton, 1878 (Stuckey, 1909, p. 381); and *G. insessa*, Gravier, 1918, p. 3.

The above list includes forms which have been erroneously placed under *Anemonia*, and had to be transferred. The two genera have been a good deal confused, and especially when dealing with preserved material it is difficult to be certain about them. I have included *Paranemonia* as represented by *P. cinerea* in this genus, because it seems to me to be simply a *Gyrostoma* without directives, and not worthy of a distinct genus. *G. glaucum*, Annan., is no *Gyrostoma*, and needs a new genus (see p. 263).

CONDYLACTIS, D. and M., 1866.

Cereaetis, Andres, 1880.

? *Ilyanthopsis* as used by Hertwig for *I. longifilis* (1888, p. 13).

Actiniidae which sometimes reach a large size (*C. passiflora* may be a foot across). Body with verrucae in the upper part, which may be well developed, or weak, or practically or even entirely absent; sometimes they are arranged in vertical rows; fragments may adhere

to them. There are no acrorhagi, but there is a well-marked margin or collar. Tentacles simple, may be long and large, their longitudinal muscle ectodermal or with occasional anastomosis. Sphincter absent or very weak diffuse. Strong retractors. As a rule the mesenteries are all or mostly perfect and fertile, directives may be sterile; more rarely only twelve pairs perfect. Brood-pouches sometimes occur in the females. The tentacles and mesenteries may run in eights or tens, &c., as well as sixes.

Species :

- C. aurantiaca*, D. Ch., 1825, p. 438. (See Andres, 1883, p. 455; Pax, 1907, p. 22.)
C. passiflora, D. and M., 1866, p. 31. (See Pax, 1910, p. 171; McMurrich, 1889, 'Journ. Morph.')
- C. georgiana*, Pfeff., 1889, p. 15. (See Carlgren, 1899, p. 13.)
C. kerguelensis, Studer, 1878, p. 524. (See Pax, 1907, p. 32.)
C. erythrosoma, H. and E., 1832, p. 257. (See Pax, 1907, p. 30.)
 ? *C. cruentata*, Dana, 1849, Syn. p. 8, pro parte. (See Carlgren, 1899, p. 10; Pax, 1907, p. 26; McMurrich, 1893 and 1904; and Clubb, 1908, p. 2.)

There is a certain amount of ambiguity about this genus, or about the way in which it has been understood. Some forms have been split off from it and established under the separate name *Parantheopsis*, and these (*P. cruentata* and *P. ocellata*) seem to have acrorhagi of some sort, and on account of this and their lack of sphincter they stand half-way between *Condylactis* and *Bunodaectis*. For *Condylactis* is essentially a genus with smooth collar and no acrorhagi; and *Bunodaectis* is wide enough in its limits already, without the inclusion of sphincterless forms. To avoid too great a fusion of genera it is perhaps wisest to retain three: *Condylactis* for forms with smooth collar and no acrorhagi or appreciable sphincter; *Parantheopsis* for such as have vertical rows of verrucae and also acrorhagi but little or no sphincter; and *Bunodaectis* for those which have vertical rows of verrucae, usually acrorhagi, and some sort of sphincter—this may, admittedly, be weak, but typically is circumscribed. It seems possible that two distinct species have been described and confused under the name *cruentata*; the descriptions rather suggest this, and that one of the two is a *Condylactis*

and the other a *Parantheopsis*. Hertwig's *Ilyanthopsis longifilis* is probably *C. passiflora*. *C. hertwigi*, Wass., is no *Condylactis*. It has, so far as one can tell, good acrorhagi and a weakish circumscribed-diffuse sphincter. If, as stated, it has no verrucae it should go to *Anemonia*. If it has, to *Bunodactis*. *C. parvicornis*, Kwiet., does not seem to be a very typical *Condylactis*, possibly that also is a *Parantheopsis* or *Bunodactis*.

PARANTHEOPSIS, McM., 1904, p. 233.

See note under *Condylactis*.

Actiniidae with verrucose body, the verrucae usually above; they are in vertical rows, ending, at least some of them, in acrorhagi (which are not necessarily nematocyst batteries); foreign bodies sometimes adhere to the verrucae. No sphincter or only a trace. All or a good many of the mesenteries perfect, and all may be fertile save directives. Good retractors. Tentacular longitudinal muscle of the simple tentacles ectodermal. Tentacles and mesenteries may be hexamerous or octamerous.

Species:

P. cruentata, Dana, 1849, pro parte. See under *Condylactis cruentata* for references.

P. ocellata, Les., 1828, p. 79. (See McMurrich, 1904.)

BUNODACTIS, Verr., 1899.

Cribrina, Ehr., pro parte; *Bunodos*, Gosse, 1855; *Aegeon*, Gosse, 1865; *Anthopleura*, D. and M., 1860; *Aulaetinia*, Verr., 1862; *Evactis*, Verr., 1868; *Bunodella*, Verr., 1899; *Actinioides*, H. and S., 1893.

Actiniidae. A large genus of forms which are some of them easily retractile, others of more lax habit and only retractile with difficulty if at all. The body has regular vertical rows of verrucae, which are sometimes graded in length, and in size of the individual verrucae, according to the cycles of mesenteries they are connected with, and this may be accompanied by colour distinctions between the verrucae. Foreign bodies are often attached to the verrucae, which may also be somewhat lobed, distally. Acrorhagi are usually developed in connexion with the upper ends of at least some of the rows; but they may be there or not even in one and the same species. They may be simple, small or large, or decidedly compound. Sphincter variable, never very

powerful, but ranging from weak diffuse to more definite diffuse and slightly or distinctly circumscribed, weak or moderate in development; the state may vary even within one species, but the circumscribed form is the more usual and may attain a good strength. (Some of the weaker *Bunodactis*-sphincters are illustrated in Part II, Text-figs. 11 D, F, and 12, D, E.) Tentacles simple, their longitudinal muscles ectodermal. Retractors often strong, diffuse to circumscribed diffuse or even circumscribed. Gonads may appear on the older mesenteries, or all mesenteries may be perfect and fertile, save sometimes the directives. Brood-pouches may occur. Siphonoglyphes and directives variable in number. Symmetry hexamer, octamer, &c., or irregular.

Species :

The genotype is *B. gemmacea*, Ellis and Solander, 1786, p. 3.
(See Gosse, 1860, p. 190; G. Y. and A. F. Dixon, 1889, p. 321.)

The other British species are *B. thallia*, Gosse, 1854, p. 283
(see Gosse, 1860, p. 195; G. Y. and A. F. Dixon, 1889, p. 310),
B. ballii, Cocks, 1849 (see Gosse, 1860, p. 198), and *B. alfordi*, Gosse, 1865, p. 41.

Foreign species numerous.

This is a genus somewhat parallel to *Sagartia* among the Mesomyaria. Its synonymy has been much discussed. The genus *Cribrina*, like *Urticina*, seems too vague to be adopted. Against one's wishes it seems necessary to let the familiar *Bunodes* lapse, since the name was pre-occupied for a Eurypterid in 1854; and Verrill's name *Bunodactis* steps into the breach with several synonyms. There may be some forms which have been wrongly placed under the genus, and their position should be reconsidered if they do not come under the above definition, which is wide enough already. The genera *Bunodactis*, *Anthopleura*, and *Aulaetia* have already been fused by Torrey (1906, pp. 47-52), and I fully agree with him that there is no valid way of separating them. I now add *Actinioides* to the list of synonyms. It has always represented the *Bunodes* species with the weaker sphincters, but apparently because of its being placed in the Actiniidae while *Bunodes* was placed in the Bunodidae, the similarity was overlooked. It is now evident that the Bunodidae and Actiniidae are one and the same family (see Part II, p. 526), and the two genera can no longer be

separated. The sphincters show a graded series from weak to fairly strong, and from diffuse to more circumscribed, so that it would be difficult to draw a boundary line (cf. Part II, Text-figs. 11, D, F, and 12, D, E, for some of the weaker ones). And there are no other special points of difference. *B. capitata* is said to have only six pairs of perfect mesenteries, in which case it should be transferred to *Macrodaetyla*, and then the latter name might have to give way to the earlier name *Aulactinia*, in a new sense. It is possible of course for young specimens of a species to have only six pairs of perfect mesenteries, but if it persists in the adult the form needs exclusion both from *Bunodaetis* and from the Actiniidae too.

TEALIA, Gosse, 1858, p. 417; 1860, p. 205.

Rhodaetinia, Agassiz, 1847, p. 679; 1865, p. 13.

Actiniidae. Body sometimes low and broad, attaining however a high cylindrical form in full expansion. Body in some specimens very mobile and changeable in form, in others less so. Column with verrucae which are not usually arranged in definite vertical rows, at any rate in the adult; they are sometimes strongly, sometimes very weakly developed, in still others quite absent—and all this within one and the same species, probably. There is a parapet and fosse, but no acrorhagi in the ordinary forms—they occur in certain Antarctic cases though. Tentacles simple, stout, their longitudinal muscle varying from ectodermal to mesogloal, the latter being perhaps the more typical condition in the common forms. Strong circumscribed sphincter. Tentacles and mesenteries often in multiples of ten in the common forms, but not invariably. Primary mesenteries may be fertile or sterile. Retractors strong or very strong, diffuse or circumscribed diffuse. All mesenteries may be perfect. (For *T. crassicornis*, see Part II, Text-figs. 7, B, and 12, B.)

Species:

T. crassicornis, O. F. Müller, 1776, p. 231. (*T. coriacea*, Cuvier, 1797, p. 653.) (See also Gosse, 1860, p. 209; Carlgren, 1893, p. 58; Carlgren, 1902, p. 38; Clubb, 1908, p. 9; McMurrich, 1911.) (*Bolocera eques*, Gosse, 1860, p. 351; *Madoniaetis lofotensis*, Dan., 1887, p. 47, pro parte.)

T. carlgreni, Clubb, 1902, p. 297.

T. sulcata, Clubb, 1902, p. 295.

Tealia seems to be the famous genus for synonymy-discussions, and I will add as little as possible. I cannot

pretend to go into detail about it, but I venture to support *Tealia* as the best name to use, even if the legality is doubtful—in any case something would be doubtful. *Urticina* is too ambiguous, and although it probably contained one of our common forms it seems justifiable to reject it in favour of the non-ambiguous *Tealia*. *Rhodactinia*, Agassiz, has priority, but although *R. davisii* seems to be identical with one of the forms more usually called *crassicornis* or *coriacea*, yet the genus is insufficiently described and is not free from ambiguity. *Tealia* is well defined and a familiar name, and seems clearly to have the advantage.

I cannot accept Carlgren's division of the genus into two distinct genera—*Tealia* and *Rhodactinia*—because it cannot (as McMurrich has already pointed out) be upheld by anything stable. With regard to species under the genus, I do not like to speak with finality; but my experience with living specimens and study of literature suggests that there is really no valid way of splitting up our British forms into species—I imagine that they are all races or forms of one variable species, but the warts and other things do vary a great deal. This has not always been my opinion, and it is the kind of point about which one is liable to change one's mind more than once—the other possibility being to regard our British forms as two species (the extremes are certainly very different from each other in appearance) with intermediate grades. Clubb's two Antarctic species are certainly distinct from ours, but seem very like each other, and in some ways verge towards *Bunodactis*—might even be members of that genus, though *Tealia*-like in build and probably best left where they are. As to the right specific name for the British species, it will perhaps always be a disputed thing, but on the idea that we have only one variable species the name *crassicornis* has priority over *coriacea*. I have seen *Bolocera eques*, Gosse, alive, and it is simply a form of *T. crassicornis*.

It has been suggested that the irregularly arranged verrucae of *Tealia* must really be in vertical rows since they

communicate with mesenterial spaces. But although this is true in a sense, it smothers the more important fact that in *Tealia* the total arrangement of warts on the body-wall as a whole presents an irregular appearance to the eye, whereas in the contrasting *Bunodactis* the warts run in regular vertical series visible as such, and often with a cyclic arrangement corresponding to the cycles of endocoels, and even coloured differently according to cycle in some cases, so that in the end the difference is a marked one.

EPIACTIS, Verr., 1868.

Epigonactis, Verr., 1899, p. 378.

Glyphoperidium, Roule, 1909, p. 10.

Not *Leiotealia*, Hertw., 1882, p. 37—which is probably a synonym of *Aureliania*; see note under that genus on p. 292.

Actiniidae in which the base may be well developed, or somewhat reduced, or may be crack-like through attachment to a spine. Wall smooth, no verrucae or acrorhagi, but well-marked parapet and fosse. There may be young ones adherent to the surface, or even shallow or deep brood-pouches. Tentacular longitudinal muscle ectodermal or with a tendency to anastomosis; disc radial muscle ectodermal or mesogloal. Sphincter well developed, circumscribed, varying in strength but usually very strong, sometimes exhibiting a good deal of anastomosis of processes. Retractors diffuse, circumscribed diffuse, or even circumscribed, often very strong. Mesenteries may be all perfect; gonads from cycle one onwards, or not; or on all, save perhaps the directives. (For two *Epiactis* sphincters see Part II, Text-fig. 12, a and c.)

Species :

E. prolifera, Verr., 1868, p. 492. (*E. fertilis*, Andres, 1883, p. 574.) See also Torrey, 1902, and McMurrich, 1901.

E. fecunda, Verr., 1899, p. 378. (*E. regularis*, Verr., 1899, p. 380.) (? *E. spitzbergensis*, Kwiet., 1898, p. 134, pro parte.)

E. marsupialis, Carlgr., 1901, p. 482.

E. badia, McM., 1893, p. 194.

E. thompsoni, Coughtrey, 1874. (See Stuckey, 1909, p. 370.)

E. novo-zealandica, Steph., 1918 a, p. 24.

E. ritteri, Torrey, 1902, p. 393.

E. bursa, Roule, 1909, p. 11.

E. vas, Roule, 1909, p. 13.

E. dubia, Wass., 1908, p. 20, may be an *Isotealia*.

ISOTEALIA, Carlgr., 1899, p. 24.

Actiniidae with smooth wall and well-marked margin provided with some sort of acrorhagi; there may be cuticle. Sphincter well developed, circumscribed. Tentacular longitudinal musculature ectodermal. Retractors fairly developed or strong. Gonads absent on older mesenteries, or present on all save directives.

Species:

I. antarctica, Carlgr., 1899, p. 25, and probably *I. dubia*, Wass., 1908, p. 20.

PSEUDOPHELLIA, Verr., 1899, p. 376.

Actiniidae in which the scapus is covered with a thick soft cuticle, the capitulum smooth and with parapet and fosse. There may be brood-pits in the scapus. Sphincter circumscribed. Strong retractors.

Species:

P. arctica, Verr., 1868, p. 328; 1899, p. 376.

This genus seems to be a sound member of the family, but more details about it are desirable.

BOLOCERA, Gosse, 1860, p. 185.

Liponema, Hertw., 1882, p. 129. (See Hertwig, 1888, p. 17; McMurrich, 1893, pp. 160 and 209; Carlgren, 1899, p. 39; Haddon, 1898, p. 429.)

Actiniidae with smooth body (no verrucae or acrorhagi) and unspecialized margin which may be tentaculate or have some sort of fold. Tentacles provided with basal sphincters so that they are deciduous; they are variable in size and number, sometimes very large, at others very numerous, or again neither unusually large nor numerous. Sphincter diffuse, may be well developed, sometimes with one or more of its processes predominating in size over the others, or with a tendency to circumscription. Diffuse retractors. Gonads may appear on the older cycles, or these may be sterile. Tentacular longitudinal muscle ectodermal. Perfect mesenteries variable in number: sometimes all are perfect.

Species:

B. tuediae, Johnst., 'Mag. Nat. Hist.', v. 163. (See Gosse, 1860, p. 186; Walton, 1908, p. 215; Stephenson, 1918 B, p. 112.)

B. longicornis, Carlgr., 1891, p. 241. (See Carlgren, 1893, p. 50; Stephenson, 1918 A, p. 20.)

- B. kerguelensis*, Studer, 1879, p. 544. (See Kwietniewski, 1896.)
B. multicornis, Verr., 1879, p. 198. (See Carlgren, 1902 (Olga), p. 36.)
B. brevicornis, McM., 1893, p. 158.
B. pannosa, McM., 1893, p. 156.
B. occidua, McM., 1893, p. 154.
B. multipora, Hertw., 1882, p. 129. (See also references given above for *Liponema*.)

The genus *Bolocera* should be limited to the above definition and list of species, as regards known forms. *B. pollens* has now a genus apart on account of its sphincter. *B. eques* is a *Tealia*. *B. norvegica* is of doubtful standing. *B. africana* is, according to Carlgren (1911, p. 21), a *Sagartid* wrongly described as a *Bolocera*.

LEIPSICERAS, Steph., 1918 B, p. 112.

Actiniidae with smooth wall, no verrucae or acrorhagi. Very strong circumscribed sphincter. Tentacles provided with sphincters, therefore deciduous.

Species:

- L. pollens*, McM., 1898, p. 230.

This genus was separated from *Bolocera* on account of the sphincter. It seemed advisable because of the wide gap between the typically diffuse *Bolocera*-sphincter and the elongate circumscribed muscle with a mesogloea axis found in *L. pollens*; there is a gap here, not a series as in *Bunodactis*.

BOLOCEROPSIS, McM., 1904, p. 255.

Actiniidae with smooth body and tentaculate margin. The only known species has large tentacles rather like those of a *Bolocera*, but without tentacle-sphincters; their longitudinal muscle ectodermal. Sphincter and retractors diffuse.

Species:

- B. platei*, McM., 1904, p. 255.

Whether there is really any sound distinction between this genus and *Gyrostoma* it is not easy to decide, but pending further knowledge it is safest to let it stand. The nature of the margin may be quite a good distinguishing point.

Dofleinia, Wass., 1908, p. 13.

Actiniidae with smooth body. The only described form has large tentacles rather like those of a *Bolocera*, and both these and the disc are covered with papillae, plainly visible to the naked eye, and which represent batteries of nematocysts. Longitudinal muscle of tentacles ectodermal. Sphincter and retractors diffuse.

Species:

D. armata, Wass., 1908, p. 14.

This is a genus not very clear in its exact relationships, but it does not seem to fuse readily with any other, and the papillose disc and tentacles are a distinct feature.

Ixalactis, Haddon, 1898, p. 443.

Actiniidae with the wall smooth below, with suckers above, and a definite crenulated parapet. Disc flat when fully expanded, but often thrown into lobes, and not fully retractile. Tentacles numerous, the aboral side of each being smooth, the oral side flattened, and with symmetrical lateral swellings, so that the whole looks not unlike a knotted cruciferous seed-pod in some conditions. Sphincter moderately developed, circumscribed.

Species:

I. simplex, H. and S., 1893, p. 123. (See Haddon, 1898, p. 443.)

A very distinct genus. Probably the form photographed by Saville-Kent as *Condylactis*, sp. It is a possibility that the genus is identical with *Ragactis* as represented by Andres's figures of *R. pulchra*—at any rate they suggest it, and it would be an interesting point to follow up.

Glyphostylum, Roule, 1909, p. 14.

Actiniidae. In the one form described the body is a long trumpet with short stout tentacles. The tentacles are thicker on one face than the other, and their longitudinal musculature is ectodermal and much stronger on the thick side than the other. Smooth body-wall and no sphincter.

Species:

G. calyx, Roule, 1909, p. 16.

Roule (1909, p. 2) has set up a sub-family Glyphactininae of the Antheidae (Actiniidae), to rank equal with two other sub-families, the Bolocerinae and Actininae. His sub-family

includes two genera, *Glyphoperidium* and *Glyphostylum*. In the first place *Glyphoperidium* seems undoubtedly identical with *Epiactis*, and is here included in that genus, with its two species, *G. vas* and *G. bursa*. Moreover, the sub-family seems to have been erected as a result of laying too much stress upon some apparently trivial characters, especially connected with the actinopharynx. It is hard to find any justification for such a sub-family, and it is not adopted here. The other genus erected, *Glyphostylum*, seems more worthy of distinction, and although its separateness is not very marked it is defined above, provisionally at any rate.

ANTHEOMORPHE (Hertw., 1882, p. 30) seems barely if at all distinguishable from *Gyrostoma*.

COMACTIS. *C. flagellifera*, Hertw., 1882, p. 32, might be almost anything. Dana's is probably *Anemonia sulcata*.

POLYSTOMIDIUM (Hertw., 1882, p. 67) can hardly stand. The stomidia seem to be the remains of torn-off tentacles and the oesophageal openings probably ruptures (I have seen the specimen). Of what genus it is a battered representative is another matter.

ILYANTHOPSIS (Hertw., 1888, p. 13) has to lapse. *I. longifilis*, Hertw., is probably a *Condylactis*, and *I. elegans*, Wass., is a *Synhalecurias* (see p. 260). Pax refers to *I. longifilis* in his 1910 paper, pp. 171, 173, &c., as probably *C. passiflora*.

MYRIACTIS (Haddon, 1888, p. 248) is not easily allocated. It may be a *Stichodaetyline* like *Radianthus*, or it may stand among the *Actiniidae* near *Condylactis*, but there are not quite enough data to make a certainty of it.

GYRACTIS (Boveri, 1893, p. 246) (see Haddon, 1898, p. 445) is very near *Bunodaetis*, if not absolutely identical with it. The fact that it has no directives or siphonoglyphes cannot keep it apart, these things are too much matters of specific or individual idiosyncrasy. The doubt about

definitely fusing it up with *Bunodactis* is that the existence of regular vertical rows of verrucae seems uncertain, although both verrucae and acrorhagi are present. Regular vertical rows are extremely characteristic of *Bunodactis*, but otherwise *Gyractis* has the organization of that genus.

TEALIOPSIS polaris, Dan., and *KYLINDROSACTIS elegans*, Dan., are, according to Carlgren's examination of the original specimens, identical with *Stomphia*. *MADONIACTIS lofotensis*, Dan., seems to be a name covering *Tealia*, *Metridium*, and *Hormathia*, and is invalid.

Family 5. ALICIIDAE, sens. strict.

Aliciidae, Duerden + Phyllactidae, Andres, as used by Haddon, 1898, pp. 433 and 435, both pro parte.

Endomyaria with definite base and more or less delicate tissues. The column may be divided into a scapus with vesicles and a smooth capitulum; or the scapus may be smooth and the vesicles occur where it joins the capitulum, and somewhat higher up as well in some cases. The form may be very changeable. Tentacles simple, variable, may be long, their longitudinal musculature ectodermal. Ectodermal longitudinal muscle may be present in actinopharynx and capitulum, also spirocysts at least in the latter. Sphincter absent or feeble endodermal diffuse. Not more than one tentacle to each exo- and endocoel. Mesenteries not divided into macro- and microcnemes. Only six pairs of mesenteries perfect.

Genera: *Alicia*, *Phyllodiscus*.

ALICIA, Johns., 1861.

Cladactis, Panc., 1868, not *Cladactis*, Verr., 1868.

Aliciidae with delicate column capable of elongation, and divided into scapus and capitulum. The scapus bears vesicles, varying in form and in detail, but at least some of which are compound and stalked. Capitulum naked; may have weak longitudinal muscle and spirocysts in its ectoderm; the muscle may also be present in the actinopharynx. The vesicles have numerous sting-cells, which may be very large. Margin may be tentaculate. Tentacles typically long and slender, retractile. The six pairs of perfect mesenteries may be sterile. Retractors not strong, diffuse. Sphincter absent or weak diffuse.

Species :

- A. mirabilis*, Johns., 1861, p. 303. (See Duerden, 1897.
A. costae, Panc., 1868, p. 30. (See Duerden, 1895, p. 213.)
A. sansibarensis, Carlgr., 1900, p. 28.
A. rhadina, H. and S., 1893, p. 127, Haddon, 1898, p. 433.
 And probably others.

PHYLLODISCUS, Kwiet., 1898, p. 407. (See Part II, Text-fig. 18.)
Hoplophoria as used by Haddon (1898, p. 438) for
H. cineta, not as used by Wilson for *H. coralligens*
 (1890, p. 379).

Aliciidae in which the lower part of the body or scapus is smooth ; at its junction with the upper part or capitulum, which may be delicate and extensile, there is at least one ring of stalked vesicles ; there may be one ring of about six vesicles only, or one complete ring containing a good many more than that, and a few outside and above the ring ; or there may be several series of them, formed by one vesicle communicating with each of the older endocoels, four or more with each of the younger endocoels and the exocoels. Form of vesicles variable as to detail, more or less compound. Capitulum may have ectodermal longitudinal muscle, its margin tentaculate. The six pairs of perfect mesenteries may be sterile. Retractors: weak diffuse. Sphincter absent or weak diffuse. At their best the vesicles form a wide frill or ruff round the body (see Part II, Text-figs. 18 and 2 A).

Species :

- P. semoni*, Kwiet., 1898, p. 407.
P. cineta, H. and S., 1893, p. 127 ; Haddon, 1898, p. 438.
P. indicus, n. sp.

I am uniting under this genus (erected by Kwietniewski for *P. semoni*) three species. One is the *Hoplophoria cineta* of Haddon and Shackleton, which is a quite distinct form, but does not agree with the type of the genus *Hoplophoria*. That type, *H. coralligens*, Wilson, is taken by Duerden (see 1898, p. 456, and 1902) to be a *Lebrunia*. *H. cineta* does, however, fit in as a *Phyllodiscus*, possibly an immature one. The third species is a new one which I have from the Maldivé Islands (out of a collection kindly lent me by Professor Stanley Gardiner), and which, though perhaps not fully developed, is much further on than *P. cineta*, and forms a link between that and *P. semoni*,

It seems not unlikely that *Phyllodiscus* is identical with *Triactis*, but it would be well to wait for the anatomy of *T. producta* before assuming that and changing the name. A species which might possibly come in here is the one described by Hargitt as *Cradactis variabilis*.

Family 6. PHYLLACTIDAE, sens. strict.

Phyllactidae, Andres + Aliciidae, Duerden + Dendromeliidae, McM., as used by Haddon, 1898, pp. 435, 433, 440, all pro parte. Including Thaumactiniae, Fowler.

Endomyaria with definite base. Body-wall variable; it may be wide and provided with vesicles below, narrower and naked above; or there may be vesicles all over it, with or without acrorhagi at the margin; or the lower part of the body may be devoid of vesicles, and provided only with verrucae, while the vesicles are confined to the sub-marginal zone, really representing foliose acrorhagi, and sometimes forming a very definite collar or ruff; or again, the sub-marginal region may bear only about six vesicles or 'pseudotentacles', which at their best form large branching bush-like structures. In spite of this variation vesicles are always present, and both they and any acrorhagi there may be can be simple or compound. Tentacles simple, provided with sphincters in one genus only; so that usually they are non-deciduous; their longitudinal musculature ectodermal or mesogloal. There may be ectodermal longitudinal muscle in body-wall and actinopharynx. Mesenteries not divided into macro- and microcnemes, more than six pairs, and usually twelve or more pairs perfect, with occasional exceptional individuals. Sphincter absent, diffuse, or circumscribed.

Genera: *Phyllactis*, *Cradactis*, *Phymactis*,
Cystiactis, *Bunodeopsis*, *Thaumactis*, *Lebrunia*.

This family and its contained genera present a good deal of difficulty. I have attempted a revision of them, but it may need carrying a good deal further in the light of new knowledge. A number of genera have been described under Phyllactidae, Aliciidae, Bunodidae, Dendromeliidae, and Thaumactidae, which need a good deal of sorting out. The principle upon which one must work, of having two families (Aliciidae and Phyllactidae) was introduced in Part II, p. 530, and the

Aliciidae, sens. strict., have been dealt with above. There remains the set of forms now to be called Phyllactidae. To begin with, one suspects that names have been needlessly multiplied, and the mass of forms seems to present seven good genera, with some synonyms. Of these seven, one can say that they exhibit the same general grade of structure as the Actiniidae, but with vesicles added; but beyond that there are differences and one notes five sets of them. At least two of these sets are the logical outcome along slightly different lines of a further development of Actiniid forms, and may be looked upon as a natural family representing a stage further than the Actiniidae. In one of these sets (*Phyllactis* and *Cradactis*) the acrorhagi of some Actiniid ancestor seem to have developed complications so as to form a sort of ruff, while the verrucae remained the same; in the other set (*Cystiactis*, *Phymactis*) the verrucae have developed into vesicles, and sometimes there are acrorhagi as well. In connexion with the first set, it is interesting to note that one gets, now and then, an abnormal individual of *Actinia equina* in which some of the acrorhagi have become compound, in just such a way as one would expect a beginning to be made in the *Phyllactis* direction.

It is when we come to the other genera that the chief difficulty arises. *Thaumactis* is a small, possibly a young form, of uncertain affinities. *Bunodeopsis* is very distinct and is now, thanks to Duerden, a well-studied genus; but it is possible to think of it on the one hand as an Aliciid (sens. strict.) which can develop more than six pairs of perfect mesenteries, or on the other as the outcome of an Actiniid which has developed along a line all its own—the ancestor being, even, a pre-Actiniid *Bolocerooides*-like form. *Lebrunia* could well enough be derived from some Actiniid or pre-Actiniid in a special way. Taking them as a whole, all these forms might be derived from forms like Actiniidae or pre-Actiniidae, the suggestion of Aliciid origin only coming in strongly in the case of *Bunodeopsis*. Since we can never know their exact history, and since it seems reasonable to think

of them diverging along different lines from somewhere near the same place, it is probably best to include them all in one family, the Phyllactidae. It will show a good deal of range as to detail, but with the fundamentals in common.

An extended discussion of other families which have been involved near Phyllactidae seems hardly necessary. It was pointed out in Part II (p. 530) that Dendromeliidae and Thaumactidae could hardly be upheld. Any inclusion of vesicled genera like *Bunodosoma* in the Bunodidae seems to have been a mistake. Some genera here placed in Phyllactidae were referred to Aliciidae before, but the revised sense in which the families are here taken necessitates an alteration.

PHYLLACTIS, M. Edw. and H., 1851.

Oulactis, M. Edw. and H., 1851; *Asteractis*, Verr., 1868; *Lophactis*, Verr., 1868; ?*Actinostella*, Duch., 1850.

Phyllactidae. Column may be capable of a good deal of elongation; it has verrucae which usually occur in vertical rows and may attach foreign bodies to themselves. Above the verrucae and below the margin proper there is a very definite ruff, frill, or collar, which may be quite wide and conspicuous, and is formed of a number of radiating series of vesicles separated from each other by grooves, and which apparently represent complicated and extended acrorhagi; the whole ruff is separated from the tentacles by a fosse; the detail of the acrorhagi or 'fronds' varies in different cases. Sphincter usually circumscribed, more or less, not very strong, but it may be diffuse. Tentacular longitudinal muscle ectodermal. Retractors typically strong, diffuse to circumscribed diffuse.

Species:

P. praetexta, Dana, 1849, p. 150. (See McMurrich, 1905 on D. and M. Actinians.)

P. flosculifera, Les., 1817. (See McMurrich, 1889 ('Journ. Morph.');

1889 (Bermudas.) (*P. fasciculata*, McM., 1889 (Bermudas), p. 108.)

P. conchilega, D. and M., 1860. (?*P. expansa*, Duerden, 1898, p. 455.) (See Pax, 1910, p. 194; McMurrich, 1905; Duerden, 1898, p. 455, and 1902.) (*P. foliosa*, Andres, 1883, p. 505.)

P. bradleyi, Verr., 1868, p. 465; 1899.

P. concinnata, Dana, 1846, p. 152. (See Pax, 1912, p. d. 12.)

P. radiata, D. and M., 1860. (See McMurrich, 1905.)

P. californica, McM., 1893, p. 196.

And probably others.

I have done the best I can with this genus, but the synonymy of its species is a matter for special study. I think McMurrich has made it clear that *Phyllactis* is identical with *Oulactis*, *Asteractis*, and *Lophactis*—probably also with *Actinostella*, in which case the latter name would have priority; but until more is known of the type, *A. formosa*, it seems better to keep to the well-known *Phyllactis*. *P. striata*, Wass., seems more like a *Cradactis*.

CRADACTIS, McM., 1893, p. 197.

Saccactis, Lager, 1911, p. 220.

Phyllactidae in which the column has verrucae, usually in vertical rows, to which foreign bodies may adhere. At the margin there are vesicles in a ring—probably modified and developed acrorhagi; they vary in form, the uppermost at least being somewhat lobed or branched or foliose, and there may be concentrations of nematocysts on them; when these 'fronds' are at their best development they may form a wide frill round the animal. Sphincter diffuse or circumscribed, usually well developed. Longitudinal musculature of tentacles ectodermal. Retractors usually strong, diffuse to circumscribed diffuse. Gonads may or may not appear from the first cycle onwards.

Species:

C. digitata, McM., 1893, p. 198.

C. plicatus, Hutton, 1878. (See Stuckey, 1909, p. 392.)

C. magna, Stuckey, 1909, p. 394.

C. memurrici, Lager, 1911, p. 220.

C. australis, Lager, 1911, p. 223.

C. muscosa, Lager, 1911, p. 223.

C. excelsa, Wass., 1908, p. 23.

? *C. striata*, Wass., 1908, p. 22.

I have joined this genus and *Saccactis* because I cannot find any very serviceable distinction between them, and I think any variation there may be in the form of the vesicles and their continuity with the rows of verrucae probably finds a parallel in *Bunodactis*, as do also the variability of nematocysts in the fronds and the variations of the sphincter. *Cradactis*

seems distinct from *Phyllactis* in that, apparently, the fronds or acrorhagi have not attained such clear distinction from the rest of the column as in that genus, where they make such a very definite zone or ruff. *C. variabilis*, Hargitt (1911, p. 51), seems of rather uncertain standing—it may come here, or, possibly, under *Phyllodiscus*.

PHYMACTIS, M. Edw., 1857.

Rivetia, Pax, 1912, p. D.5; *Bunodosoma*, Verr., 1899; *Eucladactis*, Verr., 1899, p. 49.

Phyllactidae in which the column is covered thickly with vesicles, which may be quite without arrangement, or may form more or less definite vertical rows, and sometimes the rows are of different sizes in a regular way according to mesentery cycles. The vesicles may be simple or more or less compound, and sometimes they fuse inseparably with each other. Acrorhagi, which may be compound, may be present (not always), being sometimes well developed and at others hardly distinguishable, within the same species. Above the acrorhagi a fosse. Sphincter weak or strong diffuse, circumscribed diffuse, or small to moderate circumscribed. Tentacular longitudinal muscle ectodermal. Retractors diffuse, weak or well developed, or stronger and circumscribed diffuse. The older mesenteries may be sterile, or most mesenteries may be fertile. There may be more than two siphonoglyphes, which need not correspond to directives—the latter may be absent.

Species :

- P. clematis*, Drayton in Dana, 1846, Syn. p. 6. (*P. florida*, Dana, 1849.) (See Carlgren, 1899, p. 17; McMurrich, 1904; Stephenson, 1918 A, p. 23.)
- P. granulifera*, Les., 1817, p. 173. (*Bunodes taeniatus*, McM., 1889, p. 23.) (See Pax, 1910, p. 184; McMurrich, 1889 ('Journ. Morph.'), p. 23; Duerden, 1902, p. 348.)
- P. sphaerulata*, Duerden, 1902, p. 350.
- P. küenthali*, Pax, 1910, p. 189.
- P. papillosa*, Les., 1830, p. 78. (See Pax, 1912, p. D. 6.)
- P. grandis*, Verr., 1868, p. 473; 1899, p. 49.

I have included Verrill's *Eucladactis grandis* with some hesitation in this genus, following McMurrich. It seems to have almost enough to merit distinction in its very definite cyclic rows of vesicles, rather comparable to the verrucae of *Bunodactis gemmacea* in arrangement—but there are

at any rate tendencies in this direction in other species (cf. *P. sphaerulata*), and I leave it at that for the time being. I think my fusion of *Bunodosoma* with *Rivetia* and *Phymactis* can be supported in the same sort of way as Torrey's fusion of *Bunodactis* with *Anthopleura*, &c. It has been seen from time to time that sphincter-detail alone cannot always separate species; that acrorhagi are too variable (cf. *P. granulifera* in which they may be well developed or barely discernible) to be invariable ground for separation; and there do not in this case seem to be any valid distinctions to be based on the vesicles. The siphonoglyphe-variation and lack of directives in *P. (Rivetia) papillosa* has parallels elsewhere, and is hardly in itself of generic weight.

CYSTIACTIS, M. Edw., 1857. (See Haddon and Duerden, 1896, p. 154.)

Phlyctenactis, Stuckey, 1909, p. 396.

Phyllactidae with the column covered by simple sessile or slightly pedunculate vesicles. No acrorhagi. Longitudinal musculature of tentacles mesogloal. Sphincter absent or diffuse. Primary mesenteries may be sterile. Retractors diffuse.

Species:

C. tuberculosa, Q. and G., 1833, p. 159. (See Haddon and Duerden, 1896, p. 156; Lager, 1911.)

C. retifera, Stuckey, 1909, p. 396.

C. morrisoni, Stuckey, 1909, p. 396.

?*C. Koellikeri*, Pax, 1910, p. 180.

And other older species not yet well known.

I have placed Stuckey's *Phlyctenactis* under *Cystiactis*, with which it seems to be identical, especially if the tentacular muscle is mesogloal, as his figures lead one to believe. *C. Koellikeri* may belong here or under *Phymactis*. *Cystiactis* is distinguished from *Phymactis* by its simple vesicles, constant absence of acrorhagi, mesogloal tentacle-muscle, and on the whole weaker musculature.

BUNODEOPSIS, Andres, 1880. (See Duerden, 1897 and 1902.)

Phyllactidae with the body broad and flattish below, covered with

vesicles; narrower and devoid of vesicles above, thus having a smooth extensile capitulum. The capitulum has a tentaculate margin, and both it and the long tentacles are retractile. The latter have little stinging spots on them, and moreover each has a sphincter at its base, as in *Bolocera*, so that it is deciduous. The vesicles may be simple and sphaeroidal or compound, sessile or stalked, and have nematocysts in at least parts of their ectoderm. Tentacular longitudinal muscle ectodermal. There is ectodermal longitudinal muscle in body-wall and actinopharynx, and there are no siphonoglyphes; but the mesenterial filaments have ciliated tracts as usual. Sphincter absent or weak diffuse. Number of perfect mesenteries variable, about four to twenty pairs, the irregularity probably connected with fission and laceration as modes of reproduction. Retractors diffuse. Tissues delicate. Habitat, weeds, stones, &c.

Species:

B. strumosa, Andres, 1880, p. 315. (See Duerden, 1897 and 1902, 'Trans. Linn. Soc.')

B. antilliensis, Duerden, 1897, p. 7; 1902, 'Trans. Linn. Soc.'

B. globulifera, Verr., 'Trans. Connect. Acad.', x, p. 559. (See Duerden, 1902.)

? *B. australis*, Haddon, 1898, p. 435.

B. australis may not really be a *Bunodeopsis*; its anatomy is unknown and it has only a single circle of vesicles near the base, and does not seem quite like the others. *Viatrix globulifera*, D. and M., is perhaps the same as *B. globulifera*.

THAUMACTIS, Fowler, 1889, p. 143.

Phyllactidae probably. Only described species a small form flattened like a disc, with the mouth in the middle of the upper side; perhaps free-swimming. The column has irregularly arranged simple or slightly compound vesicles. There are a few marginal tentacles, with ectodermal longitudinal musculature. Body-wall and actinopharynx have ectodermal longitudinal musculature. No siphonoglyphes. Weak diffuse sphincter. Mesenteries not numerous, with weak musculature.

Species:

T. medusioides, Fowler, 1889, p. 143.

LEBRUNIA, D. and M., 1860.

Probably *Hoplophoria* as used by Wilson, 1890, p. 379, for *H. coralligenis*, not as used by Haddon, 1898, p. 438, for *H. cincta*.

Phyllactidae with no marginal acrorhagi or fosse, but with six, or four to eight hollow outgrowths just below the margin, these at best forming large and complicated bunches or 'pseudotentacles'. They vary as to detail, but are dichotomous in their early branchings; there is usually some development of nematocysts on them, these latter being sometimes definitely concentrated into acrorhagi on the pseudotentacles, which may differ in colour from the rest of the pseudotentacles. No sphincter. Tentacles not retractile. Retractors diffuse. Mesenteries may be all fertile, save perhaps the directives.

Species :

L. danae, D. and M., 1860, p. 47. (See Pax, 1910, p. 209; McMurrich, 1889, 'Journ. Morph.', p. 31, &c.; Verrill, 'Trans. Connect. Acad.', x; Verrill, 1899; McMurrich, 1905.)

L. neglecta, D. and M., 1860, p. 48. (See same references as for *L. danae*.)

L. coralligens, Wilson, 1890, p. 379. (See Haddon, 1898, p. 437, and Duerden, 1898, p. 456, and 1902.)

Verrill considers *L. danae* and *L. neglecta* distinct species because of the acrorhagi on the fronds of *danae*; McMurrich seems to think they run into each other. Duerden thinks that *Hoplophoria coralligens* is a *Lebrunia*.

Family 7. MINYADIDAE, sens. strict.

Minyadidae, Andres, as used by Haddon, 1898, p. 463.

Endomyaria (?) in which the base forms a float. It is hollowed out and has in-drawn edges with only a slight opening, the cavity being filled by a chitinous mass which is porous and exhibits a more or less definite structure. The body is smooth in the one form best known; it has forty tentacles, one siphonoglyphe, ten pairs of perfect mesenteries with strong circumscribed to circumscribed-diffuse retractors, and ten pairs of imperfect mesenteries with more diffuse retractors. The endocoels are larger than the exocoels, this giving a curious appearance to a transverse section. Not more than one tentacle to each exo- and endocoel. Sphincter endodermal—well-developed circumscribed according to Carlgren, consisting only of a single fold according to Haddon.

Genus: *Stichophora*.

STICHOPHORA, Brandt, 1835.

S. torpedo, Bell, 1885, p. 114. (*Minyas torpedo*.)

See above definition, and also Part II, p. 533, and Carlgren, 1894, p. 19, and Haddon, 1898, p. 465.

Family 8. PHYMANTHIDAE.

Phymanthidae as used by Carlgren, 1900, p. 66.

Phymanthidae, Andres, as used by Duerden, 1900, p. 138.

Including Thelacceridae, Mitchell, 1890.

Endomyaria with definite but sometimes reduced and half-physa-like base, which is more usually, however, well developed. Form of body variable. Cinclides may be present. Verrucae usually present. No sphincter or only a trace. Tentacles of two sorts, marginal and discal. Marginal tentacles in cycles in the usual way, rarely smooth, usually with greater or less development of paired lateral swellings or outgrowths, which may be simple or ramified, insignificant or conspicuous. Oral disc with short papilliform or not much developed tentacles as a rule—they are occasionally absent; when present they may be connected with endocoels only, or with both endo- and exocoels. Mesenteries typically with well-developed retractors, which in the best cases are circumscribed. A good many mesenteries are perfect.

Genus: *Phymanthus*.

PHYMANTHUS, M. Edw., 1857.

Thelaceros, Mitchell, 1890.

Phymanthidae. Base variable, from well developed to small or reduced and capable of being half like a physa. Form of body variable—may be trumpet shaped or almost *Halcompa*-like, and so on. Cinclides may be present near the base. Upper part of body with verrucae, which may occur in vertical rows; they may attach foreign bodies; they may be insignificant. Margin crenulated or provided with acrorhagi which may even be somewhat compound; rarely no verrucae or acrorhagi; there may be a fosse. Marginal tentacles (arrangement may be hexamerous or octamerous) smooth (rarely) or provided with feebly or strongly developed lateral, usually paired, swellings, which may be merely low knobs or may amount to short ramified branches; they may meet across the oral face of the tentacle; and grades between their presence and absence are found. Discal tentacles usually sessile outgrowths of the disc; they may resemble the marginal tentacles in miniature, or may be merely papilliform, or even scarce, reduced, or absent (see Part II, Text-fig. 14, n). The whole disc may become somewhat folded. The mesenteries are a good many of them perfect, and the stronger ones have usually strong retractors, sometimes diffuse but at their best circumscribed (see Part II, Text-fig. 4, e); the older ones or all of them fertile, save sometimes the directives. Little or no sphincter. Radial musculature of disc and tentacles ectodermal or with

a mesogloea tendency. May be retractile or not. Actinopharynx and siphonoglyphes may have weak ectodermal muscle.

Species :

- P. crucifer*, Les., 1817, p. 174. (See Duerden, 1900, p. 139 ; Pax, 1910, p. 222 ; McMurrich, 'Journ. Morph.', 1889, &c.)
- P. sansibaricus*, Carlgr., 1900, p. 67.
- P. strandesi*, Carlgr., 1900, p. 68.
- P. loligo*, Ehr., 1834, p. 41. (See Carlgren, 1900, p. 70.)
- P. muscosus*, H. and S., 1893, p. 122. (See Haddon, 1898, p. 496, and Kwietniewski, 1898.)
- P. rhizophorae*, Mitchell, 1890, p. 557.
- P. levis*, Kwiet., 1898, p. 421.
- ? *P. caeruleus*, Q. and G., 1833, p. 157. (See Pax, 1912, p. 312.)

And perhaps others.

Phymanthus is an easily identified genus, and interesting as giving hints as to its evolution in the species which verge in structure in the direction of *Halcampa*. The genus *Crambactis* of Haeckel is invalid; Carlgren states that the queer inner tentacles were only extruded filaments (1900, p. 58).

Family 9. HETERANTHIDAE, Carlgr.

Heteranthidae, Carlgr., 1900, p. 72, 1900 ('Öfv. Vet.-Akad. Förh. '), p. 278.

Rhodactidae, Andres, as used by Haddon, 1898, p. 476, *pro parte*.

Endomyaria with definite base. The column in the only known form has verrucae and fosse, the distal margin with little warted lobes; the sphincter is not very strong, endodermal circumscribed; the tentacles are distinctly marked off into marginal and discal, the marginal short conical, the discal wart-like, in rows; the mesenterial musculature is well developed.

Genus: *Heteranthus*.

HETERANTHUS, Klunz., 1877.

H. verruculatus, Klunz., 1877, p. 84.

See above definition for chief characteristics. I know nothing of this family save the details here given, which are taken from Carlgren, 1900, p. 72.

Family 10. HOMOSTICHANTHIDAE, Carlgr.

Homostichanthidae, Carlgr., 1900, p. 118.

Discosomidae as used by Duerden, 1900, p. 154, *pro parte*.

Endomyaria with definite base. The known form has smooth body but for possible acrorhagi. Sphincter not strong, circumscribed diffuse. Retractors diffuse. Tentacles all of one sort, simple, may be short and more or less papilliform, in radiating series on the exocoels as well as the endocoels. Numerous perfect mesenteries.

Genus: *Homostichanthus*.

HOMOSTICHANTHUS, Duerden, 1900, p. 166.

Homostichanthidae in which the body may be elongate, smooth; distal part may be somewhat folded. Margin with elevations, possibly acrorhagi, and slight fosse. Retractable. Tentacles short, smooth, slightly capitate, knob-like, their stems glandular and heads nematocystic, their longitudinal musculature ectodermal. Slight circumscribed-diffuse sphincter. Numerous perfect mesenteries and diffuse retractors.

Species:

H. duerdeni, Carlgr., 1900, p. 117. (See Duerden, 1900, p. 167.)

Family 11. AURELIANIDAE.

Aurelianidae, Andres, as defined by Carlgren, 1900 (small paper on *Stichodactylinae*), p. 279.

Endomyaria with definite base, which may be large or small. Body may have more or less cuticle, or may have small vesicle-like verrucae below the margin. The tentacles are small vesicular outgrowths, often lobed, two or three or many communicating with each of the main exo- and endocoels. Sphincter strong circumscribed (see Part II, Text-fig. 13). Main mesenteries very strongly muscular, the retractors exhibiting at their best the extreme of circumscription and distinction from the mesenterial surface. All stronger or all mesenteries perfect and fertile. Only one siphonoglyphe. Radial musculature of disc and tentacles, such as it may be, ectodermal or mesogloal.

Genera: *Aureliania*, *Actinoporus*.

AURELIANIA, Gosse, 1860, p. 282.

Probably *Leiotecalia*, Hertw., 1882, p. 37.

Aurelianidae with a very wide base, so that the body slopes inwards more or less to the narrower disc. No verrucae. Body divided more or

less definitely (the distinction not necessarily externally clear) into an extensive scapus (to which usually adheres a roughish brown cuticle, much or little of it) and a more delicate capitulum or puffy marginal region, the ectoderm of which may contain spirocysts. Distinct fosse. The tentacles are short knobs, sometimes with stems, arranged so that two communicate with each main exocoel, and two or three with each main endocoel (see Part II, Text-fig. 14, A). Besides being in short radial rows they are so placed as to form cycles which alternate, though not in the genuine 'Actiniine' way. They are some simple, some lobed, and in a living specimen one can distinguish different tentacle-forms for the different concentric rings. Sphincter fairly to very strong, circumscribed, with a heavy central axis of mesogloea (see Part II, Text-fig. 13, B). Radial musculature of disc and tentacles where present curious, chiefly mesogloea. All stronger mesenteries perfect, fertile, with filaments and retractors; the retractors very unusual, powerfully circumscribed (see Part II, Text-fig. 4, B), and attached to the mesentery by one edge only for part of their extent, and with an axis of mesogloea. There may be additional weak mesenteries beyond the main macrocnemes.

Species :

A. augusta, Gosse, 1860, p. 283. (See Faurot, 1895.)

A. heterocera, Thompson, 1853.

A. regalis, Andres, 1883, p. 496. (See Carlgren, 1900, short paper on Stichodactylines, p. 279, &c.)

? *A. nymphaea*, Hertw., 1882, p. 38.

I have personally studied *A. augusta*, both alive and anatomically, and I do not know why Andres assumed it to be the same as his *A. regalis*. It is not impossible that Capnea, Forbes, is the young of *Aureliania*; beyond this suggestion it cannot yet be allocated. Then there is the question of Hertwig's genus *Leiotelia*. It has generally been assumed (and I shared the idea formerly) that this is identical with *Epiactis* or *Isotelia*, but this overlooks certain details of its structure. I have recently been able to investigate two species of *Aureliania*, *A. augusta* and a possibly new one, and on re-reading Hertwig's description in the light of this, it becomes evident that in all features one can be sure about the two genera really share essentials. An examination of the original Challenger specimen confirmed the idea. The one uncertain though necessary point is that it is

not known whether *Leiotetalia* has more than one tentacle to each main endocoel and exocoel or not; Hertwig apparently thought only one, but the specimen is small and so contracted (and *Aureliania* has cycles as well as radial rows) that it is hard to tell. The other things seem to point to its being an *Aureliania*, which in that case should be called *A. nymphaea*, Hertw.; whether it is *A. nymphaea*, Drayt., is another matter. It has smooth body without verrucae or acrorhagi, small button-like tentacles, pinnate sphincter with stout mesogloal axis, mesogloal radial disc-muscle; only the mesenteries of cycles 1-3 have distinct retractors, and these are great circumscribed things attached only at one edge. These things are all found in *Aureliania* (not necessarily only three cycles of mesenteries with retractors of course), as are also the wide base and pyramidal form of *nymphaea*, and some of them are very characteristic features. The sphincter is less developed in *nymphaea* than in the others. In view of the general evidence it seems probable that the *Stichodactyline* tentacle-plan may be assumed.

ACTINOPORUS, Duch., 1850. (See Duerden, 1900, p. 174; Carlgrén, 1900, short paper on *Stichodactyline*s, p. 283.)

Aureliania with definite but not specially wide base—it may even be somewhat reduced. The body may be long. There may be rather vesicle-like verrucae below the sphincter, of which the main ones sometimes form a sort of collar. Deep fosse. Disc not extensive, but notched into little permanent lobes or lappets at its margin, which correspond in number to the endocoels and exocoels. Tentacles short vesicular knobs, may be lobed, many communicating with each exocoel and endocoel, the tentaculate areas thus formed separated from each other by radial grooves. Sphincter strong circumscribed (see Part II, Text-fig. 13, A). Mesenteries all perfect and all or mostly fertile, with very strong circumscribed retractors which may be broadly or narrowly attached to the mesenteries, partly according to region. Disc and tentacle muscle very weak, ectodermal if present.

Species:

A. elegans, Duch., 1850, p. 10. (See Duerden, 1900, p. 175.)

A. elongatus, Carlgr., 1900 (small paper on *Stichodactyline*s), p. 283.

Family 12. ACTINODENDRIDAE.

Actinodendridae, Haddon, 1898, p. 488; Carlgren, 1900, p. 96.

Acremodaetylidae, Dendrianthidae, Kwiet., 1897-8.

Endomyaria with definite base, not always well marked off from the column, and it may be small. Smooth wall. No special margin. No sphincter or a slight diffuse one. Disc produced into permanent arm-like lobes, which are arranged in cycles like large tentacles, each bearing numerous tentacles or branches on it (see Part II, Text-fig. 14, K); these tentacles may be arranged all round the lobes or be more or less absent from parts of them, and may be themselves simple or branched, and in the latter case giving the whole arm a dendritic effect (see Part II, Text-fig. 19). Mesenteries all (or twelve pairs only perfect) perfect and fertile, except sometimes the directives, with strong retractors. Radial musculature of disc and tentacles ectodermal. There may be concentrations of nematocysts in the tentacle-lips, in little thickenings. The discal arms correspond one to each endocoel in the inner cycles, one to each exocoel in the outer, the size varying according to cycle.

Genera: Actinodendron, Actinostephanus,
Megalactis.

ACTINODENDRON, Blainv., 1830, p. 287.

Acremodaetyla, Kwiet., 1898.

Actinodendridae in which the tentacles are arranged all round the arms, and are themselves branched, and may have nematocystic thickenings at their tips. Very strong diffuse retractors. (See Part II, Text-fig. 19, for appearance of *A. plumosum*.)

Species:

A. plumosum, Haddon, 1898, p. 490. (See Saville Kent, 1893.)

A. glomeratum, Haddon, 1898, p. 492. (See Saville Kent, 1893 and 1897.)

A. hansingorum, Carlgr., 1900, p. 98.

A. ambonensis, Kwiet., 1898, p. 401. (See Carlgren, 1900, p. 96.)

And probably others.

ACTINOSTEPHANUS, Kwiet., 1898, p. 403.

Actinodendridae in which the tentacles are irregularly arranged on the arms and are simple. Strong retractors.

Species:

A. haeckeli, Kwiet., 1898, p. 403.

MEGALACTIS, Ehr., 1834.

Actinodendridae with the arms longer than in *Actinodendron*, the oral faces of the arms freer from tentacles, the ultimate branches of the tentacles simple and pointed, not bifid. (See Part II, Text-fig. 14, κ.)

Species:

M. griffithsii, Saville Kent, 1893, pp. 35, 147. (See Haddon, 1898, p. 493.)

And probably at least one other.

I have followed Haddon and Saville Kent in keeping *Megalactis* separate from *Actinodendron*, as I think it should be, although not yet well known.

Family 13. THALASSIANTHIDAE.

Thalassianthidae, McM., as used by Haddon, 1898, p. 482, and Carlgren, 1900, p. 86.

Endomyaria with definite base. Body with or without verrucae above. Disc circular or waved or puckered. Not more than one dendritic tentacle to each exocoel—exocoelic tentacles all dendritic. Several simple or dendritic tentacles and several (sometimes many) modified tentacles or nematospheres on many of the endocoels, these sometimes gathered up on to a definite permanent elevation or even finger-like lobe of the disc (the nematospheres being aboral), which may cover an endocoel and the two adjacent exocoels. Sphincter of variable development, from more diffuse to more circumscribed, not very strong. Mesenterial musculature well developed, but not unusually strong, retractors more or less diffuse. Numerous perfect mesenteries.

Genera: *Thalassianthus*, *Cryptodendron*,
Actinertia.

THALASSIANTHUS, Leuck., 1828.

Heterodactyla, Ehr., 1834.

Thalassianthidae with verrucae above, which may be in rows, or with none, and they may vary in distinctness. Margin may be distinct or notched. Disc may be folded or puckered up, or not. Marginal tentacles dendritic, without nematospheres, not more than one per exocoel. The endocoelic tentacles are also dendritic, but many of them arranged on elevations or permanent lobes of the disc, and occupying the more oral part of the elevation, which typically possesses aborally a bunch of grape-like stinging batteries or nematospheres (see Part II, Text-fig. 14, E). Siphonoglyphes two or several, directives present in the first case,

absent in the second. Sphincter weak to moderate, more diffuse to more circumscribed. Radial muscle of disc ectodermal. Retractors diffuse or more like circumscribed diffuse, numerous mesenteries perfect. gonads chiefly on stronger imperfects, or on the older mesenteries save sometimes the directives.

Species :

- T. aster*, Leuck. in Rüppel, 1828, p. 5. (See Carlgrén, 1900, p. 87.)
T. kraepelini, Carlgr., 1900, p. 91.
T. senckenbergianus, Kwiet., 1897, p. 337.
T. hemprichi, Ehr., 1834, p. 42. (See Carlgrén, 1900, p. 94; Haddon, 1898, p. 485.)
T. hypnoides, Saville Kent, 1893, p. 148. (See Haddon, 1898, p. 486.)

I have joined *Thalassianthus* and *Heterodactyla* because I cannot find any really important differences between them. The definition given covers both. The presence of several siphonoglyphes in some species, and no directives, of two siphonoglyphes and two pairs of directives in others, seems no valid ground of separation.

CRYPTODENDRON, Klunz., 1877.

Thalassianthidae with or without suckers on upper part of body, and with somewhat crenulated margin. Wide irregularly-folded disc. Three sets of tentacles: (*a*) a set of short exocoelic marginal dendrites; (*b*) radial rows of short, simple, and dendritic tentacles on the inner part of the disc; and (*c*) an intermediate zone of nematospheres. Sphincter weak to moderate, circumscribed. Well-developed diffuse retractors. The nematospheres especially have apical batteries of sting-cells and glandular stems. Radial musculature of disc and tentacles ectodermal. The nematospheres are in sessile packets, and they and the discal dendrites are endocoelic.

Species :

- C. adhaesivum*, Klunz., 1877, p. 86. (See Haddon, 1898, p. 483, and Kwietniewski, 1896.)

ACTINERIA, Blainv., 1830.

Thalassianthidae with vertical rows of verrucae in upper part of column, parapet notched a little. Wide folded disc, bare in the middle, with small permanent lobes at the edge. Exocoels with dendritic tentacles, endocoelic lobes with dendrites (which run inwards on the disc) on the oral side and a mass of nematospheres aborally. Sphincter

not very strong, sessile circumscribed. Numerous perfect mesenteries. Older mesenteries fertile, but probably not the directives.

Species:

A. dendrophora, H. and S., 1893, p. 123; Haddon, 1898, p. 487.

And probably also *A. villosa*, Q. and G., 1833, p. 156.

Family 14. STOICHACTIDAE.

Stoichactidae, Carlgr., 1900, p. 72; 1900 ('Öfv. Vet.-Akad. Förh.'), p. 278.

Discosomidae, Klunz., as used by Haddon, 1898, p. 469, *pro parte*.

Endomyaria with definite base. Column usually but not always verrucose above. Size sometimes very large. Tentacles simple, but for scattered bifid or multifid tentacles which sometimes occur sporadically among the others; they may be fairly long and quite ordinary, or may be short or wart-like, or even short columns with spherical heads. They are all of one sort in the same animal, and there is not more than one to each exocoel; the endocoels may in rare cases have only one tentacle each, but usually at least some of them have more—the stronger ones may have radial rows (see Part II, Text-fig. 14, F), or all the endocoels may have either one or several rows. Sphincter strong or not very strong, more or less diffuse to more or less circumscribed. Mesenterial musculature well developed, retractors weaker or stronger but not unusually strong, diffuse. Numerous perfect mesenteries. Gonads may occur on all mesenteries—usually the older ones are fertile save sometimes the directives, but not always. Tentacular longitudinal muscle ectodermal.

Genera: *Stoichactis*, *Radianthus*, *Antheopsis*.

This family is itself very clearly marked off from others, but within it, it is difficult to satisfactorily separate off genera. The difficulty is increased because some descriptions of the forms do not give enough data. At best, it seems that there are only three sound genera to be distinguished, three stages in the evolution of very similar creatures; they form a series really, and I do not feel perfectly confident that they do not all form one large genus. At any rate more than three it is unwise to insist on; some pairs of names have been given to similar forms, and some of these must now become synonyms.

In *Antheopsis* (= *Stichodactis*) the condition is sometimes purely 'Actiniine'—not more than one tentacle to each exo- and endocoel; but the more normal state is for there to be more than one tentacle: or a row, on the older endocoels but not the younger.

In *Radianthus* (= *Helianthopsis*) comes the stage where all the endocoels have radial rows, but there is only about one row on each. In *Stoichactis* (= *Discosomoides*) the last stage is attained, and there are not only radial groups on all the endocoels, but usually more than one row abreast in each group; and the tentacles have often specialized in small size. As far as sphincters are concerned, I think comparison with other families will show that their exact form cannot be used here as a generic distinction. In the lists of species given below it should be remembered that a form here and there may be allocated to the wrong genus because of insufficient data about it; but some re-arrangement has been made intentionally to get the three grades clearly separated off. The readjustments mainly mean a transference to *Antheopsis* of some forms originally described under *Radianthus*, *Stichodactis*, and *Helianthopsis*, and a consequent depletion of the true genus *Radianthus*. This has been necessary to get together all the forms with radial rows of tentacles on the older endocoels only. It is not much of a distinction, but if the two genera are to be kept apart at all it must be insisted on. That the sporadic occurrence of a few cleft tentacles in some species should be used as a generic character would be a mistake.

STOICHACTIS, Haddon, 1898, p. 472.

Discosomoides, Haddon, 1898, p. 470.

Stoichactidae. Some species attain enormous size, up to about two feet across, and often crustacea or fish are commensal with them. The body is usually wider above than below, and above with suckers which may be in vertical rows—these may, however, be rudimentary or absent, present or not even in the same species. Margin barely or slightly or distinctly marked, may be notched. Disc simple or little or much folded. Tentacles not very long at best, usually short or very short; digitiform

or subulate; or wart-like; or wider at the tip than at the base; or forming short stems with spherical heads. Only one tentacle per exocoel, a good many on each endocoel, usually more than one row abreast per endocoel; the rows may be very irregular. Sphincter weaker or stronger, circumscribed diffuse to well circumscribed. A cleft tentacle sometimes occurs among the others. Siphonoglyphes and directives variable in number.

Species:

- S. kenti*, H. and S., 1893, p. 119. (See Saville Kent, 1893, p. 144; Haddon, 1898, p. 473.)
S. haddoni, Saville Kent, 1893, pp. 32, 144. (See Haddon, 1898, p. 474.)
S. helianthus, Ellis, 1767, p. 436. (*S. anemone*, Ellis, 1767, p. 436.) (See Duerden, 1900, p. 162; Pax, 1910, p. 227; McMurrich, 'Journ. Morph.', 1889.)
S. ambonensis, Kwiet., 1898, p. 410.
S. tuberculata, Kwiet., 1898, p. 412.
S. giganteum, Forsk., 1775, p. 100. (See Carlgren, 1900, p. 77.)
S. tapetum, Ehr., 1834, p. 32. (See Carlgren, 1900, p. 74.)
S. laevis, Lager, 1911, p. 240.
S. intermedia, Lager, 1911, p. 238.
S. australis, Lager, 1911, p. 241.
S. fuegiensis, Dana, 1846. (See McMurrich, 1893, p. 200.)

RADIANTHUS, Kwiet., 1897, p. 331.

Helianthopsis, Kwiet., 1898, p. 417, pro parte.

Stoichactidae with or without suckers on upper part of body. Margin fairly well marked. Tentacles shorter or longer, but not mere papillae. More than one tentacle communicates with every endocoel (not more than one per exocoel), but only about one row on each. Here and there may be cleft tentacles. The disc may be lobed. Sphincter more or less diffuse to weak or medium circumscribed.

Species:

- R. lobatus*, Kwiet., 1898, p. 414.
R. mabrucki, Carlgren, 1900, p. 82.
 ? *R. parvitentaculatus*, Q. and G., 1833, p. 165. (See Pax, 1912, p. 314.)

ANTHEOPSIS, Simon, 1892.

Stichodactis, Kwiet., 1898, p. 415.

Radianthus, Kwiet., 1897, p. 331, pro parte.

Helianthopsis, Kwiet., 1898, p. 417, pro parte.

Stoichactidae with suckers in the upper part of the body or not; if

they are there foreign bodies may adhere to them ; margin distinct, may be crenulated. Disc circular or more or less lobed. Tentacles shorter or longer, may attain good length ; at any rate not mere papillae. Not more than one tentacle per exocoel. As to the endocoels (see Part II, Text-fig. 14, F), there are never radial rows on all of them ; usually there are radial rows on the older ones or some of them, but these vary in length—the larger ones may contain a good many tentacles or only a few ; the rows are more or less single, and sometimes they are quite absent so that the form is not 'Stichodactyline' as to tentacles at all, having only one per endocoel. Cleft tentacles may occur here and there among the others. Sphincter weak or moderate, diffuse, circumscribed diffuse, or circumscribed. Number of siphonoglyphes and directives variable.

Species :

- A. koseirensis*, Klunz., 1877, p. 77. (See Simon, 1892, and Carlgren, 1900, p. 85.)
- A. ritteri*, Kwiet., 1898, p. 417. (See Carlgren, 1900, p. 81.)
- A. kuekenthali*, Kwiet., 1897, p. 332.
- A. papillosa*, Kwiet., 1898, p. 415.
- A. macrodactylus*, H. and S., 1893, p. 120 ; Haddon, 1898, p. 471.
- A. malu*, H. and S., 1893, p. 120 ; Haddon, 1898, p. 472.
- A. carlgreni*, Lager, 1911, p. 243.
- A. concinnata*, Lager, 1911, p. 244.
- A. glandulosa*, Lager, 1911, p. 246.
- A. kwietniewskii*, Lager, 1911, p. 247.

Sub-order MADREPORARIA.

I do not wish to suggest, even vaguely, to which of the skeleton-forming corals the genera defined below are related. The ground for placing them under Madreporaria will be found in Part II, p. 510. To save repetitions, a general statement covering Corallimorphidae and Discosomidae is given first, but it is not meant as the definition of a sub-tribe, although it would serve that purpose if such a sub-tribe were needed.

Madreporaria which secrete no definite skeleton. They may live quite a solitary life, or may live together in numbers. They frequently reproduce by fission, and compound individuals with several mouths may be found, or individuals connected by a coenosarc. There is a definite base. The body is smooth, and variable in form and consistency. The

tentacles are arranged so that more than one communicates with some at least of the endocoels, and sometimes more than one with exocoels also; they may be simple, knobbed, or branched, and so on, and there may be more than one sort in the same species; they may be reduced and wart-like (Part II, Text-fig. 3), or even reduced to nothing externally visible. There are typically no siphonoglyphes—these are recorded in some cases but their existence probably needs confirming. The mesenterial filaments have no ciliated tracts. Sphincters are absent or weak diffuse. Sting-cells of a size characteristic more of *Madreporaria* than of *Actiniaria* are usually present somewhere in the body (see Part II, Text-fig. 6). There are usually a good many perfect mesenteries, as a rule twelve or more pairs, and there is no distinction of them into macro- and microcnemes. The longitudinal mesenterial musculature consists typically of a feeble layer, not forming the sort of sheet or retractor characteristic of *Actiniaria* (see Part II, Text-figs. 4 and 5). Basilar muscles are absent. Ectodermal muscle present at least sometimes in the body-wall, sometimes probably absent. Directives usually present, varying in number. The large sting-cells may occur in tentacles, actinopharynx, mesenteries, body-wall.

Family 1. CORALLIMORPHIDAE.

Corallimorphidae, Hertw., 1882, p. 21; Carlgr., 1900, p. 19.

Size larger or smaller; habit solitary or gregarious, individuals may be connected by coenosare. Ectodermal muscle in body-wall present at least in some cases. Tentacles simple, knobbed at the tips. Not more than one tentacle per exocoel, more than one on at least the older endocoels.

Genera: *Corallimorphus*, *Isocorallion*,
Corynaectis.

CORALLIMORPHUS, Moseley, 1877, p. 299.

Corallimorphidae with weak musculature throughout. Body-wall ectoderm has weak longitudinal musculature. No sphincter. Body-wall and oral disc may be very thick and cartilaginous, and animal may attain fairly large size. Tentacles simple, and all knobbed at the tip (see Part II, Text-fig. 14, G), divided into two sorts, marginal and discal. There is never more than one tentacle of each sort arising from one and the same endocoel. The exocoelic tentacles are the smallest of the marginal series, taken on the whole, and the discal tentacles correspond to the endocoels of the inner marginal tentacles. There may be a good deal of irregularity.

Species :

- C. rigidus*, Moseley, 1877, p. 301. (See Hertwig, 1882, p. 23, and 1888, pp. 9, 10; Stephenson, 1920 B, p. 178.)
C. profundus, Moseley, 1877, p. 300. (See Hertwig, 1882, p. 28, and 1888, pp. 9, 10; Stephenson, 1920 B, p. 178.)
C. obtectus, Hertw., 1888, p. 9. (See Stephenson, 1920 B, p. 178.)
C. ingens, Gravier, 1918, p. 23.

The above definition of the genus is practically that given in my short note on the genus *Corallimorphus* ('Proc. R. I. Acad.', 1920, B. 9). I began it there with the words 'Stichodaetyline Actiniaria', this being provisional, as I had not then worked out my idea of its being a skeleton-less coral fully enough for publication. I have listed the four species here for reference purposes, but as before suggested, I am inclined to think they are all one, and the more so since dealing with another specimen from an Antarctic collection and looking at the Challenger specimens. *C. ingens* is probably the same as the others. If the four listed are to be separate, my Irish form would make a fifth.

ISOCORALLION, Carlgr., 1900, p. 19.

Chalmersia, Del. and Hér., 1901, p. 536.

Corynactis as used by Hertwig for *Corynactis*, sp., 1888, p. 10.

Corallimorphidae differing from *Corallimorphus* in having the ectodermal muscle in the body-wall stronger, and with normally two disc-tentacles on each of the oldest radii of the disc.

Species :

1. *hertwigi*, Carlgr., 1900, p. 19. (See Hertwig, 1888, p. 10, *Corynactis*, sp.)

I feel doubtful of the distinctness of this genus from *Corallimorphus*, but hardly enough is yet known of it to justify their fusion.

CORYNACTIS, Albm., 1846. (See Duerden, 1898, p. 635, &c.)

Corallimorphidae of small size, often gregarious in habit, sometimes forming large sheets of individuals; often clusters or pairs of individuals are found attached to each other by a basal coenosarc; fission is a usual

method of increase. The individuals are very variable in form, often trumpet shaped in expansion, and more or less retractile. The tentacles are knobbed, the outer larger than the inner, and the exocoelic tentacles largest of all. Some or all of the endocoels have more than one tentacle connected with them. Tentacle-heads usually with large sting-cells and little or no muscle, shafts with ectodermal longitudinal muscle. Perhaps very weak ectodermal muscle in the body-wall. Sphincter absent or weak diffuse.

Species :

- C. viridis*, Allm., 1846, p. 417. (See Gosse, 1860, p. 289, and Rees, 1915, p. 543.)
- C. globulifera*, Ehr., 1834, p. 39. (See Carlgren, 1900, p. 20, and Haddon, 1898, p. 467.) (? *C. hoplites*, H. and S., 1893, p. 118.)
- C. myrcia*, D. and M., 1866, p. 124. (See Duerden, 1900, p. 181.)
- C. carnea*, Studer, 1879, p. 542. (See McMurrich, 1904, and Kwietniewski, 1896.)
- C. australis*, H. and Duerden, 1896, p. 151.
- C. haddoni*, Farquhar, 1898, p. 532. (See Stuckey, 1909, p. 390.)
- C. mollis*, Farquhar, 1898, p. 534. (See Stuckey, 1909, p. 390.)
- C. gracilis*, Farquhar, 1898, p. 534. (See Stuckey, 1909, p. 390.)
- C. albida*, Stuckey, 1909, p. 390.

And perhaps others.

Possibly *haddoni*, *mollis*, *gracilis*, and *albida* are all one species.

Family 2. DISCOSOMIDAE, sens. strict.

Discosomidae as used by various authors, *pro parte*.

Used here in the sense taken by Carlgren, 1900, p. 58.

Including Phialactidae, Fowler, 1889.

Rhodaetidae, Andres, as used by Haddon, 1898, p. 476, *pro parte*.

Size variable. Living singly or in patches. With one or more mouths. Sphincter absent or weak diffuse. Tentacles simple or dendritic (see Part II, Text-fig. 14, B, C) or somewhat capitate or curious and urn-like, or reduced to warts (see Part II, Text-fig. 3), or to little or nothing, so that they do not show above the surface of the disc at all; more than one sort may occur in the same species, and more than one may communicate with endocoels and exocoels or with endocoels only, there being often radial rows. Presence of ectodermal muscle in body-wall doubtful.

Genera : *Discosoma*, *Paradiscosoma*, *Ricordea*,
Orinia, *Rhodactis*, *Actinotryx*.

DISCOSOMA, Leuck., 1828.

Discosomidae with tentacles all of one sort, not branched, not knobbed, may be swollen towards the tips : short, usually wart-like, sometimes reduced or even vanished, so that only traces of them remain as endodermal evaginations in the mesogloea of the disc. Margin of body straight or more or less notched or irregular. Tentacles in radial rows on at least some endocoels, sometimes on exocoels too. Sphincter absent or weak diffuse.

Species :

D. nummiforme, Leuck., 1828, p. 3. (See Simon, 1892, and Carlgrén, 1900, p. 62.)

D. Yuma, Carlgr., 1900, p. 63.

D. Unguja, Carlgr., 1900, p. 64.

And probably others.

I do not feel clear that all the genera that follow are really distinct from *Discosoma*, but am listing them in full. Taking the family as a whole, the two clearest genera are *Discosoma* and *Actinotryx*. Beyond this there is less certainty. *Rhodactis* is probably distinct but is little known. *Ricordea* and *Paradiscosoma* seem doubtfully distinct from *Discosoma*. Even *Orinia* might be only a curious state of *Discosoma*, but is more likely to be distinct than the others : even in *Paradiscosoma* one sometimes sees the tentacles collapse on themselves so that they form little double-walled cups, and it would not take much to make this into *Orinia* ; and McMurrich says some of the more peripheral of them are tuberculiform and not crateriform. If there is a naked zone between the marginal and discal sets, however, that will clinch the distinction. There are other genera and species which have been referred at one time and another to this family, before it was properly understood, but these have been cast out as time went on, and are in this paper referred to their new positions, e. g. *Stoichactidae*. **PARADISCOSOMA**, Carlgr., 1900, p. 60. (n. nom. for *Isaura*.)

Discosomidae with margin of disc thrown into small lobes. Otherwise like *Discosoma*. (See Part II, Text-figs. 3, 6, B, 5.)

Species :

P. neglecta, D. and M., 1860, p. 51. (*Isaura neglecta*, D. and M.) (See Carlgren, 1900, p. 60, and Pax, 1910, p. 214.)

A vertical section of a species of *Paradiscosoma* is given in Part II, Text-fig. 3.

RICORDEA, D. and M., 1860. (See Duerden, 1898, p. 635, &c.)

Discosomidae which often live aggregated together in patches. The majority of individuals have more than one mouth, there may be up to seven or so, the disc being consequently sinuous in outline. Sometimes individuals are found connected by a basal membrane. No sphincter, though the animal is retractile. Tentacles short and may be somewhat capitate or rounded at the tip, in radial rows on at least some endocoels. Stems of tentacles may be glandular, their tips nematocystic.

Species :

R. florida, D. and M., 1860, p. 42. (See Duerden, 1900, p. 156; Pax, 1910, p. 219; McMurrich, 1889, 'Journ. Morph.')

ORINIA, D. and M., 1860.

Discosomidae with tentacular, simple structures in the periphery of the disc. Inner part of the disc provided with characteristic large urn-like outgrowths. Between the simple tentacles and the urns a tentacle-free area. (See Carlgren, 1900, p. 60.)

Species :

O. torpida, D. and M., 1860. (See Carlgren, 1900, p. 60, and McMurrich, 1905.)

RHODACTIS, M. Edw. and H., 1851.

? *Phialactis*, Fowler, 1889.

Discosomidae with tentacles of two sorts, simple ones round the mouth and the edge of the disc, branched ones in the middle, which may arise from pits in the disc; the two sorts not gathered up into sharply-separated zones, and no naked area between marginals and discals. Tentacles may be somewhat capitate in certain states. The animals may live massed together in patches.

Species :

R. rhodostoma, Ehr., 1834.

R. howesii, Saville Kent, 1893, p. 150. (See Haddon, 1898, p. 478.)

? *R. neglecta*, Fowler, 1889, p. 148. (See Carlgren, 1900, p. 59-61, &c.)

And perhaps others.

ACTINOTRYX, D. and M., 1860, p. 321. (See Duerden, 1898, p. 635, &c.)

Discosomidae which may occur in scores together, crowded so as to form a carpet, and some individuals have two or more mouths. More or less retractile. There are simple or nearly simple tentacles or tentaculiform outgrowths connected with the margin; within these is a well-marked clear zone, then the main part of the disc has dendrites, some at least in radial rows. Sphincter absent or weak diffuse. (For details of an *Actinotryx* see Part II, Text-figs. 14, B and C, 4, D, and 6, A.)

Species:

A. sancti-thomae, D. and M., 1860, p. 45. (See Duerden, 1900, p. 148; McMurrich, 1889, 'Journ. Morph.')

A. bryoides, H. and S., 1893, p. 121; Haddon, 1898, p. 479.

And probably others.

2. APPENDIX.

There are some anemones recently described by Professor Gravier, whose papers I did not know about, unfortunately, when Part I of this paper was written, and which should be mentioned now. I am at the same time giving a few further details which seem worthy of note about some of Verrill's genera which can hardly be finally allocated yet, but are interesting as showing the direction which some future work should take to clear them up. I regret that by a mischance I overlooked the genus *Euphelia* of Pax before, and that also is included here, together with a few other points.

(i) Professor Gravier's forms.

Professor Gravier has established five new genera and some new species, as follows:

1. *Nectactis* (1918, p. 18). *N. singularis*, 1918, p. 18.

This has the form of a disc thicker in the middle than at the edge, where the capitate tentacles are, the lower surface of it representing the column and having a little pit-like base in its middle. Smooth wall and no sphincter. A good many mesenteries with indiscernible muscles.

It is very difficult to even suggest a position for this form in classification. Gravier suggests *Minyadidae*, but it would not do for that family as understood here. If there were disc-tentacles one might suggest *Corallimorphidae*, and possibly that would be best even without them—but more details are needed.

2. *Thoracactis* (1918, p. 12). *T. topsenti*, 1918, p. 12.

A small form living on the surface of a sponge. It is disc shaped, incrusting, the foreign bodies even getting embedded in the mesogloea. Sphincter mesogloea, seemingly double. No acontia or cinclides. Weak mesenterial musculature.

Gravier believes that the gonads develop from the endoderm of the body-wall. There is not much guide, but the form may be a tiny Paractid or even, possibly, a Zoanthid?

3. *Telmatactis* (1916, p. 236). *T. valle-flori*, 1916, p. 236.

This seems to me to be probably identical with *Phellia*, in which case the species becomes *Phellia valle-flori*.

4. *Sicyopus* (1918, p. 21). *S. commensalis*, 1918, p. 21.

This lives on a Holothurian, in a hollow of its skin near the mouth. It has the form of a thick disc, strong mesogloea sphincter, no acontia or cinclides, diffuse retractors, all mesenteries fertile. It seems like a small Paractid of uncertain affinities.

5. *Gliactis* (1918, p. 7). *G. crassa*, 1918, p. 7.

Here the base envelops *Acanella*. There are no verrucae, the column wall is thick. Good mesogloea sphincter. Apparently twenty pairs of perfect mesenteries, probably diffuse retractors. If there are no acontia or cinclides this seems eligible for one of the Paractid genera, and probably does not merit generic distinction.

I have not suggested very definitely about the above forms, but they are not all very fully studied as yet, and the time has not come to decide for or against them; but they will probably fit into known families.

In addition Professor Gravier has described new species in old genera as follows:

1. *Paractis flava* (1918, p. 4). Either a *Paractis* in the strict sense, or belonging to a neighbouring genus.

2. *Paractis vestita* (1918, p. 5) may have some sort of investment on the column, and seems to have only six pairs of perfect mesenteries, no acontia and cinclides, mesogloea sphincter; in which case it is no *Paractis*, but an Actinosephyd near *Paranthus*, perhaps eligible for that genus.

3. *Actinernus verrilli* (1918, p. 6) is not an *Actinernus* (= *Porponia*), since it has a mesogloea sphincter and is apparently

not endocoelactous. Nor is it an *Actinoseyphia* since it has numerous perfect mesenteries. It must therefore belong to *Catadiomene* or *Polysiphonia*, as it has basal swellings to the tentacles; and from the description I gather that it is more likely to be *Polysiphonia* than the other, but further details are needed for decision.

4. *Sagartia sociabilis* (1918, p. 10). No cinclides. Seemingly six pairs of perfect mesenteries with weak musculature. If it has acontia it must be a *Sagartiomorphe*—certainly not a *Sagartia*.

5. *S. sobolescens* (1918, p. 11) is perhaps a *Sagartiomorphe* also.

6. *Chitonanthus incubans* (1918, p. 11) is very exceptional as a *Chondractinian* in having the three oldest cycles of mesenteries fertile. Since *Chitonanthus* is only a synonym of *Hormathia*, the right name for the species is *Hormathia incubans*.

7. *Chitonanthus indutus* (1918, p. 12) should, similarly, be *Hormathia induta*.

8. *Chitonanthus abyssorum* (1918, p. 13) seems to be either *Hormathia abyssorum* or an *Actinauge*.

9. *Hormathia elongata* (1918, p. 14) seems correctly named.

10. *Hormathia? muscosa* (1918, p. 15) has apparently no acontia, so cannot be a *Chondractiniid*. It has numerous perfect mesenteries and a mesogloal sphincter, which bring it to *Paractidae*; its circumscribed retractors and some of its externals suggest *Hormosoma* or *Tealidium* or *Pseudoparactis*, but this is uncertain, and it may need a new genus.

11. *Stephanactis impedita* (1918, p. 16) becomes *Stephanauge impedita*, since Verrill has shown that the name *Stephanactis* was pre-occupied.

12. *Stephanactis inornata* (1918, p. 17) becomes *Stephanauge inornata*.

13. *Corallimorphus ingens* (1918, p. 23); see this paper, p. 302.

14. *Anemonia inessa* (1918, p. 3) is more likely a *Gyrostoma*.

(ii) Details from Verrill.

1. Verrill (1899) has explained that the name *Stephanactis* is pre-occupied (1868), and renamed Hertwig's genus *Stephanauge*. There are now recorded, as forms with mesogloal sphincter, six pairs of perfect mesenteries (not macroenemes), no acontia, and a very few (up to about eight) cinclides, *Stephanauge impedita*, Grav., *S. inornata*, Grav., *S. abyssicola*, Hertw. (= *Actinauge nexilis*, Verr.), *S. tuberculata*, Hertw., &c. In Part I of this

paper I mentioned (p. 487) this genus without being very definite about it. I do not think a final decision can be made even now, but if these cinclidal non-acontiated forms are established they will probably need a family Stephanaugidae, one of the further combinations foreshadowed in Part I. From the fewness of their cinclides, and from their general characters one imagines the cinclides to be vestiges not to last much longer, and probably the forms are descendants of Metridiid ancestors which have lost the acontia before all the cinclides; but it is not even certain yet that there are not really rudimentary acontia, easily overlooked, present, in which case the forms are actually queer Metridiidae on the way to forming Chondractiniid or Actinosephyhiid or other stages. *S. tuberculata*, at least, has basal mesogloal swellings to some of the tentacles. If the others have not they need separation, and the whole genus and its relations need careful revision. The related (?) *Amphianthus* seems to be an Actinosephyhiid, so far as it is at present known.

2. *Synanthus*, Verr., is probably *Paranthus*.

3. *Ammophilactis*, Verr., 1899, p. 213.

Body may be long, with small base, divided into smooth scapus with a collar in which is the mesogloal sphincter, and capitulum with suckers which can attach grains of sand. Tentacles in more than two cycles in the adult. Numerous perfect mesenteries. Strong apparently diffuse retractors. Older mesenteries fertile.

A. rapiformis, Les., 1817, p. 171. (See Verrill, 1899, p. 213.)

This seems clearly a Paractid, differing from *Pseudoparactis* in its single sphincter and suckers.

4. *Archactis*, Verr., seems very near or identical with *Antholoba*.

5. *Raphactis*, Verr., 1899, p. 144.

Definite base, broadly expanded or stem-clasping. Column with a capitulum which may be more or less ridged, and a scapus which is often ridged at the top, where the mesogloal sphincter lies, and may also be tuberculate. Twelve or more pairs of perfect (and at least mostly) fertile mesenteries, others may be fertile too. Diffuse retractors. Tentacles in more than two cycles in the adult.

R. nitida, Verr., 1899, p. 144.

R. caribaea, Verr., 1899, p. 205.

This may be the same as *Pseudoparactis*, in which case it takes priority. But it seems distinguished by its single sphincter, and distinct from *Ammophilactis* in its lack of suckers.

6. Verrill says *Stomphia* may have fertile perfect mesenteries, perfect mesenteries 16-24 pairs in large specimens.

7. *Antiparactis*, Verr., is probably a synonym of *Paranthus*.

(iii) Other details.

1. *Euphellia*, Pax, 1908, p. 475.

Diadumenidae with definite base. Wall may be wrinkled. No papillae or suckers. Distinctly divided into scapus and capitulum, the scapus with an easily-shed investment. No acrorhagi or fosse. Long strong mesogloal sphincter. Six pairs of macrocnemes. Acontia not specially strong. There are cinclides in longitudinal rows.

E. cinclidifera, Pax, 1908, p. 475.

The definition of Diadumenidae will need slight alteration of detail to admit this form. It seems to be, if it really has cinclides, a link between Diadumenidae and Phelliidae, a Diadumenid on the way to becoming a Phellia.

2. Pax describes a *Paraphellia polyptycha* (1908, p. 493), which may be a *Paraphellia* or possibly a *Sagartiomorphe*.

3. *Andvakia* is of quite uncertain standing and more needs to be known of it.

4. *Allantactis* seems to be the same as *Sagartiomorphe*, and if this is so the name has priority.

5. *Octineon*, Moseley, M. S. (See Fowler, 'Quart. Journ. Micr. Sci.', vol. 35, 1894, p. 461.) (= *Ammodiscus*, Carp., 1871, p. 159.)

The body has the form of a thin disc a little raised in the middle, and encrusted with sand and other things which may get into the mesogloea. Sphincter seemingly mesogloal. Probably twelve tentacles. Twelve larger primary and perfect mesenteries, but only the eight *Edwardsia* mesenteries provided with true retractors. Very few of the mesenteries beyond the twelve primaries perfect, and these are thin, with no gonad or filament and little muscle. Of the two couples of primaries over and above the *Edwardsia* eight, one couple has a modified kind of muscula-

ture and no filaments, and the other couple has no gonad, filament, or well-developed muscle. The eight *Edwardsia* mesenteries have huge circumscribed retractors of curious form, which seem to be tending to shift off the mesenteries; they also have gonads and filaments.

O. Lindahli, Carp. 1871, p. 159. (See Fowler, 1894, p. 461.)

This genus seems to be eligible for *Marsupiferidae*. As far as I can understand the account of it, I take it that it has a mesogloeal sphincter, and the rest fits in fairly well. It is a queer form with a reduced number of macrocnemes; cf. *Decaphellia* and some *Haleampas*.

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On the Post-Embryonic Development of certain Chalcids, Hyperparasites of Aphides,

with Remarks on the Bionomics of Hymenopterous Parasites
in General.

By

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With 7 Text-figures.

INTRODUCTION.

IN the summers of 1919 and 1920, certain hyperparasitic Chalcidoidea were reared from material collected in the field for the study of two hyperparasites of aphides, the Proctotrypid, *Lygocerus* (5), and the Cynipid, *Charips* (6).

The following is an account of the post-embryonic development of two common forms, which were obtained in considerable numbers from the cocoons of the Braconid, *Aphidius ervi*, Hal., a parasite of *Macrosiphum urticae*, Kalt., an aphid that infests the stinging nettle.

I would here express my sincere thanks to Professor Stanley Gardiner, who gave me facilities to carry out the work in the Zoological Laboratory, Cambridge: and to Mr. J. Waterston of the British Museum (Natural History), who kindly determined the species of Chalcidoidea submitted to him.

BIONOMICAL AND SYSTEMATIC POSITION.

The two species now considered belong to the sub-family Sphegigasterinae of the family Pteromalidae, which is, according to Ashmead, the largest group of the Chalcidoidea, and the most difficult to classify.

Asaphes vulgaris, Wlk., belongs to the tribe Asaphini, the majority of which are said by Ashmead to be parasitic on Aphidiidae and Coccidae (1).

Pachycrepis clavata, Wlk., is included in the allied tribe Pachyneurini, which Ashmead says are regarded as chiefly parasites of the same Rhynchota, but he adds that these insects have other hymenopterous parasites, through which the Pachyneurini are probably hyperparasitic.

In addition to *Asaphes* and *Pachycrepis*, two females of a species of *Pachyneuron* were reared. The eggs and early larval stages of the two former species are indistinguishable. The egg of the *Pachyneuron* is characteristic, but its development was not observed.

Various Chalcidae have been recorded as reared from aphides, and it is possible that some of them may yet prove to be primary parasites; but the forms described here are hyperparasites of the plant-lice through the larvae of *Aphidius*, and allied genera of Braconidae, which develop internally in aphides. The Chalcidae do not oviposit until the aphid is dead and the *Aphidius* has woven its cocoon, and is ready to transform inside the empty skin of its late host. Their true relation to the aphid was shown as long ago as 1834 by Nees ab Esenbeck for *Asaphes* or a similar form, and his observations have been confirmed by Walker and Buckton, and subsequently by other writers.

These hyperparasites do not appear to be specific for different Aphidiidae or aphides. In 1919 I reared *Asaphes vulgaris* from an *Aphidius* in *Rhopalosiphum sonchi*, Kalt., and also from *Aphidius salicis*, Hal., a parasite of *Aphis saliceti*, Kalt. This Braconid and aphid are less than half the size of *A. ervi* and *M. urticae*, but the Chalcid seems to adapt itself to either form, and thus probably has considerable latitude in the choice of a host.

PAIRING.

All observed ovipositions of *Asaphes* and *Pachycrepis* took place after pairing. Only two examples of *Pachyneuron* were obtained, and both were females.

One laid a single egg parthenogenetically and died soon afterwards. The other lived for some days but did not oviposit.

OVIPOSITION.

The female Chalcid selects a cocoon containing an *Aphidius* larva on the point of metamorphosis, but sometimes a newly-transformed pupa may be chosen. The hyperparasite shows considerable excitement in her search, and runs round the cocoon, tapping it eagerly with her antennae. Finally she mounts upon it, facing the head of the aphid, and, boring through the integument with its silk lining, she deposits a single egg upon the upper surface of the body of the *Aphidius* larva, as it lies curved head to tail within the cocoon. The whole operation lasts from one to three minutes. Only one egg is inserted at each oviposition, and when more are found they are the result of different attacks. The number of eggs laid by each female seems to be between thirty and forty, but it is difficult to be precise on this point as the insects will live for some days in captivity, and the eggs in the ovarian tubes do not all mature at the same time.

THE EGG.

The eggs of *Asaphes* and *Pachycrepis* are indistinguishable from one another. They are white, elliptical bodies

TEXT-FIG. 1.



Egg of *Asaphes vulgaris*.
×100.

TEXT-FIG. 2.



Egg of *Pachyneuron* sp.
×100.

with a smooth chorion, having dimensions, $.29 \times .12$ mm. (Text-fig. 1).

The single example of the egg of *Pachyneuron* was long, oval, and slightly curved. On the concave side, the

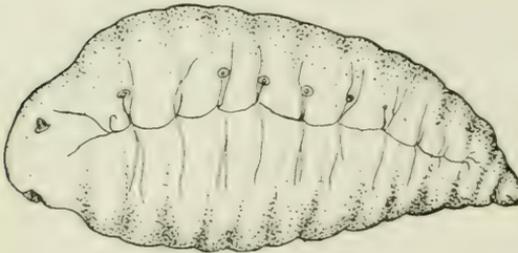
chorion is smooth, but the rest of the surface is covered with minute scales or papillae. Dimensions, $.31 \times .10$ mm. (Text-fig. 2). This egg is very similar to that of *Pachyneuron gifuensis*, Ashm., figured by Howard and Fiske (7).

THE FIRST INSTAR.

Dimensions $.45$ mm. $\times .23$ mm.

The egg hatches about sixty hours after oviposition. The larva in the first instar much resembles in general form that of the *Lygocerus* previously described (Text-fig. 3). It is

TEXT-FIG. 3.



The larva of the first instar. $\times 300$.

TEXT-FIG. 4.



Mandibles of the newly-hatched larva. $\times 600$.

white, semi-transparent, and consists of thirteen segments in addition to the head, which is furnished with two tactile papillae. The mouth is small and oval, and the mandibles are somewhat more curved than those of the larva of *Lygocerus* (Text-fig. 4).

The tracheal system consists of a pair of longitudinal trunks, united by an anterior commissure between the first and second segments, and a posterior commissure in the eleventh segment. At this stage there are four pairs of functional spiracles, namely between the first and second segments, and on segments 4-6 inclusive. These segments are supplied with dorsal and ventral lateral branches, and the developing spiracular trunks of segments 3 and 7-9 are visible. The larva makes an incision in the skin of the host, and as the

body-fluids of the latter fill the midgut the hyperparasite is tinged pale yellow.

INTERMEDIATE STAGES.

The exact number of ecdyses of these Chalcids was not determined. There is no marked change of form during development, but the body becomes more globose, and the head less conspicuous. The cephalic papillae do not disappear as in *Lygocerus*, but persist until metamorphosis. The spiracles on segments 7 and 8 become functional, and those on segment 3 open shortly afterwards. The ninth pair (on segment 10) open as development proceeds, but the tenth pair are closed until shortly before metamorphosis.

The host dies a day or two after the Chalcid larva has begun to feed, and decomposes rapidly. These hyperparasites penetrate more deeply into the decaying tissues than do the larvae of *Lygocerus* at the same stage. The larvae are also more fragile and transparent, and are easily crushed or ruptured when handled.

THE FULL-GROWN LARVA.

Dimensions, 1.26 mm × .60 mm.

The larva when fully fed is creamy white and opaque, slightly curved, and with a smooth glabrous cuticle. The body tapers somewhat to the anus, and the segmentation is well marked. The head bears a pair of conspicuous papillae, and a pair of similar, though smaller, appendages are found on the first segment. In addition, each segment from the first to the fifth or sixth is furnished with one or two pairs of minute spines (Text-fig. 5).

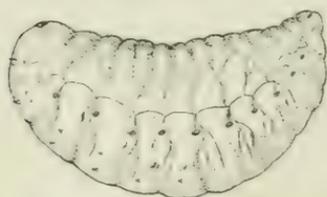
The labrum and labium both bear palps, as do also the maxillae. The mandibles are simple, and strongly chitinized, though less massive than in *Lygocerus* (text-fig. 6).

The ramifications of the tracheal system are more elaborate than in the preceding stages, and the tenth spiracle (on segment 11) becomes functional.

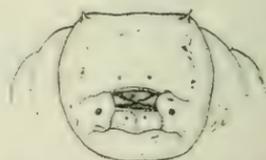
The internal structure is of the type usual among hymeno-

pteroous larvae. The narrow oesophagus opens by a valve into the vast mesenteron filled with food, which is churned to and fro by muscular contractions. The mesenteron is closed posteriorly and does not communicate with the proctodaeum until immediately before metamorphosis. A pair of short Malpighian tubules enter the hindgut at its anterior end. The salivary glands extend backwards to the ninth segment, and lie on either side of the gut ventrally as a pair of long straight tubes. Behind the head their ducts unite to form the common salivary duct, which opens on the floor of the mouth. The ventral nerve-cord appears as a broad uncontracted band extending backwards into the tenth segment. The rest of the internal structure calls for no particular comment.

TEXT-FIG. 5.

The full-grown larva. $\times 25$.

TEXT-FIG. 6.

Head of the full-grown larva.
 $\times 75$.

In a cocoon opened carefully when the hyperparasite was almost full grown, it was possible to watch the transformation into the pupa, and by this means it was determined that the mature larvae of the two forms examined were identical in appearance. Attempts to follow the earlier development in the same way always failed, because exposure to the air caused the decaying tissues of the *Aphidius* to dry up and thus brought about the death of the hyperparasite. The larval development of the Chalcidoidea has been more studied than that of other Hymenoptera parasitica, but so much diversity exists within the family, owing to secondary modifications induced by various hosts and habits, that a comparative account can throw little light on their affinities. The forms here described agree very closely with that of *Torymus propinquus*, an ectoparasite of certain *Cecidomyiidae*,

studied by Seurat (10). The general form and the number and order of opening of the spiracles are the same in both cases. Certain parasites of Coccidae, described by Inms (8) show a reduction in the number of spiracles from behind forwards; but in one, *Aphyeus melanostomatus*, rudimentary trunks of the tenth pair appear during development, though they never become functional. Ectoparasitic Chalcidae, such as *Asaphes* and *Torymus*, have probably retained certain primitive features, such as the full number of spiracles, which have been lost in the more specialized and frequently hypermetamorphic forms, found among the endoparasitic members of the super-family.

PUPATION AND EMERGENCE.

When the remains of the *Aphidius* have been completely devoured, the gut of the hyperparasite opens, the meconium is voided, and the Chalcid pupates within the cocoon previously woven by the *Aphidius* inside the skin of the aphid. The pupal stage lasts from fourteen to sixteen days, for *Asaphes* and *Pachycrepis*; but in a single observed instance of *Pachyneuron* the period of pupation was only ten days. When ready to emerge, the imago gnaws a hole in the cocoon and creeps out. The adults lived in confinement for from four to seven days, and fed on the sap oozing from cut leaves, and on honey-dew which had fallen from the aphides.

At least two generations may occur in the year, but the exact number was not ascertained: it is probably dependent on the number of hosts obtainable. There is no evidence to show how these Chalcids pass the winter.

REMARKS ON THE BIONOMICS OF HYMENOPTEROUS PARASITES IN GENERAL.

The relations of any animal to its enemies, predatory or parasitic, form what may be termed a bionomical complex; although the limits of such a complex are often difficult to determine, especially when the enemy has a wide choice of alternative food or host species.

Aphides, with their parasites and hyperparasites, form a biological complex of considerable intricacy; but its limits are well defined, and it is thus convenient for the study of the bionomics of parasitism. The Aphidiidae, which are a large and distinct sub-family of Braconidae, are all obligative parasites of Aphides, and have no alternative hosts; and the hyperparasites, which belong to the three super-families of Cynipoidea, Chalcidoidea, and Proctotrypoidea, are exclusively confined to the Aphidiidae, with the exception of certain Cynipids (Charipinae) and Chalcids, which possess allied forms parasitic upon Coccidae.

The bionomics of some members of this complex are comparatively simple. Thus, the species of Charips (Cynipidae) described elsewhere (6) are invariably parasites of Aphidius, and thus hyperparasites of the aphid, and, so far as is known, never prey upon another hymenopteron. The status of such Proctotrypids as *Lygocerus* (5), and Chalcids such as *Asaphes* and *Pachycrepis*, is more difficult to determine, because although usually parasites of Aphidius, and therefore standing in the same relation to the aphid as Charips, they may on occasion be parasitic on each other. The interrelations of these forms are shown in the accompanying diagram (Text-fig. 7). An Aphidius cocoon is sometimes found to contain two hyperparasites of either, or both these species, the result of two successive ovipositions. Fiske (3) has called this phase of parasitism 'superparasitism'; but as the word means neither more nor less than hyperparasitism, a term already employed in cases where the parasite is itself attacked by a parasite, I would suggest replacing this etymological hybrid by 'epiparasitism'. In such a case, in the aphid complex, only one imago emerges from the cocoon. Either one parasite is sufficiently advanced to devour the host before its rival can compete with it; or else, if both parasites are of the same age, there is insufficient food to nourish both up to metamorphosis, and they starve to death. One seems never to make a direct attack on the other.

But in certain instances a Chalcid hyperparasite larva,

generally one three-quarters grown, may be found with the egg or larva of a Proctotrypid, or of another Chalcid, on its body. It may be that the second hyperparasite deliberately oviposits upon the larva of the first, if the *Aphidius* host

TEXT-FIG. 7.

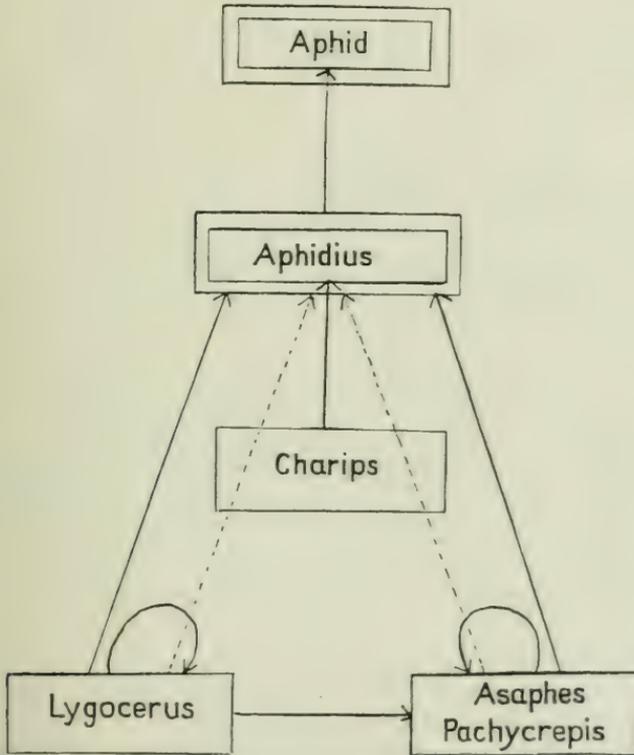


Diagram to illustrate the biological complex of an aphid, its parasites, and hyperparasites. Endoparasitism is indicated by a double margin to the host.

has already succumbed to the attack, and originally I thought that this was the case; but further observation led me to modify this conclusion. Thus instances of this kind are rare compared with those of simple epiparasitism and attempts to induce the Chalcid or Proctotrypid to oviposit on the full-grown larva of another hyperparasite that had already devoured

the host, always failed. The more probable explanation seems to be that the intention of the second hyperparasite is to oviposit upon the *Aphidius*, but if by chance her ovipositor comes in contact with the larva of the first, she is unable to distinguish between it and the proper host, and places her egg upon it. Certain observations support this view. For instance, young larvae were never found thus parasitized, possibly because they escaped discovery owing to their small size; and the mature larva of *Lygocerus* was never found to be infected. There is very marked increase in the size in this species between the early and late stages, and the latter is of peculiar form with a dorsal conical appendage to the last segment. The full-grown larva and the pupa are capable of active movement, and jerk the abdomen violently when irritated. It is possible that this action warns off the ovipositor of another hyperparasite. I have observed only three instances where *Lygocerus* was parasitized, and then always by its own species. In two cases, larvae were observed on newly-transformed pupae, and here, contrary to the usual rule, the egg must have been placed on the larva when nearly full grown. In the third case, an egg was found upon a younger larva, whose power of movement was not yet developed.

The Chalcid larvae, which are sluggish at all stages, are more frequently attacked in their later instars by *Lygocerus* and by other Chalcids.

The incidence of mortality from epiparasitism is high in the Cynipidae, since they invariably perish within the host when the latter is attacked by an ectoparasite. Exceptionally, a full-grown larva of *Charips* may be found epiparasitized by a Chalcid or by *Lygocerus*, and in such cases it is probable that the oviposition of the second hyperparasite coincided with the emergence of the Cynipid from the host, and before it had demolished the remains of the latter.¹

¹ It should be pointed out that other forms not dealt with here are involved in this biological complex. Thus Silvestri ('Contribuzioni alla conoscenza biologica degli Imenotteri Parassiti', 'Boll. Lab. Scuola Agric. Portici', vol. iii, 1909) has described the development of a Chalcid,

It is clear that this phase of parasitism differs somewhat from ordinary epiparasitism. It has been called 'accidental superparasitism' by W. D. Pierce, quoted by Fiske (3), but might better be termed 'metaparasitism'. Epiparasitism then may be defined as successive infestations of a single host by two or more species, or by several individuals of the same species, of parasite. Metaparasitism is a development of epiparasitism, and may be defined as the direct attack of one epiparasite upon another. Objection may be taken that the distinction is too fine to warrant the coining of a new word in a science already burdened with technical names; but of late years the practice of introducing parasites to control insect pests, in countries or continents where the latter have become troublesome, has been much extended; and, before importing a parasite into a new area, it is of the first importance to ascertain to what extent it is potentially metaparasitic upon other species.

Thus, suppose that two forms of primary parasites A and B are imported into a certain locality. There will be a slight reduction of their total efficiency, in proportion to the incidence of epiparasitism between them; but as long as plenty of hosts are available, the loss due to this will be small, and in any case little harm will result, as a pest destroyer will be reared ultimately. But supposing that B is potentially metaparasitic, while A is not, then in course of time, B, since it will always be successful in contest with A, will reduce the latter species, or even supplant it altogether. The mischief will be even greater from an economic standpoint, if B should prove to be less efficient than A in destruction of the host pest.

In fact, this is what has actually taken place in Hawaii, *Eucyrtus aphidivorus*, Mayr., which like *Charips* is an endoparasite of *Aphidius*; but as its other bionomical relations are not known, it has not been included in this discussion, and the same applies to other Chalcidae, recorded as reared from Aphides, but many; if not all of which, are probably hyperparasites. However, as Arrow ('Entomologist's Monthly Magazine', vol. lvii, September 1921) observed *Aphelinus chaonia*, Wlk., ovipositing in aphides, this form may prove to be a primary parasite.

according to the recent investigations of Pemberton and Willard (9). Among the parasites introduced to control the Mediterranean fruit-fly (*Ceratitis capitata*, Wied.) were two species, *Opius humilis*, Silvestri, and *Diachasma tryoni*, Cameron. It has now been shown that epiparasitism is common between *Opius* and *Diachasma*, and that in such a case *Diachasma* is nearly always victorious. Thus *Diachasma* is gradually suppressing *Opius* in Hawaii; and, as the authors point out, this result is the more deplorable in that *Opius* is not only equally efficient as a parasite, but is actually more prolific than its rival, and if left to itself would destroy a larger number of fly larvae. The situation has been further complicated by the introduction of a Chalcid, *Tetrastichus giffordianus*. This form is very prolific; but, as it is almost always epiparasitic, it is ineffective as a control of the pest, and generally causes the death of the *Opius* or *Diachasma* larva when it comes into competition with them.

Fiske and Thompson (4) have shown that the larvae of certain Saturniidae are parasitized by the hymenopterons, *Ophion*, *Theronia*, and *Spilocryptes*. All three are primary parasites, but epiparasitism is frequent, and when it occurs, *Theronia* and *Spilocryptes* respectively overcome *Ophion*. In competition between *Spilocryptes* and *Theronia*, the first generally is the conqueror; but *Theronia*, it appears, dies of starvation from destruction of its food-supply rather than by direct attack.

Timberlake investigated the bionomics of *Coccophagus lecanii*, Fitch (14), a parasite of *Coccus hesperidum*, which is more frequently reared as a hyperparasite from another primary parasite, *Microterys*. According to this observer, *Coccophagus* is thelytokous when a primary parasite, producing generations of females only; but when it is reared as a hyperparasite, the resulting imagos are all males—a state of things so far unparalleled.

Howard and Fiske (7) in their report on the measures taken to control the gipsy and brown-tail moths in the United States, record many interesting observations on the bionomics

of native and imported parasites. Thus the Chalcid *Schedius Kuvanae*, How., is primarily an egg parasite of the gipsy moth, but it will also oviposit in *Anastatus bifasciatus*, Forst., another egg parasite. In this complex, two other species, *Tyndarichus novae*, How., and *Pachyneuron gifuensis*, Ashmead, are hyperparasitic upon *Anastatus*, but epiparasitism is frequent, and they have been reared not only from *Schedius*, but also from one another.

Monodontomerus aereus, Walk., and *Pteromalus egregius*, Forst., are also primary parasites of the gipsy moth and brown-tail moth respectively; but both forms are also hyperparasitic through certain Tachnidae, and, in addition, the latter form is sometimes reared from other hymenopterons, such as *Mesochorus* and *Apanteles*.

Smith (12) has shown that *Perilampus hyalinus*, Say., although strictly speaking an obligative hyperparasite of certain lepidopterous larvae, through their hymenopterous and dipterous parasites, may, when epiparasitism occurs, become metaparasitic. Thus in one instance a cocoon of the Ichneumonid, *Limnerium validum*, was first parasitized by *Perilampus*, and subsequently by the Pteromalid *Dibrachys boucheanus*. The latter devoured the *Limnerium* host, but was shortly afterwards itself destroyed by *Perilampus*.

The following table gives the synonyms used by previous writers on the bionomics of the Hymenoptera parasitica for the terms suggested here.

Parasitism	{ Primary parasitism. Parasitism.
Epiparasitism	{ Superparasitism. Secondary parasitism. Secondary hyperparasitism.
Metaparasitism	{ Accidental superparasitism. Tertiary hyperparasitism. Superparasitism.
Hyperparasitism	{ Secondary parasitism. Hyperparasitism.

These terms may be illustrated with examples from the aphid complex as follows :

Parasitism	aphid+Aphidius	
Epiparasitism	aphid+Aphidius+	{ Lygocerus and Asaphes
Metaparasitism	aphid+Aphidius+	Asaphes+Lygocerus
Hyperparasitism	aphid+Aphidius+	{ Asaphes or Lygocerus or Charips

The possibility of 'hyper-hyperparasites' has been suggested by some writers, but although obligative hyperparasitism of the second degree may occur, I am not aware that it has been definitely proved. The records that seem to point to it are probably due to epiparasitism among hyperparasites.

Apart from their economic importance, cases such as those described are of much biological interest, as throwing light on the origin of parasitism in the Hymenoptera parasitica.

Thus the epiparasitism of *Lygocerus* and *Asaphes* may exceptionally become metaparasitism, if, by chance, one species oviposits directly upon the larva of the other; and a stage further has been reached in *Coccophagus* and *Theronia* which are as often hyperparasites as parasites. Fiske says of the latter (3) that it is so frequently a 'super-parasite' that it is in danger of becoming a hyperparasite. From such forms as these it is not a great step to the obligative hyperparasitism of, for example, *Charips*.

Epiparasitism is brought about by a high proportion of parasites to the host population. Fiske (3) has made an ingenious calculation, showing that as the incidence of parasitism rises, the chances of epiparasitism rise likewise. Thus, given a hundred hosts, by the time that the parasite has laid ten eggs, there is an even chance that one will have been placed in a host already infected, and so on, until with fifty eggs the odds are even that no less than ten ovipositions will have been duplicated in this way. But although hyperparasitism may have arisen from epiparasitism, through metaparasitism, primary parasitism cannot be accounted for thus.

Wheeler (15) has put forward a theory of the origin of parasitism in the Aculeata. He supposes that parasitism arose within the species, when certain individuals acquired the habit of laying their eggs in the brood cells of their neighbours, instead of working for themselves : and he supports his suggestion by the significant fact that the existing parasites are frequently generically allied to the host species.

But this theory can hardly be extended to the Parasitica, even if we regard them as a heterogeneous group, derived from different ancestral stocks, and classified together in virtue of characters acquired independently by members of different families in adaptation to parasitic life. The existing Parasitica are a vast class, of infinite variety of size, structure, and habit ; and with the exception of most of the Cynipids and a few Chalcids, which are gall-formers on plants, all are parasitic upon insects, frequently upon families distantly related to them.

To suppose that the parasitic habit arose spontaneously in a common ancestor, and was perpetuated by natural selection, involves the assumption of a considerable initial mutation. If, as among the bees and wasps, we found that phylogenetic relationship between host and parasite was the usual rule, we might suppose that parasitism arose within the species in the Parasitica, as Wheeler suggests for the Aculeata ; but there is as much to be said against as for this view, since the modern Parasitica include, not only their own allies, but almost every stage of almost every family of insects among their hosts.

Nevertheless, parasitism must have had a beginning, and the suggestion may be put forward that the parasitic habit arose among these Hymenoptera from the inquiline habit. In other words, the proto-Parasitica were phytophagous, and oviposited on plants. A further stage was reached, when, for the better protection of the eggs, they resorted to the shelter of galls and other deformities produced by members of their own tribe, and by other insects. Here they became established as commensals or inquilines, and from the inquiline habit to the parasitic habit is possibly not a great step. The Chalcid,

Torymus propinquus, previously alluded to, is now an ectoparasitic of a gall-forming Cecidomyiid of the nettle, but if this view is correct, its ancestors inhabited this, or a similar gall, as inquilines, and later acquired the habit of devouring the maker of the growth that harboured them.

The intra-specific origin of parasitism in bees may find a parallel among inquilines, for it is quite conceivable that certain individuals may have adopted the habit of ovipositing in a ready-formed gall, and thus became inquilines to their own species. Cameron (2) remarks that among the Cynipidae, the known inquilines are species of *Synergus*, *Ceroptres*, or *Sapholytus*, which are all forms nearly related to the true gall-formers.

The view that parasitism is derived from inquilinism would account for the diversity of the hosts of the Parasitica. Galls, and similar plant deformities, are caused by insects of other groups, such as many Hemiptera, Diptera, and Lepidoptera. The ancestors of the Parasitica may have used these as well as the galls produced by members of their own family, and later become parasitic upon the insects which formed them.

It will be very desirable in future to investigate fully the bionomics of the forms reared from, for example, Cynipid galls. If any, generally found to be inquiline, are proved on occasion to devour the maker of the gall, it will support the suggestion that the Parasitica are descended from inquiline ancestors.

SUMMARY.

1. *Asaphes vulgaris*, Wlk., *Pachycrepis clavata*, Wlk., and *Pachyneuron*, sp., are hyperparasites of aphides through the larvae of certain Braconidae (*Aphidius*).

2. Oviposition took place after mating for *Asaphes* and *Pachycrepis*, and parthenogenetically for *Pachyneuron*.

3. The eggs are deposited upon the body of the host when the latter is fully fed and about to undergo metamorphosis within the skin of the aphide.

4. The larvae feed ectoparasitically upon the host, which soon becomes a decomposing mass.

5. The newly-hatched larvae are maggot-shaped forms, with four pairs of open spiracles and two cephalic papillae.

6. In the later stages small tubercles are developed on the prothorax and succeeding segments, and there are ten pairs of functional spiracles.

7. The total period of development is a little over three weeks, and at least two broods may occur in the summer.

8. The bionomics of aphides and of their parasites and hyperparasites are discussed.

9. The term 'epiparasitism' is proposed instead of 'superparasitism' which has been used by other writers, and it is suggested that it should be restricted to cases where two or more species, or two or more individuals of the same species, independently attack the same host.

10. The term 'metaparasitism' is suggested for cases where one parasite or hyperparasite in epiparasitism, becomes secondarily hyperparasitic upon the other.

11. Instances are given of the occurrence of epiparasitism and metaparasitism among other hymenopterous parasites.

12. The origin of parasitism in the Hymenoptera parasitica is discussed, and it is suggested that it arose from an earlier inquiline mode of life.

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Animal Chlorophyll: its Relation to Haemoglobin and to other Animal Pigments.

(Contribution from the Bermuda Biological Station for Research, No. 132).

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PART I. THE PIGMENTS OF ANIMALS HAVING
NO BLOOD-VASCULAR SYSTEM.

1. INTRODUCTION.

IN the study of marine invertebrates one of the most impressive things encountered is the great richness and variety of colour; it is not surprising, therefore, that the question of animal coloration has long engaged great attention. Investigated at first superficially by those who sought an explanation of the so-called phenomenon of 'protective coloration', the problem attracted, during the latter part of the nineteenth century, the attention of several English physiologists, and it is to the investigators of this group—Lankester, Sorby, MacMunn, Mosley, Griffiths, Poulton, and Halliburton are the more important names—that we are indebted for very real contributions to our knowledge of animal pigments; especially for the introduction of the microspectroscope into this field of biological research. Since 1900 the work on pigmentation has been to a very large extent spasmodic, and while certain valuable additions have been made (in particular, those of Gamble and Keeble) there are still many problems which invite further investigation.

The present paper aims to show that the pigment which is responsible for the colour of certain representative invertebrates comes from the blood-stream, and that in many cases the pigment cells of the blood arise (while in circulation) from unpigmented corpuscles. This view concerning the origin of pigment occurred to the writer after noting that a pigment of the blood appeared to be identical with the body pigment in three representative phyla: (1) in another paper the writer (Fulton, 1921 *b*) has shown that the pigmented corpuscles in the blood of a Bermuda tunicate, *Ascidia atra*, arise in the blood from colourless cells, and that the blue pigment cell, so common in the blood-stream, is identical with the blue cells of the tunic—the cells which give to this ascidian its intense blue colour; (2) Crozier (1916 *b*) has demonstrated that the blue

pigment granules which colour the nudibranch *Chromodoris zebra* are also to be found in great abundance in the pigment cells of the blood—the identity of the two pigments having been determined by the spectroscope; (3) lastly, while examining the body-fluid of one of the common sea-urchins, *Tripneustes esculentus*, it was found that the large red amoeboid cells (so well described by Geddes, 1880) gave strong indication that their pigment is the same as that which colours their spines and tube-feet. Thus, in a tunicate, a mollusc, and an echinoderm there seemed to be very good evidence that the body-pigment is found also in the blood.

The experimental work was carried on at the Bermuda Biological Station for Research during the summer of 1920, and I wish to express my warmest thanks to Dr. E. L. Mark, who gave me the facilities of the laboratory, and who made possible my trip to Bermuda.

2. PROTOZOA AND PORIFERA.

With any effort to trace animal pigment back to the blood-system there arises at once a very serious difficulty. Pigmentation as such appears phylogenetically long before the rise of the blood-system. How, then, is it possible to assume that all coloration comes from the blood?

Among the Protozoa the only class—with rare exceptions—which contains chromatophores is the Mastigophora (Minchin, 1912, p. 13). Here, however, the pigment is probably in every case chlorophyll, or a substance closely allied to chlorophyll. A typical species of chlorophyllogenous protozoa is *Archeria boltoni*, which was described by Lankester (1885). One notable exception to the assumption that all protozoan pigments are closely related to chlorophyll is found in *Stentor cæruleus*, which possesses a blue pigment called by Lankester (1873) 'stentorin'. Spectroscopically the absorption bands of this pigment, quite unlike chlorophyll, resemble those of the blue algal pigment, phycocyanin, which, according to Phillips (1911, p. 596), when present even in minute amounts, greatly alters the spectrum of chlorophyll. Consequently

stentorin is not to be considered an important exception to the rule that all protozoan pigments are chlorophyllogenous in nature.

But how does the chlorophyll of protozoans originate? Is the animal itself capable of manufacturing chlorophyll, or is it the result of outside infection? Geddes (1882) and Lankester (1882 *a*, and 1882 *b*) maintained strenuously that *Hydra viridis* and *Spongilla fluviatilis* were capable of synthesizing their own chlorophyll: ¹ that the green deposits found in these animals are chloroplasts belonging to the animal and consequently are not of plant origin. In support of his contention Lankester asserted the absence of nucleus and cellulose wall in the green corpuscles. Though no histological evidence has been adduced to show the presence of a nucleus in these bodies, it seems fair to conclude, since Beyerinck (1890, note 1, p. 784) has succeeded in obtaining cultures of algae from the green corpuscles of *Hydra viridis*,² that the chlorophyll of *Hydra* is algal in nature and due to an infection from the outside. The algae probably represent a phase in the life-history of *Chlorella viridis*. A similar condition undoubtedly holds for most of the green protozoa: Famintzin (1889, 1891), Dantee (1892), and Dangeard (1900) all report having obtained colonies of algae from the macerated tissues of *Stentor*, *Paramoecium*, and *Frontonia*; Schewiakoff (1891) found that if colourless *Frontonia* are fed upon macerated green specimens they become infected with green algae which subsequently divide within the cell. Similar results have been reported for *Paramoecium* (Dantee). Certain contrary evidence is also on record for the Protozoa—the puzzling cases of *Vorticella campanula* (Englemann, 1883), and of *Pelomyxa viridis* (Bourne, 1891)

¹ For a more complete discussion of this question see Gamble and Keeble (1903), Keeble and Gamble (1907), and Fulton (1921 *a*).

² Entz (1881 and 1883) reports similar results, but in his experiments little precaution was taken against infection and consequently the results are to be accepted with caution. Recently Goetch (1921) has succeeded in infecting colorless *Hydra* with *Chlorella*, and in so doing has corroborated in a very substantial way the views of the earlier investigators cited above.

are the most significant examples—which favours Lankester's hypothesis of the intrinsic nature of certain of the animal chlorophylls. Nevertheless the balance of evidence seems to favour the algal theory to account for chlorophyll in animals; and it is probable, moreover, that further investigation with a more refined technique will show conclusively that Lankester was wrong. Particularly does this seem probable in the light of the classic researches of Gamble and Keeble (which will be discussed later) on *Convoluta*, the green cells of which were shown to be intruding algae. In short, the Protozoa do not present any real difficulty to assuming that body-pigments arise in the blood.

The sponges possess nothing in the nature of a blood-system: nutrition and respiration being accomplished by water currents within the body. What, then, is the nature of their coloration? Is it an animal pigment, which has arisen independently of the blood-system; or is it, as in the Protozoa, a chlorophyllous substance? Though it has not been demonstrated that every species of sponge contains chlorophyll, the spectroscopic investigations of Sorby (1875 *a*), Krukenberg (1884), and MacMunn (1888) have established the presence of a chlorophyllous pigment in eighteen species of sponge, it being most common in the genera *Halichondria* and *Halina*. The more highly-coloured sponges possess pigments the absorption bands of which (as indicated by the figures of MacMunn and Krukenberg) resemble in many respects those of certain of the pigments from blue and red algae, the pigment spectra of these sponges is, in addition, quite different from the spectra of chlorophyll. Inasmuch as it has been shown that the presence of small quantities of such algal pigments as phycoeyanin, phycophaein, and phycoerythrin (Phillips, 1911; also Willstätter and Stoll, 1913) greatly alter the spectrum of chlorophyll, the fact that the pigment of certain coloured sponges fails to show the bands characteristic of the plant pigment does not necessarily indicate its absence. As in the Protozoa, one is also confronted in the Porifera with a question concerning the nature of the pigment itself—a point which is as yet unsettled. Lankester (1882) and his school were vigorous in upholding the animal origin

of sponge chlorophyll, while Brandt (1881 *a*, 1881 *b*, 1882, and 1883) supported the view that chlorophyll in the Porifera and other animals results from a symbiotic association with green algae.¹ Zooxanthellae—symbiotic holophytic flagellates—have been reported for several sponges, also recently by Kirkpatrick (1912) for *Merlia normani*.² Cotte (1904) likewise gives an account of an interesting association of this sort.

There is no doubt but that Lankester had every reason to question the evidence of Brandt—which in the light of later investigations was most decidedly inconclusive—and he has done valuable service by his championship of the opposed view, that of the intrinsic nature of the corpuscles under discussion. For his view compels those who hold the “algal” theory to investigate each case separately and to vindicate their view by the synthesis of the green animal’ (Keeble and Gamble, 1907, p. 171). Now, however, there is little question but that true chlorophyll in animals owes its existence in every case to plants. It seems evident, therefore, that the pigmentation of most sponges has resulted from an association, symbiotic or otherwise, with plant cells; and that, as with the Protozoa, the Porifera present no serious obstacle to the assumption that animal colour arises in the blood. Consequently a discussion of the phylogenetic aspect of pigmentation must of necessity commence with the coelenterates.

3. COELENTERATA.

Since the coelenterates are organisms having but two cell layers, ectoderm and entoderm, it is at once obvious that they

¹ Since the present writing the work of Van Trigt (1918) has been brought to the writer’s attention. He has shown that in *Spongilla* the green cells very clearly are invading organisms, and has made an extensive series of experiments with cultures of the green cells derived from the macerated sponge tissue. He has also given conclusive proof of an oxygen-carbon dioxide exchange between the algal cells and the sponge tissue. His evidence further corroborates the view just expressed concerning the symbiotic nature of the green cells in sponges.

² Winter (1907) has shown that Zooxanthellae are symbiotic in the foraminifer *Peneroplis*.

can possess no blood-system in the sense in which it is used for the higher animals. Nevertheless, as Griffiths (1892, pp. 128 and 184) has emphasized, the nutritive or 'chylaqueous' fluid is analogous to the blood of the higher forms in that it carries nourishment, supplies oxygen, carries off the waste products of metabolism, and in many cases, as Kollmann (1908) and others have since shown, is a corpusculate fluid. The importance of this analogy between the chylaqueous fluid and the blood of higher animals is uncertain; however, a question immediately presents itself concerning the relation of this fluid to the pigmentation of the coelenterates. It is therefore desirable to consider first the nature of coelenterate pigments. The animals on which the greater share of my work has been done are two species of actinians common in the Bermuda Islands: *Condylactis passiflora* Duch. and Mich.,¹ and *Actinia bermudensis* Verill.

(a) *Condylactis passiflora*.

Condylactis occurs in great abundance in all parts of the Bermudas, and is usually found firmly attached to the under side of rocks and in crevices just below the level of low tide.

If the gastrovascular (chylaqueous) fluid of *Condylactis* be withdrawn at any point on the body with a hypodermic syringe and examined, two types of cell are usually to be observed: one a yellow cell with several large granules, and the other, an unpigmented element. The pigment cells of this body-fluid might easily be confused with wandering pigment cells of the body-wall. In reality, however (as Rand, 1909, has noted) these cells are Zooxanthellae, and it is this fact which in part explains a very striking phenomenon presented by a fresh smear of the gastrovascular fluid: viz. the very marked oscillation of the individual cells. These yellow cells (Zooxanthellae) gyrate usually in a counter-clockwise direction on a single axis, while the colourless cells as a rule

¹ For an excellent description of this species McMurrich's (1889) paper should be consulted.

vibrate much less regularly. The latter, inasmuch as they are motile, have in all probability been torn from the ciliated lining of the gastrovascular cavity; with acetic acid or neutral red their cilia may very easily be demonstrated.

The coloration of *Condylactis*, it should be emphasized, is due largely, but not entirely, to this yellow flagellate. The tissue of the tentacles is itself colourless (Rand, 1909; Parker, 1917), as is shown when a tentacle is transected. The brownish-yellow colour, which is a constant feature of the uninjured tentacle, is therefore due to the presence of the flagellate organism in the internal fluid. This may readily be shown by examining the liquid contents of the tentacle. When an animal is withdrawn from the aquarium with the tentacles in expanded condition the internal pressure on the gastrovascular fluid causes minute streams of water to issue from the terminal pore of each tentacle. If some of the fluid so exuded be caught in a watch-glass it occasionally contains the 'symbiotic' organisms; under normal conditions of exudation, however, they probably do not escape when the tentacle contracts.

Not only are the tentacles coloured by the presence of *Zooxanthellae*, but the column itself owes much of its colour to this organism. However, the column also possesses large collections of red pigment granules, some patches being as much as 2 mm. in diameter. These are more highly concentrated in the lower parts of the column than in the upper, which gives to the basal region an intense red colour, while the upper parts tend toward the brownish yellow of the tentacles.

In two *Siphonophora*, *Velella spirans* and *Porpita umbella*, Kuskop (1921) has found *Zooxanthellae* in abundance; they reside chiefly in the 'hepatic canals', so called, and in the gonophores. Their occurrence in the latter organs strongly suggests that the association of the *Zooxanthellae* with these coelenterates is continuous from one generation to another. Before discussing the significance of these observations the condition of the pigment in *Actinia bermudensis* will also be described.

(b) *Actinia bermudensis*.

Actinia bermudensis is a deep red anemone, which is found on the rocks just about the level of low tide. At high tide, when the water splashes over them, their tentacles open up for feeding; when out of contact with the water they draw their tentacles into the interior of the column and have the appearance of a deep-red gelatinous mass hanging limply from the rocks. The specimens used in the present study were obtained from the caves on the north side of Long Island (Bermuda) where they occur in considerable numbers. This species is distinguished by a very remarkable power of resisting unfavourable surroundings; as an example, it will remain alive sealed in a 100 cc. of sea-water for from six to seven days (Fulton, 1921 a).

A. bermudensis is coloured uniformly by red pigment granules, which are spread through the entire ectoderm. The granules are not of a definite size, however, and the outlines of the cells which contain them are never clear in the living tissue and can be discerned only with great difficulty in tissue which has been fixed. In shade, the pigment is precisely the same as the red pigment patches of *Condylactis*. Consequently a series of experiments was performed with a view to determining whether or not the two pigments are identical. Small pieces of tissue from each species were teased out and placed side by side upon a slide. Their action in the presence of an acid was first tested. In both cases when either 10/N hydrochloric acid or 10/N valeric acid¹ were added a decided increase in the depth of colour took place. When treated with alkalis (NH_4OH and NaOH , 10/N and 50/N) no change could be observed. Neither of the pigments could be dissolved with any of the following solvents: ether, chloroform, methyl, ethyl or amyl alcohol, petroleum ether, xylol, or pyridine. In acetone, however, the pigment of *A. bermudensis*

¹ It has been shown by Crozier (1915, 1916 a, and 1916 c) that, of twenty-two of the more common acids, valeric is the most penetrating to tissues.

proved readily soluble; the condylactid pigment was also dissolved by acetone, but not so readily. The difference in rate of dissolution is probably to be accounted for by the greater thickness of the *Condylactis* tissue. Therefore, since the two pigments are found in species of the same order, since they are of the same colour, since in the presence of acid they are uniformly deepened in shade, and since they are each to be extracted by only one (the same one) of nine solvents, it seems reasonable to conclude that the two pigments are identical.

Among the earlier authors the observations of Moseley (1873) upon actinian pigments are perhaps the most significant. He described a red colouring matter (from *Actinia mesembryanthemum* and from *Bunodes crassicornis*) which he called 'actiniochrome'; MacMunn found this pigment insoluble in many of the ordinary solvents for animal pigments.¹ All of the solvents which he employed also gave negative results when applied to *A. bermudensis* and *C. passiflora*. This suggests that the pigment of the Bermuda actinians is probably identical with Moseley's actiniochrome, but only a spectroscopic examination can determine this with certainty. The two species worked on by Moseley were subsequently investigated by MacMunn (1885 c) with the result the Moseley's actiniochrome was identified, but, in addition, a haematin-yielding pigment was isolated and given the same 'actiniohaematin'.² This pigment, which, it should be emphasized, is closely related to haemoglobin, performs a respiratory function, being capable of existing in a state both of oxidation and reduction. On finding these two pigments together in one animal MacMunn drew the conclusion that one is a respiratory substance (actiniohaematin), and that the other (actiniochrome) is purely for ornament. The most notable contribution of MacMunn, however, was his observation concerning the relation of *Zooxanthellae* ('yellow cells')

¹ MacMunn (1885 c, p. 643), alcohol, ether, chloroform, and carbon bisulphide.

² This pigment, on treatment with a metallic hydroxide and sodium sulphide, gives haemochromogen which on oxidation gives haematin.

to the respiratory pigment. He found that in actinians which were not infected with Zooxanthellae a respiratory pigment is present, but that in forms which are 'packed with "yellow cells"' the pigment had ceased to perform a respiratory function. The most striking case which he observed was that of *Bunodes balli*, in which occurs a facultative association between itself and the Zooxanthellae: the larger variety of that species has many Zooxanthellae and, as a result, is almost without trace of any of the pigments common among other forms: the smaller variety of the same species is uninfected, and as a result possesses a respiratory pigment. The results of MacMunn's work are briefly as follows: (1) that a respiratory pigment is present in most actinians: (2) the pigment is not a carrier of oxygen, but serves simply to store oxygen in the tissue which is subsequently to use it: (3) in those actinians in which yellow cells are present the chlorophyllous pigment of these organisms seems to replace the respiratory pigment; (4) besides this respiratory substance there are other pigments (such as Moseley's actiniochrome) which serve for decoration.¹

In a recent paper on *Actinia equina* and *Anemonia sulcata* Ehnhirst and Sharpe (1920) record several observations which are not in agreement with those of MacMunn. They find that the non-haematin pigment, instead of being purely ornamental, produces oxygen, possibly by photosynthesis. However, they report the presence of Zooxanthellae and fail also to find any haematin derivative, which accords with the results of MacMunn. In addition, these authors hold that the intensity of colour in *A. equina* varies with exposure to light, and the pigment, therefore, functions as a light screen. Certain observations made in the course of the present study support this latter conclusion; when *A. bermudensis* is kept in the dark (three days) the animal loses its deep red tinge and acquires a brownish-red shade. Conversely, if an individual is exposed to direct sunlight, its colour changes to a brilliant carmine.

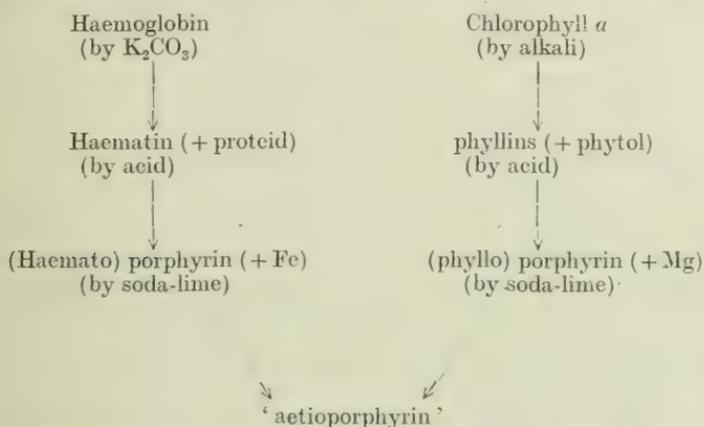
¹ Quoted also by Griffiths (1892); an excellent description of MacMunn's work will be found in Griffiths' book, especially in Chapter VIII.

Having considered the physical and chemical aspects of the actinian pigments, what deductions can be drawn as to their origin?

It is clear, inasmuch as there is no internal circulatory system between the ectoderm and entoderm, that the pigment must be manufactured from substances of the outside world which come actually into contact with the individual cells. Likewise it is evident that these substances are absorbed from within, carried, that is, in the chylaqueous fluid, since the ectoderm of an anemone serves for protection rather than for absorption. It is highly improbable that the pigment comes directly as food (as Crozier (1917) holds for a species of polyclad and Poulton (1893) for certain insects); if that were true, it should exist in solution in the gastrovascular fluid; but this seems definitely not to be the case (MacMunn). The more reasonable hypothesis, it seems to me, is that the cells containing the colour themselves synthesize the pigment from certain food substances. This means that in the absence of a blood-system each actinian cell has to elaborate its own pigment.

Little is known concerning the nature of the food from which the cells manufacture pigment. Various speculations, however, have been made on this point, particularly in the case of the insects. In this class of animals, as Poulton (1893) has shown, the pigment of the body appears to be a modified chlorophyll. That such a condition should obtain among the actinians seems at first impossible. Many actinians live in an obligate association with Zooxanthellae, an association in which the anemone is probably parasitic upon chlorophyllous cells (Fulton, 1921 *a*); that is, in times of starvation they turn upon the cells from which they possibly receive nourishment (by photosynthesis) and engulf them. Also in many actinians the 'yellow cells' are lodged directly in the tissue of the ectoderm and entoderm (Hertwig, 1883). It is a matter of common knowledge, too, that actinians feed upon pelagic forms which contain chlorophyll. From these facts it is evident that actinians use chlorophyll as food. As has already (p. 348)

been stated MacMunn (1885 *c*) demonstrated in many actinians the presence of a haematin-yielding pigment, which was designated as actiniohaematin. Recent investigations have shown that haemoglobin and chlorophyll are similar chemically, each having as a base a substance known as a porphyrin. This derivative is composed of four pyrrol groups in complex linkage. The exact similarity which exists between haemoglobin and chlorophyll may be shown by the following :¹



Thus haemoglobin, by the loss of a proteid (globin) and its iron, forms a porphyrin; in the same way chlorophyll *a*, by the loss of phytol and its magnesium, forms several porphyrins, one of which (phylo) is spectroscopically and chemically very closely related to haematoporphyrin; both phylloporphyrin and haematoporphyrin by the action of soda-lime give the same substance, aetioporphyrin. From this it seems evident that there is no *a priori* reason for assuming that the tissues of actinians could not convert chlorophyll into such a substance as actiniohaematin, which is closely related to haemoglobin. The obvious objection is that exchange of metals (iron for magnesium) would make such a transformation impossible. But one should recall that it is a far more simple

¹ The best discussion in English of the chemistry of haematoporphyrin and chlorophyll is that of Plimmer (1915); Bayliss (1918) is good, but the standard work is that of Willstätter and Stoll (1913).

process to drop off an atom of magnesium and add one of iron than it is to build up an enormously complex molecule such as the porphyrins present.

If such a view is capable of experimental proof it will have an important bearing upon the phylogenetic origin of animal pigments; it will give fair indication that many animal pigments were derived originally from plant chlorophyll as the result of some symbiotic association (perhaps for the purpose of facilitating respiration) of an animal with a chlorophyll-bearing organism—a condition probably similar to that found to-day among the sponges and certain of the Protozoa. On the basis of this theory it is interesting to speculate concerning the origin of haemoglobin in the higher animals. Is it not possible, for instance, that our blood-pigment is derived from the chlorophyllous substances which are taken in as food, a condition not unlike that which the writer believes to exist in the coelenterates? The recent feeding experiments of Bürgi and his co-workers (1919) indicate that such is the case, for they give strong indication that the animal body is dependent upon chlorophyll for the building of haemoglobin; of three sets of anaemic rabbits, one was fed alone upon a chlorophyll diet, the other upon iron pills, and the last group upon a mixture of iron pills and greenstuffs. The anaemic condition of the first two groups was very slow to improve, whereas the animals in the last group within a short time lost all symptoms of anaemia and the haemoglobin content of their blood came back to normal. This means that chlorophyll with its four pyrrol groups is quite as necessary for the manufacture of haemoglobin as elemental iron. This conclusion is further substantiated by Grigoriew (1919), who has repeated Bürgi's feeding experiments with positive results. If this be true, methods can very well be devised to control the formation of haemoglobin in disease.

What, then, must be the conclusion as to the origin of pigment in the coelenterates? In the first place the chylaqueous fluid, which in function at least is the analogue of the blood of higher animals, carries to the tissue the components

from which it elaborates its pigment; the components, in addition, are probably highly-organized substances. The gastrovascular fluid derives these pigment-making substances from the chlorophylls which enter as food. The pigment, therefore, in addition to bearing a close relationship to haemoglobin, is probably itself derived from chlorophyll. This applies to MacMunn's actiniohaematin; and it will also be recalled that Elmhirst and Sharpe (1920) have shown that the non-haematin pigment of *A. equina* releases oxygen as a result of photosynthesis, which likewise suggests an intimate relation to chlorophyll.

4. PLATYHELMINTHES.

Huxley (1877, p. 57), writing of the digestive cavity in the Coelenterata, remarked that the 'fluid' which it contains represents blood'. Concerning the next higher group he states: 'In the Turbellaria, Trematoda, and Cestoidea, the lacunae of the mesoderm and the interstitial fluid of its tissues are the only representatives of a blood-vascular system.' The observation of Huxley is interesting, but it must be recalled that the mesodermal lacunae represent merely the morphological homologue of the blood-system;¹ the functional precursor of the vascular system, as in the coelenterates, is to be found in the gastrovascular cavity.

Pigmentation is common among the flat worms; many of the marine polyclads, in particular, are distinguished by a brilliant coloration. The only investigations concerning the pigment of the animals belonging to this class with which the writer is acquainted are those of Gamble and Keeble, and Crozier. The latter author (Crozier, 1917) has shown that the polyclads

¹ Though the supply of pigment-forming substances is undoubtedly given by the gastrovascular system, it is interesting to note that among the Rhadocoele Turbellaria the parenchyma (in which the lacunae are formed) is the seat of the body-pigment (Parker and Haswell, vol. i. p. 265). This seems to be the first instance in which the function of providing pigment has been taken over by the morphological fundament of the future blood-system.

commensal with the orange colonies of *Ecteinascidia turbinata* and the purple colonies of *Rhodozonia picta* are themselves orange and purple, respectively, and of a shade very similar to that of the animal with which they are commensal. On starvation (i.e. when removed from the colony of tunicates) these polyclads lose their colour, but when allowed to feed again with the tunicate colonies they regain their colour in a very short time. This, Crozier believes, is an example of a pigment which is formed directly from food, and it accounts for the colour being the same as that of the animal with which the polyclad is commensal. The writer has made certain other observations which in part support Crozier's conclusion. The colouring matter in the tunic of *Ecteinascidia turbinata* is made up of stellate orange chromatophores. Now, if the body-cavity in one of the polyclads recently taken from a colony of *Ecteinascidia* be observed under the microscope, not infrequently small pieces of orange pigment can be observed, many of which show clearly that they are portions of the chromatophores from the tunic. Owing to the great frailty of the polyclads, the fate of these small pieces of pigment could not be followed completely; as a result it was impossible to settle definitely whether the pigment was ingested bodily by the entodermal lining as Congo red is ingested by the young of *Convoluta roscoffensis* (Gamble and Keeble, 1903), or first went into solution. If the former assumption were true, it might be possible to raise a race of colourless individuals of this species (as Poulton, 1893, has done for certain of his insect larvae) by preventing their association with *Ecteinascidia*. The writer, however, is inclined toward the belief that the fragmentary pieces of chromatophore go into solution in the water-vascular system and are subsequently taken up by the cells which need them. The latter explanation, if correct, would accord with the fact that the pigment does not extend promiscuously over the body, but is found in definite and regular designs.

Among some Turbellarians (*Convoluta* and *Vortex*) the green or yellow colour is occasioned by the presence of symbiotic algae.

5. ECHINODERMATA.

It is generally accepted that the first phylogenetic appearance of a vascular system is to be found among the echinoderms. The animals of this phylum are provided with distinct organs of circulation consisting of two radiating canal systems (haemal and perihæmal), the most important of which arises from a ring surrounding the oral end of the digestive tube.

Although these vessels always contain a corpusculate fluid, it is certain that they are incapable of either peristalsis or of any other contractile manifestation. Also the sinus which accompanies the madreporic canal, while usually looked upon as a rudimentary heart, certainly performs no pumping function. Consequently serious doubt has arisen as to the correctness of looking upon the haemal and perihæmal systems of the echinoderms as true blood-vascular systems.

The corpusculate fluid of the haemal and perihæmal vessels is likewise found throughout the entire peritoneal cavity. The corpuscles are nucleated cells, which exhibit amoeboid movements; and the fluid so obviously represents the blood of higher animals, that I know not why the preposterous name of "chylaqueous fluid" should have been invented for that which is in no sense "chyle", though, like the other fluids of the living body, it contains a good deal of water' (Huxley, p. 480).

(a) The Sea-urchin *Tripneustes esculentus*
Leske.

The red pigment cells are the most noticeable constituent of the body-fluid of this animal. With them are found non-pigmented cells, vibratile cells which are supposed to facilitate circulation, and, less frequently, yellow cells which are not unlike *Zooxanthellae*.¹ The red cells are closely packed with small granules; when protruding its pseudopodia, the cell first sends forth a thin, transparent lamella of hyalin ectoplasm.

¹ The most recent work on the body-fluid cells of sea-urchins is that of Kollmann (1908). He recognizes five types of cell.

and into this the round red granules subsequently flow. The conformation of the pseudopodia resembles closely that described by Goodrich (1919) for the coelomic corpuscles of *Asterias glacialis*. Thus (as first shown by Geddes, 1880) the cell is truly amoeboid, being able both to protrude and to withdraw its pseudopodia.

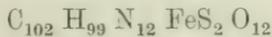
It seemed a curious fact that the cells within the body-fluid of the sea-urchin should be of identically the same colour as the pigment granules which give to the animal its characteristic coloration. Consequently an effort was made to determine whether or not the two pigments are identical. The same technique was employed as was made use of in settling the identity of the pigments from *A. bermudensis* and *C. passiflora* (p. 347), viz. that of testing their action in the presence of certain solvents, and the results seemed to indicate clearly that the two pigments are one and the same. In examining the external pigmentation, small pieces of the tube-feet were employed, since the behaviour of their coloured granules can be watched much more closely than can those of the spines. For testing the perivisceral fluid, fresh smears were used to which the reagents were added with a capillary pipette while under observation. Of the alcohols, amyl was the only one which dissolved the pigments, they being very readily soluble, however, in this reagent. Neither of the pigments were extracted by the lipochrome solvents, ether, chloroform, petroleum ether, or xylol. The colouring-matters are readily dissolved by 10/N solutions of the acids, dissolving with particularly great rapidity in valeric acid.¹ In the presence of alkalis both pigments were darkened, but not extracted.

After these experiments had been made, it came to the author's attention that Geddes, in a personal communication to Gamgee (1880, p. 134), stated his belief that the pigment of the red amoeboid cells was identical (in *Echinus*) with that of the epidermal spines. This view resulted from a very thorough study of the body-fluid of sea-urchins (Geddes, 1880). Con-

¹ This again corroborates Crozier's (1916 a) conclusion that valeric is the most penetrating of all acids.

cerning the origin of these pigment cells Geddes and Prouho (1887) agree that the yellow cells¹ which are found in the body-fluid give rise to the red cells; they base their conclusion upon the fact that at times many intermediate stages between yellow and red cells are to be observed. In sea-urchins which had been weakened on the preceding day by the loss of perivisceral fluid, the writer also noticed intermediate stages between these two corpuscles.² The observations regarding the origin of the red cells are of importance, since they give direct evidence that the body-pigment arises in the blood-system.

Very little is known regarding the nature of the yellow cells from which the red ones arise. It is quite possible that they are chlorophyllous cells, as Geddes has suggested. But if this is the case, we have before us a phenomenon of great importance, since it would afford direct proof that a chlorophyllous substance gives rise to a haematin pigment. MacMunn (1883 *c*) has found chlorophyll in the integument and certain tissues of many invertebrates, including the echinoderms. In addition he (1883 *a*) has described the red pigment of echinoderms as being a definite chemical substance, which he has named 'echinochrome'. This pigment was believed both by him and by Griffiths to be respiratory in nature, but this has since been denied by Saint-Hilaire (1896), and more recently by McClendon (1912). Echinochrome has been analysed chemically by Griffiths (1897) with the following result:



On boiling, the pigment is converted into haematoporphyrin (p. 351) and haemochromogen, which shows that echinochrome is related to haemoglobin; the relationship, moreover, is probably close, since it has in the molecule both Fe and S, and also because it breaks down into haemochromogen, which

¹ Found most abundantly in *Dorocidaris papillate*, *Arbacia*, and particularly in *Spatangoidea*.

² In the study of the pigmented cells of *A. atra* (Fulton, 1921 *b*) a similar phenomenon was observed; in animals which previously had been weakened by the loss of blood many intermediate stages between the colourless cells and the pigmented corpuscles were observed.

is a reduction product of haemoglobin. It is interesting also to note that MacMunn (1883 *b*) has shown the presence of free haematoporphyrin in the tissues of echinoderms (especially in *Uraster rubens*) which, as he suggests, is probably an intermediate product in the formation of echinochrome.

From the preceding facts it is evident that in most echinoderms there are present simultaneously chlorophyll, haematoporphyrin, and echinochrome, the last being closely related to haemoglobin. If the diagram on p. 351 be consulted it will be observed that this represents almost the complete circle from chlorophyll to haemoglobin: thus we have chlorophyll, a porphyrin, and an iron-containing pigment which breaks down into a reduction product of haemoglobin. This evidence greatly emphasizes the hypothesis (p. 352) that many of the animal pigments, including haemoglobin, are derived originally from chlorophyll.

(*b*) Other Echinodermata.

The body-fluids of numerous other echinoderms possess coloured amoeboid cells whose pigment in many cases appears to be identical with that of the epidermis. The fact that the colour of these cells is similar to that of the body is in itself significant; in addition, however, there is frequently chemical evidence which tends also to establish their identity. To avoid tiresome repetition only a few of the more important examples from several of the classes of echinoderms will be given.

Asteroidea.—The amoebocytes from the coelomic fluid of starfish possess granules which vary in colour from yellow to a deep brown (Cuénot, 1901). It is noteworthy, moreover, that the cells with the darker granules are found in the animals with a deeper body-colour. This is particularly noticeable in some of the Bermuda starfish.

Ophiuroidea.—The only species examined was *Ophiocoma pumila*. In this form the body-fluid was marked by the presence of many yellowish-brown pigment cells which were closely allied in colour to that of the disc.

Echinoidea.—So far as the writer is aware, in every

species of echinoid whose blood-cells have been reported upon, the existence of a red, or red-brown pigment cell (such as was described for the Bermuda sea-urchin) has been noted: and a careful study of the epidermal pigment would undoubtedly reveal, as it did in *T. esculentus*, that it is identical with that of the coloured amoebocytes.

Holothuroidea.—The paper of MacMunn (1889) on animal chromatology contains many valuable observations concerning the pigments of *Holothuria nigra*. He has shown the presence of a red lipochrome in the 'blood' of this form and has found an identical substance in the integument. He identified the same pigment in the digestive gland and concluded that it 'is built up in the digestive gland and carried in the blood current to be deposited in other parts of the body, though what its rôle may be when deposited there it is difficult to say'. The fact that this pigment is found in the digestive gland is of particular importance, and should be borne in mind when the pigmentation of the crustaceans is considered. In passing, it seems worth while to note that this red lipochrome described by MacMunn is the one which Harvey (1915) found in the testis of *Stichopus ananas*, on which he has performed an interesting series of permeability experiments, using the lipochrome within the cells as an indicator.¹ The writer has observed amoebocytes with brown pigment granules in the body-fluid of *Holothuria surinamensis*—pigment granules which are similar to those of the epidermis. In addition Hérouard (1889) and Cuénot (1891) have reported brilliant brown and yellow amoebocytes for many holothurians common along the French coast. One holothurian (*Cucumaria planci*, Marenz.) is distinguished by the presence of haemoglobin in the cells of the perivisceral cavity. Kollmann (1908, p. 188) endeavoured to find the origin of these cells, but was unsuccessful.²

¹ The substance turns blue in acid (MacMunn, 1889; Harvey, 1915).

² Haemoglobin is found also in the ophiuroid, *Ophiaetis virens* (Foettinger, 1880); derivatives of haemoglobin (actiniohaematin), moreover, are found in many actinians.

PART II. THE PIGMENTS OF ANIMALS WHICH HAVE A BLOOD-VASCULAR SYSTEM.

1. INTRODUCTION.

In the first half of this study consideration was given to those invertebrates which possess no blood-vascular system, and the general conclusion was reached that the body-pigments, in the absence of such a system, are deposited in the organism by its nutritive fluids. It was concluded also that the materials used in the production of pigment are derived from certain substances—usually chlorophylloid—which are taken in as food. It seemed, therefore, that in the lower invertebrates the body-fluids (which serve the nutritional function of the blood of higher animals) serve also to supply the organism with the substances from which it elaborates its pigment. In echinoderms, for example, the pigment is formed in the perivisceral fluid, and is subsequently carried to the epidermal regions. As a result of such observations one might reasonably expect the blood of the higher animals also to furnish the supply of materials for the body-pigments. The writer, in consequence, has examined the evidence to determine whether such a deduction is justifiable; the results of the investigation are presented in the present half of the paper.

2. NEMERTINA.

Though the nemertean worms possess certain annelid affinities (nephridia, &c.), the balance of morphological evidence places them close to the Turbellaria. In one very important characteristic, however, they are distinct from all of the Platyhelminths, and that is in the possession of a well-developed blood-vascular system which is entirely closed. The vessels originate from a fusion of the spaces which arise in the mesoblast. The circulatory fluid contains flat, nucleated corpuscles, and it is propelled through the body, probably by bodily contractions, though there is some evidence of vascular peristalsis. Few investigations have been made upon the

pigmentation of nemerteans; one form, *Polia sanguirubra*, however, is distinguished by the presence of haemoglobin in the blood-cells (Hubrecht, 1874). Previously Lankester (1872) had found haemoglobin dissolved in the plasma of this form. In *Cerebratulus urticans* (Hubrecht and Shipley, 1911) haemoglobin is found in the wandering body-fluid cells. It is reasonably certain, therefore, that the red colour of certain nemerteans is due to the presence of haemoglobin in the blood and in the body-fluids. The origin of the haemoglobin is not known, but it is interesting to note that the food of these worms is largely chlorophyllous.

3. MOLLUSCA.

Among the molluscs the most highly-coloured groups are the opisthobranchs and the cephalopods, and to these brief consideration will be given.

(a) The Opisthobranchs.

Crozier (1914, 1916 *a*, and 1916 *d*) found that the coloured substance in the mantle of the nudibranch *Chromodoris zebra* is very sensitive to the presence of acids and alkalis, being blue in its natural state (alkaline) and pink in acid. He (1916 *b*) observed further that the blood-cells contain a pigment which is similar in colour to the mantle pigment and likewise turns pink in the presence of acid. An examination of the absorption spectra of the two coloured substances showed that they are identical. Thus the pigment of the blood is the same as the pigment which is responsible for the external coloration.¹ Inasmuch as it has been shown that the pigment cells in the blood of other forms—echinoderms (Part I of this paper, p. 357) and Tunicates (Fulton, 1921 *b*)—arise while in circulation, it is not unreasonable to assume, until further evidence is afforded, that the blood-pigment of *C. zebra* is also synthesized by the moving blood.

¹ The writer attempted to secure specimens of *C. zebra* when in Bermuda in order to find the origin of the pigment cells of the blood, but owing to the scarcity of this species during the summer months none were obtained.

Little is known concerning the chemical nature of the pigments of opisthobranch molluscs ; however, certain observations of Crozier and others are of interest. Crozier reported the presence of manganese in the pigment of *C. zebra*, though he did not believe that the manganese facilitated respiration. Paladino (1908) detected both magnesium and iron in the pigment of *Aplysia punctata*.¹ It appears to the writer that Paladino's observations are of particular importance in their relation to the origin of animal pigments. In the first part of this paper, evidence has been given to show that the pigment haemoglobin is derived from chlorophyll. The chief objection to such a contention is the difficulty of exchanging the magnesium of chlorophyll for the iron of haemoglobin. It would seem from Paladino's work that the coloured substance of *A. punctata* is a pigment which is intermediate between chlorophyll and haemoglobin since it possesses both magnesium and iron. It probably is not entirely justifiable to draw any conclusion until more is known concerning the chemical nature of the molecule to which the iron and magnesium are attached. However, the pigment showed absorption bands which are similar in some respects to those of haemoglobin ; moreover, nitrogen was detected in the molecule. Both of these facts bear evidence that the pigment of *A. punctata* possesses the same chemical base as haemoglobin.

The blood of a closely-allied form, *Aplysia depilans*, has a distinct rose colour, due to the presence of an albuminoid (Cuénot, 1890) which is precipitated by alcohol and acids. The coloration of the body is due in part to the presence of this substance in the epidermal regions.

(b) The Cephalopods.

Among the earlier observations on the blood of cephalopods were those of Rouget (1859), who described coloured corpuscles

¹ MacMunn (1899 *b*) has made a spectroscopic investigation of the pigments of *Aplysia punctata*. He also found (as did Crozier and Paladino) that the pigment is very sensitive to a change from alkalinity to acidity.

in an octopus, and of Bert (1867), who gave an interesting description of the epidermal circulation. Rabuteau and Papillon (1873) obtained a pigment from *Octopus vulgaris* which failed to give absorption bands. The classic researches on this form, however, are those of Fredericq (1878), who was the first to demonstrate the presence of a respiratory pigment (on haemocyanin, see below) in the blood. In addition to the haemocyanin he found in the blood a red lipochrome. Though there is no direct proof (as there is in the case of the red lipochrome found in the blood of crustaceans) that the substance is identical with the red pigment in the chromatophores, it is highly probable that such is the case. The colour itself strongly suggests the similarity, while the fact that similar pigments are found in the blood of other animals as well as in their chromatophores, gives added evidence that the two pigments are identical. The best description of the chromatophores in *Octopus* is that of Cowdry (1911).

The inky fluid of the cuttle-fish *Sepia officinalis* is a pigment which is chemically identical with certain melanins of the higher animals (Piettre, 1911 *b*). Though melanin-like substances have been reported in gorgonian stems and in certain mollusc shells, their identity with vertebrate melanins has never been proved. Consequently it is a very curious fact that the invertebrate which possesses the most highly organized nervous system should also possess melanin, which in the higher animals is so intimately connected with the nervous system. The question of the origin of melanin and of its chemical constitution will be considered in a later section.

(c) Other Mollusca.

Many detailed investigations have been made on the respiratory pigments of the mollusca, but since these pigments seldom function in the production of coloration, they will not be considered in detail.

Haemocyanin.—The substance responsible for the blue colour of the blood of certain mollusca was first isolated and described by Fredericq (1878). Haemocyanin has since been

found in representatives of every class of mollusc save the Amphineura,¹ and it is also common among the crustaceans and the arachnids. Recently the chemical and physiological properties of haemocyanin have been extensively investigated by Alsberg and Clark (1914), who find that the pigment lacks many of the remarkable properties of haemoglobin; particularly to be noted is its small binding power for oxygen.

Pinnaglobin.—The blood of the lamellibranch *Pinna squamosa* is distinguished by a respiratory pigment which contains manganese. This metal is linked to a protein which contains the usual elements of blood pigments, viz. C, H, N, S and O₂, and these in proportions similar to the corresponding elements of haemoglobin (Griffiths, 1897).

Haemoglobin.—The colour of the 'blood-clam' (*Arca*) is due to the presence of haemoglobin in the blood; the pigment is found also in other Lamellibranchs: *Solen legumen*, and *Cardita*, and in the gastropod *Planorbis* (Lankester, 1872; Leitch, 1916).

Chlorophyll has been detected in many of the Mollusca. The colour of the green oyster is due to an algal infection (Lankester, 1886). Enterochlorophyll has been described by MacMunn (1883 *c*, 1885 *b*) as existing in the liver of many crustaceans and molluscs. For further discussion of enterochlorophyll, see below, p. 369.

4. THE ANNELIDA.

One of the most interesting characteristics of the coloration in annelids is the fact that the coloured substances found in this phylum are usually respiratory pigments; few of the coloured substances serve alone for ornament. The segmented worms have two circulatory fluids: the perivisceral (coelomic)

¹ The chitons possess a non-metallic respiratory pigment, β -achroglobin, which was described by Griffiths (1897). In addition to this pigment a yellow lipochrome is present in the blood, which is more common in the female of the species than in the male, and is thus responsible for the deep yellow colour which the female chiton assumes during periods of reproduction.

and the pseudohaemal, or blood-vascular fluid. The fluid of the coelom contains corpuscles, and carries food substances derived from the intestine. The blood, on the other hand, functions in respiration and probably has little to do with the transportation of food. The respiratory pigments, therefore, are found in greatest abundance in the blood-vascular system, though they are sometimes met with in coelomic fluid.

There are four varieties of pigment found in annelid worms : haemoglobin, chlorocruorin, chlorophyll, and the lipochromes.

Haemoglobin.—In the more primitive annelids, haemoglobin is found dissolved in the blood plasma, the corpuscles themselves being colourless and serving as phagocytes (most chaetopods and Hirudineae ; Lankester, 1872). In the Capitellidae and in Glyceridae the haemoglobin is packed in individual cells.¹ Much more extraordinary, however, is the occurrence of haemoglobin in the nervous chain of *Aphrodite aculeata*, and as Lankester (1872, p. 79) remarks it is difficult to account for its appearance there as ‘ we have no knowledge that this Annelid is remarkable for nervous energy ’.

Annelid haemoglobin (particularly that of *Lumbricus terrestris*) is almost identical with the respiratory pigment of vertebrates. Griffiths (1892, p. 147) made a detailed chemical analysis of the haemoglobin of *Lumbricus* and found that the only considerable discrepancy between it and the haemoglobin of a dog lay in the amount of iron present, there being slightly less iron in the pigment of the annelid.

Concerning the differences found between the various haemoglobins, a statement which Griffiths (1897, p. 101) makes in another work might very aptly be quoted. ‘ If species have been modified in the course of long eras, the haemoglobins—though fulfilling all the conditions of a true chemical compound—must have become modified step by step with the species. . . . Hence we see that the conception of evolution must necessarily find a place in chemistry, not merely as

¹ In many text-books—Parker and Haswell, Hegner, &c.—it is stated that the haemoglobin of annelid blood is found dissolved in the plasma. Though true for certain species it is quite incorrect as a general statement.

regards the elements, but as regards the formation of highly complicated compounds.' Though the earlier writers considered that there were no spectroscopic variations between the haemoglobins of various animals, more recent work has shown that there are minute differences even between the spectral bands of ethiopian haemoglobin and that of the white races. Recently Vlès (1919) made a careful investigation of the haemoglobin of *Arenicola piscatorum*, Lk. and of *Marphysa sanguinea*, Quatref., and has found certain pronounced differences between their spectra and that of vertebrates.

With such facts as these one cannot avoid agreement with Griffith's contention that haemoglobin is the product of a long and complicated phylogenetic development. Sorby (1876), in a paper on the evolution of haemoglobin, held that the substance had its beginning as a bile pigment (non-respiratory) such as exists in *Helix aspersa*. The next step was found in the haemoglobin-like pigment of *Planorbis*, which is respiratory. The final stage is seen in the concentration of haemoglobin into individual cells, a condition such as one finds first in the blood corpuscles of *Gephyrea*, and later in the vertebrate erythrocytes.

Reichert and Brown (1909), in their monumental work on the crystallography of the haemoglobins, have urged, since there is a characteristic crystal form for the haemoglobin of every species, that the haemoglobin of each species is chemically different from that of every other, and that a specific crystal form is an expression of a specific chemical molecule. But, 'it is well known that crystal-habit is modified by the alterations of the medium from which the crystals are deposited', and since it is a matter of common knowledge, particularly in the light of modern bacteriology, that the blood plasma of animals differs profoundly between individuals (even of the same species), and since the haemoglobin crystals of each species studied by Reichert and Brown were deposited from a different medium, 'it is not improbable that the observed differences in the crystals are attributable to these known differences in the media in which they were formed' (Robert-

son, 1920). This, however, does not in any way destroy the value of their work, which is a most fundamental contribution to our knowledge of the crystal structure of proteins.

Chlorocruorin.—This pigment, though green in colour, is closely allied to haemoglobin. The substance was noted by Milne-Edwards (1838), but was first isolated by Lankester (1869) from *Sabella ventrilabrum* and *Siphonostoma*. MacMunn (1883 *a*) subsequently identified it in *Serpula contortuplicata*.¹ Though the absorption bands of chlorocruorin present a striking similarity to that of haemoglobin, the green pigment cannot be broken down into any of the decomposition products of the red pigment. Chlorocruorin, however, is definitely respiratory and is capable of existing in two states of oxidation, oxychlorocruorin and reduced chlorocruorin. The pigment is found both in the blood and in the epidermis, and the animals possessing chlorocruorin owe their colour almost entirely to its presence.

Chlorophyll.—The only authentic example of a chlorophyllous pigment existing in annelids is that of *Bonellia viridis*. Sorby (1875 *b*) showed that this pigment gave absorption bands which were practically identical with chlorophyll, but Geddes (1882) failed to find any evolution of oxygen in the tissues of *Bonellia* when it was exposed to strong sunlight. Krukenberg likewise obtained negative results (Geddes). However this may be, it is most probable that bonellein is a derivative of chlorophyll, particularly in view of the recent investigations of Hans Przibram (1913), which have demonstrated striking spectroscopic similarities between the two pigments.

Lipochromes.—The blood of *Arenicola piscatorum* has, in addition to haemoglobin, certain lipochromes, some of which give absorption bands (MacMunn, 1883 *a*, 1889). MacMunn, in addition, found that a lipochrome present in the digestive tract was found also in the epidermis. Fauvel (1899)

¹ It is a curious fact that the pigment of *Serpula*, though spectroscopically identical with that of *Sabella*, is red in colour. Griffiths (1897) believes that the two pigments are isomeric.

has likewise reported the presence of a yellow lipochrome in the epidermis of *Arenicola*. Inasmuch as Racovitza (1895) has shown that the amoebocytes of many polychaet annelids (including *Arenicola*) carry fatty pigments from the blood and deposit them in the epidermis, the unavoidable conclusion remains that the fatty pigments of *Arenicola* are derived from food, and transported to the epidermis by the blood-system. It is worthy of note also that many of the lipochromes are closely allied to carotin, which means that they are derived from chlorophyllous food substances. Further consideration of carotin will be given in the following section (p. 373).

5. ARTHROPODA.

In the phylum Arthropoda, not only is the blood-system well developed, but there are present, as in the cephalopods, large chromatophores which facilitate colour change. A vast amount of work has been done upon the pigments of the arthropods, and since it has been reviewed very completely by Fuchs (1914, crustaceans), and Biedermann (1914, insects), the writer will limit himself to a discussion of the origin of the pigments in this phylum.

(a) Crustacea.

The presence of chromatophores in the carapace of crustaceans was first noted by Focillon (1851). The classic work on crustacean pigment, however, is that of Gamble and Keeble (1900) on the colour phases of *Hippolyte varians*, in which the structure and activities of the chromatophores are described in great detail. The structure of the chromatophores from other species of Crustacea has been investigated more recently by Franz (1910). The pigments which have been found in the blood of crustaceans are very nearly the same as those found in the molluses, viz. chlorophyll (enterochlorophyll), lipochromes, carotin, haemoglobin,¹ and haemocyanin. As the

¹ Haemoglobin has been detected in several crustaceans by Regnard et Blanchard (1883).

copper-containing pigment of the crustaceans is identical with that of the molluses, its further consideration is unnecessary.

Enterochlorophyll.—MacMunn (1883 *c*) discovered that a pigment which was spectroscopically identical with plant chlorophyll occurred in the liver of many molluses and crustaceans. He found that this pigment—which he named enterochlorophyll—occurs dissolved in oil globules or in granular form, but sometimes even in the protoplasm of the secretory cells of the liver. For a long time MacMunn believed that the occurrence of enterochlorophyll in the livers of invertebrates demonstrated that animals are capable of synthesizing chlorophyll. Finally, however, he was forced to abandon this position when he (MacMunn, 1899 *a*) found that enterochlorophyll is present in the intestine and blood of the forms in whose livers it also occurs. He was thus forced to admit that enterochlorophyll enters the animal as food and is stored in the liver. In his own words (p. 438): ‘I have been forced, I must confess against my inclination, to believe that enterochlorophyll is a pigment which primarily has been taken up from the intestine dissolved in a fatty medium, and is carried either by leucocytes, or in some other way to be deposited with this fat, and perhaps other reserve products, in the gastric gland. Whether it is utilized for the production of other pigments or not is a question for future investigation. That it is a chlorophyll derivative I now believe to be proved.’

The same conclusion was reached by Dastre (1899, p. 120), who states: ‘La chlorophylle hépatique n’est pas un produit animal fabriqué par le foie: c’est une chlorophylle végétale, venant des aliments, fixée seulement et conservée d’une façon remarquable dans le tissu hépatique.’ Further investigations were made by Dastre and Floresco (1898, 1899 *a*, and 1899 *b*) whose conclusions were the same. What is the significance of the occurrence of chlorophyll in the livers of crustaceans and molluses?

The most obvious fact is that it is a food substance stored there for future use. But of even greater importance is the fact that chlorophyll is shown by these observations to be

capable of absorption into the animal body without being materially changed in the process. Hence if animals employ chlorophyll in building up their pigments, they have it on hand unchanged by the processes of digestion. This conclusion is strongly reinforced by more recent work. Dhéré et Vegezzi (1916) have shown that all of the chlorophylloid pigments pass without change into the liver of *Helix pomatia*. But the more important work is that of L. S. Palmer (1915, 1916), who has shown that certain plant pigments are absorbed from the intestine into the blood of a cow and from there pass unchanged into the milk. This investigation will be discussed in greater detail under carotin (p. 373). One is reminded at this point of the observations of Moglia (1910), that the depth of coloration in certain gastropods decreases in the winter, due as he believes to a lack of food, and also of M. E. Johnson's (1913) contention that *Rana* depends for its colour, not upon the amount of nutrition but upon being nourished by food substances which are properly pigmented.

MacMunn (1883 *c*) has shown that in certain molluscs—as *Patella*—a haematin is present in the liver in addition to enterochlorophyll, and there is every probability he adds, that the haematin finds its origin in that tissue. Though the haematin might have arisen independently of the chlorophyll in the liver, the more logical assumption (in view of the chemical similarities between chlorophyll and haematin, alluded to in Part I of this study) is that it is derived from the chlorophyll. As with the origin of echinochrome, the origin of molluscan haematin is highly uncertain, but the hypothesis just presented is interesting and certainly merits further investigation.

From the observations of Paladino (1910) one is led to believe that haematin is present not only in molluscs but likewise in the livers of certain crustaceans. Apparently unacquainted with MacMunn's work Paladino has reported the presence of a water-soluble ferruginous pigment in the livers of several crayfish. In addition, he finds a yellow lipochrome (probably carotin) which contains no iron. The iron-containing pigment which he finds is undoubtedly either haematin or a derivative.

Lipochromes.—Halliburton (1885), in an investigation of the blood of decapod Crustacea, found a red lipochrome present in addition to haemocyanin, which had been described previously. He extracted the red substance by first precipitating the proteins of the blood with alcohol and then extracting the red pigment with ether. He noted also that the amount of the red pigment varied in different specimens, but was unable to find an explanation for the fluctuation. In the same year MacMunn (1885 *a*) investigated Halliburton's red lipochrome and concluded that it was identical with the pigment of the chromatophores in the exoskeleton. Several years later Miss Newbigin (1897) reinvestigated the question. She corroborated MacMunn's results, but in addition found evidence that the red lipochrome was derived from the yellow pigment (probably the carotin contained in the enterochlorophyll; see below, p. 373) of the liver. Consequently, if Miss Newbigin's results are reliable, one would trace the origin and development of the red pigment in the chromatophores of crustaceans as follows: chlorophyllous substances enter the body as food; they are absorbed by the intestine and stored by the liver, in which tissue they probably undergo slight modification. The carotin contained in the chlorophyll of the liver, when called upon, is transformed into a red pigment substance—a lipochrome—and transported by the blood to the epidermis, where it is used in building up the chromatophore. The recent analyses of Verne (1920) have shown that the red pigment of the decapod Crustacea is a hydrocarbon identical chemically and spectroscopically with vegetable carotin, and possess the same empirical formula ($C_{40}H_{56}$). This investigation removes any doubt which may have existed regarding the identity of the two pigments.

The fact that the red lipochrome present in the blood is subject to great fluctuation (Halliburton), is possibly to be explained by the moulting period of the animal. Immediately after the moult there is great demand for pigment to build up new chromatophores, which results in a diminution of the amount present in the blood. In this connexion the recent

observation of Paul and Sharpe (1919) is of interest. They have shown that immediately before the moult the contents of the liver greatly increase in quantity, and after the moult the amount returns to normal. This would certainly affect the amount of pigment free in the blood. G. W. Smith (1913) has called attention to the fact that the amount of red pigment (which he called 'tetronerythrin') varies in the female (crab) during periods of reproduction, being most abundant shortly before the eggs are produced.

Before proceeding to the consideration of carotin a word should be said concerning the red pigment, tetronerythrin. This substance was first described by Wurm (1871), who reported that it existed in several animals. Mérejkowski (1881) reinvestigated the pigment, and came to the conclusion that it was widely distributed throughout the animal kingdom, since he found it in every phylum. Mérejkowski's criteria for tetronerythrin (also known as zoerythrin) are as follows: the substance must be (1) insoluble in water; (2) soluble both in alcohol and ether; (3) blue in acid; (4) red in carbon disulphide; and (5) decolorized by light. Both Mérejkowski and Gautier held the pigment to be respiratory. In a later paper, Mérejkowski (Krukenberg) reported that he had demonstrated zoerythrin in 117 species. Krukenberg¹ found it also on the eye-lids of birds and in their feathers. MacMunn (1889), however, showed that a number of different pigment substances would respond to the tests used by Mérejkowski.

The substance which responds to the test for tetronerythrin in crustaceans is the red lipochrome described by Halliburton (Halliburton, 1885; MacMunn, 1889), and, as tetronerythrin, it has been extracted both from the epidermis and from the blood; this further substantiates the conclusion reached above, that the red lipochrome of the blood is identical with that of the chromatophores. In addition, MacMunn also held that tetronerythrin arises in the liver.

Carotin.—This substance is a yellow pigment found in

¹ Vergleich. physiol. Studien, Abth. 5, and (2. Reihe) Abth. 1, p. 151; Abth. 3, p. 128.

association with chlorophyll. It was first isolated from carrots (sometimes spelled 'carrotin'), from which it received its name.

Willstätter und Meig (1907) have shown that carotin is a crystalline unsaturated hydrocarbon ($C_{40}H_{56}$) melting at 174° . It is of wide occurrence not only in the vegetable, but also in the animal kingdom, being found in the blood sera of most birds and mammals (Schunck, 1903; Palmer, 1915, 1916; and Hymans van den Bergh und Miller, 1920), and in mammalian milk;¹ the yellow pigment of butter fat likewise is composed largely of carotin (Steenboch, Sell, and Buell, 1921). It is also found in mammalian ovaries; however, the 'lutein' of the corporea lutea themselves is isomeric with xanthophyll, the oxide of carotin. The colored substance of egg-yolk is made up largely of xanthophyll, though it too contains some carotin (Palmer and Kempster, 1919 *a*, *b*, and *c*); carotin also is sometimes present in human urine, appearing there after carotin has been ingested (Hess and Meyers, 1919), and it has been isolated from gall-stones (Plimmer, 1915, p. 532). But of much greater interest is the fact that carotin is present abundantly in mammalian nerve-cells (Dolley and Guthrie, 1919 *a* and *b*), and in the fovea centralis of the human eye. Also the yellow pigment cells (xantholeucophores) in the epidermis of *Fundulus* contain carotin, but what concerns us more immediately is its occurrence in the chromatophores of crustaceans.

Blanchard (1890) made it evident that the epidermal pigment of the copepod *Diaptomus lacillifer* contains a large percentage of carotin. Keeble and Gamble (1902, 1904, and 1905) have corroborated this result, finding that the chromatophores of many other crustaceans also possess the pigment. They noted, too, that mobile fat globules are usually to be found in the branching pigment cells. It has been shown by Kohl (1902) that carotin was capable of photosynthesizing fats. With this fact before them, Keeble and Gamble made

¹ Palmer and Eckles (1914) have shown that the amount of carotin and xanthophyll in milk (human and cow) is in exact proportion to the amount of green food consumed.

an effort to find the origin of the fat globules in the crustacean chromatophores. The investigation proved that the carotin present in these cells produces the fat globules by photosynthesis. The work was carried out on *Hippolyte varians*, and the experiments were briefly as follows:¹ when *Hippolyte* is starved in the dark practically all of the oil disappears from the chromatophores; when starved in sunlight, however, the globules continue to exist as before; when a 'dark-starved' *Hippolyte* is exposed to sunlight (without feeding) the fat returns. Thus the crustaceans present a remarkable phenomenon: the plant pigment which has been eaten by the animal, is stored in the liver; later it is carried by the blood-system and deposited in the epidermal chromatophores, where it functions exactly as in the plants from which it was derived! The only other instance of such a phenomenon known to the writer is that (already referred to on p. 373, note) described by Palmer and Eckles (1913), who have shown that carotin and xanthophyll pass in the blood from the intestine to the mammary glands. It is extremely interesting, also, to note the recent observation of Findlay (1920), that carotin and xanthophyll are found in the mammalian adrenals, and are largely responsible for the colour of the glands.

(b) The Insecta.

In many ways the insects offer a more favourable condition for the study of animal pigments than any other group of animals. They are small, many are brilliantly coloured, and in addition the physiological processes of insects are on the whole less complicated than in other forms; moreover the more important pigments of insects are concentrated in the wings, which are thin and therefore well adapted for the purposes of observation.

The greater share of the work on the pigmentation of insects

¹ It is not within the scope of this paper to attempt to summarize Keeble and Gamble's results on the mechanism of colour change in this animal. They will be found in the papers cited above and in Gamble (1910); a more recent study of colour variation in crustaceans is that of Potts (1915).

has been carried on by the English investigator Poulton,¹ who found that the pigment of a large number of insects (in all stages of development) is a modified chlorophyll derived from the plant on which the animal feeds. The chlorophyllous substances are eaten, absorbed into the blood-system, and deposited in the regions of the body exhibiting pigmentation. The most valuable results in Poulton's work came from a spectroscopic examination of insect blood. His (1884) work on the pigment of *Sphinx ligustri* is interesting. The blood from the pupae of this form was examined and its spectrum recorded; then an extract was made of the *calceolaria* leaves upon which the larva feeds. When the spectrum of the extract was superimposed upon that of the blood, the bands were found to correspond in a very striking way. In Poulton's own words (p. 290):

'Considering the chemical change which must have taken place in the chlorophyll during digestion, rendering possible the passage of the walls of the digestive tract, and considering its chemical union with the proteid constituent of the blood, the resemblances of the spectra are very striking; in fact, the two spectra are far nearer each other than the ordinary spectrum of chlorophyll in alcoholic solution is to the unaltered chlorophyll of leaves'.

It was held by Poulton that the power of utilizing chlorophyll in building up pigments is an adaptation on the part of the insect which enables it to assume the colour of the leaves on which it feeds. As evidence for this he brought forward the fact that if larvae of *Trypoena pronuba* are fed respectively on green, brown, and white cabbage leaves, green, brown, and white larvae result according to the colour of the leaf on which they were fed. This result has since been corroborated by Levart et Conte (1902), who worked on *Attacus orizaba* and *Bombyx mori*.

Peterson (1913) has found that the chlorophyll which passes into the intestine of certain red caterpillars is modified into

¹ Poulton has written a large number of papers on this subject. The more important ones will be found in the bibliography under Poulton, 1884, 1889, 1892, and 1893.

a red substance (vanessa red), which is later absorbed and transported to the epithelium, where it is deposited and becomes the pigment of the wings and of the other body-parts. The unabsorbed portion of the red pigment is voided. The investigations of Gortner (1911 and 1912 *b*) on insect melanins are also of interest, since he has shown that they are sometimes formed from chlorophyllous substances (oxidizable chromogens) acted upon by the plant ferment, tyrosinase. More recently Schmidt (1919) has conducted similar investigations on insect melanins, and his results accord with those of Gortner.

It appears to the writer that the most significant part of all Poulton's work on insects is the demonstration that chlorophyll resists the digestive enzymes, and passes practically unchanged into the blood-system. Evidence has been cited to prove that this also is the case in the Mollusca and Crustacea, but the evidence is not conclusive (except in the case of carotin). In insects, however, there is undisputable proof, and it is of particular importance, since it shows that the theory that haemoglobin as a derivative of chlorophyll must not be ruled out by the fact that chlorophyll is incapable of passing through the digestive tract. In general, then, the epidermal pigments of Crustacea are derived from food and are carried to their destination by the blood-system.

6. THE TUNICATA.

The strongest evidence in support of the view that the epidermal pigments are deposited by the blood-system is to be derived from a study of the tunicates, where it is possible to predict that there will be found in the blood-stream pigment cells of a colour corresponding to that of the tunie. Moreover one can show that the pigmented corpuseles arise from colourless cells while they are in the circulation. The tunicate to be first considered is *Ascidia atra*.

(*a*) *Ascidia atra*.

This species is distinguished from other tunicates, and in fact from almost all other animals, by the possession of an

exceptionally large variety (ten distinct types) of blood-cells. In the vascular fluid of this animal there are three kinds of highly-pigmented cells : green, orange, and blue ; in addition there are four kinds of non-motile white corpuscles, and three other types which are distinctly amoeboid (Fulton, 1921 *b*). The writer has shown that all of the pigmented cells in *A. atra* arise directly in the blood-stream from unpigmented corpuscles. In the case of the green 'chromocyte' the metamorphosis from the colourless cell may be stimulated artificially and the complete process watched under the microscope. When an acid, preferably an organic acid of N/10 to N/20 strength, is added to a fresh smear of blood, all the non-motile colourless cells of one variety may be observed to take on a light shade of green, which gradually deepens ; at the same time the cell fragments into large green lumps and finally assumes the characteristic form of the green pigment cell. Therefore, it may be inferred that in nature the green pigment cell arises from an unpigmented corpuscle as a result of an increase in acidity.

The orange and the blue cells also arise from unpigmented corpuscles, but in a slightly different manner. Various methods were employed in an effort artificially to stimulate the change from the colourless to the orange and to the blue cells. No response was secured from acids or bases, but it was observed in smears of blood taken from an animal which previously had been weakened by the loss of blood, that there occurred many intermediate stages between the unpigmented corpuscles and the orange cell or the blue cell. If one of the intermediate stages be carefully watched, under very favourable conditions, there is some indication that it gradually increases its depth of colour. With this evidence ¹ the conclusion is unavoidable that all of the pigmented cells arise in the blood-stream directly from colourless ones.

The deep purple-blue colour of the tunic of *A. atra* is

¹ The details of the experiments and a more complete statement of the evidence for this conclusion will be found in the paper by Fulton (1921 *b*) on the blood of *Ascidia atra*.

caused by the presence of large blue pigment cells (Hecht, 1918 *a*; Crozier, 1916 *d*) containing spherical granules, which migrate from one part of the cell to another. The blue corpuscles of the blood-stream are like the pigment cells of the test in every detail of their structure. It remains, therefore, to establish the identity of these cells. In the first place, the presence in both cells of a very prominent vacuole is strong evidence in favour of their being identical. In testing the cells with various reagents another interesting resemblance was noted. It is known that calcium chloride in minute amounts has the power of greatly accelerating the activity of phagocytes (Hamburger, 1910 and 1916); it also causes a decided increase in the activity of the blue pigment cells both of the blood-stream and of the test. After a M/10 solution of CaCl_2 has been added to a fresh smear of blood the blue cells, which in their quiescent state are nearly spherical, immediately send forth pseudopodia, and at the same time the blue granules within the corpuscles commence to move from one end of the cell to the other. In the test, however, the pigment cells, inasmuch as they are fixed within the substance of the test, cannot move their processes; there is, nevertheless, following the addition of CaCl_2 to a section of the test, a decided activity on the part of the blue granules,¹ an activity which is similar to that displayed by the corresponding granules of the blood-cells. The conclusion, therefore, seems to be warranted that the two cells are identical, and consequently that the blue cells in the blood give rise to the pigment cells of the test.²

From these two kinds of evidence it is a reasonable conclusion that in *Ascidia atra* the coloration of the animal is eventually traceable to the colourless cells of the blood; for, as has been shown, the unpigmented cells give rise, while in circula-

¹ A description of a phenomenon of this kind is also to be found in Pizon's (1898, 1901) papers on the pigment granules of tunicates.

² Hecht (1918 *a*) states that when *A. atra* regenerates a portion of its test, there are a great many of the blue blood-cells in the area of the regenerating tissue.

tion, to the blue corpuseles; these finally become lodged in the tunic, and in that way give rise to the surface pigmentation of the animal.

(b) Other Tunicates.

There are many other ascidians in the Bermuda waters which are highly coloured. In a cave on the west side of Agar's Island¹ five specimens of the brilliant red tunicate, *Microcosmus miniatus*, Verrill, were found. An examination of the blood revealed that its most prominent constituent was an amoeboid cell, containing many brilliant carmine-coloured granules, which was very similar to the pigment cell that colours the test. The animal from which the blood had been extracted was examined on the day following, and, as in *A. atra*, there were many intermediate stages between the colourless cells and the pigmented corpuseles. These observations confirmed in a substantial way those made upon *A. atra*.

Other species of ascidians have been examined and in every case the colour of the pigment cells in the test was duplicated by the coloured cells of the blood. The colonial form, *Ecteinascidia turbinata*, Herdman, which is brilliant orange in colour, has as its only coloured cell in the blood-stream a corpusele possessing orange granules.

(c) Discussion.

Concerning the origin of the pigments in the blood-stream of ascidians, no definite statement can be made. It has been observed that the pigment cells arise while in circulation from unpigmented corpuseles. Just what is the process involved in that colour change it is difficult to explain. Griffiths (1897) has described a colourless respiratory proteid (γ -achroglobin) in the blood of ascidians, and the chromogen of *Phallusia* has been stated by Henze (1911, 1912) to be a proteid in combination with the element vanadium. It is possible, therefore, that the change from the colourless cell to the 'chromocyte'

¹ Where the laboratory is situated.

is occasioned by the chemical union of the proteid with the metal. This seems extremely unlikely, however, since vanadium does not exist in the blood in its elemental state—the differences in colour of the corpuscles being due to vanadium in different states of oxidation—and also because the vanadium probably has the rôle of catalyst in the respiratory phenomena of ascidians (Fulton, 1921 *c*). A more likely explanation is that in the colourless antecedents of the pigment cells there exists some colourless vanadium compound which, either on oxidation or reduction, is converted into one of the coloured oxides of that metal.

7. DISCUSSION—PIGMENTATION OF VERTEBRATES.

The results obtained in a study of the pigmentation of invertebrates cannot be entirely without application to the problem of coloration in vertebrates. Particularly does this seem true in view of the question regarding the origin of melanin. In recent years the origin of pigments in vertebrates has been much discussed. One school holds that the epidermis is capable of elaborating its own pigment ;¹ another maintains that the pigment is carried into the integument by wandering leucocytes ;² still another holds that melanin is derived from the haemoglobin of the blood.³ For a very complete account of the historical development and the present status of the biological theories regarding the origin of melanin, the reader is referred to Dawson's (1920) paper on the integument of *Necturus*. But, in addition to the purely biological discussion of the question, there are certain chemical investigations on melanin which demand attention.

The chemical analyses which have been made upon melanin tend on the whole to support the view that the pigment is

¹ Hooker (1914) and Eycleshymer (1906) are the more important advocates of this view.

² See the papers of Reinke (1906), Nègre (1906), and Borrel (1913).

³ Rabl (1894) maintained that the leucocytes phagocytized red blood-cells and converted their haemoglobin into melanin. This view is also supported by certain chemical investigations.

a derivative of haemoglobin. In the first place the same elements are present in melanin and in haemoglobin (Hammarsten and Hedin, 1915, p. 84). Aside from nitrogen and sulphur the most noticeable element present is iron. The earlier observers (Scherer, 1841; Berdez und Nencki, 1886) failed to detect iron, but, as Halliburton (1898, vol. i, p. 121) points out, their failure was due to the fact that they extracted the pigment with hydrochloric acid and thus removed the iron. Mörner (1887), and Brandl und Pfeiffer (1890) found that melanin contained a large amount of iron, and believe as a result that melanin is a derivative of the blood-pigments. Schmiedeberg (1897) obtained similar results for the sarcomelanin from a sarcomatous liver, finding that it contained 2.7 per cent. iron. The more recent work on the subject likewise corroborates the observation that iron is present in melanin (Gortner, 1912*a*; von Fürth und Jerusalem, 1907; Piettre, 1911*a*).

There is, therefore, both biological and chemical evidence in favour of the view that melanin is derived from the blood-pigments. Moreover, as the present paper has attempted to show, the great majority of invertebrate pigments are not only derived from the pigments of blood-systems but the invertebrate blood-pigments are themselves derived from food. Unless a profound change has occurred in the physiological processes of the vertebrates as compared with those of the invertebrates—which is not probable—it appears reasonable to the writer to admit that some at least of the vertebrate pigments likewise owe their origin to the pigments of the blood.

Urochrome.—The recent feeding experiments of Roaf (1921) have given strong evidence that the output of urochrome from the urine of guinea-pigs and of man is roughly proportional to the amount of chlorophyll taken in as food, and Roaf suggests, in view of the chemical similarity between the two pigments (the pyrrol reaction), that urochrome is derived from chlorophyll. It is evident that this observation throws quite a new light upon the debated question of urinary pigments, and it gives an added instance of the dependence of animals

upon the pigments of plants. The further relation of chlorophyll to the bile and urinary pigments is a subject which will well repay further investigation.

In conclusion it may be said with reasonable certainty that many animals and probably man do normally use the four pyrrol groups of the chlorophyll molecule to synthesize haemoglobin and allied pigments; however, though most evidence points in this direction, no one has actually demonstrated that the animal body is itself incapable of synthesizing haemoglobin in the absence of chlorophyll.

8. CONCLUSIONS.

Part I.

What deductions may be made concerning the animals which have thus far been considered? In the first place it must be recalled that in no case has the writer dealt with forms which have a true blood-vascular system.

The most important function of the blood-system in the higher animals is that of carrying nutriment to the tissues. In the lower invertebrates this function is accomplished either by the direct contact of the tissue with the surrounding sea-water, or by circulatory fluids—less highly specialized than blood—which move within the body. These fluids, therefore, are the ones which represent the functional antecedents of the blood-vascular system; and it is to these that one should look in seeking the origin of many of the body-pigments. From the foregoing pages the following conclusions seem reasonable:

1. The pigmented protozoans owe their colour, probably in every case, to an algal pigment which has resulted from an outside infection.

2. Though the evidence in the Porifera is not conclusive,¹

¹ The evidence recently adduced by Van Trigt (1918) removes any reasonable doubt concerning the chlorophyllous nature of the pigments of a large number of sponges.

it is probable that their pigment is chlorophyll or a substance closely allied (chlorophyll has been demonstrated in more than twenty species of sponge), and there is evidence which indicates that it, too, is obtained from external sources.

3. The constituents from which actinians manufacture their pigment are carried to the tissues by the gastrovascular fluid ; it is likely that the constituents themselves are derived from the chlorophyllous substances which enter as food ; thus they are in a highly-organized state when they reach the tissues, making the synthesis of the pigment less difficult. Certain of the actinian pigments—aside from being derivatives of chlorophyll—are closely related to haemoglobin (actiniohaematin).

4. In certain flatworms which are not coloured by algal symbionts, the pigment is derived from food and is carried to the tissues by the gastrovascular system.

5. The pigment of the red cells of *Tripneustes esculentus*, so numerous in the perivisceral fluid, is identical with the pigment of the epidermis, and since it has been shown by Geddes and others that the pigmented cells arise while in circulation from yellow cells, direct evidence is thereby afforded that the body pigment arises in the nutritive fluid.

6. The red pigment, echinochrome, though probably not respiratory (McClendon), nevertheless bears a close chemical relationship to haemoglobin (Griffiths).

7. Since there is every probability (in *T. esculentus*) that the yellow cells from which the reds arise are chlorophylloid corpuscles, it seems clear that chlorophyll is capable not only of giving rise to an animal pigment but to a pigment which is closely akin to haemoglobin (echinochrome breaks down into haemochromogen, a reduction product of haemoglobin). Bürgi's feeding experiments show that chlorophyll facilitates the formation of haemoglobin in anaemic rabbits.

8. The theory that haemoglobin is derived from chlorophyll is further strengthened by the fact that in many echinoderms there is present, simultaneously with haemoglobin and chlorophyll, a substance, haematoporphyrin, which is an intermediate product chemically between chlorophyll and haemoglobin.

9. In many other echinoderms Asteroidea, Ophiuroidea, Echinoidea, and Holothuroidea, there are found pigmented body-fluid cells which likewise give rise (in part at least) to the external pigmentation by becoming deposited in the epidermis.

Part II.

In the preliminary observations of the present work, it was found that in nearly every invertebrate investigated there occurs in the blood a chromogen—either completely formed, or in the process of formation—which is similar to, and not infrequently identical with, the pigment of the epidermis. Though this in itself is an interesting fact, it is at once apparent that behind the phenomenon there lies something of much greater significance: Why should the body-pigments occur in the blood-system, and whence do they come? The pigments of the invertebrates, so far as they have been investigated, appear to be derived very largely from food, being absorbed into the blood-stream and carried by that tissue to the epidermal regions, where they are deposited.

The more specific conclusions are as follows:

1. In the absence of a blood-system, as in the Echinoderms and lower forms, the nutritive fluids supply the epidermis with its pigments (Part I).

2. The coloration of several nemertean worms is due to the presence of haemoglobin in the epidermis and in the blood.

3. In certain nudibranchs (*Chromodoris zebra*) and cephalopods, pigments are found in the blood-stream which are identical with the epidermal pigments. There is strong evidence that the pigments of the blood-stream of the Mollusca owe their origin to the chlorophyllous substances taken in as food.

4. The lipochromes of annelids are derived from food substances, being absorbed into the blood-stream and transported to the epidermis. Annelid haemoglobin is found both in the blood and in the epidermis.

5. The enterochlorophyll found in the liver of many crustaceans and molluscs is of vegetable origin. There is evidence

that it is the base from which the animal synthesizes many other of its pigments, including haematin.

6. The red lipochrome of the blood and the chromatophores of crustaceans are derived from carotin, a pigment associated with chlorophyll (found also in the liver). Carotin and the red lipochrome of crustaceans are chemically identical (Verne).

7. Many pigments of insects are modified chlorophylls derived directly from the chlorophyll of the food (Poulton).

8. The pigments of the tunicates are found first in the blood-system. The pigmented cells arise, while in the circulation, from unpigmented corpuscles, and certain of the pigment cells which arise in this way are subsequently deposited in the test.

9. Strong evidence exists that the respiratory pigment haemoglobin is derived both phylogenetically and physiologically from chlorophyll.

I cannot close this paper without mentioning my great indebtedness for advice and inspiration to Professor Benjamin Moore whose recent untimely death will be most keenly felt in America as well as in England. He more than any one has helped to clarify the perplexing question of the relation which chlorophyll bears to the protoplasmic system of plants and animals.

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Further observations on Chromosomes and Sex-determination in *Abra xas grossulariata*.

By the late

Professor L. Doncaster, F.R.S.,

University of Liverpool.

THE following paper was left by the late Professor Doncaster in an incompleated condition, and, as I was one of his assistants during his last year's work, it has been entrusted to me to prepare for publication.

The paper as it now stands is exactly as Professor Doncaster left it, except that I have added the account of the staining methods used to test the nature of the elimination plate. I was familiar with his staining methods because I was myself testing for chromatin in some entirely different work when Professor Doncaster was testing the elimination plate, and he kindly passed on all his stains to me as he used them, showed me his preparations, and discussed the whole matter with me. In his rough notes I find a full account of all the stains used, and carefully labelled figures showing the results obtained from the different staining methods: there is, therefore, no uncertainty about the facts which I have added.

A summary of the paper included amongst Professor Doncaster's rough notes shows that he intended to add three other sections on 'Conjugation, &c., of polar nuclei', 'Binucleate eggs', and 'Gynandromorphs'. These sections were unfortunately not written even in note form, and therefore cannot possibly be produced; but the paper as it stands is of such obvious interest that its publication even in this very incomplete form is more than justified.

RUTH C. BAMBER
(Mrs. Bisbee).

FURTHER OBSERVATIONS ON CHROMOSOMES AND SEX-
DETERMINATION IN *AbraXas grossulariata*.

In a previous paper ('Journ. of Genetics', iv, 1914, p. 1) I described the inheritance of a tendency to produce families consisting chiefly or entirely of females in *AbraXas grossulariata*, and attempted to correlate it with the behaviour of the chromosomes. It was found that females of the strain in which unisexual families occurred have fifty-five chromosomes as the somatic number, while all males and most other females have fifty-six. In the maturation of the fifty-five-chromosome strain, twenty-eight chromosomes travel to one pole of the first polar spindle and twenty-seven to the other. Since all spermatozoa were found to have twenty-eight, it seemed evident that eggs with twenty-seven must be female-determining, since the union of an egg having twenty-seven with a sperm having twenty-eight would give fifty-five, the number found in females of the strain in question, while eggs with twenty-eight meeting sperms with twenty-eight would give the fifty-six found in the male. Evidence was also given that in families in which great excess of females was produced, a majority of the eggs matured in such a way that twenty-eight chromosomes were extruded in the first polar nucleus, and twenty-seven remained in the egg-nucleus, and it was therefore inferred that the condition in some families, in which only females were produced, was caused by the invariable extrusion of the twenty-eighth chromosome in the polar body, leaving all eggs with only twenty-seven, and therefore female-producing. This hypothesis was supported by the observations of Morgan on *Phylloxera*, in which one chromosome is always extruded in the polar body of male-producing eggs, although it is already determined in some other way that these eggs will become males.

When the paper referred to was published, I had been able to obtain no completely conclusive evidence that in families consisting wholly of females all the eggs had only twenty-seven chromosomes in the egg-nucleus, and I spent the next two seasons in collecting material which it was hoped would give

an unequivocal result. The method adopted was to pair females belonging to all-female families, allow them to lay eggs as far as possible under observation, and to preserve the first 50 or 100 eggs at an age (about two hours) when the maturation-divisions would be in progress. The moths were then allowed to continue laying, the eggs counted, and reared either to the imago or to larvae in which the sex could easily be determined by dissection. Some of these families produced both sexes, others either females only or females in great excess. The preserved eggs of families which proved all-female were then sectioned, and counts made of the chromosomes in the polar division-spindles.

By the summer of 1915 I had already enough material to show that the hypothesis put forward in the 1914 paper was almost certainly incorrect, and since work in connexion with the war prevented the immediate continuation of the investigation I published a preliminary note in a letter to 'Nature' (June 10, 1915) in which I wrote as follows: 'I have now examined the eggs of several such families [i. e. all-female families], and find, contrary to expectation, that the equatorial plate of the inner spindle contains twenty-eight chromosomes about as frequently as twenty-seven. The new material confirms the observation that twenty-seven occur in one spindle and twenty-eight in the other, but it seems to make it certain that the presence of twenty-eight chromosomes in the inner spindle does not necessarily cause the production of a male—at least in the strain which produces all-female families. A possible explanation of the anomaly is that in all-female families a chromosome is eliminated at a later stage, but at present I have no direct evidence for this.'

From that time to the summer of 1919 the work was interrupted, but enough material had been collected to provide the required observations, and examination of the sections confirms the account shortly given in the letter quoted. There are two questions at issue: (1) whether the all-female families are so because all the fertilized eggs are truly female, or whether they arise through non-viability of male zygotes; (2) if all

zygotes in such families are female, whether the egg-nuclei before fertilization contain always twenty-seven chromosomes, or sometimes twenty-eight.

EVIDENCE THAT ALL-FEMALE FAMILIES ARE NOT CAUSED
BY NON-VIABILITY OF MALE ZYGOTES.

In the earlier papers a number of families were recorded in which considerably over half the eggs were reared either to imagines or to larvae in which the sex was definitely determinable, but there still seemed some slight chance that all-female families might arise through death of male zygotes at an early age. This, however, seems to be definitely excluded by the results of later experiments, as is shown in Table I, which gives a list of the all-female, or almost exclusively female families in which at least two-thirds of the eggs were reared to larvae or adults of ascertainable sex.

TABLE I.

<i>Family.</i>	<i>Number of Eggs.</i>	<i>Eggs Hatched.</i>	<i>Larvae or Imagines.</i>	
			♀	♂
1912.1 . . .	97	97	66	1
1912.8 (2) . . .	40	31	28	—
1912.29 . . .	110	110	77	—
1912.29 B . . .	83	82	66	—
1913.30 . . .	77	77	54	2
1914.9 . . .	37	not recorded	28	—
1914.16 . . .	47	..	37	—
1914.18 . . .	63	..	58	—
1914.7 . . .	14	..	9	1
1914.28 . . .	62	..	45	—
1916.7 . . .	61	46	42	—
1916.9 . . .	27	22	22	—
1916.10 . . .	62	56	39	4

In view of the fact that in most cases almost all the eggs hatch, and that as soon as the larvae are old enough to be dissected the sex is already clearly distinguishable, these results make it practically indubitable that the all-female families do not arise in consequence of the death of male eggs or larvae. But the matter can be tested in another way,¹ which makes this conclusion doubly sure.

¹ It seems as though a paragraph has been omitted here, but there is no

The evidence just given seems to prove beyond the possibility of reasonable doubt that all zygotes in the all-female families are female, and that these families do not arise by the death of male zygotes. The problem then presents itself whether all eggs of these families before fertilization contain twenty-seven instead of twenty-eight chromosomes. In the letter to 'Nature' referred to I announced that I found evidence that this was not so, and further work has confirmed this conclusion. In 1914 I preserved eggs from four pairings, of which the eggs subsequently laid yielded only females. The data with regard to these families are as follows, excluding the eggs preserved for microscopic examination:

<i>No. of Family.</i>	<i>No. of Eggs.</i>	<i>Eggs Hatched.</i>	<i>Males Reared.</i>	<i>Females Reared.</i>
14.9	37	not recorded	0	28
14.22	74	nearly all	0	28
14.28	62	62	0	45
14.29	38	38	1 ?	21

It will be noticed that in families 14.9 and 14.28 over two-thirds of the eggs kept for rearing were reared to imagines (or in 14.28, thirty-six imagines and nine pupae). The eggs of these same families preserved for microscopic examination gave the following chromosome counts in the equatorial plates of the second maturation division.

14.9. In the inner spindle 27, in the outer 28—four cases recorded as 'good'.

In the inner spindle 27, in the outer 28—two cases recorded as 'probable'.

trace of it in the manuscript unless it be the following, which I find on a page of note-paper along with the manuscript: 'Summaries to 1916 show that all-female families are not due to mortality, due to "lethal" or other causes, of male. Apart from such cases as 14.16, and 14.18 (37 and 58 females from 47 and 63 eggs),* the fact that in all-female families in which over 50 per cent. of the eggs are reared to imagines there are twice as many females per cent. of eggs (64.6 per cent.) as compared with percentage of females in bisexual families (32.3 per cent.) proves this.'

* See table given on previous page, 1914.16 and 1914.18.

In the inner spindle 27, outer not countable—one case recorded as 'good'.

In the inner spindle 28, in the outer 27—three cases recorded as 'good'.

In the inner spindle 28, in the outer 27—four cases recorded as 'probable'.

In the inner spindle 28, outer not countable—one case recorded as 'good'.

In the outer spindle 27, inner not countable—one case recorded as 'good'.

Total, seven cases with the inner spindle containing 27, five of these being 'good' cases in which there is no reasonable doubt as to the number, and nine cases, in which the inner spindle has 28, four of these being 'good' cases.

14.22. The counts were less satisfactory; they gave three cases in which the inner spindle had 27 or the outer 28, and five in which the inner had 28 or the outer 27, but in only one could both inner and outer be counted with confidence in the same egg; in this egg the inner spindle had 28 and the outer 27.

14.28. In the inner spindle 27, or the outer 28—four cases (three in which both plates could be counted with fair certainty).

In the inner spindle 28, or the outer 27—four cases (one countable in both plates).

14.29. In the inner spindle 27, or the outer 28—three cases, in two of which both inner and outer plates were countable with fair certainty.

In the inner spindle 28, in the outer 27—one case (fairly good).

Although the number of 'good' counts in which the chromosomes could be counted with confidence in both inner and outer spindles is not large, some of them, especially in family 14.9, are so certain that no doubt can remain that in many eggs of all-female families the inner spindle contains twenty-eight chromosomes, and adding up all counts in the four families we get seventeen cases in which the inner spindle had twenty-

seven (or the outer twenty-eight) and nineteen with the converse arrangement. In the bisexual family 14.35, in which 15 ♂♂ and 19 ♀♀ were reared from forty-six eggs, four eggs were found in which the inner spindle had twenty-seven, the outer twenty-eight chromosomes, and four with the converse arrangement (all 'good' counts including both spindles of each egg), so it does not appear that the all-female families have twenty-seven in the inner spindle with any greater frequency than in bisexual families of the same stock.

It seems evident from the facts given above that the determination of sex in the fifty-five-chromosome strain of *Abra x a grossulariata* does not depend on the passage of the odd chromosome to one or other pole of the first polar division. At the same time, since females of this strain have fifty-five chromosomes in their diploid nuclei and males have fifty-six, a chromosome must be eliminated at some stage from those eggs in which twenty-eight travel to the inner pole of the first polar spindle. Attempts to find a chromosome which does not divide in the second maturation division have not been successful, and it seems clear that the elimination does not occur at that stage. Only two possibilities remain: either a chromosome is eliminated at some division after fertilization—presumably the first segmentation division, or the odd chromosome must degenerate so that the twenty-eight chromosomes present in about half the eggs at the inner pole of the first polar spindle are reduced to twenty-seven by the degeneration of one of them. Neither possibility seems likely on general grounds, but there are some facts which make the hypothesis of the degeneration of a chromosome less entirely improbable than would appear at first sight. These will be discussed in the next section. With regard to the hypothesis of the elimination of a chromosome in the first segmentation division, I can only say that I have not succeeded in obtaining figures in which the chromosomes in this division can be accurately counted: in the few segmentation divisions present in my material the chromosomes tend to become aggregated into small groups, apparently of two or three, so that counts give numbers not

much greater than the haploid complement (twenty-eight). Probably mitotic figures embedded deeply in the yolk are fixed less rapidly than the maturation mitoses near the surface of the egg, with the result that observations on the number and behaviour of the chromosomes in the segmentation divisions become untrustworthy.

‘CHROMATIN ELIMINATION’ IN THE MATURATION DIVISIONS
OF THE EGG.

In my 1914 paper¹ I mentioned that ‘during the first polar division, a mass of granules which stain deeply with iron haematoxylin is left in the equatorial plate as the chromosomes travel to the poles’ (fig. 14 of that paper). No further investigation was made at the time on the nature or mode of origin of these granules, but in a paper published almost simultaneously² Seiler describes them in considerable detail in the eggs of the moths *Phragmatobia fuliginosa*, *Orggia antiqua*, *Lymantria monacha*, and *L. dispar*. He gives evidence that these granules are separated from the chromosomes in the early anaphase of the first polar division, and maintains that in favourable cases it is possible to see that each chromosome, as it divides, leaves behind on the equator of the spindle a chromatin mass which for a time at least preserves its identity, so that in sections of a mitosis in anaphase cut at right angles to the axis of the spindle it is possible to see three plates each containing the same number of chromatin bodies similarly arranged—the two anaphase groups of chromosomes and between them an ‘elimination plate’ consisting of chromosome-like bodies having the same number and arrangement as the chromosomes in the true chromosome plates. Careful search among my preparations—both old ones and new sections made for the purpose—has not revealed the existence of plates with such definite, chromosome-like granules in *Abraaxas*, but in other respects my sections, when stained

¹ Doncaster, L., ‘Journ. of Genet.’, 1914, p. 1.

² Seiler, J., ‘Archiv für Zellforschung.’, xiii. Band, 2. Heft (p. 159). Leipzig und Berlin, 1914.

with iron haematoxylin, give very nearly the same series of figures as are represented by Seiler. I have a few cases in very early anaphase (just after metaphase) in which each chromosome seems to be leaving behind, as its halves diverge on the spindle, a mass of staining substance (cf. Seiler's figs. 19-22), and in later anaphase there is always an equatorial plate of staining granules lying across the middle of the spindle. Not infrequently these granules are elongated, so as to appear like short threads, and some or all of them seem to lie on or in the spindle fibres. Towards the end of the anaphase they generally form a plate of fine-stained dots, of varying size, and always more numerous than the chromosomes, as if they had become broken up and scattered. During the second division they sometimes become aggregated into a sort of network (cf. Seiler's fig. 35), or they may apparently have become more finely divided and comparatively inconspicuous.

Like Seiler, I find great variation among different polar mitoses in respect of the amount of this eliminated substance. In some spindles there is a dense equatorial mass, staining with iron haematoxylin almost as deeply as the chromosomes around the poles. In others the granules are much less conspicuous, in others again so few and small as hardly to be noticeable. The amount of staining matter in the 'elimination plate' varies in different eggs of the same female, and even in eggs mounted on the same slide, though on the whole it is more abundant in the eggs of some females than in those of others. It is important to notice, however, that the apparent amount varies with depth of staining, and when sections of several eggs are mounted together on a slide it may happen that some spindles are fully washed out, so that the chromosomes alone remain clearly stained, while a spindle in a neighbouring egg may retain so much stain as to be useless for the study of chromosomes. This variability probably arises from differences of fixation due to variation in the penetrability of the egg-shells to the fixative, and therefore it is not impossible that the variation in the apparent amount of eliminated sub-

stance may be in part at least due to the technique of fixing and staining.

Seiler has no doubt that the stained matter in the elimination plate is chromatin, and reviews the literature of maturation divisions of insect eggs, and also such examples of chromatin elimination as those seen in the segmentation of *Ascaris* and *Miastor*, in order to discuss the significance of the process. He does not, however, discuss at all fully the question whether the substance eliminated is in fact chromatin, or if it is, whether it is of the same nature as the chromatin of the anaphase chromosomes. His account of his staining methods is meagre—'Gefärbt wurde vorwiegend mit Heidenhains Eisenhämatoxylin und Kontrollfärbungen wurden mit Kernfarbstoffen vorgenommen. Als Plasmafärbstoff verwendete ich S.-Fuchsin'. Unless the elimination process is in reality an artefact, which seems very unlikely in view of the almost invariable presence of staining granules in the equator of the spindle and the definite manner in which they appear to be left behind by the diverging chromosomes, it appears to be of considerable importance to determine the true nature of the eliminated substance, for if it be chromatin, it seems not impossible that the process may supply the clue to the anomaly presented by the presence of twenty-eight chromosomes in eggs which nevertheless yield females. If the chromosomes do in fact leave behind on the polar spindle a considerable part of their substance, it is at least conceivable that the sex-chromosome, in the eggs of all-female broods, eliminates so much that it becomes functionless as regards sex-determination, and that, having lost so large a part of its substance, it ceases to function and disappears, so that in the oogonia only fifty-five instead of fifty-six can be counted. With the object of determining whether the elimination plate does or does not consist of chromatin I stained eggs with a number of combinations of stains, the more important of which were as follows:¹

[All the sections used had been previously stained with iron hæmatoxylin and were decolourized with acid alcohol.]

¹ Professor Doncaster's manuscript ends here.

I. Ehrlich's Triacid Stain.

Sections were immersed for eighteen hours in the stain, blotted, and passed through absolute alcohol and xylol into balsam. The chromosomes and the elimination plate were stained purple, the surrounding protoplasm brown.

II. Safranin and Lightgreen.

Sections were placed in safranin for from twelve to twenty-four hours, followed by lightgreen for one to two minutes. The chromosomes were stained a bright red, the elimination plate a lighter red in some cases and in others green with a distinct admixture of red. The spindle-fibres were green.

Both the above methods gave very clear results which strongly suggest that chromatin was present in the elimination plate.

III. Mann's Methyl Blue Eosin.

Sections were stained for a few minutes only. This method gave very erratic results. In most cases the spindle-fibres were blue, but whereas in some sections the chromosomes and elimination plate were also blue, in others the chromosomes were purple and the elimination plate red; and in others again the chromosomes were red with a bluish tint here and there, and the elimination plate purple.

In spite of the varying results obtained with this stain it is clear that in any given section there is a very close correspondence between the chromosomes and the elimination plate.

IV. Ehrlich's Haematoxylin.

Sections were stained for eighteen hours with Ehrlich's haematoxylin, differentiated for from one to two minutes in acid alcohol, washed in 70 per cent. alcohol and counterstained with eosin in 90 per cent. alcohol for about one minute. The spindle-fibres always stained pink, the chromosomes were usually black, and the elimination plate pink with grey dots: sometimes, however, the chromosomes were a bright pink and the elimination plate a paler pink, and at other times the chromosomes and the elimination plate were purple.

Here again, as with Mann's methyl blue eosin, the chromo-

somes and the elimination plate in any given section correspond very closely in their staining properties, although in different sections very different results were obtained from the same combination of stains.

V. Borax Carmine and Picro-indigo-carmine.

Sections were stained in borax carmine for forty-eight hours, followed by picro-indigo-carmine for ten minutes.

The chromosomes were found to be dark red, and the elimination plate and spindle-fibres yellowish. Although this method did not appear to give support to the view that chromatin is present in the elimination plate, it does not disprove that hypothesis, for in some sections even the chromosomes themselves were barely stained with the carmine, so that it is not surprising to find the elimination plate unstained even though it may contain chromatin, for this eliminated chromatin would almost certainly be undergoing disintegration. In sections stained by other methods as given above, it was not unusual to find that the elimination plate was unstained, even though it had previously stained deeply with iron haematoxylin.

These staining experiments, although not conclusive, give a considerable weight of evidence in support of the hypothesis that there is a certain amount of chromatin left behind, on the equator of the spindle, by the chromosomes when they move apart at anaphase. If this be true 'it is at least conceivable that the sex-chromosome, in the eggs of all-female broods, eliminates so much that it becomes functionless as regards sex-determination',¹ and that here may lie the explanation of the production of all-female families from eggs some of which contain twenty-seven and others twenty-eight chromosomes.

From conversation with Professor Doncaster, as well as from his own argument in this paper, I know that this was the conclusion at which he himself had arrived.

R. C. B.

¹ p. 406.

The Infra-cerebral Organs of *Peripatus*.

By

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With 4 Text-figures.

ATTACHED to the ventral surface of the supra-oesophageal ganglion of *Peripatus* and hanging therefrom are two small vesicles. They were discovered as far back as 1853 before *Peripatus* was regarded as an Arthropod, and Grube, their discoverer, considered them to be auditory organs (8).

In 1883, Balfour, in his classical description of the anatomy of *Peripatus capensis* (2), described the structure of these vesicles, and after a statement detailing their shape and position added that each consisted mainly of ganglion cells. He continued with the following words: 'In its interior is a cavity with a distinct bounding membrane. . . . At its free end is placed a highly refractive, somewhat oval body, probably forming what Grube describes as a dark spot, half embedded in its substance, and kept in place by the sheath of nervous matter surrounding it. It is difficult to offer any interpretation of the nature of this body. It is removed considerably from the surface of the animal, and is not, therefore, so far as I can see, adapted to serve as an organ of hearing.'

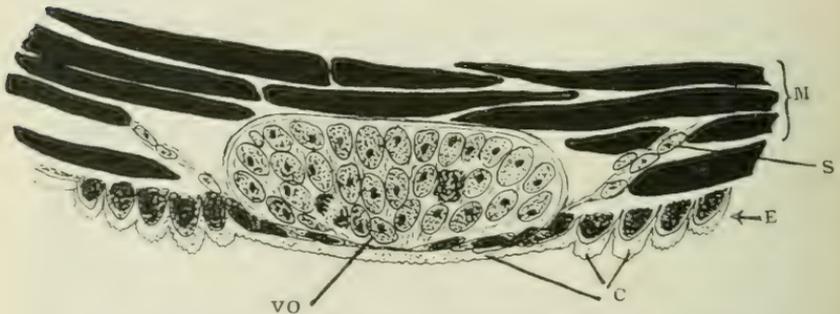
Three years after the appearance of Balfour's paper, Kemel (11) followed the development of the infra-cerebral vesicles and discovered that they were apparently homologous with certain groups of cells situated between the legs—and known as the 'ventral organs' (see Text-fig. 1).

This was confirmed by Sedgwick (15) in 1888, and the

discovery has very considerably modified the views of the function and meaning of the infra-cerebral vesicles.

If any definite theory of the function of these structures can be said to be generally accepted, it is that they represent the ectoderm from which the nervous system arose in the embryo, and in a recent paper by Duboseq the suggestion is made that the infra-cerebral vesicles remain, even in the adult stage, structures for the renovation or increase in size of the supra-oesophageal ganglia. Cells are supposed to be cut off from the vesicle cells and to migrate into the ganglia, there to become either new nerve-cells or supporting cells.

TEXT-FIG. 1.



Peripatoides occidentalis: section of so-called ventral organ, vo; c, cuticle; e, ectoderm; m, muscles of body-wall; s, strand connecting ventral organ with lateral nerve-cord.

The present note has been written because in several of our best preparations from the head of *Peripatoides occidentalis*, Dendy, of Western Australia (5), the histology of the organs in question is not the same as that illustrated by Duboseq (6). And a little more may be said in explanation of the presence of these curiously definite structures.

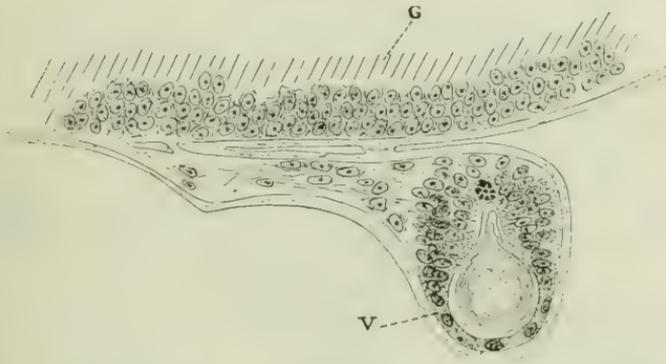
According to Duboseq (who examined *Opisthopatus cinctipes*, Purell) one can distinguish in these organs two distinct regions, (a) the vesicle, (b) the ganglion intermediaire. The latter is the part which former writers have called the stalk or peduncle of the vesicle. The term 'ganglion intermediaire' is unsuitable, especially since it appears that Duboseq himself

is not sure whether the cells of this part are nerve-cells or merely supporting cells.

There is nothing that might be termed the ganglion intermediaire in *Peripatoides occidentalis* (Text-figs. 2 and 3).

The vesicle is described by Duboseq as containing nothing within the cavity but serous fluid, there being no refringent oval body of the kind referred to by Balfour. (Unfortunately Balfour's figure gives no idea of the histology of the infra-cerebral vesicles.) Now we have found occasionally that

TEXT-FIG. 2.



Infra-cerebral vesicle, with enclosure, from adult *Peripatoides occidentalis*. $\times 530$. G, ganglion; V, infra-cerebral vesicle.

bodies do occur within the cavity, reminding one of Balfour's description, and the Text-fig. 2 is from the best preparation of this character. It is part of a transverse section through the head. The structure is referred to below.

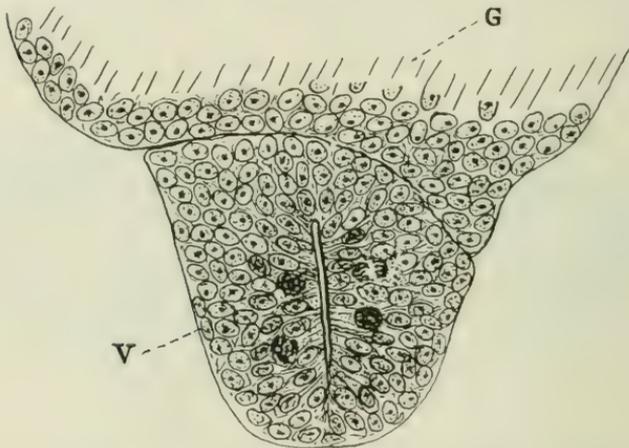
The infra-cerebral organs appear in dissections to hang from the supra-oesophageal ganglion by short stalks. In sections, however, they appear more closely attached. The difference in appearance is due to the transparency of the suspending membrane which is the structureless, almost non-staining, sheath of the ganglion. In *Peripatoides occidentalis* there is generally a region that one might term the peduncle, within which are a few scattered nuclei and a small number

of delicate fibre-like strands. They might be nerve-fibres or, on the other hand, merely processes of non-nervous cells.

In young and small *Peripatoides* (Text-fig. 3) the conditions are somewhat different, however, and the walls of the vesicles are not so distinctly separated from the cerebral ganglia (see Text-figs. 2 and 3).

In the adult the infra-cerebral vesicle is covered by the neurilemma or sheath of the supra-oesophageal ganglion, and

TEXT-FIG. 3.



Infra-cerebral organ from young *Peripatoides occidentalis*. $\times 400$.

this layer almost cuts it off from the latter. According to Saint-Rémy (14) and Duboseq (6) the sheath is pierced by numerous pores, through which bipolar cells are to be seen migrating into the brain. This is hardly the case in the adult *Peripatoides*, as the figure shows. There are only a few fibres passing from the vesicle into the supra-oesophageal ganglion and but a few nuclei occur here and there.

The cells of the vesicle itself are not of the same depth throughout. Ventrally the walls are thin whilst laterally they are thick, and the cells are slender, so that the nuclei lie at different levels. The nuclei resemble closely those of the ganglion cells of the brain mass.

The cavity of the vesicle is most usually empty, but sometimes contents are present, and the most conspicuous example of this kind has been figured (Text-fig. 2). In this specimen there is a non-granular mass surrounded by a number of concentric lamellae—almost like a decalcified concretion. The occasional presence of an enclosed body is very interesting.

TEXT-FIG. 4.

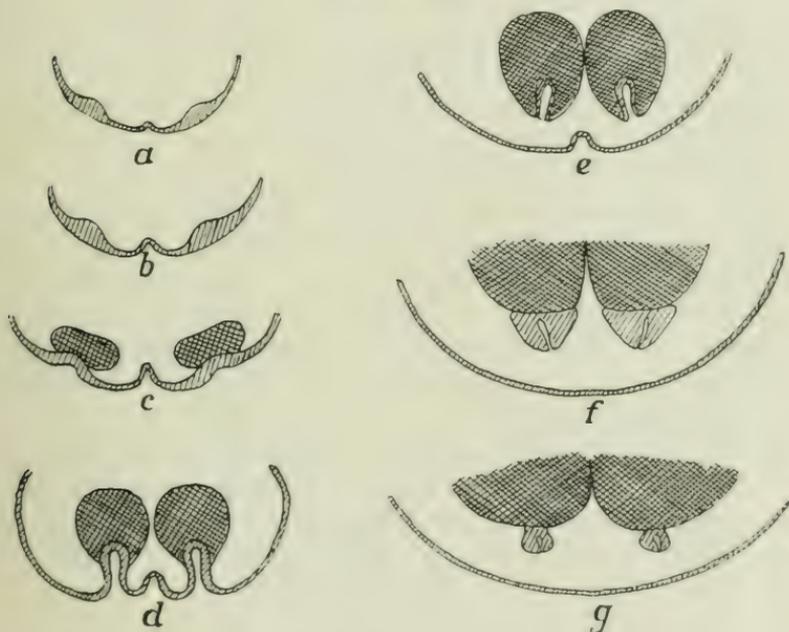


Diagram illustrating development of infra-cerebral vesicles in *Peripatus occidentalis*.

At first it was thought to be due to fixation, but there is no reason to believe that such is the case. Evidently the vesicle cells may sometimes secrete into the central cavity. Thus a feature recorded by Balfour has again been found and in another species. The few writers who have mentioned the infra-cerebral vesicles since the date of Balfour's paper seem to have doubted its occurrence. No cilia are found within the vesicle nor is there any very definite lining membrane.

The development of these infra-cerebral vesicles is now well known. They arise as invaginations of the ectoderm (Text-fig. 4) which is concerned in the formation of the supra-oesophageal ganglia, and at first they are open to the exterior (to the buccal cavity or near it), so that at this stage the supra-oesophageal ganglia possess cavities which are open below. The cavities become closed and then, whilst increase in size of the ganglia takes place, they remain almost of the original size (a slight decrease takes place if anything), and the surrounding cells, which are indistinguishable from the ganglion cells, become separated and pinched off from the brain mass, until finally two small distinct vesicles lie appended as we have seen. The diagrams illustrate how this takes place.

One other feature of considerable importance remains to be stated. In the adult one may occasionally find cells in the infra-cerebral body undergoing mitotic division. This was first described by Duboseq. We find, however, that the number is much reduced as the animal becomes larger, and they are only occasionally found in the full-sized specimens. In the small *Peripatus*, not long born, they are more numerous (see Text-fig. 3). It may be stated here that the same feature is to be met with in the so-called ventral organs (Text-fig. 1). This fact has not been recorded before and it completes the resemblance between the ventral organs and the infra-cerebral vesicles. There can be little doubt of their homology.

DISCUSSION.

The infra-cerebral vesicles of *Peripatus* were once considered to be sense organs concerned in hearing. Probably this was by analogy with the little vesicles often found close to the supra-oesophageal ganglia in the Polychaets and certain other Invertebrata and once termed Otocysts. They are now usually regarded as Statocysts or organs of orientation. Duboseq concludes, however, that in *Peripatus* they are not sense organs, nor glandular structures, but that in the adult as well as in the embryo they are organs for the production of either nerve-cells or neuroglia cells (supporting cells).

The vesicles known as statocysts or otocysts in the Invertebrates are still of questionable function in many cases. This is particularly so in the case of certain Nemertines (*Metanemertines*) (3), where the walls of the vesicles are surrounded by the ganglion cells of the brain mass and no cilia are present. In fact they are not unlike the infra-cerebral vesicles of *Peripatus*. On the other hand, in Molluscs such as *Pterotrachea*, where each statocyst contains a statolith supported on bunches of cilia, the circumstances are altogether different, as experiment has shown. Amongst Polychaet worms statocysts are known in Sabellidae, some Terebellidae, Arenicola, Aricidae, and some Alciopidae. In some cases cilia are found within the vesicles, and statoliths are present (either secreted, or consisting of sand grains from the exterior). In the *Arenicola* species, however, the state of development of the 'statocysts' varies within very wide limits and it is difficult to express any opinion about the function of these organs. They appear to develop from invaginations of the ectoderm, but there is not the close connexion with the development of the cerebral ganglia which is so characteristic of *Peripatus*. Are these vesicles homologous?

It is interesting to look at the condition of things amongst the Tracheata.

In none of the Tracheata do the organs of orientation take the form of statocysts associated with the supra-oesophageal ganglion. But in the development of the supra-oesophageal ganglion of the Myriapoda it is certainly very striking that pit-like depressions occur on the ventral surface which afterwards become closed vesicles and later disappear (10). The same thing is true of the Insecta and Arachnida (1 and 12).

In the Crustacea there is, so far as I am aware, no evidence of pit-like depressions of this kind during development. Statocysts are found, but these are not at all homologous with the organs we are considering and occur in very different situations. Curiously enough, there is a striking exception. Thus, according to Claus (4), two otocysts are found connected with the cerebral ganglion in certain Amphipoda—the *Platyseelidae*. The same author mentions two vesicles as of similar function in the

brain of a Copepod—*Eucalanus attenuatus* (Dana), but Esterly (7) considers these to be optical in function and apparently their structure is quite different from that of the other brain vesicles we have dealt with.

We would suggest, therefore, that the infra-cerebral vesicles of *Peripatus* are homologous with the cephalic pits of other Tracheate embryos. In these cases the pits become closed off, the walls become parts of the cerebral ganglion, and the cavities disappear altogether. In *Peripatus*, on the other hand, the vesicles remain, but they are gradually constricted off from the rest of the supra-oesophageal ganglion. The adult condition in *Peripatus* is, then, an embryonic stage in the Myriapoda. In the adult it is probable that the infra-cerebral vesicles serve no special function—they are not really 'organs' at all—they may still be regarded as parts of the supra-oesophageal ganglion. Possibly their wall contains a few ganglion cells from which fibres pass into the deeper parts of the brain mass. The occasional presence of bodies within the cavity is interesting, but this suggests nothing more than the ectodermal origin of the cells of which the vesicle is composed, and the tendency for the secretion of a chitinous cuticle.

In the earlier stages before growth is complete this portion of the supra-oesophageal ganglion retains some of its former power of growth and continues to give rise to cells by division (as observed by Duboseq), but it is not a special organ for this purpose and loses its function in the adult.

Whether this character, apparently common to the Tracheates, is homologous with the statocysts found occasionally in the worms is another matter—quite impossible as yet to decide. It has been affirmed, however, that in certain Annelids (*Lopadorhynchus*) the supra-oesophageal ganglia develop in connexion with ciliated pits, which degenerate somewhat afterwards (13). This is very suggestive.

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A Critical Study of the Facts of Artificial Fertilization and Normal Fertilization.

By

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With 1 Text-figure.

FACTS concerning the process of fertilization and of artificial parthenogenesis have steadily accumulated during the past thirty years, and although numerous suggestions have been put forward as partial explanations of this imposing mass of experimental evidence, yet there are only two theories which claim to give an adequate picture of even a majority of the known facts. These theories we owe to J. Loeb (24) and to F. R. Lillie (20). According to Loeb, the activation of an unfertilized egg is effected by the introduction into the egg of two substances, (i) a specific cytolytin, which brings about the destruction of the surface layer of the egg, and (ii) a substance which limits or controls the destructive influence of the cytolytin. On the other hand, Lillie holds that the union of the egg and spermatozoon is only possible in the presence of a specific substance or fertilizin which is secreted by the unfertilized egg; if all three elements are present fertilization and normal development take place.

Loeb's theory is based upon the facts of artificial parthenogenesis; Lillie's theory is based upon the behaviour of the normal gametes. It is not surprising to find that each theory encounters its chief difficulties when confronted with the facts which constitute the main argument of its rival. Both theories are essentially chemical, although the door is left open, at rare intervals, to the intervention of physical factors. In 1915 (8) I suggested that although the theories urged by R. S. Lillie (21)

and by McClelland (25) were inadequate, yet the facts appeared to indicate that the activation of the egg, by a spermatozoon, or by artificial parthenogenetic agents, was essentially a physical rather than a chemical process. It now seems possible to put forward a more comprehensive scheme.

The activation of a resting cell, by contact with another cell in a state of activity, is not limited to the reproductive cells. All contractile cells exhibit the same phenomenon; if localized fibres at the surface of a large muscle are stimulated, the whole of the muscle is rapidly thrown into a state of activity; the ciliated combs of *Pleurobrachia* illustrate the same fact (Gray, 10); also cells in contact with each other usually divide at the same moment. There can be no doubt that such co-ordination of activity is due to the responsive cells themselves, and is not due to any nervous or controlling influence. Thus spermatozoa in contact with each other rapidly acquire a synchronous rhythm; similar examples are readily found in the case of ciliary or muscular elements.

There can be but little doubt that the influence of one cell upon the activities of its neighbours has a very profound bearing on the behaviour of the animal as a whole. There is, however, no reason to regard such co-ordination as essentially vital, since a ready parallel is found in inorganic systems. Ostwald (27) found that when a strip of chromium was placed in hydrochloric acid the hydrogen was evolved at regular periods; each period of activation was followed by a period of inactivity. This periodic condition of activity and inactivity was quite regular for each strip of metal: different strips of metal were, however, characterized by periods of different length. If several such strips are placed in a bulk of hydrochloric acid, the periodicity of each strip exhibits itself; if, however, the strips are in contact with each other then all the strips exhibit the same uniform periodicity. The activation of a passive strip of iron by contact with an active piece of the same metal has been discussed by R. S. Lillie (23), and has a close bearing on the present problem.

The activation of a passive cell or metal by contact with an

active unit is invariably accompanied by an electrical disturbance; and there seems good evidence for the belief that the electrical change is the essential condition of activity. When an inactive unit comes into contact with an active unit, an electro-motive force is established between the two; the active unit is electro-negative to the inactive unit, and if activation of the latter occurs, the state of negativity is not restricted to the region of contact but spreads from it all over the originally inactive surface. Such facts are, of course, well known in the case of fibres in the same muscle, but Kühne (18) showed that the action current of one muscle could stimulate another muscle if the two were in close electrical contact. Now the E.M.F. set up between two cells in contact depends on, and is an expression of, the difference in the activity of the two units; the greater the difference in activity the greater is the E.M.F. set up on contact.

In the opinion of the writer an application of the above principles to the problem of fertilization is not without value. In the unfertilized egg metabolic activity is reduced to a minimum, and unless fertilization takes place the cell dies without any recovery from its inert condition. The spermatozoon, on the other hand, is radically different: it is exceedingly active and metabolism proceeds at a rapid rate (Cohn, 3). When the two cells come into contact it seems legitimate to conclude that an E.M.F. will be set up between the two, and if the conditions be right it is to be expected that some form of activity will be induced in the inert egg. If the process of activation be analogous to that of other cells, then the egg will be activated whenever the E.M.F. set up by contact with a spermatozoon reaches a certain minimum value in a minimum time. It is, therefore, not surprising to find that a certain minimum of activity on the part of a spermatozoon is necessary for fertilization. Mobility and proximity of the egg are not sufficient—a fact difficult to explain on any chemical conception; there must be a definite and rather high degree of activity on the part of the sperm, and this degree of activity differs between individual spermatozoa. Glaser (7) observed

that the eggs of *Arbacia punctulata* can be activated by means of highly active minute Infusoria. It is, therefore, the degree of activity of the sperm which determines one condition of fertilization and not its structure or chemical constitution.

Whereas the normal activity of a spermatozoon is usually adequate for the activation of eggs of the same species, yet it requires to be increased to an abnormal degree to fertilize the eggs of another species. Now the activity of spermatozoa can readily be controlled by the hydrogen-ion concentration of the medium (Gray, 9), and correspondingly it is found that the addition of hydroxyl ions removes the block which normally exists between the eggs of *Strongylocentrotus* and the sperm of *Sphaerechinus*. These and similar facts are difficult to explain on the basis that fertilization depends on the existence of specific chemical substances in the egg or sperm (see Loeb, p. 204). They appear to the writer to be less formidable when subjected to physical arguments. According to the present physical hypothesis, if it be mechanically possible for the sperm of one species to gain contact with the egg of another, then activation will take place if the E.M.F. set up between the two cells reaches a certain critical value within a minimum time. Consider two species, A and B. When normal fertilization of egg A is effected by sperm A, let the E.M.F. be E_1 and let it be developed in unit time; similarly when egg B is fertilized by sperm B let the E.M.F. at contact be E_2 . Let $E_1 > E_2$; then when egg B comes into contact with sperm A, the E.M.F. will probably be more than enough to activate the egg; when egg A comes into contact with sperm B, no activation will occur unless the activity of the sperm is artificially increased, since otherwise the requisite E.M.F. will not be reached. Such irreciprocal hybridizations have already been mentioned and are by no means uncommon [see Vernon (28), Doncaster (5)]. Further, the conditions which affect the ease with which hybridization can occur are such as support the view that some such physical factors are involved, e. g. seasonal variation of gametes, degree of maturity, staleness or freshness of gametes.

hydroxyl ions, dilution of sea-water, &c. It is exceedingly difficult to apply the chemical conceptions of Loeb or of Lillie to such facts. It is obvious that the self-sterility of the gametes of *Ciona* can also be analysed by a similar physical argument to hybridization.

Under normal conditions only one spermatozoon enters an egg. In view of the very large number of spermatozoa which may be in the immediate vicinity of the egg-surface at the moment of fertilization, it is almost inconceivable that any chemical change could be set up, and carried to a conclusion between the time that two successive spermatozoa touch the egg. Neither Lillie nor Loeb has offered what would seem to be a reasonable explanation of monospermic fertilization. Once more, the facts appear to be amenable to physical treatment. Assuming that the rate at which an electrical change can travel round the egg is approximately that at which it travels along a piece of smooth muscle then within 0.00001 sec. after the effective spermatozoon has made its contact with the egg, no other spermatozoon will have any effect: if, however, the eggs are treated in such a way as to reduce the rate of propagation of an electrical disturbance, e.g. by incomplete anaesthesia (cf. nerve or muscle), then the wave will not pass completely over the egg before other spermatozoa can effect contact with unaffected portions of the egg-surface, and polyspermy will result. Hertwig (17) showed that unfertilized eggs treated with chloral hydrate and other anaesthetics were markedly polyspermic.

The first visible sign that fertilization has occurred, is at the surface of the egg. In the case of the echinoderm or annelid egg, fertilization is attended by the formation of a 'fertilization membrane'. It must be remembered, however, that the essential change at the egg-surface is completed long before any visible change is possible. What is the nature of the fertilization membrane? In the case of the egg of *Nereis* it seems certain (Lillie, 20) that this membrane is the vitelline membrane of the unfertilized egg, which is pushed away from the egg-surface by the disintegration and hydration of the

egg-surface immediately under the vitelline membrane. In the case of the echinoderm egg it seems probable that essentially the same change takes place.

Many years ago Loeb (25) showed that the fertilization membranes collapsed when placed in sea-water containing albumen, and that on transference to normal sea-water the membrane regained its normal spherical shape. He concluded

TEXT-FIG. 1.

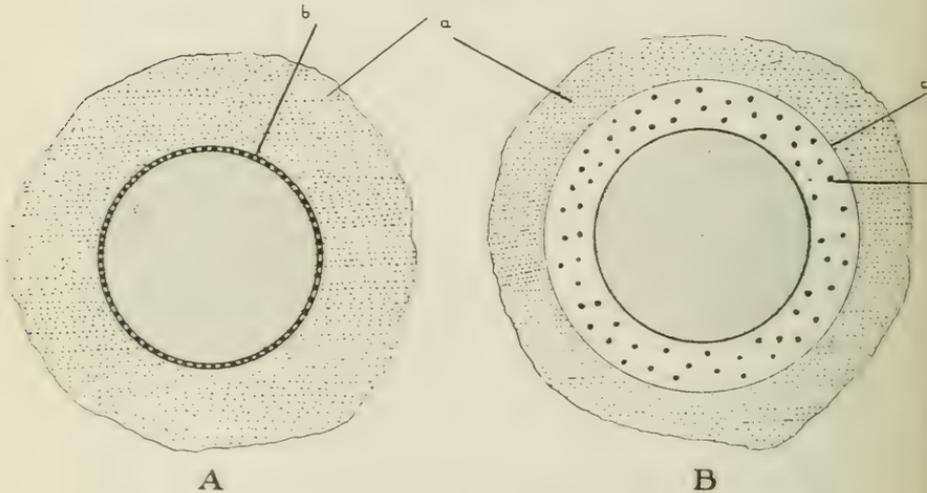


Diagram illustrating the origin of the fertilization membrane of *Echinus miliaris*. A. Unfertilized egg. B. Fertilized egg.
a, Zona pellucida containing an electro-positive colloid.
b, Vitelline membrane of unfertilized egg.
c, Fertilization membrane formed by the interaction of *a*, and an electro-negative colloid, *d*, which is set free when *b* is emulsified.

that the extrusion of the membrane was therefore due to the existence of an osmotically active colloid (proteid) within the fertilization membrane. Now the osmotic properties of such a colloid are markedly affected by the presence of hydrogen and hydroxyl ions: hence, if Loeb's conclusion be correct, the degree to which the fertilization membrane is extruded should be altered by altering the acidity or alkalinity of the sea-water. This is actually the case. If the water be made acid

the membrane remains close to the egg; the higher the alkalinity the more water is absorbed and the farther out is the membrane pushed.

<i>Ph.</i>	<i>Relative Extrusion of Membrane.</i>
9.2	121
7.6 (normal)	100
7.3	44
6.9	16

It seems tolerably clear, therefore, that the extrusion of the fertilization membrane is due to the existence of an osmotically active electro-negative colloid between the egg-surface and the fertilization membrane.

The origin of the fertilization membrane and of the enclosed colloid is, however, more difficult to determine.

The relative impermeability of the unfertilized egg to water and to all substances not soluble in oils or fats leads to the suggestion that, like the protoplasmic surfaces of many cells, the vitelline membrane contains a continuous lipid phase. Such a conclusion cannot be substantiated by direct experimental evidence, but, if it be correct, then many facts receive a reasonable explanation. It is, of course, well known that the formation of the fertilization membrane can be brought about by a great variety of artificial agents. Of these, the simplest and most efficient are saponin, benzol, fatty acids, esters, and soaps. All these substances are essentially emulsifying agents for a lipid surface in contact with water.¹

Since the use of fatty acids is most commonly adopted as a means of artificial membrane formation, it is interesting to analyse their action in detail. The process is as follows :

- (i) Unfertilized eggs are placed in 50 c.c. sea-water + 1.5 c.c. N/10 Butyric Acid for 1½ mins.
- (ii) They are then transferred to sea-water, containing a minimum concentration of hydroxyl ions.

¹ And for this reason these substances in higher concentrations destroy the normal protoplasmic surface of most cells: in other words, they are also 'cytolytic' agents.

Whilst in the acid solution no change, either visible or physiological, takes place, apart from the fact that the acid rapidly penetrates into the egg and can be detected by means of indicators (Gray, 8). It may be noted that in such a solution the activity of normally fertilized eggs is completely stopped.

<i>No. of c.c. N/10 Butyric Acid in 50 c.c. s.w.</i>	<i>Approx. Ph.</i>	<i>No. of Fertilized Eggs of Echinus miliaris which Segmented.</i>
<i>c.c.</i>		<i>Per cent.</i>
0	7.9	100
0.4	7.2	100
0.7	6.8	10
0.9	6.3	0
1.0	6.1	0
1.5	5.0	0

It is only when the unfertilized eggs are removed from the butyric-acid solution to sea-water containing a definite concentration of hydroxyl ions, that fertilization membranes appear; and that the physiological properties of the fertilized eggs are acquired and are carried on at a speed equal to that produced by normal fertilization. It is now that the eggs become more permeable to water (R. S. Lillie, 22) and to ions (McClendon, 25; Gray, 18), and that there is a marked increase in oxygen consumption. If the vitelline membrane be regarded as a continuous lipid film, then an interesting parallel experiment can be carried out by the emulsification of olive oil.

Olive oil+no fatty acid forms no emulsion with distilled water.

Olive oil+no fatty acid forms no emulsion with alkaline water.

Olive oil+fatty acid forms no emulsion with distilled water.

Olive oil+fatty acid forms a complete emulsion with alkaline water.

The process of artificial membrane formation and that of oil emulsification are dependent on the same factors: (a) the existence of a fatty acid in the oil, (b) the existence of a minimum concentration of hydroxyl ions in the neighbouring aqueous phase. In each case the resultant product shows a marked increase in permeability to water and to ions.

There seems, therefore, reasonable grounds for believing that the unfertilized egg is surrounded by a continuous lipid film, and that membrane formation occurs when this film is emulsified. When this occurs, however, we have seen that an electro-negative colloid is liberated; we must suppose, therefore, that either (a) this colloid exists in the lipid layer as a dispersed phase or (b) lies immediately between the lipid layer and the protoplasmic surface.

We have still to determine the origin of the fertilization membrane itself. Each unfertilized egg is surrounded by a wide gelatinous zona pellucida: this substance appears to be of a proteid nature—it is readily soluble in dilute acids, and so we may infer that it is electro-positive. If the zona pellucida is not removed before fertilization then the electro-negative colloid set free when the lipid layer of the vitelline membrane is emulsified will come into contact with a colloid of opposite electrical charge. Mutual precipitation must occur—and this, it is here suggested, is the origin of the fertilization membrane. If, however, the zona pellucida be removed prior to fertilization, then no fertilization membrane is formed (McCleendon, 25: Gray, 8). Nevertheless the egg develops normally.¹

The complete mechanism of 'membrane formation' may therefore be as follows:

The unfertilized egg is surrounded by two membranes—the hyaline vitelline membrane, and the gelatinous zona pellucida. The vitelline membrane consists of a continuous lipid structure, in which an electro-negative protein (*d*) [see Text-fig. 1] exists in solution as a dispersed phase (or below the lipid structure is a layer of electro-negative protein). The zona pellucida consists of an electro-positive protein. When the continuous lipid phase of the vitelline membrane is destroyed by emulsification, the enclosed protein (*d*) comes into contact

¹ Since writing the above I have found that a similar conclusion had been reached by McCleendon (see 'Internat. Zeit. für Phys. Chem. Biologie', vol. i, p. 163, 1914); although the eggs of *Toxopneustes* examined by this author appear to have the reverse charges on the respective colloids when compared with those of *Echinus*, which were used by the present writer.

with the zona pellucida, i. e. with a colloid of opposite sign. Mutual precipitation must occur, giving rise to the fertilization membrane. This membrane is impermeable to the remainder of the protein (d), which draws in water through the fertilization membrane by osmosis. In this way the fertilization membrane is extruded from the protoplasmic surface of the egg.

The evidence for such an analysis of membrane formation is strong. (i) There is no doubt that the protein within the fertilization membrane has an opposite charge to that of the zona pellucida. (ii) If the zona pellucida be removed prior to fertilization no fertilization membrane is formed, but the egg is activated in a perfectly normal way. (iii) By micro-dissection it can be shown that the fertilization membrane is much tougher than any membrane possessed by the unfertilized egg.

It must again be emphasized that the essential act of activation is the emulsification of the vitelline membrane, and not the formation of the fertilization membrane or the extrusion of the latter by absorption of water.

If the above analysis of 'membrane formation' be accepted, the question arises how can the spermatozoon act as an emulsifying agent? Loeb holds that the sperm introduces a specific 'cytolysin' into the egg-surface. The evidence is, however, against this view: (i) in order that artificial membrane formation may occur as quickly as a normal fertilization membrane, fairly high concentrations of emulsifying agents are necessary: it is quite impossible for one spermatozoon to introduce sufficient quantities into the egg. (ii) Membrane-forming substances are not in any way specific, whereas spermatozoa are markedly so. The only alternative seems to be that the spermatozoon emulsifies the egg-surface by a different means to that effected by artificial agents. The action of the spermatozoon is at first local, and evidence has already been put forward in support of the view that its activating action on the egg is essentially a physical process. The suggestion made is that the destruction of the surface lipid film is brought about by the spermatozoon electrolytically. Thin lipid films are, according to Hardy, electrically charged, and are sensitive to the electric current.

It seems likely, therefore, that a disturbance of the electrical properties of the film will result in a loss in the stability of the film. Such reactions are well known in the case of non-lipoid films, e. g. in rhythmical catalysis (Bredig, 1), and in the activation of passive iron¹ (Lillie, 23).

Before passing on to the later stages of fertilization in the echinoderm or annelid egg, reference must be made to the activation of the eggs of Amphibia. There are two methods of artificial activation, (a) by mechanical puncture, (b) by electrical stimulation. These facts seem to indicate most clearly that the process is essentially physical in nature. In the first case the egg is subjected to an injury current with an inevitable wave of negativity sweeping over the egg-surface. In the second case the electrical disturbance is set up precisely as in the stimulation of a muscle or nerve. The only difference is that no recovery process ensues. It seems almost impossible to harmonize these facts with the theory of Loeb, or with that of F. Lillie.

The beautiful experiments of F. Lillie (20) enable us to be quite sure that the changes induced in the echinoderm or annelid egg by artificial 'membrane formation' are precisely those changes which are set up by contact of the egg with a spermatozoon. Lillie showed that the cortical changes in the egg of *Nereis* are completed on contact between the egg and spermatozoon: if the latter be now removed by means of the centrifuge, the eggs undergo a series of changes essentially similar to eggs which have been activated by means of artificial membrane-forming agents. Development of the egg depends in each case on events which take place subsequent to this phase. The whole process of normal fertilization is divisible into two well-marked phases, (a) activation, due to cortical changes produced by contact with the spermatozoon,

¹ The activation of an egg and a piece of passive iron appear parallel phenomena; and in this respect the activation of echinoderm eggs by means of metallic silver (Herbst, 13) may warrant further investigation, although it is not clear whether the metal or one of its salts is the activating element.

(b) development, which usually depends on the exposure of the egg to hypertonic sea-water. Let us now consider the second or developmental phase of fertilization.

In the normally fertilized egg, the visible change which attends the second phase of fertilization, consists in the inclusion into the egg-substance of the head and middle piece of the spermatozoon; soon after this an aster appears in the vicinity of the sperm-nucleus. Eventually the male and female pronuclei, having approached each other, fuse together, and cell-division begins. We must suppose that as soon as the cell wall of the spermatozoon and that of the egg at the point of attachment are broken down¹ then the body of the spermatozoon will be very rapidly drawn into the egg by surface tension. On the mechanism of this process no experimental facts are available.

Once the sperm has been drawn into the egg, we can continue our experimental analysis. The experiment of Kupelwieser (19) shows that the sperm-nucleus plays no essential rôle, since although in certain cases it rapidly degenerates yet normal segmentation occurs: again in artificial parthenogenesis complete development takes place without any male pro-nucleus. Now, the only other visible structure which is associated with the male nucleus is the male aster. In Lillie's experiment with the centrifuged eggs of *Nereis*, it was found that eggs from which the sperm had been removed failed to develop a bipolar mitotic spindle; only one aster—the female aster—was present; otherwise the nuclear behaviour of the egg was normal. Again, in Kupelwieser's experiment, although the male nucleus degenerated the male aster remained functional. From a study of normal fertilization we therefore suspect that the developmental phase of fertilization is associated with the existence of two asters, one belonging to the female nucleus, and one introduced into the egg by the spermatozoon—or which comes into being when the male nucleus enters the egg.

¹ Until this occurs the two cells will remain essentially distinct from each other. Mere agglutination in a common matrix would not produce actual incorporation of the sperm and the egg.

Since the initial phases of fertilization and of artificial parthenogenesis are alike, and since the subsequent phase of development depends on the existence of a sperm-aster, is it possible that the process of artificial parthenogenesis can only be completed by treating the egg in such a way as to induce the formation of a second aster?

As is well known, eggs which have been subjected to 'membrane formation' will proceed to normal development if treated with hypertonic sea-water. The recent work of Herlant (16) shows clearly that such treatment does actually lead to the formation of a second aster. Without such treatment activated eggs behave in exactly the same way as eggs from which spermatozoa have been removed after normal initial activation.

The formation of accessory asters within fertilized, or within artificially activated eggs, when exposed to hypertonic sea-water, is now quite well established (Herlant, 16; Vles and Dragoiu, 29). Herlant has shown that one of these asters comes into communication with the female aster and forms a normal mitotic spindle in the case of artificially activated eggs; further, he has shown that the optimum conditions for accessory aster formation and the optimum conditions for development are exactly equivalent. When it is remembered that Morgan (26) and Wilson (30) showed that similar treatment led to the formation of asters within unfertilized eggs, it will be realized that the work of Herlant has thrown much light on the whole process of artificial parthenogenesis. We are now able to give a reasonable explanation of the fact that membrane formation may either precede or follow treatment with hypertonic sea-water. There is no need to postulate the 'corrective substance' of Loeb. Since the egg is more permeable to water after membrane formation than before, it is equally clear why treatment with hypertonic sea-water is more rapidly effective after membrane formation than before (Loeb).

We can, therefore, summarize the process of artificial parthenogenesis as follows. There are two phases. (i) An activation of the egg, by the destruction of a lipoid film at the surface.

This process raises all the physiological activities of the egg to the values reached in a normally active cell. (ii) A developmental phase, whereby the necessary machinery for development is introduced into the egg in the form of an artificially produced aster.

It has been stated, however, that the theory of fertilization advanced by F. R. Lillie is based on a very different series of facts to that of Loeb. Lillie's theory is based on the behaviour of the normal gametes. It is necessary for any alternative theory to cover the whole of the facts.

Lillie has shown that sea-water which has been in contact with the unfertilized eggs of the same species has a remarkable effect on the spermatozoa. Such sea-water contains a substance which (i) usually causes a marked increase in the activity of the spermatozoa, (ii) causes them to form macroscopic clusters—usually rounded in shape—of intensely active sperm, (iii) in some cases causes the spermatozoa to adhere to one another for a considerable time, in large immobile clumps. The essence of Lillie's theory is that fertilization is effected by the union of the egg and the sperm by this intermediate and specific substance (given out by the unfertilized egg) which Lillie calls 'fertilizin'. The sperm contains a substance which is agglutinated by fertilizin, and so the sperm becomes attached to the egg. Immediately fertilization has been effected the production of fertilizin ceases, and so no more spermatozoa can adhere to the egg.

I think it is a just comment to say that the above theory (with its marked analogy to the side-chain theory of Erhlich) does not purport to indicate the nature of the forces, physical or chemical, which underlie the various processes of normal fertilization. It does, however, stress the necessity of the existence of specific substances, 'fertilizin, &c.', without which union between egg and sperm is impossible; further, Lillie makes no attempt to extend his theory to the process of artificial parthenogenesis. Let us attempt to examine the properties of 'fertilizin' from a physico-chemical point of view.

The presence of fertilizin usually stimulates normal sperma-

tozoa to a high degree of activity: it is not, however, the sole means whereby an increased activity may be brought about. The same effect can frequently be obtained by exposing the spermatozoa to a slight increase of hydroxyl ions in the surrounding medium, in fact the activity of spermatozoa can readily be regulated by this means (Gray, 9; Cohn, 3); the gametes of *Luidia* have been frequently observed to show no activity when exposed to egg-secretions, whereas intense activity is aroused by hydroxyl ions (Gray, 11). Again, the sperm of *Sphaerechinus* will not fertilize the eggs of *Strongylocentrotus*, unless hydroxyl ions are added to the medium. These facts indicate that either (i) the efficiency of fertilizin depends upon the concentration of hydroxyl ions present, or (ii) 'fertilizin' is itself a weak base which stimulates the spermatozoon by virtue of its basic properties. The second hypothesis covers all the facts, has the advantage of simplicity, and is supported by the fact that the essential constituent of the egg-secretion is readily destroyed by acids, but not by alkalis.

Since, however, there is unanimous agreement that a certain degree of activity on the part of the spermatozoon is necessary for fertilization, let us consider the more unique effects of egg-secretions on the sperm, viz. aggregation and agglutination. There can be no doubt that the aggregation of the sperm into active clusters must be due to some effect which the sperm have on each other: the observations of Lillie on the spontaneous aggregations of *Nereis* sperm provide strong evidence that aggregation is due to the production of CO_2 and that the sperm aggregate at regions of optimum CO_2 tension. There is nothing to indicate that such clusters are due to any other cause than the clusters of protozoa which have been described by Jemings (12). It is, therefore, reasonable to suppose that whenever the activity of the sperm is sufficient to produce the critical amount of CO_2 in the medium immediately surrounding each sperm, then aggregations will form: naturally, they will only be temporary owing to (i) a gradual abatement in CO_2 production in an increasingly acid solution, (ii) a gradual

abatement of movement owing to the supply of available energy being used up within the cells.

Again, Loeb's comment on the significance of sperm aggregations is important. There is no evidence that a spermatozoon must take part in an aggregation before it can effect fertilization. Aggregation need be nothing more than an interesting corollary to the activation of the sperm by the egg-secretion.

Subsequent to forming active aggregations in water containing egg-secretions, the sperm may adhere to each other in dense masses (e. g. *Nereis*); in certain species no such agglutination takes place. A consideration of the agglutination effect of egg-secretions would involve a discussion of the whole mechanism of cell agglutination: such a discussion is not possible, but the lucid summary given by Buchanan (2) may be recommended to the notice of any who feel disposed to follow Lillie's argument of specific agglutinations. The fundamental fact is that agglutination depends primarily on the presence of free ions. This can readily be demonstrated in the case of spermatozoa or of eggs. The addition of a trivalent cation such as cerium causes a very marked agglutination (Gray, 9), which Lillie regards as comparable to the effect of heterofertilizin (i. e. the fertilizin of one species on the spermatozoa of another). Again, the addition of a small amount of sodium phosphate to normal sea-water causes a marked agglutination of the sperm of *Echinus miliaris*. These phenomena depend upon the deposition of an insoluble precipitate on the surface of the cells; in the case of cerium agglutination, insoluble cerium hydroxide (probably in the colloidal form) is deposited on the cell-surface and the cells adhere to each other by virtue of this common matrix—just as particles of oil are agglutinated by ferric hydroxide (Ellis, 6). Any substance which breaks up or dissolves this matrix reverses agglutination; thus acid dissolves $\text{Ce}(\text{OH})_3$ forming cerous chloride, while alkali or sodium citrate peptises or breaks up the film into non-coherent parts.¹

¹ It may be noted that this explanation differs from that offered in a previous paper (Gray, 9). The conditions for the deposition of such

Agglutination by sodium phosphate is somewhat different : in this case agglutination is due to the formation at the cell-surface of insoluble calcium phosphate. This agglutination only occurs in the presence of calcium ions, and is reversed by any substance which dissolves calcium phosphate, e. g. acids.

The parallel drawn by Lillie between the phenomena of agglutination of germ cells and those of bacterial cells is no proof that the agglutinative properties of the germ cells is an essential condition for fertilization. Both bacteria and germ cells exhibit the phenomena of spontaneous agglutination. The latter phenomenon has been described by the writer (8) for the eggs of *Strongylocentrotus lividus*, and the physiological properties of the cells indicates clearly that the same factors are involved as in experimental agglutination.

Apart from such considerations it does not follow that because the eggs give off a substance which causes spermatozoa to adhere to one another, the same substance will cause a spermatozoon to adhere to the egg. As pointed out elsewhere, a mere agglutination of the egg and sperm is an inadequate picture of the events which ultimately lead to the inclusion of the sperm into the cytoplasm of the egg ; it is only when the cell-membranes break down at the point of contact that an actual fusion can occur much as a small drop of orthotoluidine is drawn into a large drop of di-methyl-aniline (Darling, 4).

According to the present view, therefore, the only essential effect of egg-secretions upon the spermatozoa lies in the capacity of these substances to increase the activity of the sperm. In certain cases egg-secretions appear to have no effect on spermatozoa and yet fertilization readily occurs ; this fact is obviously explicable on the physical analysis outlined elsewhere in this paper.

SUMMARY.

1. The theory of artificial parthenogenesis put forward by Loeb meets with considerable difficulties when applied to the precipitates depends on the charge on the cell-surface, but the mechanical effect of flocculation seems certainly due to these precipitates acting as a common matrix for the cells.

facts of normal and hybrid fertilization. The theory of fertilization put forward by F. R. Lillie does not appear to be applicable to the facts of artificial parthenogenesis.

2. The facts of normal fertilization appear to indicate that the action of the spermatozoon on the egg is essentially of a physical nature.

3. Evidence is advanced in favour of the view that the activation of an unfertilized egg by a spermatozoon is due to the electro-motive force set up when the two gametes come into contact. The inert egg is activated by the spermatozoon in the same way as any other resting cell is activated when in intimate contact with an active neighbour.

4. After activation normal development only occurs if two asters are present in the egg. Under normal circumstances the second aster arises in the egg in conjunction with the male pro-nucleus; in artificially activated eggs the second aster arises when the egg is treated with hypertonic solutions.

5. In the case of the echinoderm egg the formation of the fertilization membrane is discussed. One essential step in the activation of these eggs is the removal of a continuous lipid film from the surface of the unfertilized egg.

6. The view is expressed that the only essential effect of egg-secretions on spermatozoa is the capacity of these substances, in certain cases, of increasing the activity of the male gametes.

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Calma Glaucoides: A study in adaptation.

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With Plate 11 and 3 Text-figures.

A DETAILED description of certain portions of the anatomy of a small British mollusc is here submitted, not so much as an extension of our knowledge of molluscan structure, as an account of the general biological interest of a unique metabolic type.

Whilst retaining the shape and general structural plan of an aeolidiomorph Nudibranch, *Calma* presents a combination of important departures from that type which may all be directly or indirectly referred to its specialized diet, namely, the eggs and embryos of the smaller shore fishes. So close is the external resemblance to the Aeolid that Alder and Hancock originally (1, Pl. xxii, letterpress) placed it in Cuvier's genus *Eolis*, whereas the modifications to be described are in some respects so great as to be comparable with those associated with a parasitic life.

The genus has been recorded only from European waters, and contains *Calma glaucoides* of Alder and Hancock, commonly taken at Plymouth, Roscoff, and Concarneau, the *Eolis albicans* of Friele and Hansen (5) from the North Atlantic, and the *Forestia mirabilis* of Trinchese (9) from the Mediterranean. All three will probably be found on re-examination to belong to one species, *C. glaucoides*.

At Roscoff, Hecht (6) found the animal feeding during June and July on developing eggs of *Cottus*, *Lepadogaster* and *Liparis* under stones, and in September in the swollen radical

sacs of *Laminaria flexicaulis*. The cavities of these sacs are rendered accessible to the smaller fish by the boring activities of various Prosobranchs, notably *Heleion*. The author collected *Calma* in August at Concarneau on *Lepadogaster* eggs in *Laminaria* sacs, while Dr. Allen reports that at Plymouth the animal is taken during the summer months on eggs of *Blennius ocellaris* and *Gobius minutus*.

EXTERNAL APPEARANCE (fig. 1).

When full grown *Calma glaucoides* is about half an inch in length, specimens obtained from Plymouth varying from 0.25 cm. to 1.3 cm. The body is depressed except when much distended with food. The very broad foot (0.3 cm. in large specimens) has a curved thick anterior rim, passing laterally into processes capable of extension. The rhinophores (*rh.*) and cephalic tentacles (*c.t.*) are smooth and of moderate length. Linear cerata, sometimes thrown into a pyriform shape by the contained food, are arranged in ten or eleven pairs of lateral groups, varying in number of cerata from four in front to two or one behind. The members of a group are not arranged in a row as in the *Aeolididae*, but irregularly, the smaller ones being ventral. In even quite young individuals the pale yellowish rosettes of the gonads (*g.*) can be seen in the angles between the bases of the groups of cerata, the grey centre of the rosette being the large male acinus, round which the numerous female acini are set. The pericardial hump (*pc.*) lies on the right opposite the space between the second and third groups of cerata, and with a strong lens the renal opening (*r.o.*) can be made out to the right of it in front. This was mistaken by Trinchese for the anal opening. With the exception of silvery white dots on the tentacles and cerata, representing gland-cells, and the opaque whiteness of the pedal glands which are especially richly distributed anteriorly, the integument is transparent, and the colour of the animal varies with the condition of the stomach contents and the gonads. In general, it is a dull greyish white in which the pigment of embryonic eyes may show as black dots. Hecht (*loc. cit.*) makes much of the protective value

of this inevitable colour resemblance of Calma to the spawn on which it feeds, and in one English text-book the argument for protective coloration is enhanced through careless quotation of Hecht, the fish spawn being thereby represented as laid on stones and roots of Laminaria. As, however, the spawn is laid under the stones and within the radical saes, the value of the colour resemblance seems very questionable, especially if the cause of it be also considered.

INTERNAL ANATOMY.

Although Alder and Hancock (1) had referred to the simple wide alimentary tract and the regular lateral repetition of the gonads, the only considerable description of the internal anatomy is that of Trinchese (9). Excluding certain errors such as the identification of the renal pore as the anus, and the saccular kidney as the great dorsal vein, Trinchese's account, so far as it goes, applies well to the British species. His description of the radula and the contents of the gut added to that of the external features places the generic identity of *Forestia* and *Calma* beyond question. In fact there appears to be no reason for giving the Mediterranean form separate specific rank. It is curious that Trinchese did not recognize the spheres which he saw in the semi-digested food as the lenses of embryonic fishes. Hecht (6) gives a faithful description of the kidney in its relation to the pericardium, but represents the former as extending to the end of the body, whereas in all the numerous specimens examined for this paper the kidney lay entirely in front of the seventh ceratal group. Sir Charles Eliot's revision of the genus (3 and 4) served to establish its generic character, to collect together the scattered *Calmas* of the literature and to exclude from among them *Calma cavolini* of various authors which possesses none of the special anatomical characters of a *Calma*. He emphasized the peculiar nature of the radula, the great size of the stomach, the absence of enidosaes, and the mode of grouping of the gonadial units as modifications correlated with the specialized diet. To him the author's thanks are due for an introduction

to Calma. In the account that follows the digestive system claims first place, not only because it shows the most extensive aberration from type but because it provides the key to the meaning of other topographical and histological changes.

The Digestive System.—The facial aspect of the animal in repose is rather flat and directed forwards and downwards. Below the middle of its smooth surface is a conical depression leading to the small oval mouth. In the act of eating the face fits like a hood on the egg and is capable of considerable extension. In this position the animal looks very aggressive, especially when the pressure which results in the swallowing of the embryo is exerted. In the meanwhile the narrow odontophore bearing the saw-like radula has been protruded into the oral opening and the act of slitting the egg-membrane performed. This muscular odontophore (fig. 2, *rad.*), which is very narrow at the protruded edge, is broadly based behind and on the floor of the buccal cavity. It is covered by a cuticle which is continuous under the radula and with the general buccal lining. Laterally the buccal cavity is largely occupied by a pair of muscular pads bearing smooth jaws, which are local thickenings of the buccal cuticle (*j.*). These come into action in the act of swallowing. Between them is a strongly cuticularized ventral groove in which the odontophore moves. The groove continues forward into a cavity in the ventral lip which acts as a reservoir for the very massive buccal glands.

Previous descriptions of the radula (fig. 3) have represented it as a continuous ribbon finely serrated at the edge like a bent saw, thus contrasting it strongly with other uniseriate radulae consisting of separate teeth carried on a basal dentigerous strip. The examination of transverse sections, however, shows that the profile view obtained in potash preparations is misleading, and that this radula is less of a neomorph than was supposed. It is constructed on the fret-saw principle, teeth not unlike those of the Acolids being borne on a stout bent cylindrical rod (*b.r.*) secreted by the bed-cells of the radular sac. The teeth are as usual formed by the roof-cells of the sac and sit

closely equitant on the rod. In sections stained with iron haematoxylin and acid fuchsin this rod, which is the homologue of the basal membrane of other radula, takes the acid dye, while the teeth are a deep black. Even in potash preparations the faint lines of demarcation of the individual teeth can be made out under an oil immersion lens (see fig. 4). To the stoutness of the basal rod is due the fact that the radula is always obtained complete and undistorted in preparations, as well as its efficiency as a cutter of membranes. The most interesting feature of the Calma radula, however, is the preservation at its anterior end of the small first-formed teeth to the number of four or more. These minute persistent teeth (fig. 4, 1-5) are spaced out on their thin basal membrane and closely resemble those of the uniseriate radula of Favorinus, being without lateral denticulations. The basal membrane is continuous in front with the thick rod of the later radula. Between this early normal Aeolidian radula and that of the adult is a gap in which the dentigerous strip is already thickening, but the teeth themselves are imperfect. Numbers 5 and 6 of the figure look like imperfect Aeolidian teeth, while the remainder of the gap contains irregular serrulations suggesting the incipient adult structure. As this sequence is remarkably constant, it is evident that here in the radula of Calma we have a concise record of the change that occurs in the feeding methods of the animal, for it is unimaginable that the minute adult at the beginning of its career is capable of feeding on the eggs of fishes. It is still more interesting as the preserved record of the evolution of the Calma type from a more generalized carnivorous Aeolid.

The post-bulbar salivary glands (Text-fig. 1, *s.g.*) consist of a pair of simple tubes, the walls of the distal part of which contain very large granular cells. These bulge out singly or in groups of two or three, and their cell-contents stain deeply with the basic dyes. The salivary ducts pass through the nerve-ring to open into the buccal cavity at the posterior ventral edge of the lateral pads.

The oesophagus is short and narrow, but its walls are

thrown into longitudinal folds so that whole fish embryos pass through it unmutilated. Good serial sections of these are often obtained in microtome preparations.

The rest of the alimentary system consists of a spacious bag (Text-fig. 1, *g.s.*) extending to the end of the body together with its glandular diverticula into the cerata. In a well-fed specimen

TEXT-FIG. 1.

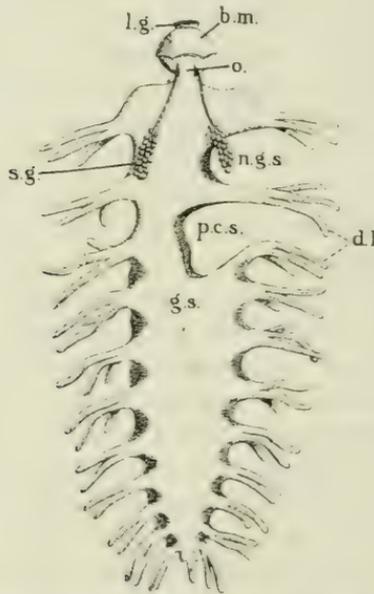


Diagram of the alimentary system. *b.m.*, buccal mass; *dl.*, hepatic diverticula; *g.s.*, gastric sac; *l.g.*, labial glands; *n.g.s.*, space occupied by the nidamental part of the oviduct; *p.c.s.*, space occupied by the pericardium and anterior part of the kidney; *o.*, oesophagus; *s.g.*, salivary gland.

this sac is so distended as to displace such loose structures as the salivary glands and the male duct into the head region above the brain and buccal mass, while the swollen ceratal diverticula may give the cerata an ovoid shape. The appearance of a common ceratal stalk observed by Alder and Hancock and suggested as a characteristic of *Calma* is also a temporary result of distension. On the right the sac is deeply constricted

and indented by the pericardium (*p.c.s.*) and anteriorly to that by the ootype (*n.g.s.*). Thus two chambers are connected by a narrow tube, but neither their histological structure nor their function justifies their being regarded as other than mechanically separated portions of a continuous food reservoir. The lining-cells of the gastric-sac are low and flat even when it is more or less empty, while the cells of the ceratal diverticula are very large and extensively vacuolated during active digestion. A comparison of these hepato-pancreatic cells in the active state (fig. 5, *d.c.*), and the tenuous squames that line the full stomach (fig. 8), strongly suggests that the former are responsible for the bulk of the digestive juices in Calma. The fish embryos (fig. 8, *s.e.*), whether very young or considerably developed when eaten, are, however, partly disintegrated in situ in the stomach, probably by enzymes delivered from the ceratal glands (fig. 8, *d.l.*), which at this stage are uninvaded by the food. Later the stomach contains a semifluid mass in which lenses of eyes (fig. 8, *l.*) and scattered lumps of undigested nuclei are the only remaining evidences of the nature of the food. During further digestion this thick fluid is continuously delivered into the cerata, where it undergoes solution (fig. 9, *d.l.*). An animal fixed at this stage is difficult to cut on account of the extremely hard consistency of the precipitated proteins. The gland-cells of the ceratal outgrowths (fig. 5, *d.c.*) project deeply into the food; no evidence of ingestion could be found, though fine brown granules similar to the eventual residuum in the whole system accumulate in them and are extruded into the lumen.

There is no trace of anus or intestine. The small amount of undigested matter remains as a dark-brown core (fig. 9, *d.*) throughout the alimentary system, so that the shape of this system can be made out in a fasting animal by clearing alone. On account of a certain amount of compression of this faecal residue during the fast there is no admixture with a subsequent meal. In connexion with the digestive system must be mentioned certain special connective-tissue cells (figs. 8 and 9, *c.s.*) of the cerata which differ widely from those of the rest of the

body in structure and function. These, while retaining their connecting processes and their position as lining-cells of the ceratal blood-spaces (fig. 5, *b.s.*), are at times among the largest cells in the body and exhibit remarkable secretory activities during the period of digestion of a meal in the neighbouring gut diverticula. At the same time they increase greatly in size till, finally, their identity as cells of the connective tissue is obscured, and only a thin envelope continuous with the processes (fig. 6, *e.*) is free from accumulated secretion staining deeply with basic dyes. Simultaneously with the deposition of stainable material in the cytoplasm, a clear non-staining sphere (fig. 6, *n.b.*) grows within the nucleolus, which in fixed tissue is so hard as to be frequently displaced or torn out by the microtome knife. In specimens with empty stomachs these cells are found in various stages of reduction in size, an early stage of reduction and solution of the deutero-plastic contents being shown in fig. 6, while fig. 5 shows normal, faintly granular cells in which the nucleolar body is absent. Hecht (*loc. cit.*) notifies these cells as 'cellules spéciales', the significance of which he discusses without offering a final judgement. He draws them as loose cells and seems not to have recognized their essential conjunctive nature, but compares them with the large rounded or oval cells found in the ceratal connective tissue of *Galvina* and other *Aeolidiomorpha* previously described by Herdman (7). Comparison of sections of animals at different stages of the alimentary cycle appears to provide convincing evidence that both the secretum of the cell-body and the refringent spherule of the nucleolus grow during digestion and disappear during a fast. On account of their structure, the readiness with which they take up both basic and acid dyes, their position, on the one hand close to the absorptive cells of the gut, and on the other on the walls of the blood-spaces, and lastly on account of the significant variation of their contents during a digestive cycle, it is here proposed to regard them as protein storage cells. The agreement in phase between the granular deutero-plasm and the nucleolar secretion is in keeping with this explanation, and

suggests a zymogenic character for the latter. The cell of fig. 6 is on the metabolic down-grade; the more centrally placed secretion has been brought into solution, and the streaming enzyme from the nucleus has also attacked the periphery. The necessity of means of storage must be present in all organisms depending on a precarious food supply, but a peculiar spatial relationship exists in *Calma* between gut and gonad, which makes it advisable to postpone the discussion of the utility of these cells until after the reproductive system has been described.

The Nervous System (fig. 7) resembles closely that of *Facelina* and other *Aeolids* with uniseriate radulae. The following points are to be noted :

- (1) The large dorsal ganglia (*cp.g.*) contain the cerebral centres and all the ganglionic elements of the visceral commissure. The short unbeaded visceral loop (*v.l.*) gives but one visceral nerve which sends a branch to the gastro-oesophageal anastomosis and continues into the reno-cardiac plexus, and probably the gonad.
- (2) The rest of the reproductive system is innervated from a stout nerve (*g.n.*) arising from the right dorso-pedal connective. This nerve consists of fibres derived chiefly from the dorsal ganglion, and some pedal fibres.
- (3) There are large rhinophorial ganglia (*rh.g.*), and the optic ganglia (*o.g.*) are also outside the dorsal mass.
- (4) The parapedal commissure (*pp.c.*) is distinct from the pedal.
- (5) The eyes and statocysts (*ot.*) are placed, as in most *Aeolididae*, dorso-laterally in the angle between the pedal and dorsal ganglia.

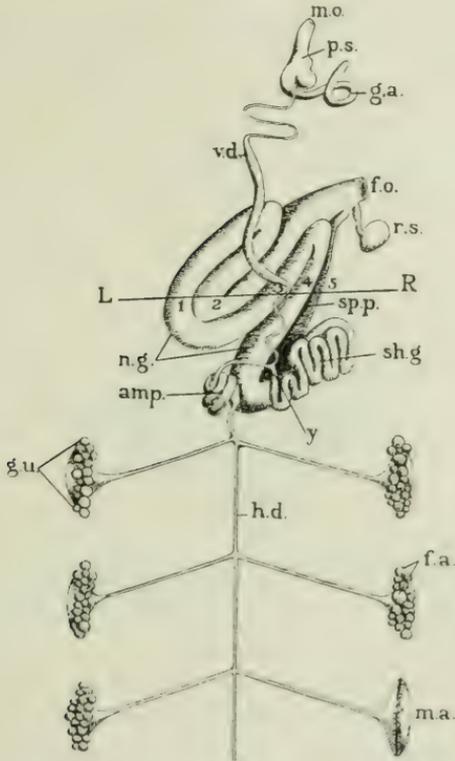
The vascular system does not call for special description, and the renal and pericardial coelomic spaces have, as Hecht (*loc. cit.*) has shown, the normal relations and openings, but the kidney is unusual in being a simple dorsal sac extending backward from the pericardium to the level of the sixth or seventh ceratal groups.

The Reproductive System (Text-fig. 2).—The works of Alder and Hancock, Bergh and Trinchese provide an abundance of surface views of incompletely dissected reproductive systems of the Aeolidiomorpha, but the complexity of the oviducal glands (ootype) is such that none is satisfactorily described. The attempted reconstruction of the oviduct of *Doto fragilis* by Dreyer (2) shows how a tangle may be made worse by this method. In *Calma*, however, the structure of the ootype is so simple that a little displacement of its parts, aided by reference to serial sections, is sufficient to disclose its mode of formation. With the knowledge thus gained as key it was found that the much more intricate ootypes of Aeolidia, *Antiopella*, and *Pleurophyllidia* are elaborations of the same general plan.

As in Aeolidiomorpha generally, the gonadial unit consists of a large central male acinus, bearing a number of female acini, first as solid outgrowths, later as hollow diverticula of its wall. Here, however, the gonadial units (*g.u.*) are not as elsewhere united together into a compact mass, but serially distributed in all the interceratal spaces except the first. Thus six to eight pairs are present according to the size of the animal. Paired efferent ducts (*e.d.*) lead into the spermoviduct, which swells into a coiled ampulla before the bifurcation into male and female ducts at *y*, Text-fig. 2. The vas deferens (*v.d.*) is very long, with a thick, glandular wall in its middle portion. All other Aeolidiomorpha have their male and female openings approximated in a common atrium, except *Fiona*, but the male opening of *Calma* is placed in front of and below the level of the right rhinophore (see fig. 1), while the female opening occupies the usual position between the first and second ceratal groups on the right. It is curious that, along with this primitive position, the male organ itself has a primitive structure reminiscent of the Bullids, especially *Haminea*. It is a partial pleurembolic introvert, the penis sac in the retracted condition containing the unchanged apex of the penis as a conical papilla on which opens the vas deferens. Close to the end the deferent duct receives that of a long tubular gland (*g.a.*) resembling the

organ named prostate in the Bullids. When retracted this gland lies along with the terminal coils of the vas deferens tucked away in the head above the brain, but is partly drawn

TEXT-FIG. 2.



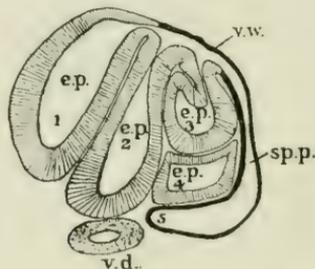
Dissection of the reproductive system of a small, nearly mature individual. *amp.*, ampulla of *h.d.*, the sperm-oviduct; *m.a.* and *f.a.*, male and female acini; *m.o.* and *f.o.*, male and female openings; *g.a.*, glandular appendage of the penis; *g.u.*, genital unit; *n.g.*, nidamental gland; *p.s.*, penis sac; *r.s.*, receptaculum seminis; *sh.g.*, shell-gland; *sp.p.*, sperm-path from the receptaculum to the commencement of the oviduct at *y*; *v.d.*, vas deferens; 1.2.3.4.5, coils of the oviduct similarly numbered in Text-fig. 3.

into the everted penis, giving it its shape and firmness. The large cells lining it contain a clear secretion which, unlike the granular contents of the prostatic cells of the vas deferens.

takes up no dyes, acid or basic. The fact that no other Aeolidio-morph possesses such a glandular appendage of the penis adds to the interest of this coincident acquisition of primitive position and structure.

The female duct is in very young specimens a straight broad tube leading from the bifurcation of the sperm-oviduct to the exterior. During growth this is differentiated into a dorsal, much-coiled oviducal passage and a ventral straight pathway for the introduced spermatozoa. The dorsal coils (1.2.3.4 and *sh.g.*) are folds of the greatly enlarged female duct, and in the figure are shown to form a continuous tube. In

TEXT-FIG. 3.



Section through the female complex at LR in Text-fig. 2. *e.p.* 1.2.3.4, folds of the oviduct through which the eggs pass; 5, path of sperm migration downwards; *v.w.*, thin ventral wall of the oviduct; *v.d.*, vas deferens.

surface view the coils project as two bulges, one on the left in front and the other behind and on the right. The former has been by common consent called mucus or nidamental gland, and appears in pickled specimens of the Opisthobranchs as a brittle white mass, swelling in water, while the latter is named albumen gland. Many authors have stated or conveyed the impression that these lobes are dependent glands opening by ducts into the oviduct and pouring their secretion on the eggs as they pass. The long continuous tube here described, however, comprises both lobes, and is actually the functional oviduct through which the eggs pass, and in which they receive the successive layers of nidamentum. The first or posterior lobe (*sh.g.*) is composed of a coiled portion of the tube which is

opaque in life and more slender than the rest. In it single eggs or small groups of eggs receive a separate investment of a substance giving the chemical tests for mucin. This is at first laid on in a fluid condition, while the later layers are dense and firm. Shell-gland would therefore perhaps be the appropriate name for this portion. In the rest of the oviducal passage (*n.g.*) which is pellucid in the living animal, the eggs in their shells are enclosed in the substance of the nidamental ribbon, also a mucin. The outer layer of this, like that of the shells, is firmer and denser than the rest. Hitherto we have considered only those changes that affect the dorsal wall of the original oviducal sac, and result in the formation of a twisted egg-passage (see also Text-fig. 3, *e.p.*). The ventral wall (Text-fig. 3, *v.w.*) remains flat, thin, and non-glandular. Distally, near the atrium, the flask-shaped receptaculum seminis (Text-fig. 2, *r.s.*) is formed as an evagination of it. From the atrium it extends back as the floor of a broad, shallow chamber (*sp.p.*) which narrows as it becomes continuous behind with the initial part of the female duct at the point of departure of the functional oviduct (*y*). The impression so far conveyed is that the original sac-like female duct has been divided into two passages by a process resembling the pinching off of the vertebrate semicircular canals, namely, a long coiled dorsal one, ciliated and glandular for outgoing eggs, and a short thin-walled ventral one for incoming sperms from the receptaculum, which is neither ciliated nor glandular. Such a complete female diauly is, however, not strictly true. Text-fig. 3 of a section through the nidamental region in the plane LR in Text-fig. 2 shows that three (1.2.3) out of the four oviducal loops thus cut across are incompletely separated from the vaginal chamber below, while the fourth or proximal loop (4) is a discrete tube. Thus for a considerable length of ribbon-forming oviduct a facultative but not a structural diauly is present. The tube of the shell-gland is, however, completely separated except at its commencement, as mentioned above. This is essential since it deals with loose eggs, or with eggs receiving a fluid envelope. The continuity at *y* of the undivided oviduct with the vaginal

channel (*sp.p.*) is also essential for the passage of the backwardly migrating spermatozoa. It is, therefore, seen that dialy of the female duct in *Calma* is just so far expressed as to constitute passages that are functionally efficient.

The foregoing rather detailed account of the female reproductive ducts, though a digression from the main thesis of the paper, has been introduced because the supposed female monaul of the Aeolidiomorpha forms an important item in the definition of that group.

The reproductive system as a whole presents three anatomical departures from the Aeolidian type, namely (1) the displacement of the male opening and its accessories into the head, (2) the substitution for a muscular penis of one which derives its bulk from a gland which grows at sexual maturity, and (3) the breaking up of the massive aeolidian gonad into serially arranged pairs of gonadial units which are so placed as to intrude least on the body-space available for distention of the alimentary system. They are, moreover, placed where digestion of a meal begins, and in the path of food-laden blood from the cerata, so that their growth proceeds *pari passu* with the adjacent reduction in bulk of the stomach. All three modifications may be regarded as correlated with the demand for space to receive the maximum meal when food is plentiful. The last further enables *Calma* to replace that meal by its own enlarging gonads with the greatest structural convenience. Figs. 8 and 9 are sections through the interceratal regions of two animals of similar size at nearly opposite poles of the metabolic cycle. In fig. 8 digestion has begun, the gonads are small, and the special cells (*c.s.*) in the cerata are almost at minimum size, while in fig. 9 the meal has largely disappeared, the special cells are greatly enlarged, and the ovarian acini (*f.a.*) are distended with nearly full-grown eggs. In this specimen the black granular remains (*d*) of a previous meal are also visible on the floor of the gastric sac. As animals of various sizes are found in both of these metabolically antithetic conditions, it is almost certain that the rhythmic succession of alimentary and reproductive activity takes place several times in the individual lifetime. Dr. Orton (8) has shown by

his raft experiment that the life-cycle of such a much less advantageously placed animal as *Galvina* is far shorter than had been imagined. What must it be then in a case where food is plentiful if found at all, and its nutritive value so great that a hind-gut is useless; where, moreover, the chemical constitution of the food and the gonad which it nourishes must be so similar as to reduce the entailed metabolic conversion to a minimum? In fact, such an economical metabolic system is equalled only by parasites that absorb the gonads of their hosts.

As to the special cells of the cerata, the supposition that they act as reservoirs of food during a fast is supported by the incidence of their periods of growth and diminution, while the fact that only the connective tissue cells of the cerata are so employed agrees with the principle observed throughout in *Calma*, namely, that the whole available body-space should be reserved for the alternation of food and gonad.

GENERAL CONSIDERATIONS.

The *Aeolidiomorpha* are all carnivorous, and the *Aeolididae* all eat *Cocelenterates*. The smaller ones live on *Hydrozoa*, but supplement that diet by eating other members of their own and neighbouring species or their eggs. Such are *Facelina* and *Favorinus*, and it was among these most probably that *Calma* arose, and, in spite of its extensive aberration from type, it is to be hoped that no systematist will think fit to separate it from them. The contours of the body are still typically *Aeolidian* in detail. Examination of the least plastic of bodily systems, the nervous system, by itself would place *Calma* in the genus *Facelina*. During the precarious early days of settling down on the sea bottom it is highly probable that the little animal actually uses its initially uniseriate *Aeolidian* radula as a generalized carnivore. All the departures from the *Aeolididae* in the structure of the alimentary and other systems have been shown to be closely associated with the adoption of a diet different from and even more specialized than that of its polyp-eating relatives. In doing this it provides an exception to the rule that, as Dr. Willey expresses it, the adoption of a specialized diet marks the culmination of a phyletic career.

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EXPLANATION OF PLATE 11.

Fig. 1.—Dorsal view of the mature animal. *a.m.f.*, anterior margin of the foot; *c.t.*, cephalic tentacle; *E.*, eye; *g.*, gonad seen through the integument; *pc.*, pericardium; *pe.*, extruded penis; *rh.*, rhinophore; *r.o.*, renal opening.

Fig. 2.—Buccal mass laid open dorsally by turning back the flap *f.* *j.*, muscular pads thinly chitinized, called jaws when the chitin is locally thickened; *m.*, mouth; *o.*, gullet; *rad.*, odontophore with radula.

Fig. 3.—Side view of the radula. *b.r.*, basal dentigerous rod.

Fig. 4.—Anterior end of the radula, highly magnified. 1.2.3.4.5, the primary teeth. Note the reduction in size from 1 to 4, and the loss of shape at 5.

Fig. 5.—Part of a section of a ceras. *a.g.*, epidermal gland-cell with contents taking acid dyes; *b.g.*, ditto taking basic dyes; *b.s.*, blood-space; *c.c.*, ciliated cell of the epidermis; *c.s.*, connective-tissue cell; *c.t.*, dense dermal connective tissue; *d.c.*, digestive cell; *f.*, food with vacuoles; *m.f.*, muscle-fibres.

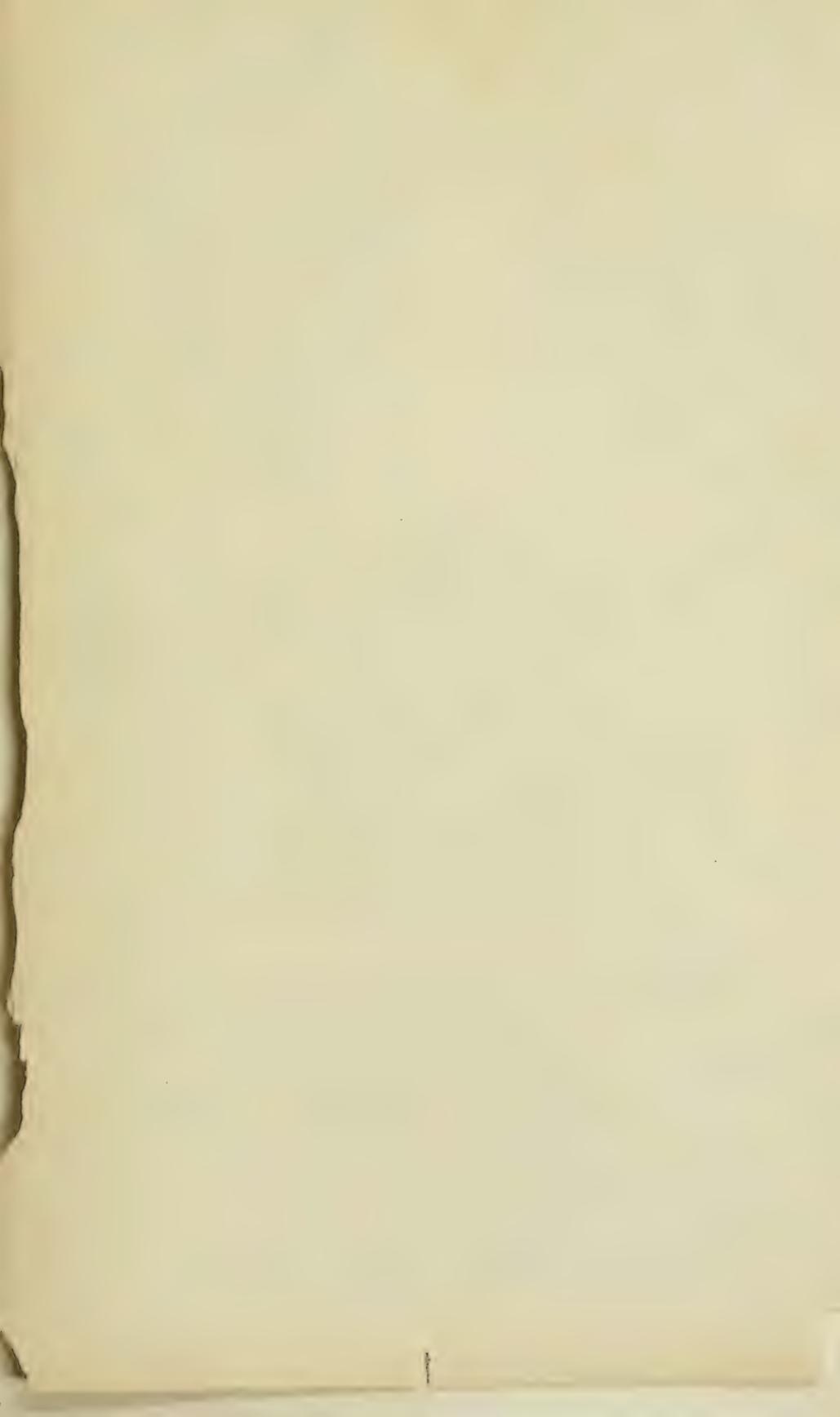
Fig. 6.—A connective-tissue cell, special cell of Hecht, during solution of its contents. *e.*, clear envelope free from granular material; *n.b.*, nucleolar body.

Fig. 7.—Central nervous system. *cp.g.*, cerebro-pleural ganglion;

p.g., pedal ganglion; *st.g.*, stomatogastric ganglion; *o.g.*, optic ganglion; *g.o.g.*, gastro-oesophageal ganglion; *rh.g.*, rhinophorial ganglion; *a.p.v.*, anterior pedal nerve; *b.m.n.*, nerve to buccal mass; *b.u.n.*, nerve to the lips; *E.*, eye; *g.n.*, genital nerve of cerebro-pleural origin, continuing beyond the genital complex as a pleural nerve; *n.c.t.*, nerve to cephalic tentacle; *ot.*, otocyst; *p.c.*, pedal commissure; *p.p.c.*, parapedal commissure; *pl.n.*, pleural nerves, innervating the cerata and the dorsal body-wall; *p.n.*, pedal nerves (*N.* no branches to the cerata from these were discovered); *rh.n.*, rhinophorial nerve; *s.n.*, nerve to salivary duct.

Fig. 8.—Section through an interceratal space of an animal killed shortly after a full meal.

Fig. 9.—Ditto of an animal of the same size taken when the meal was nearly all digested. *a.v.* and *e.v.*, afferent and efferent veins of the ceras; *c.s.*, the special cells of the ceratal connective tissue; *d.*, residual faecal mass on the floor of the gastric sac; *d.l.*, hepatic diverticulum; *f.a.* and *m.a.*, female and male acini; *h.d.*, sperm-oviduct; *pl.n.*, pleural nerve; *p.n.*, pedal nerve; *v.*, median dorsal vein leading to the auricle.



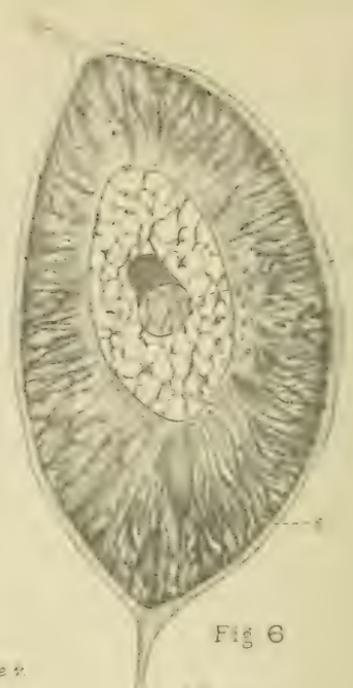
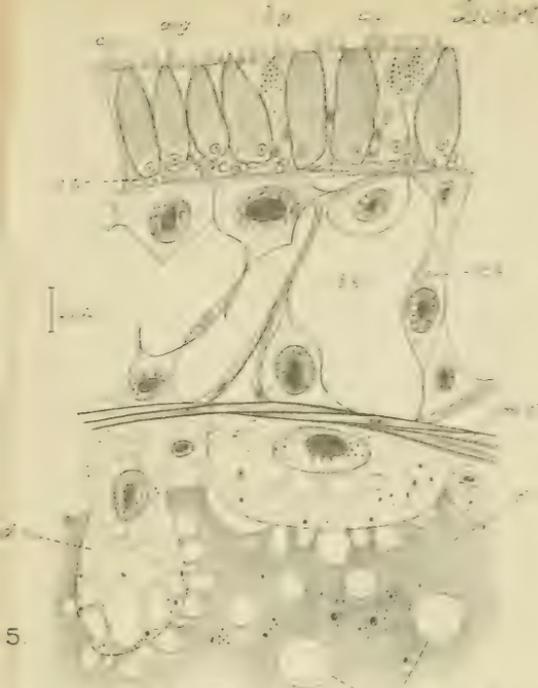


Fig 6



Fig 6



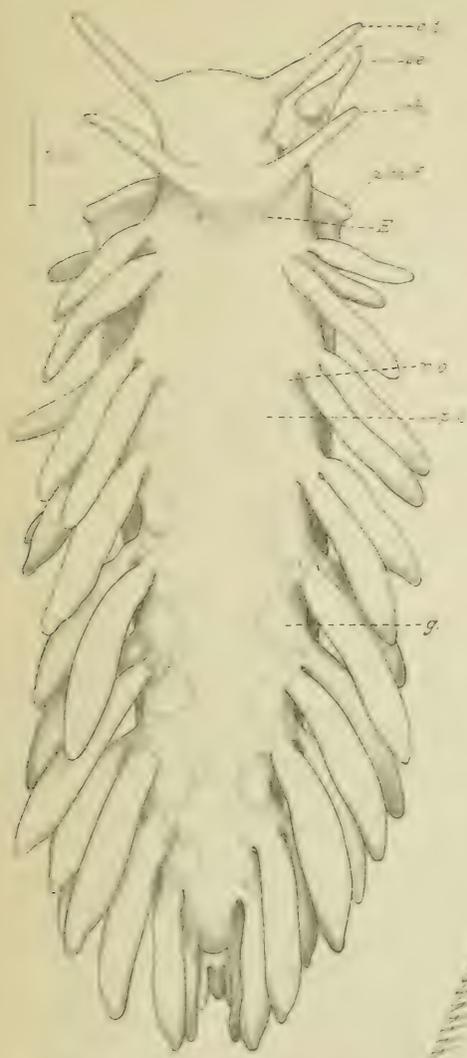


Fig. 1.

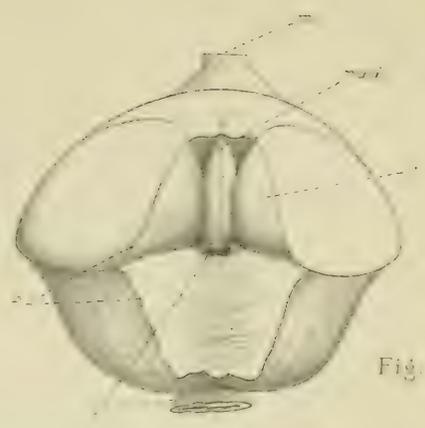


Fig. 2.

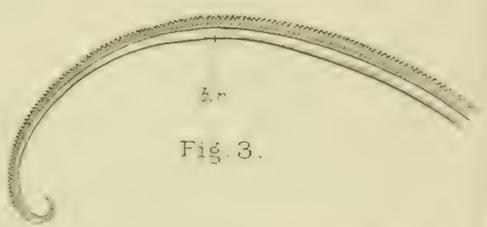


Fig. 3.



Fig. 4.

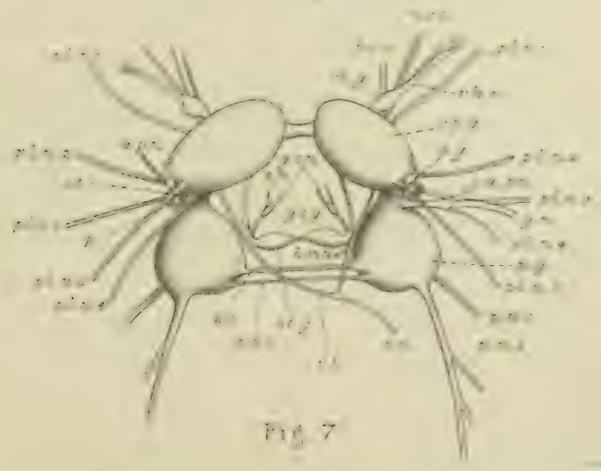


Fig. 7.

The Segmentation of the Head in *Squalus acanthias*.

By

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With 13 Text-figures.

THERE are two views with regard to the segmentation of the head. One has arisen out of Balfour's (1) pioneer work, the other is due to Van Wijhe (18). They both agree on many points, and the difference between them lies in the interpretation of the numerical relations of the different segmented structures, as pointed out by Goodrich (8). In order that they may be compared, a short account of these views will now be given.

Balfour, in his classical 'Development of Elasmobranchs', expressed the opinion that the six visceral clefts are related to six consecutive somites situated dorsally and posteriorly to each respective cleft, the clefts being intersomitic. In front of the spiracle he recognized two somites (premandibular and mandibular), so that in all, from the anterior extremity to behind the last gill-slit, there are eight somites, of which the six posterior are simply and harmoniously related to six visceral arches and clefts.

The recognition of the nature of the cranial nerves is due to the work of Marshall and Van Wijhe. Five nerves are regarded as dorsal roots, viz. ramus ophthalmicus profundus, trigeminal, facial, glossopharyngeal, and vagus, the latter being really compound and probably representing four segments. There are then eight dorsal nerve elements, and if each is related to

one of Balfour's eight somites the following relations will result, as shown in Table I (ventral roots also included).

This is the theory which has grown out of Balfour's work, and the somites bearing the relations described above may be called Balfour's somites. Among the supporters of this view are Ziegler (19), Koltzoff (10), Goodrich (8).

TABLE I.

<i>Segment.</i>	<i>Dorsal Nerve.</i>	<i>Ventral Nerve.</i>	<i>Somite.</i>	<i>Visceral Arch.</i>	<i>Visceral Cleft.</i>
1	R.O.P.	Oculomotor	Premandibular	—	—
2	Trigeminal	Patheticus	Mandibular	Mandibular	Spiracle
3	Facial	Abducens	Hyoid	Hyoid	Gill-slit 1
4	Glossopharyngeal	—	4th	3rd	Gill-slit 2
5	Vagus 1	Hypoglossus	5th	4th	Gill-slit 3
6	Vagus 2	Hypoglossus	6th	5th	Gill-slit 4
7	Vagus 3	Hypoglossus	7th	6th	Gill-slit 5
8	Vagus 4	Hypoglossus	8th	7th	
9	1st Spinal	—	9th	—	

TABLE II.

<i>Segment.</i>	<i>Dorsal Nerve.</i>	<i>Ventral Nerve.</i>	<i>Somite.</i>	<i>Visceral Arch.</i>	<i>Visceral Cleft.</i>
1	R.O.P.	Oculomotor	Premandibular	—	—
2	Trigeminal	Patheticus	Mandibular	Mandibular	Lost
3	Facial	Abducens	Hyoid	Hyoid	Spiracle
4	Facial	—	4th	Hyoid	Gill-slit 1
5	Glossopharyngeal	—	5th	3rd	
6	Vagus 1	—	6th	4th	Gill-slit 2
7	Vagus 2	Hypoglossus	7th	5th	Gill-slit 3
8	Vagus 3	Hypoglossus	8th	6th	Gill-slit 4
9	Vagus 4	Hypoglossus	9th	7th	Gill-slit 5
10	1st Spinal	—	10th	—	

But the majority of authors have followed Van Wijhe (18) in the interpretation of the relations. He regards the facial nerve as really of double-nature, the existing nerve being related to the fourth somite while the nerve of the third somite has either disappeared or become merged with that of the fourth. The relations of these somites (Van Wijhe's somites)

are shown in Table II. Braus (2), Hoffmann (9), Sewertzoff (17), and Neal (11) adopt this view.

The difference between the two interpretations is centred in the region of the spiracle and hyoid arch. On the first view all the segmented elements are harmoniously and consecutively related without gaps or discrepancies: on Van Wijhe's there is one somite too many in the region of the spiracle. For since the mandibular or second somite corresponds to the first (mandibular) visceral arch, if the second (hyoid) arch corresponds to the fourth somite, as Van Wijhe supposes, then the third somite has no arch or cleft. Van Wijhe suggests that these have been lost.

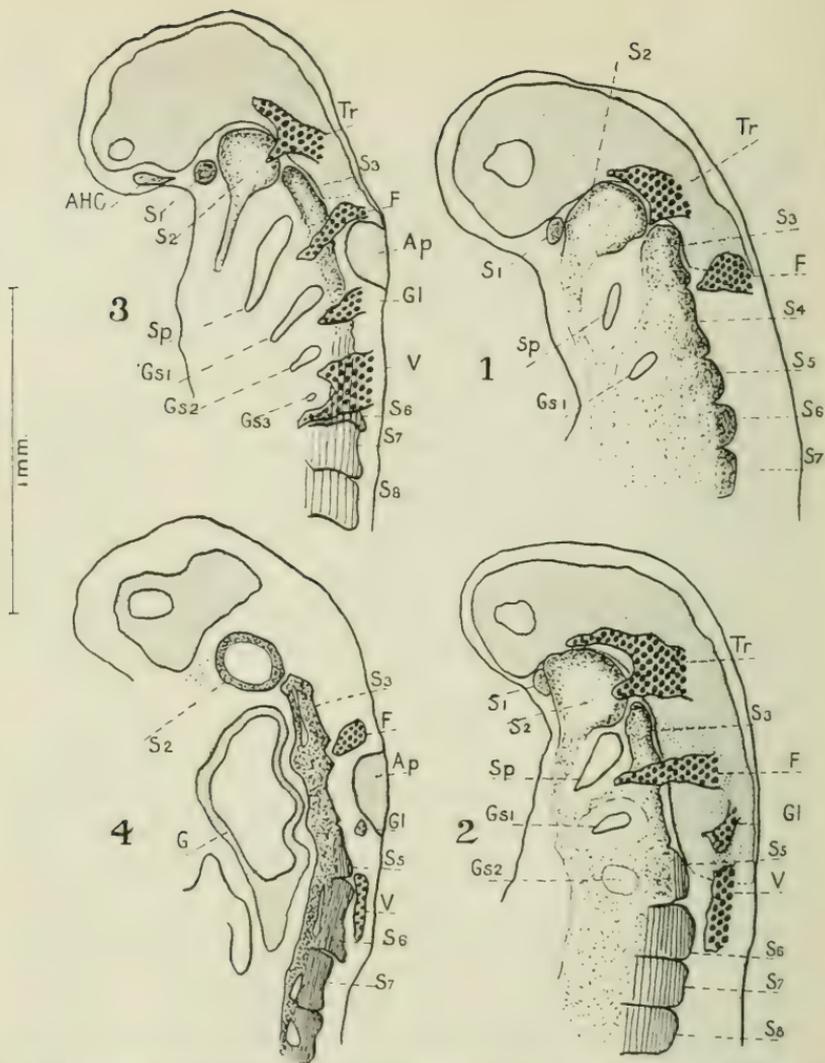
The question has been gone into thoroughly in the case of *Scyllium canicula* by Professor Goodrich (8), and it was at his suggestion that I undertook to investigate *Squalus acanthias* (*Acanthias vulgaris*) in order to see whether the conditions were similar in this related form.

The first part of this paper deals with the question of the correspondence in the region of the hyoid arch, and which somite forms the first permanent myotome. This is followed by a brief description of the occipital region, for the purpose of comparing the extent of the cranial region in *Squalus* and *Scyllium*.

The work was done in the Department of Comparative Anatomy at Oxford. To Professor Goodrich, for advice and encouragement, I wish to offer my grateful thanks. I also had the privilege of consulting Professor Neal in person and to him, for valuable assistance and material, I express my deep gratitude.

In a 4.5 mm. embryo (Text-fig. 1) reconstructed from longitudinal vertical sections all the somites of the head can be discerned. Two visceral clefts are present—spiracle and gill-slit 1 situated beneath the third and fourth somites respectively.

In the next stage (Text-fig. 2, 5 mm.) the second gill-slit has appeared beneath somite 5. Of the dorsal nerves trigeminal is related to the second somite. facial is situated between



Text-fig. 1.—Reconstruction of the head of an embryo of *Squalus acanthias* 4.5 mm. long seen from the left side.

Text-fig. 2.—Embryo 5 mm. long. First appearance of muscle-fibres in somite 5. The relation of somites to gill-slits and dorsal nerves is plainly seen.

Text-fig. 3.—Embryo 6 mm. long. The fifth somite is indistinct, the sixth is covered by the vagus except for the posterior dorsal corner, which begins to assume the hook-shaped appearance.

Text-fig. 4.—A single section through an embryo 5 mm. long, showing the single nature of the somite under the auditory capsule.

EXPLANATION OF LETTERING.

Ab, Abducens nerve. *A.H.C.*, Anterior head cavity of Platt. *Ap.*, Auditory Placode. *A.S.*, Auditory Sac. *F*, Facial nerve. *G*, Gut. *Gl*, Glossopharyngeal nerve. *G.S.* 1-5, Gill-slits 1 to 5. *H*, Heart. *N.* 1-7, Neuromeres 1 to 7. *Oc.*, Oculomotor nerve. *S.* 1-10, Somites 1-10. *Sp.*, Spiracle. *Sp. Gn. 2*, Second Spinal Ganglion. *Tr.*, Trigeminal nerve. *V*, Vagus nerve. *V.* 7-10, Ventral nerve-roots of segments 7 to 10. *V.C.L.*, Vena Capitis Lateralis.

somites 3 and 4, glossopharyngeal between 4 and 5. The rudiment of the first branch of the vagus overlies somite 5, which is the most anterior of the post-otic somites to develop muscle-fibres.

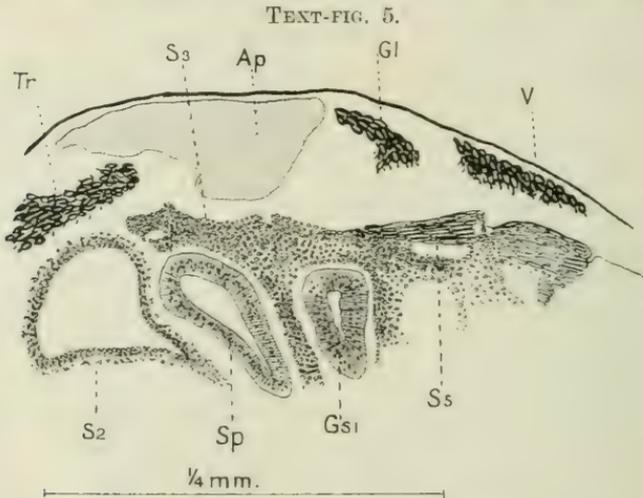
Text-fig. 3 shows clearly the relation of the vagus to the somites. It covers the posterior border of the fifth somite and most of the sixth. By tracing up through later stages I have arrived at the conclusion that it is the sixth somite which forms the first permanent myotome. For the greater part median to the vagus, its posterior dorsal corner is prolonged into a hook-shaped process which, lapping round the posterior edge of the vagus, extends forwards laterally to it. The hook-shaped process can be seen in an incipient condition in this (Text-fig. 3) and in subsequent stages; likewise that the sixth somite corresponds to the third gill-slit, which is of course related to the second branch of the vagus. There is also serial correspondence between spiracle, gill-slits 1 and 2, and the third, fourth, and fifth somites respectively. The establishment of this correspondence is important, for some authors (Dohrn, Froriep) have described a varyingly large number of somites under the auditory capsule. I am convinced that there is only one somite between the facial and the glossopharyngeal in *Squalus*. Text-fig. 4 is a drawing of a single section, and the region beneath the auditory capsule from two sections of another embryo is shown in Text-fig. 5 and under higher magnification. The peculiar nature of the posterior corner of the sixth somite also is shown.

Each cleft lies between two visceral arches. The first or mandibular arch contains a prolongation of the second or mandibular somite. Therefore, since clefts and somites correspond, the next posterior visceral arch (hyoid or second) must correspond to the next somite (hyoid or third somite). This is corroborated by the fact that these two consecutive arches, first and second, are related to two consecutive dorsal nerves, trigeminal and facial. Similarly the third visceral arch corresponds to the fourth somite and the glossopharyngeal nerve. This interpretation implies that the dorsal roots are related to

the somites lying anterior to them, and it will be shown that this is the only view which avoids weighty assumptions and discrepancies.

As development proceeds the interpretation becomes more difficult, and for two reasons :

1. The fourth and fifth somites lose their distinctness and the fourth breaks up unrecognizably into mesenchyme. This is possibly due to the pressure of the auditory sac, which appears

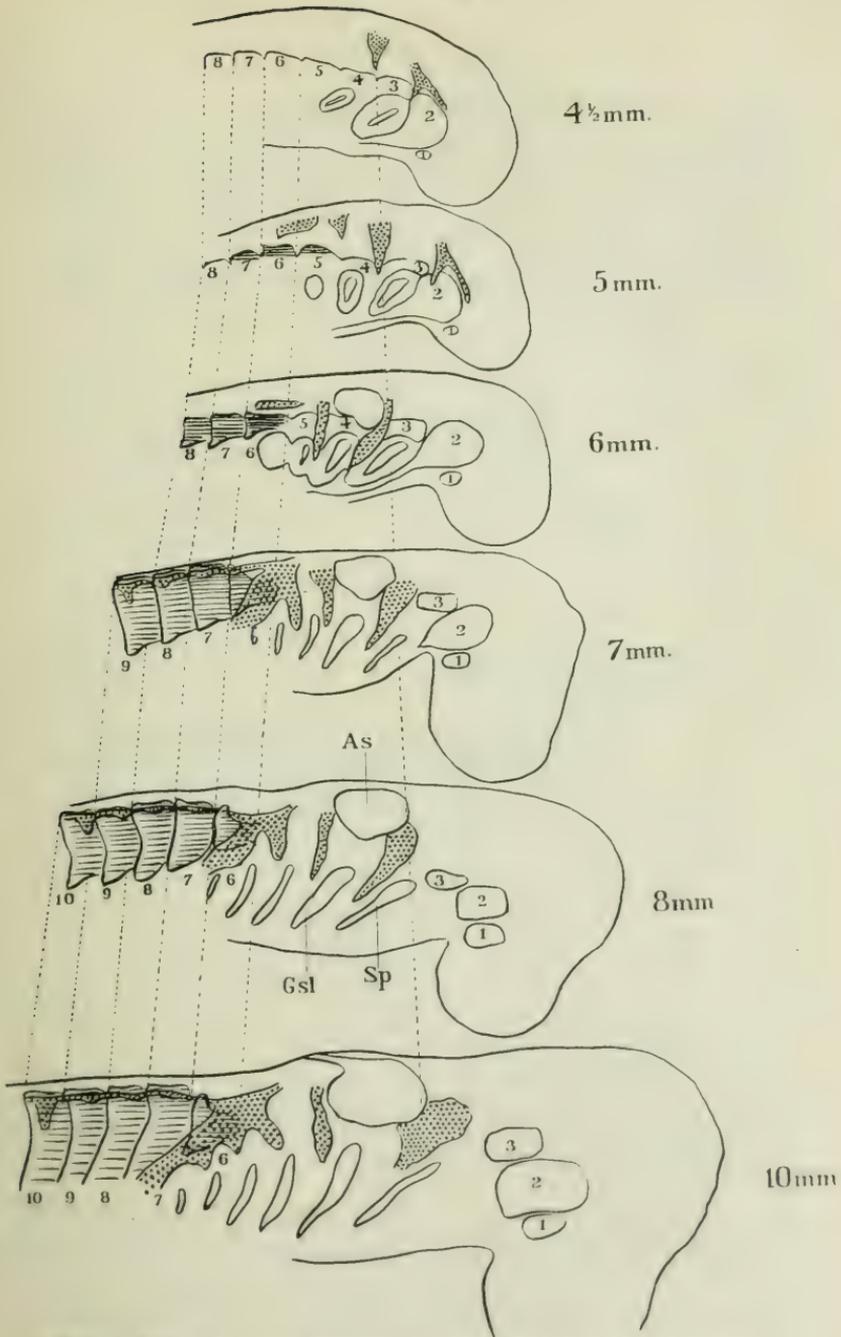


The somite beneath the auditory capsule.

between the facial and glossopharyngeal nerves, overlying the fourth somite. As the sac extends backwards the fifth somite also begins to break up, though some remnants of its muscle-fibres persist.

2. The somites appear in later stages to be situated more posteriorly with regard to the gill-slits. This is due partly to the development of the latter, which push them backwards, and partly to the fact that owing to the slight curvature which the head undergoes, the line of mesodermic somites finds itself situated on the outer side of the circumference of this curvature. Since the centre region of the head mesoderm (somites 4 and 5) is broken down into mesenchyme, the more anterior somites 3

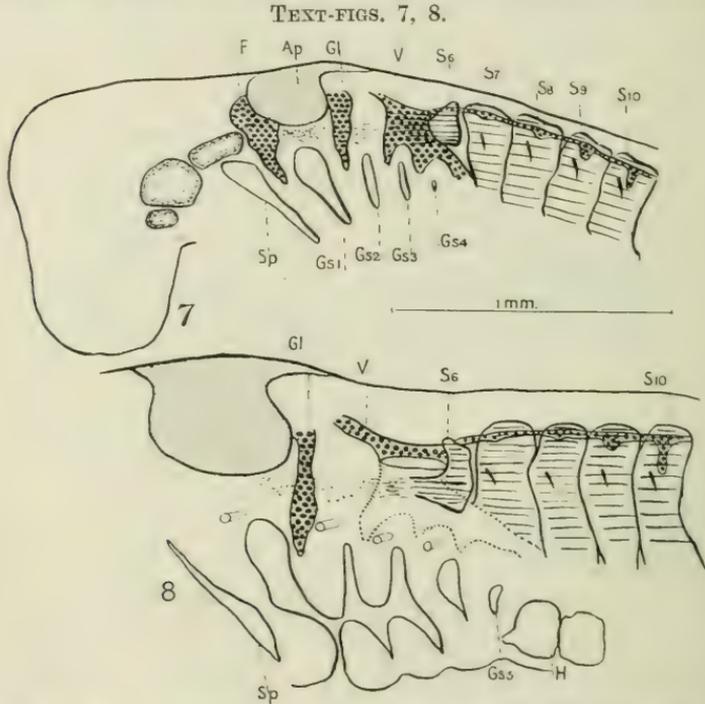
TEXT-FIG. 6.



Series of diagrammatic reconstructions of embryos of the lengths of 4.5, 5, 6, 7, 8, and 10 mm, drawn to the same scale. The rudiments of the facial nerve are joined by a chain line, the corresponding somites of the different embryos by dotted lines.

2, and 1 (which will be drawn off into the service of the eyeball), acquire a more anterior position.

Similarly the more posterior somites, 6, 7, &c., move relatively backwards. By measuring somites in the region of the fifth and sixth, it can be seen that they are stretched and occupy more space along the long axis of the embryo than the remainder.



Text-fig. 7.—Embryo 8 mm. long. Ventral roots are present from the seventh somite backwards.

Text-fig. 8.—Embryo 10 mm. long. The tenth segment is the most anterior to develop a fully-formed mixed nerve. The vagus is represented as truncated to reveal the ventral portion of the sixth somite, which lies median to it.

Text-fig. 6 shows a number of embryos in successive stages of development, all drawn to the same scale. The corresponding somites of each embryo are interconnected by dotted lines. It will be seen that the somite which laps round the vagus and forms the first permanent myotome in the 10 mm. and all later stages

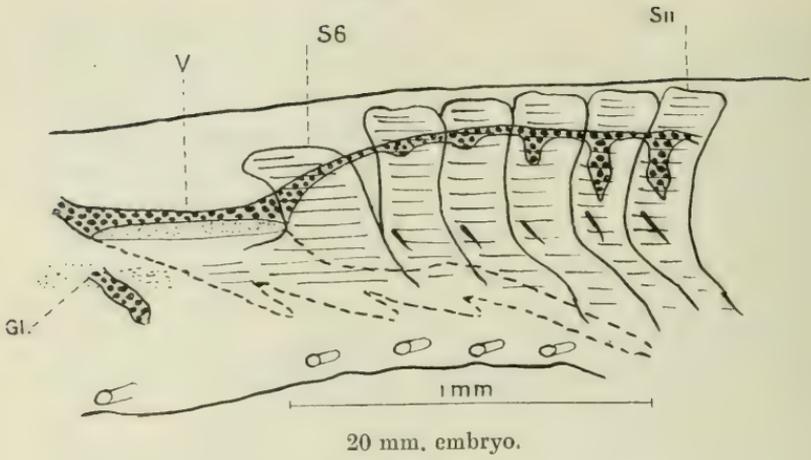
cannot be any other than the sixth, provided that no great migration on the part of the somites has taken place. Several authors regard the seventh somite as the one which gives rise to the first permanent myotome, and this suggests a migration forwards, as described by Braus (2). But, as stated above, any relative movement which the somites undergo is backwards and not forwards, and is entirely passive. Text-figs. 7 and 8 are reconstructions of embryos in the 8 mm. and 10 mm. stages respectively. The sixth somite is very obvious, with its anterior margin drawn out and indistinct, anterior to which there are remnants of muscle-fibres of somite 5, and the same is true of the 20 mm. stage (Text-fig. 9).

Up to a stage between 8 mm. and 10 mm. a ventral root can be traced to the myotome of the sixth somite. In later stages, however, I have been unable to find it. This is in agreement with Neal (11) and Hoffmann (9), both of whom state that it disappears. Presumably the sixth somite is innervated by a branch from the next posterior ventral root and somite (seventh), or the two nerves may combine, but I have not succeeded in determining this point.

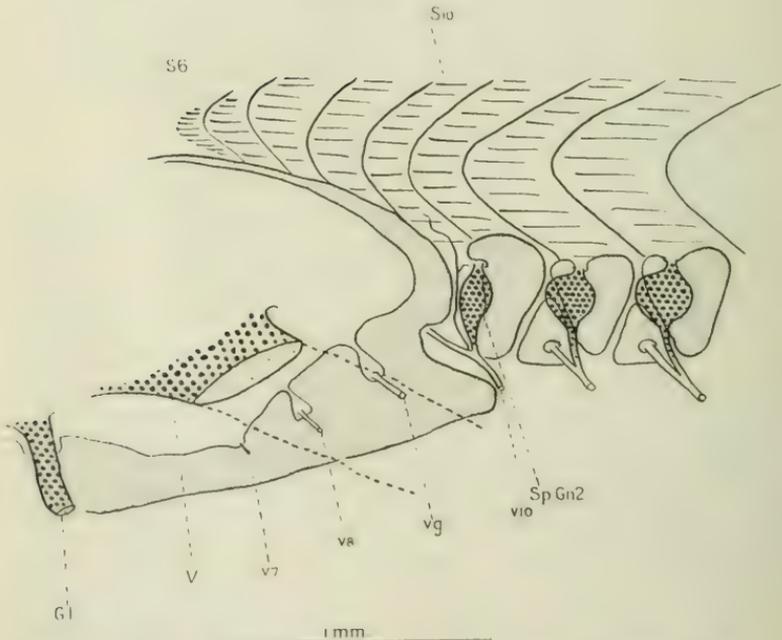
The fate of the ventral roots of the post-otic myotomes is of importance in determining the posterior limit of the cranium. Cartilage begins to appear in the L stage (Sewertzoff, 17: Gaupp, 5). Text-fig. 10 shows a reconstructed embryo about 50 mm. long. Three ventral roots are present, emerging through foramina in the cranial cartilage. The anterior one is very thin and belongs to the seventh somite, the remaining two are stout nerves. The foramina through which they pass become confluent with the vagus foramen, the vagus lying immediately lateral of their point of exit from the neural tube. These two nerves I regard as belonging to somites 8 and 9. These results are in agreement with those of Hoffmann (9). Fürbringer (4) did not study very young specimens of *Squalus*, so that it is probable that his *y* and *z* are the same as Hoffmann's *c* and *d* and my eighth and ninth.

The next ventral root, the tenth, comes out of a deep notch in the posterior wall of the cranium, but behind the occipital

TEXT-FIG. 9.



TEXT-FIG. 10.



arch so that it is not included in the cranium. Its fibres join those of the spinal ganglion to form the first mixed nerve, branches of which I have traced to the tenth somite. Since the eighth somite is the last of the vagus segments the ninth is morphologically the first spinal or post-vagal (in the case of *Squalus* included in the skull), and the tenth, which in *Squalus* forms the first mixed root, is really the second spinal or post-vagal. Rudimentary dorsal ganglia are present belonging to the seventh, eighth, and ninth somites (Text-fig. 9). In *Squalus*, therefore, there are nine segments included in the skull. Hoffmann (9), Sewertzoff (17), and others state that there are ten, but since they adopt Van Wijhe's somites, and like him intercalate a somite between those related to the fifth and seventh nerves, the number of their somites from the third backwards are the same as those of Balfour, plus one. Hence their results and mine are really in accordance since we both regard the same segment as being the last one included in the skull, though the numbers attributed to it are different.

As compared with *Seyllium Squalus* has two more segments included in the skull. But it is interesting to note that in both forms it is the tenth segment (second spinal) which gives rise to the first mixed nerve. It is another proof that homology does not depend on numerical correspondence (Goodrich, 6).

DISCUSSION.

The acceptance of Van Wijhe's scheme of segmentation renders it necessary that two somites, the third and fourth, should be associated with a single cleft and visceral arch: the spiracle and hyoid arch.

There is in this region one dorsal nerve, the facial, and this Van Wijhe regards as double and representing elements belonging to the third and fourth somites. The hyoid arch he assigns to the fourth somite, and in order to account for the third he assumes that a visceral cleft and arch have been lost. We shall return to this assumption later. Further, he regards the somites from the fourth to the eighth as related to the visceral arch and dorsal root lying in front of them. Now the

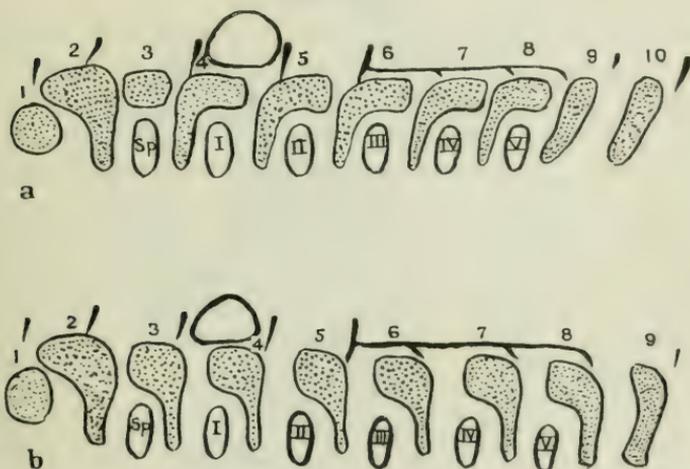
mandibular somite corresponds undoubtedly to a visceral arch (the first) and a dorsal nerve (trigeminal) lying behind it. Similarly the ramus ophthalmicus profundus is situated posteriorly to the first somite. Again, in the trunk region the ventral root of a somite joins the dorsal root posterior to that somite to form a mixed nerve. Therefore Van Wijhe's scheme involves two discrepancies, viz. that in the regions between the mandibular and hyoid arches and between the posterior somite of the head and the first of the trunk there has been a reversal of the relations between somites and dorsal nerves. The first of these discrepancies concerns the trigeminal and facial nerves. The trigeminal is situated behind the mandibular somite, whereas the facial lies in front of the fourth. To be consistent one would have to attribute the trigeminal to the somite posterior to it (third) and the ramus ophthalmicus profundus to the second, but this would leave the first somite without a corresponding dorsal nerve. Similarly, in the region between the trunk and the head, the last branch of the vagus would lie anterior to its somite (Van Wijhe's ninth), whereas the first spinal ganglion is situated posterior to its somite. Not only would the nerves from the facial to the vagus lie anterior to their somites, but they would also lie anterior to their corresponding ventral roots. In the trunk the ventral root is always more anterior than its corresponding dorsal root (Goodrich, 8). These relations of Van Wijhe's somites are diagrammatically represented in Text-fig. 11.

We see then that this scheme has to contend with serious difficulties, all of which are the outcome of regarding the third somite as having lost its visceral cleft and arch. Let us now examine this assumption. In the first place the missing gill-slit is not indicated by any of the structures which it must have involved and of which it is reasonable to expect that some vestige would remain. There is no trace of arch, cleft, afferent or efferent blood-vessels or nerve. This in itself is significant in view of the fact that the anterior visceral arches are conserved with constant regularity all through vertebrate phylogeny. And even when the clefts disappear they leave traces of

their former existence in the form of blood-vessels, nerves, skeletal elements or modified structures. Then authors are not agreed as to the exact position of this missing cleft. Whereas Van Wijhe considers the hyoid arch as double, Hoffmann and Platt (14 and 15) regard the mandibular arch as representing two elements fused.

With regard to the ventral roots there is no question about

TEXT-FIG. 11.



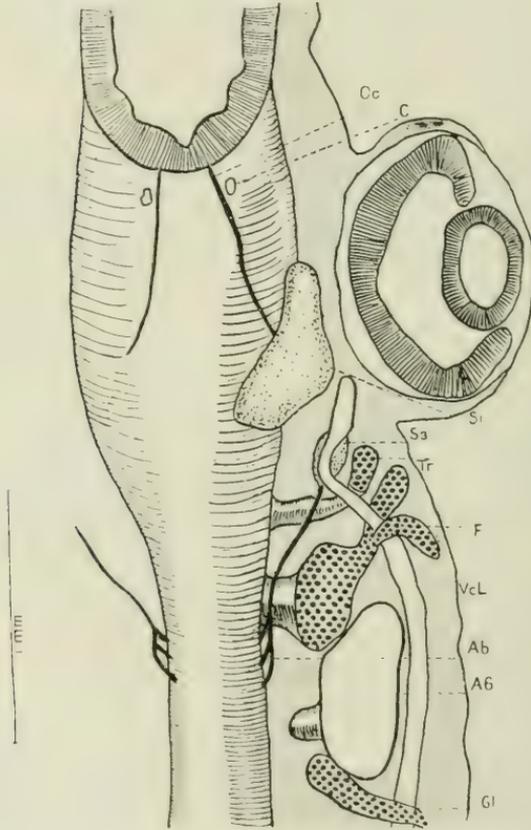
Diagrammatic representation of (a) Van Wijhe's somites, (b) Balfour's somites.

the oculomotor being the premandibular somite's nerve, and the patheticus, in spite of its curious course, doubtless belongs to the second and mandibular segment. The fourth and fifth somites since they disintegrate have no ventral roots as such (though the fifth is present in *Seyllium*). To the sixth somite a ventral root can be seen up till about the 10 mm. stage.

The abducens has usually been regarded as the nerve of the third somite and therefore as the ventral root corresponding to the facial. It certainly innervates the external rectus muscle, but Neal (12) states that in *Squalus* this muscle is of composite origin, consisting of elements derived from the mandibular as well as the hyoid somite.

The abducens is held to arise from the neural tube by many roots; according to Neal four, corresponding to Van Wijhe's somites 3, 4, 5, and 6. I have been able to discern three roots which in a 23 mm. embryo arise not very far behind the facial

TEXT-FIG. 12.



Ventral view of an embryo 23 mm. long to show the origin of the abducens nerve.

(Text-fig. 12). In earlier stages their origin appears to be slightly more posterior. It has been suggested that the hypoglossus and abducens roots form a continuous series, implying that the abducens is a compound nerve derived from elements belonging

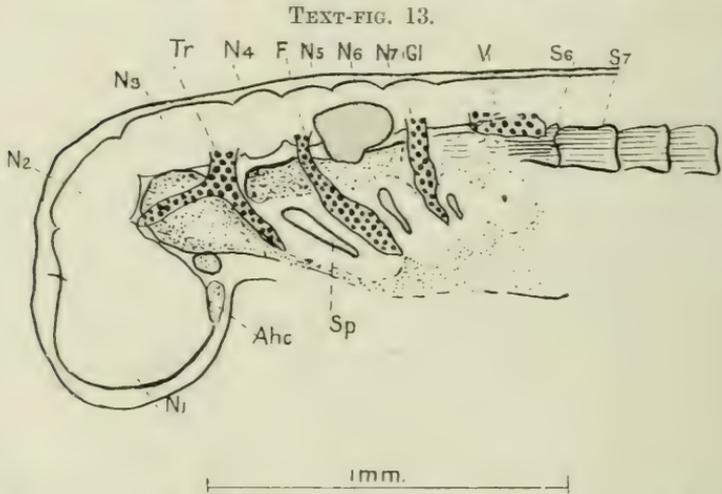
to three or more segments, but even going by topographical relations alone it is not unreasonable to regard the abducens as being the genuine third ventral root. At any rate I do not see that the condition of the abducens furthers the assumption that a gill-slit has been lost. If Neal's contention is true the whole question of the eye muscles innervation and segmentation will require revision.

Lastly, one more train of thought has been brought to bear on the supposedly lost organs, and that is the question of the relation of neuromeres to the other segmental structures.

Neal (11) describes seven neuromeres, of which the first corresponds to the anterior head cavities with the olfactory as its dorsal nerve: this is, of course, assuming that the anterior head cavities have the value of a somite anterior to the pre-mandibular somites. To the second neuromere correspond the ramus ophthalmicus profundus premandibular somite and oculomotor. The third or cerebellar neuromere later undergoes subdivision (which Neal regards as secondary) and to it belong trigeminal, patheticus, and mandibular somite. The next dorsal root, the facial, arises from the fifth neuromere, and this led to the idea that the nerve of the fourth neuromere (which has none) has disappeared and that it was this nerve which was related to the lost gill-slit. To the sixth and seventh neuromeres belong the glossopharyngeal and vagus, though their topographical correspondence has been lost. The glossopharyngeal appears to arise from the seventh neuromere, and the vagus behind it; but this is explained as being due to the pressure of the auditory sac and relative shifting of the elements of the neural crest and neural tube. These relations are shown in the embryo (6 mm.) reconstructed in Text-fig. 13.

If it be granted that neuromeres have a primary segmental value, then it may be said that there is one neuromere too many overlying the hyoid arch; but that such a segmental value exists remains to be proved. To start with it rests on the assumption that the anterior head cavities represent a somite. These are present only in Galeus and Squalus, but in Amia.

Reighard, and Phelps (16) have described the sucker as arising from muscle anterior to the premandibular somite. Goodrich (7) has produced good evidence to show that the anterior head diverticula of *Amphioxus* are homologous with the premandibular somites of Craniates, and it is more reasonable to agree with Dohrn (3) that no segmental significance must be attached to Platt's anterior head cavities. Then, supposing that the



Embryo 6 mm. long showing the relations of the neuromeres to the remaining segmented structures.

subdivision of the third neuromere is not secondary but primary and retarded (which it might be, for the third neuromere is just about twice as long as the following ones), it would be necessary to postulate yet another gill-slit lost, to correspond to the extra neuromere. But perhaps the greatest objection to the segmental value of neuromeres lies in the fact that they are altogether absent in *Amphioxus*, scarcely developed in *Petromyzon*, irregular and asymmetrical in *Bdellostoma*, and that they are best developed in the higher craniates, birds, and mammals (Neal, 13). This strongly discountenances their paligenetic value and suggests that they are neomorphs. And so I cannot believe that the evidence from neuromeres favours the assumption of a lost gill-slit.

The simplest explanation then, that originating from the work of Balfour, is the most suited to the facts: the third somite is related to the hyoid arch and the fourth to the third arch, &c.; somites correspond to the dorsal roots lying immediately behind them, as in the pre-otic and trunk regions, and there are no discrepancies.

In dealing with such a subject as segmentation, the essence of which is a simple and orderly repetition of parts, if a simple explanation can be deduced which does not produce inconsistencies or go against facts, the *onus probandi* must lie with those who would reject such an orderly state of affairs.

SUMMARY.

1. Balfour's interpretation of the somites of the head is correct and free from the objections which accompany Van Wijhe's.

2. No gill-slit or arch has been lost in the neighbourhood of the hyoid arch.

3. Nine segments are included in the head of *Squalus*, of which three are pre-otic (first, second, third) and six post-otic. Of these

One (fourth) breaks down completely into mesenchyme;

One (fifth) forms muscle-fibres but later breaks down;

Four (sixth, seventh, eighth, ninth) produce permanent myotomes;

The tenth somite (first of the trunk, and second post-vagal) corresponds to the first mixed nerve.

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Some Notes on the Gametogenesis of *Ornithorhynchus Paradoxus*.

By

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With Plates 12, 13, 14, and 1 Text-figure.

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1. INTRODUCTION.

In this paper I have described in as much detail as was possible the oogenesis of the duck-billed platypus of Australia. Owing to the unique position of the Prototheria, any new facts

with regard to their germ-cells is sure to be of value. I must take this opportunity of thanking Professor J. P. Hill, F.R.S., for allowing me to study his material of *Ornithorhynchus*, without which I could not have published these notes.

There is no account extant of the detailed structure of the ovarian egg of *Ornithorhynchus*, of the yolk formation, of the maturation stages, or of the corpus luteum. Such accounts of the ovary as are published are scrappy and full of errors, this, however, being chiefly due to the scanty and poor material at the disposal of the various observers who have attacked these problems. The material at my disposal, while not having been prepared by the most modern technique, is well preserved by routine methods and allows of a fuller description of various problems than hitherto given. The material consisted of one ovary preserved in Flemming's strong fluid, and of several ovaries preserved by a variety of picric and bichromate fixatives. Most of the new results were procured by examination of the Flemming-fixed ovary.

This work was partly carried out in the Embryological Laboratory, University College, London, and was finished in the Zoological Laboratory, Dublin University. Apart from his kindness in lending me the material, I have to thank Professor J. P. Hill for assisting me by lending some of the literature on *Ornithorhynchus* and *Echidna*.

2. PREVIOUS WORK ON GAMETOGENESIS OF ORNITHORHYNCHUS AND OF ECHIDNA.

Thirty-seven years ago E. B. Poulton, in his paper on 'The Structures connected with the Ovarian Ovum of Marsupialia and Monotremata', gave some account of the general appearance of the ovary and follicle of *Ornithorhynchus* and *Echidna*. Poulton's material consisted only of ovaries removed from spirit specimens, and he was consequently much handicapped. Nevertheless, he succeeded in establishing several facts of great importance. The ovary of *Ornithorhynchus*, according to Poulton, is flat or compressed, oval, and about 13 mm. long, 7 mm. wide, and 2 mm. thick. The follicles are

confined to the edge of the transverse section of the ovary, i. e. on the surface of the ovary; there does not seem to be any distinct arrangement of follicles, according to size, but the small ones always seem to be near the surface. Poulton noticed that there was evidence that the large follicles were constricted off in the presence of a deep furrow encircling some of them. By this I believe he means that the egg (and follicle) is constricted from outside, and tends to hang somewhat freely on the surface of the ovary.

Poulton identified a follicular epithelium, which he considered to be of one layer, 'the whole of the time the ovum remains in the follicle'.

This author also describes faithfully the zona pellucida, follicle, basement membrane, and tunica fibrosa, and establishes the fact that the 'ova of Monotremes practically fill their follicles, and are of considerable size'. The nucleus Poulton considered to be central in the small ova. He recognizes in the older egg a peripheral stainable granular area, and, deeper down, a lighter granular area, beneath which lies the yolk.

It is remarkable that Poulton should have been able to describe so many interesting facts from such poor material.

Three years later, in 1887, Caldwell published a paper on 'The Embryology of the Monotremata and Marsupialia', in which he pointed out that Poulton and Guldberg had wrongly stated that the follicular epithelium remains always a single layer of cells.

Guldberg and Beddard both described the ovary of *Echidna*. They showed that it resembled in its oogenesis the condition already described by Poulton for *Ornithorhynchus*.

Probably the finest collection of Monotreme material is that procured by Semon about 1893; this observer had at his disposal a large number of eggs in all stages. He gives no account of the oogenesis, and his description of the structure of the egg consists of thirty-five lines of general comment, without any detailed account of his material. It is therefore difficult to know how much Semon understood of the structure of the egg. Certain appearances drawn in his figures of the egg

are undescribed in the text. In some cases it is impossible to know whether Semon's figures of supposed nuclei are cells or nucleoli; this applies especially to his *Tafel IX*, figuring early stages of development.

Writing of the full-grown egg, Semon says: 'Die Keimscheibe ruht auf einem Lager von feinkörnigem, weissem Dotter, und dieser entsendet nach innen eine strangförmige Fortsetzung, einen "Dotterstiel", der im Centrum sich flaschenförmig zu einer Latebra aufbläht. Die Elemente des gelben Dotters sind kugelförmig; gegen den weissen Dotter zu, besonders in der Gegend der Keimscheibe, nimmt der Durchmesser der Kugeln des gelben Dotters continuirlich ab. An der Grenze erblickt man häufig die Kugeln des gelben Dotters in allen Stadien des Zerfalls zu kleineren und kleinsten Elementen. In gleichem Maasse wie das Blastoderm den Dotter unwächst, breitet sich an der Oberfläche des letzteren und ersteren eine Schicht von weissem Dotter aus.'

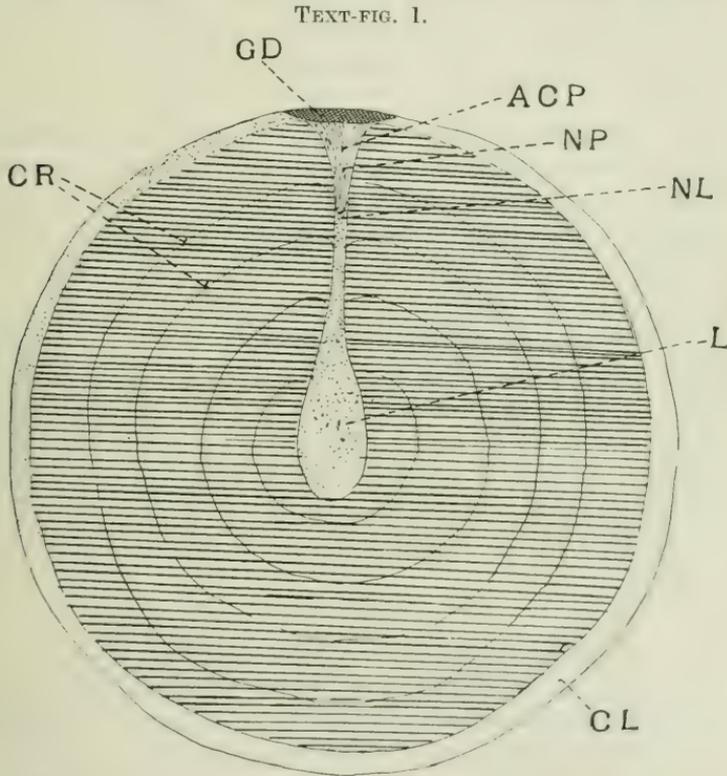
In his figures of sections of the eggs of both *Echidna* and *Ornithorhynchus*, Semon draws, within the more central part of the cross-section of the egg, from one to as many as three concentric rings lying in the yellow yolk, as is well known to occur in the hen's egg, but he does not describe these rings in his text. His description of the structure of the egg is very poor. It would probably be worth while for a capable cytologist to re-describe the sections of eggs of both *Echidna* and *Ornithorhynchus* procured by Semon's party in Australia.

3. GENERAL NOTE ON THE STRUCTURE OF THE EGG OF THE SAUROPSIDA.

In both the *Aves* and the *Reptilia*, the egg, as is well known, has a very complicated structure, and for the purpose of comparison with that of *Ornithorhynchus* I have given a diagram in *Text-fig. 1*.

The germinal disk (GD) is formed of pure protoplasm free of any but the smallest yolk-spheres; this protoplasmic disk contains a very granular, generally somewhat basophil, type of protoplasm, which can readily be distinguished from the

clear cone of protoplasm (ACP) which lies below. This clear cone of protoplasm is not granular, and passes insensibly into the disk above, on the one hand, and into the neck of the latebra (NL) below, on the other hand (Nucleus of Pander). The latebra (L) is formed of a clear substrate containing numbers



of fine yolk-granules. Completely surrounding the egg, and forming a peripheral area, is a thin layer of clear protoplasm containing very fine yolk-spheres (CL). All the internal substance of the egg, excepting that part occupied by the latebra (L), is filled with enormous numbers of large coarse yolk-spheres; and within this substance can be found concentric rings of clear material (CR) which are said to mark areas of growth of the yolk (see Riddle, 5).

The peripheral clear area (CL), the cone of protoplasm (ACP), and the latebra are generally described as containing white yolk-spheres, the rest of the egg mainly yellow yolk-spheres.

The clear thin layers of concentric stratification (CR) have been said to contain white yolk-spheres, though this has not been settled satisfactorily. Riddle (5), however, believes that the concentric layer does contain white yolk, and is a growth-mark.

Semon's description of the yolk of the egg of *Ornithorhynchus* does not include any mention of these concentric layers of stratification within the egg, but in his figures he shows eggs of *Echidna* and *Ornithorhynchus* which contain one (Tafel VIII, fig. 23), two (fig. 25 and fig. 19), and three (fig. 20) layers, as depicted in Text-fig. 1 (CR) of this paper.

It is possible that the egg of *Ornithorhynchus* might contain these concentric lines of growth, if such they be. The varying number of lines are probably significant of the different periods or epochs of the year during which the eggs grew most; that with two rings possibly grew in two sudden well-marked periods, and so on. This opinion is supported by Riddle's work on feeding Sudan III to laying fowls (5).

4. GENERAL ACCOUNT OF THE FORMATION OF EGG-MEMBRANES IN SAUROPSIDA AND MAMMALIA.

In a recent paper (8) Miss Alice Thing has studied the formation of the zona pellucida in various turtle eggs. When the young oocyte of the turtle has reached a size two or three times that of the oogonium, it becomes surrounded by a flattened epithelium which persists as one layer throughout the course of development of the egg. With the gradual growth of the oocyte, the epithelial cells take on a definite prismatic shape and increase in height in the axis perpendicular to the surface of the egg. Occasional mitoses prove, that to accommodate the increasing volume of the egg, the epithelium extends itself by division of its constituent cells; in very large eggs numerous mitoses occur. The epithelial cells forming the follicle are

sharply marked off from one another by intercellular channels filled with intercellular substance. The latter undergoes early a change of constitution and becomes transformed at the level of the surface of the cells into the special cement known as the terminal bars.

The zona pellucida is formed by two or three different elements. It takes its origin as a veil-like formation consisting of a mosaic of terminal bars and polygonal fields within which may be recognized the small, pale areas, future canals of the adult membrane separated by pale and dark filaments giving origin to the future fundamental substance of the adult membrane. The fundamental substance of the zona pellucida is developed as a cuticular element, by the terminal bars or primary network, that is by a definite special intercellular cement possessing the property of extension over the free surface of the epithelial cells and forming connexions there with the delicate secondary network apparently produced directly by the superficial cytoplasm of the epithelial cells.

With regard to the origin of the zona pellucida in mammals, I believe that there are three possible methods of development: the zona might develop from the follicular epithelium, it might develop from the egg-cytoplasm, or it might be developed under the influence of, and from, both egg-cytoplasm and follicular epithelium.

The majority of present-day workers appear to believe that the zona of Mammalia develops from the follicular epithelium alone. This is the view of such well-known older observers as Flemming, Retzius, Fischer, Von Ebner, Bonnet, and Rubaschkin. But Van Beneden, Sobotta, Waldeyer, and Kolliker all believe that the zona pellucida is secreted by the egg-cytoplasm. In support of this view are such observations as that of Van Beneden, who described in a bat the fact that while there may be two eggs in such close contact that at one place the follicle is interrupted, yet at this region the zona is properly developed. This is not of very rare occurrence in ovaries of placentals, and is certainly difficult to explain if one believes that the zona is of purely follicular origin.

5. GENERAL ACCOUNT OF THE YOLK FORMATION IN BIRDS AND AMPHIBIA.

In both birds and amphibians the egg is richly provided with yolk, i. e. macrolecithal. The formation of yolk in the egg of amphibians does not seem to have been followed out with any detail or pains by a modern worker, and though I have made numerous preparations by the best methods, it has been difficult to determine the exact source of origin of the yolk-spheres (see Gatenby, **15**, p. 139).

In the amphibian oogenesis the mitochondria spread out mainly to form a deep cortical zone on the periphery of the egg. It is in this zone that the first sign of yolk-granules appears, but it is wellnigh impossible to give an opinion as to whether the yolk originates directly from the mitochondria, or whether the latter only elaborate materials which, precipitating in the ground cytoplasm, come to form the separate spheres of substance we recognize as yolk.

Van Durme, in his monumental work on the oogenesis of birds (**10**), has entered into the subject with care, and has produced a paper which may be accepted as an authentic account of the steps in the formation of yolk in birds. He recognizes in the oocyte just before the beginning of the extensive yolk formation: (1) an attraction sphere containing a centrosome, (2) a yolk-forming region or vitellogenous cloud, (3) a quantity of fatty yolk. The vitellogenous cloud is formed of mitochondria of various types, e. g. chondriomites, chondriosomes, and it soon undergoes a process of dissociation. This dissociation of the 'couche vitellogène' invokes the appearance throughout the egg-cytoplasm of a uniform layer of mitochondria. This uniformity does not last long, for soon afterwards three distinct mitochondrial zones appear; a cortical dense zone, an inner deeper, and an internal still deeper zone.

The first vestiges of yolk formation are the appearance of clear yolk-vesicles (vacuoles) in the neighbourhood of the cortical fatty layer, thus constituting a peripheral vacuolated area, which spreads gradually towards the centre of the yolk:

a second vacuolated area appears around the nucleus, known as the perinuclear vacuolated zone. These two zones meet at the animal pole of the egg, above the nucleus, forming the vacuolated nuclear cap.

Subsequently in this second phase of vitellogenesis the first true yolk-spheres put in an appearance firstly in the region of the exoplasm, then more deeply in the endoplasmic region. Van Durme unhesitatingly states that these yolk-spheres partly arise from the larger mitochondria, and partly from the contents of the clear yolk-vesicles (vacuoles).

From this stage onwards the more deeply-lying mitochondria become fewer, the yolk-elements more numerous, but the cortical mitochondrial zone persists throughout all stages.

6. THE STRUCTURE OF THE OVARY OF ORNITHORHYNCHUS.

On taking up a slide of sections of the ovary of *Ornithorhynchus* and examining it with the naked eye, one is first of all struck by the enormous size of the riper eggs. These are much larger than the full-grown ovarian oocytes of the frog, and of course infinitely larger than those of a rabbit or dog. As in the ovary of a *Sauropsidan*, the eggs project out around the surface of the organ in a way familiar to any one who has examined the ovary of a fowl or turtle. Thus, while the eggs may be very large, the stroma and general extent of the whole ovary is relatively small. This will be best understood by reference to Pl. 12, fig. 3: in this ovary there was at least one egg nearly if not quite ripe (o), which measured 4.36 mm. in diameter in its shortest way, by 4.52 mm. in its longest way.

In the ovary drawn in Pl. 12, fig. 3, no corpora lutea were to be found, and when these occur they protrude from the surface of the ovary almost as much as the full-grown egg. In several of the ovaries I have examined there are two corpora lutea close together, and these form by far the most prominent structures in the ovaries in question.

Examined under the low power of a microscope the most striking features of the *Ornithorhynchus* ovary are the immumer-

able lacunae or spaces in the ground-work or stroma. The biggest of these spaces are drawn in Pl. 12, fig. 3, being cross-hatched (CA), but to gain a better understanding of this peculiarity one must examine fig. 4 of Pl. 13. Here the extraordinary structure of the ovary is demonstrated, a well-marked germinal epithelium is recognizable (GE), and beneath it are a row of oocytes in various stages; on the right of Pl. 13, fig. 4, the oocytes are found to lie in a more solid cortical area of the ovary, which is marked off at this region quite sharply by the wide and numerous lacunae, with their trabeculae in between (TR). These cavities do not contain blood, or lymph corpuscles, but seem to have been occupied by a non-corpuscular fluid, which leaves no trace of coagulum in the finished sections.

As the young oocytes grow older they tend to become completely surrounded by strands of much vacuolated tissue, as is indicated in the largest oocyte drawn in Pl. 13, fig. 4. This feature is certainly one of the most remarkable in the ovary of *Ornithorhynchus*. It will therefore be clear that by the time an egg has reached the stage drawn in Pl. 13, fig. 4 (roughly one-eighth of its full size), it is already floating in a basket-like area formed by connective-tissue trabeculae and intervening lacunae filled with liquid.

7. THE APPEARANCE OF THE IMMATURE OVARY OF THE PLATYPUS.

In Pl. 12, fig. 2, is drawn an immature ovary measuring 3.250×1.0 mm. This shows remarkably well the almost amphibian character of the ovary at this stage. As was pointed out above with reference to the mature ovary, there is also to be seen in this immature specimen a cortical arrangement of oocytes; around the ovary the eggs tend to lie in a thickened area, beneath which is a space occupying the centre of the organ. This cavity is only partly filled with loose strands of connective tissue.

One is forced to look upon this peculiar structure of the immature ovary of *Ornithorhynchus* as a very primitive feature.

In subsequent development the cavity becomes more and more filled with connective tissue, and this, together with the growth of the cortical walls of the ovary, caused the primitive type of arrangement to be disguised and partly obliterated; but it should be pointed out that the lacunae figured on Pl. 13, fig. 4, are largely the remains of the early cavity within the gonad.

8. THE SIZE OF THE LARGEST AND SMALLEST OVARIAN OOCYTES OF THE PLATYPUS.

In the adult ovary of *Ornithorhynchus* no oogonia are to be found; all these seem to have undergone their maturation prophases and to have become oocytes certainly long before the animal is full grown. Even in one very small immature ovary in Professor Hill's possession there were no oogonia: this ovary measured only 3 mm. in depth (see Pl. 12, fig. 2), whereas the adult ovary is at least 12 mm. in depth. Possibly during an embryonic period all the oogonial divisions, as well as the prophases of the maturation division, have taken place, so that when the animal hatches there are already formed all the oocytes which it will possess and use during its life.

This feature, with regard to the absence of true oogonia in the ovary, does not occur in forms like the frog, where numerous pockets of true oogonia exist in the ovary of the adult (vide *Gatenby*, 9). Were it not for these pockets of cells which continually proliferate new oocytes, the frog would be unable to lay three to five thousand eggs for so many seasons. In the case of *Ornithorhynchus* and other *Mammalia*, the number of offspring produced is so small as not to necessitate a continuous new supply during each breeding season.

Measurements have been taken of a number of the oocytes of the smallest dimensions I could find. The smallest was 0.07 mm., the average among the smaller being 0.08 mm. In the adult ovary the smallest oocytes measured from 0.08 to 0.09 mm.

With regard to full-grown ovarian oocytes the largest I found was 4.5 mm. in diameter, not counting the theca (Pl. 12, fig. 3): 4 mm. seems an average diameter for the ovarian oocyte of

Ornithorhynchus. The one complete egg- and shell-membrane of which I examined sections was only from 4.5 to 5 mm. in diameter, though it was difficult owing to the wrinkling to make an accurate measurement (Pl. 12, fig. 1).

9. THE YOUNG OOCYTE OF THE PLATYPUS.

Some oocytes which had just undergone the prophases of the heterotypic division were discovered in the Flemming-fixed material; two such oocytes are drawn on Pl. 14, figs. 7 and 8. The nucleus is nearly always spherical, but occasionally irregular as shown in fig. 8; there is a well-marked nucleolus, NU in fig. 7, of the fragmented type; in some nuclei the nucleolus can be seen to be formed of two parts—a lightly-staining region, NUP in fig. 10, and a darkly-staining region, NUB. In fig. 11 the nucleolus consists of a very large darkly-staining sphere and a number of smaller pale elements; the chromatin is feebly staining and dispersed in all these nuclei.

In nearly all the young oocytes observed a centrosphere is present, cs in figs. 7 and 8; the centrosphere at this stage lies near the nucleus, often within a dent in the nuclear membrane, as in Pl. 14, fig. 8. In some cases centrioles or small granules within the centrosphere can be made out, as in fig. 8, cs. In the youngest oocytes the centrosphere may be surrounded by a cloud of granules which have been identified as mitochondria (M), fig. 7.

In older oocytes the mitochondria, as happens in all vertebrate eggs, gradually pass away from the centrosphere, and become spread out into the cytoplasm (fig. 8); they tend to collect as matted granules and filaments, particularly in the region of the periphery of the egg, and become difficult to demonstrate at and after this period.

10. ON THE EARLY ESTABLISHMENT OF A POLARITY IN THE PLATYPUS OOCYTE.

All the oocytes examined showed a distinct polarity, in that the nucleus had taken up a position to one side of the oocyte cytoplasm. I believe that this polarity has no relation-

ship to the plane of the surface of the ovary, nuclei being found lying inwards, outwards, or sideways to an axis drawn directly down at right angles to the surface of the gonad.

From the material examined it is impossible to understand completely the mode of origin of the polarity in the young oocytes, but from our knowledge of many vertebrate oogonia we are aware that when in this early stage the nucleus tends to lie to one side of the cell. The polarity of the Ornithorhynchus oocyte is therefore probably established during the oogonial stage, either as the accidental result of the position of the centrosomes and centrospheres of the daughter-cells during oogonial divisions, or as a subsequent and more expressly determined movement of the oogonial nucleus within the cytoplasm, at a stage just before the inception of the prophases of the heterotypic divisions. The former is most likely.

This polarity of the oocytes persists throughout their entire growth, marking permanently the position of blastoderm and vegetative pole of the full-grown oocyte, and of the part of the egg in which the latebra will be formed.

11. FORMATION OF EGG-MEMBRANES.

The egg-membranes on the ovarian oocyte of Ornithorhynchus are a theca (externa and interna), a follicle, and a zona pellucida.

In all the youngest oocytes that have been observed the follicle is well formed; it is shown in Pl. 14, figs. 7 and 8, *FOL.* and much enlarged in fig. 9. In the latter figure the follicle is seen to consist of one layer of flattened cells, overlying the substances of the oocyte (*OC*). In good preparations it is possible to recognize clearly a limiting or true cell-membrane around the egg-cytoplasm, *OM*, in Pl. 14, fig. 9. Distinct cell-walls between the individual cell elements of the follicle were generally difficult to find, but are probably always present.

In Pl. 14, fig. 12, the same region of an older oocyte is drawn. The follicle cells as such could not be identified in this preparation, but the nuclei and general cell-substance have increased greatly in size. Just at this stage a new arrangement of the

individual elements of the follicle begins to take place; the nuclei dividing rapidly, soon become too large and too numerous to lie all in one row in the follicle, and gradually certain nuclei are displaced, as shown in Pl. 14, fig. 12, and ultimately a two-layered follicle results (Pl. 14, fig. 11, FOL). Two-layered the follicle remains all through its subsequent life.

Now comes one of the events most difficult to understand and interpret—namely, the formation of the zona pellucida. Possibly, however, judging from the accounts of workers who have studied other material, *Ornithorhynchus* presents the problem in a less difficult form, though there are some points which are still far from clear to me.

A glance at Pl. 14, fig. 9, gives one an impression of the condition of the egg-membrane (OM) at this early stage—the membrane is a true cell-wall, and nothing else at this period.

Now in Pl. 14, fig. 12, the egg is considerably older, and two new structures have appeared: one is the substance marked pz, the other the fibrillae marked cf. The substance marked pz is the precursor of the zona pellucida, while the fibrillae, cf, grow to form the much larger structures shown in Pl. 14, fig. 14, at cf. The fibrillae serve as connecting elements between the zona pellucida and the outer cell-membrane (OM) of the oocyte cytoplasm.

In none of the best slides I examined could I be sure that cell-walls existed at the stage drawn in Pl. 14, fig. 12, just when the pre-zona substance is becoming clearly marked. The follicle nuclei appear to lie within a syncytium, but in my mind there exists no doubt that the pre-zona material is formed in or by the follicle cells. The substance might possibly be intercellular, as described by Miss Thing, but it is certainly derived from the follicle; moreover, up to the last step in the development of the oocyte the follicle cells lie in close relationship with the zona, as in Pl. 14, fig. 13, and when the egg is extruded the naked edges of the follicle cells are left, apparently supporting the view that the zona and the follicle were previously most intimately related. This is all I can write with reference to the development of the zona.

In a well-advanced oocyte the zona and the underlying structures appear as drawn in Pl. 14, fig. 14. The zona has stained black with haematoxylin; beneath the zona is the true cell-wall of the egg (OM), which is quite thick. I call this the true cell-wall of the egg because I believe it can be traced back to the undoubted cell-wall of the earliest oocyte, marked OM, in Pl. 14, fig. 9. In Pl. 14, fig. 14, the cell-wall (OM) is connected to the zona by a large number of cortical fibrillae; these, marked CF in Pl. 14, figs. 12 and 14, probably serve the dual purpose of attaching the zona firmly to the egg, and of acting as living protoplasmic connexions between the nutrient bringing follicle and the receptive interior of the egg.

Outside the theca itself is possibly another layer of less closely packed, often obscurely defined cells, which can be recognized as a theca externa, distinguishable from the true theca, or theca interna (Pl. 14, fig. 13, TH and OSTR). The theca externa, like the true or inner theca, is formed by cells which, sympathetic to the development of oocyte, become slightly flattened and help to form a supporting and vascular capsule for the egg.

12. YOLK FORMATION IN THE PLATYPUS.

The egg of Ornithorhynchus is macrolecithal and an extremely difficult object to section. Its yolk, like that of the frog's egg, stains densely in iron alum haematoxylin. Pl. 12, fig. 3, gives an idea of the appearance of a section stained by this method. In another paper¹ on the full-grown egg (shortly in press), in Pl. 1, fig. 1, the enormous number of yolk-granules can be noted. After fixation of the ovary in acetic acid fixatives, the formation of the yolk is seen apparently to be heralded by the appearance of a number of vacuoles beneath the periphery of the egg. These vacuoles, which are shown in Pl. 13, fig. 4, at B, are probably filled with a lipoid substance of some sort, for at this stage the egg preserved in chrome-osmium does not exhibit such vacuoles.

¹ In this paper is described the polar body formation and minute structure of the latebra of the maturing ovum.

Now at a later stage of oogenesis as seen after non-osmicated fixatives, the yolk-granules are observed to appear beneath or within the wall of vacuoles, as shown in Pl. 13, fig. 4, c. This stage is drawn at a higher magnification in Pl. 13, fig. 5; the vacuoles are at v, and lie below the non-vacuolated clear outer zone of the egg (oz); here and there on the trabeculae between the vacuoles, but mainly beneath the vacuoles themselves, are found in all stages of development yolk-spheres, YA, YB, YC. Beneath this row of yolk-elements the egg-cytoplasm again becomes non-vacuolated, forming a distinct inner zone at this period iz.

At a still later stage the inner zone free from yolk still persists, but smaller in extent comparatively with the rest of the egg (Pl. 13, fig. 4, d).

The individual yolk-granules may be noted to become formed within certain of the clear vacuoles. In Pl. 13, fig. 5, the vacuole at YA contained a partially formed yolk-granule, or in other words was filled *intra vitam* with yolk-substance so thin in quality as not to be firmly coagulated by the fixative, and thus gave the shrunken appearance noticed in YA and YB. The yolk-sphere at YC was older and became fixed more intensely, not undergoing shrinkage.

I feel sure that many of the yolk-granules form by additions, from the surrounding cytoplasm, to the fluid contents of the vacuoles. The latter appear first, and then their contents become richer and richer, till the yolk-granule is completely formed. From the material available I was unable to say whether the mitochondria take any part in yolk formation.

13. FORMATION OF LATEBRA.

In Pl. 13, fig. 4, are several stages in the formation of the latebra; in fig. 4, B, the oocyte cytoplasm presents a ring of vacuoles which divide the egg into two parts, an outer (B) and an inner (BL); the latter forms the main part of the latebra. The latebra is that part of the inner region of the egg where no coarse yolk-granules are ever formed.

In Pl. 13, fig. 4, c, a later stage is shown; the yolk-granules

have begun to form beneath the layer of vacuoles, and the clear space inside will form the body of the latebra. Reference to Pl. 13, fig. 5, will show that not all the inner non-vacuolated area (1z) forms latebra, for at YA to YC is an area in which yolk-granules appear in this region.

In Pl. 14, fig. 13, a still later stage is drawn; this oocyte is interesting because it shows how the formation of coarse yolk-granules (oz) never takes place in the region beneath the nucleus (NC). It is just in this region beneath the nucleus that the cone of protoplasm (CP in Text-fig. 1) and the upper part of the latebra meet to form the so-called Nucleus of Pander.

The latebra, at a later stage, is shown in Pl. 13, fig. 4, D. At UAL the neck of the latebra is distinguishable and the substance of the latebra itself (BL) has become very vacuolated, as indeed has the whole egg, especially after preparation in acetic acid corrosive fixation.

In another forthcoming paper the appearance of the latebra in the fully-formed egg has been described. It should be noted that the latebra is not formed by the path left by the movement of the nucleus, as is thought by some to be the method of origin of this structure.

14. A FULLY-FORMED EGG (diameter 4 mm.).

In Pl. 12, fig. 1, is a figure, only slightly diagrammatic, of a fully-formed egg of the duck-billed Platypus. On the outside is the thin shell-membrane (SM), which owing to the contact of the fixatives had become somewhat bent and irregular. Beneath the membrane is a layer of albumen or white (W), which is seen in finished sections as a flocculent lightly-staining substance. The egg-white has been pushed out of place on one side by the bending of the shell-membrane.

The rest of the egg is formed of the ovum (oocyte) proper. It is bounded on the outside by a very thin membrane (Z) called by Caldwell the vitelline membrane, and which I believe to be the zona. In the egg drawn are two distinct areas, an outer (OZY) and an inner (IRY) yolk-zone.

The latebra passes up from the centre of the egg (LZL) to

the region generally called the Nucleus of Pander (UAL) beneath the blastodisk (BD), in which the nucleus (NU) is situated.

For further details of the egg proper the other paper should be consulted. The neck of the latebra and the region of the Nucleus of Pander has been described therein more fully.

Faithful drawings of the shell-membrane and its underlying areas have been made by Caldwell, and will be found in his paper.

The average size of the fully-formed egg of the platypus is about 4 mm.; but, as Wilson and Hill have pointed out, it soon absorbs liquid from the uterine walls, and grows to 12 or 14 mm. at the time of laying.

15. NOTE ON SPERMATOGENESIS.

Among Professor J. P. Hill's material were some sections of *Ornithorhynchus testis*, and in Pl. 13, fig. 6, I have given a drawing of a part of one semeniferous tubule and some interstitial tissue. Very good figures of the spermatozoa have previously been made by Retzius and Benda.

Two of the most striking facts about the histology of the platypus testis are the large size of the cells of the interstitial tissue (INT) and the remarkable development of the Sertoli cells (sc). The latter seem to be derived directly from the basement cells or primitive spermatogonia, and I could find nothing suggestive of the presence of any kind of Sertoli cell determinant as described by Montgomery for man. In the platypus the primitive spermatogonium probably becomes a Sertoli cell merely in sympathy with the development of a group of spermatocytes above it. At spt (lower) are a group of spermatocytes nearly full grown, and beneath them, at ysc, is a cell which is in the same series as the primitive spermatogonia above and below (spg), but which is hypertrophying step by step with the group of spermatocytes near by. At sc is a Sertoli cell ready for the fixation of the spermatids (spd¹) which is just beginning, and at spd² is a Sertoli cell with the later spermatids all attached. At spz is a group of ripe sperms attached to a fully-formed Sertoli cell. The sperms are not

spatulate, but resemble those of reptiles and birds, except that the cytoplasmic part is relatively shorter.

16. DISCUSSION.

Probably the most interesting fact ascertained by an examination of this material of the Platypus is the presence of a large hollow cavity in the young ovary. This is undoubtedly a primitive character, which is noticeable even in the adult ovary, in the form of numerous lacunae throughout the stroma of the ovary.

The stroma of the ovary of the Platypus evidently appears early as a number of separate chords of cells which probably grew into the hollow sac-like ovary at a late stage of embryonic history. The ovary of the original vertebrate seems to have been a sac-like structure, the stroma being a new formation; the cells which in *Ornithorhynchus* constitute this loose stroma seem to have been formed by a retro-peritoneal invasion, but as has been pointed out above, they never quite fill the cavity even of the adult ovary.

The egg of *Ornithorhynchus* is discharged from the ovary in quite a different way from that of the placental mammal. In the latter the oocyte, with a corona of follicle cells, breaks loose from the glomus proligerus and the release of the egg from its cellular bed involves only part of the follicular elements. In the case of the egg of the Platypus breakage involves the entire follicular layers, as in the case of the frog's ovary, and no liquor folliculi is present or takes part in the expulsion of the egg.

The formation of the yolk resembles that of the bird described by Van Durme, and the latebra forms in the same manner. Some, at least, of the yolk-spheres are formed as in birds, i. e. by the appearance of watery vacuoles in the ground cytoplasm, and the subsequent loading up of the contents of these vacuoles with fatty and proteid substances, thus constituting coagulable and 'solid' yolk-spheres.

The zona appears to be formed from a substance which is intracellular at first; but it must be admitted that the

matter was difficult to decide. In none of the preparations could distinct cell-walls be found in the follicle at the period when the zona substance was beginning to appear. There is no doubt in my mind that the substance of the zona is formed in direct relationship to the cells of the follicle, and the cytoplasm of the egg probably takes merely a secondary or stimulatory part in the production of this important membrane.

The mitochondria, so far as they could be followed out, act in the same manner as in both the fowl and the frog, and the young oocyte contains the same formed elements as that of the fowl, i. e. sphere, centrioles, and cloud of mitochondria.

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EXPLANATION OF LETTERING.

A, youngest oocyte found. AT, area of attachment of ovary to body-wall. B, oocyte at beginning of formation of latebra. BD, blastoderm. BL, inner region of egg, which will form part of latebra. BV, blood-vessel. C, egg at stage of beginning of yolk formation. CA, cavities in ovary. CF, cortical fibrillae, beneath zona. CH, chromatin. CS, centrosphere. DY, dark-staining yolk. FOL, follicle of egg. GE, germinal epithelium. INT, interstitial tissue of testis. IRY, inner region of yolk. IVZ, inner vacuolated zone of egg. IZ, innermost zone of oocyte. LZL, lower zone of latebra. M, mitochondria. NU, nucleus. NUB, darkly-staining nucleolus. NUP, faintly-staining nucleolus. OC, cytoplasm of oocyte. OFOL, outer limiting membrane of follicle. OM, cell-wall of oocyte. OO, oocyte. OOX, small oocyte compressed by growth of a larger one. OSTR, outer region of theca (theca externa). OZ, outer or peripheral zone of oocyte. OZY, outer zone of yolk. PY, pale yolk. PZ, pre-zona, or substance which forms zona pellucida. SC, Sertoli cell of testis. SM, shell-membrane. SPD, 1 and 2, two stages of spermatids. SPG, spermatogonium. SPT, spermatocyte. SPZ, spermatozoon. TH, theca (interna). TR, ovarian trabeculae. UAL, upper area of latebra. V, vacuole. W, egg white. X, material formed probably by degeneration of oocytes. XY, enigmatic plasmatic body in young oocytes. YA, YB, YC, stages in formation of yolk-spheres. Z, zona.

DESCRIPTION OF PLATES.

PLATE 12.

Fig. 1.—Fully-formed egg of *Ornithorhynchus paradoxus*, in vertical section. Shows latebra, yolk, albumen, and shell-membrane.

Fig. 2.—Transverse section of a young ovary showing cavity (CA) and loose trabeculae (TR), and cortically arranged oocytes (OO).

Fig. 3.—Fully-developed ovary of *Ornithorhynchus*, oocytes blacked in. Cavities in stroma (CA) cross-hatched.

PLATE 13.

Fig. 4.—Part of adult ovary more highly magnified showing oocytes in different stages. The numerous cavities in the stroma are evident, and several stages in the formation of the latebra are given (B, C, D). In the egg D, the follicle is only put in below.

Fig. 5.—Part of the egg at an early stage of yolk formation, as in fig. 4, c. Cortical vacuoles and deeper yolk formations are shown.

Fig. 6.—Part of the testis of *Ornithorhynchus*. For description see p. 492 of text.

PLATE 14.

Figs. 7 and 8.—Two of the youngest oocytes of *Ornithorhynchus*, showing sphere and mitochondria.

Fig. 9.—Edge of young egg showing relationship of follicle to cell (oocyte) membrane.

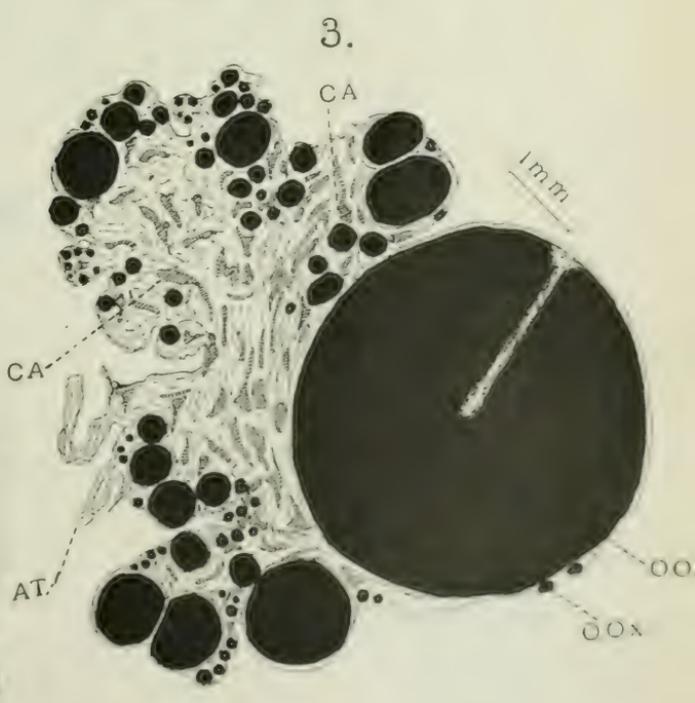
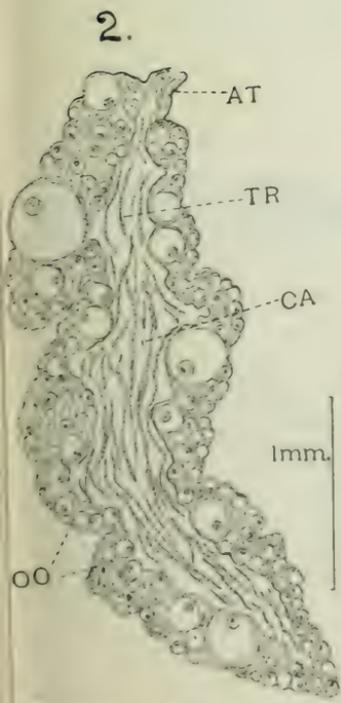
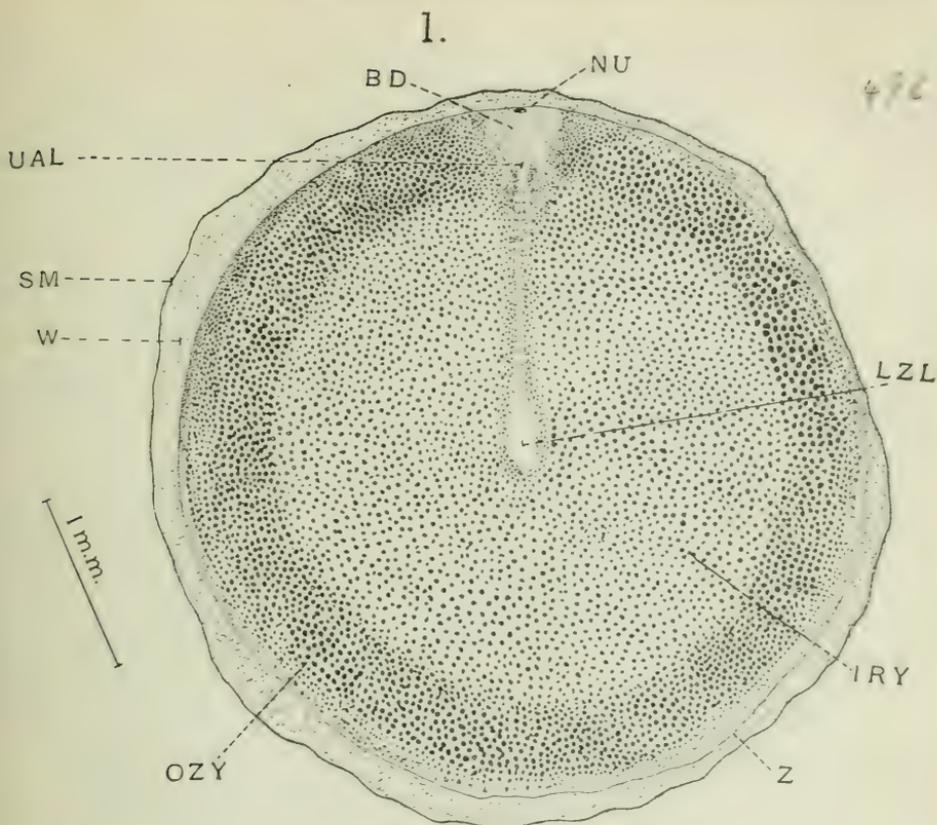
Fig. 10.—Egg at time of formation of pre-zona (pz) follicle, one-layered.

Fig. 11.—Later stage, zona formed, follicle two-layered.

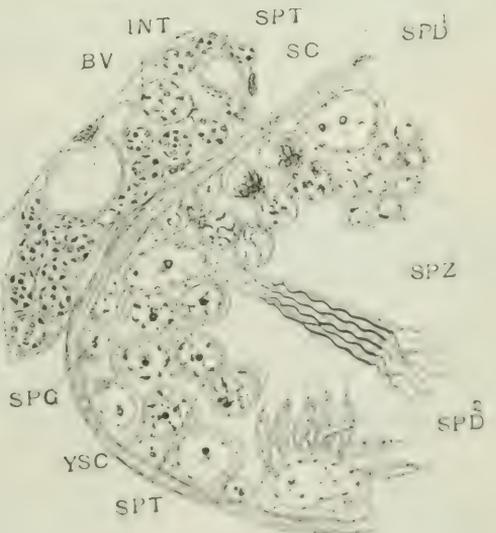
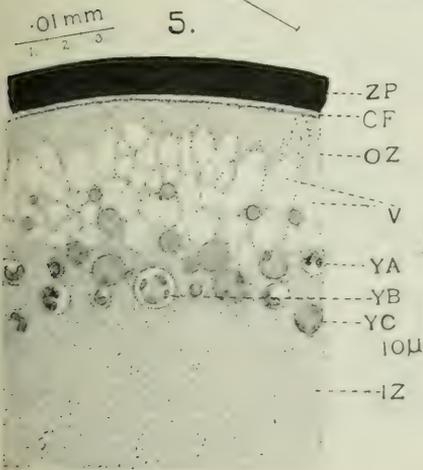
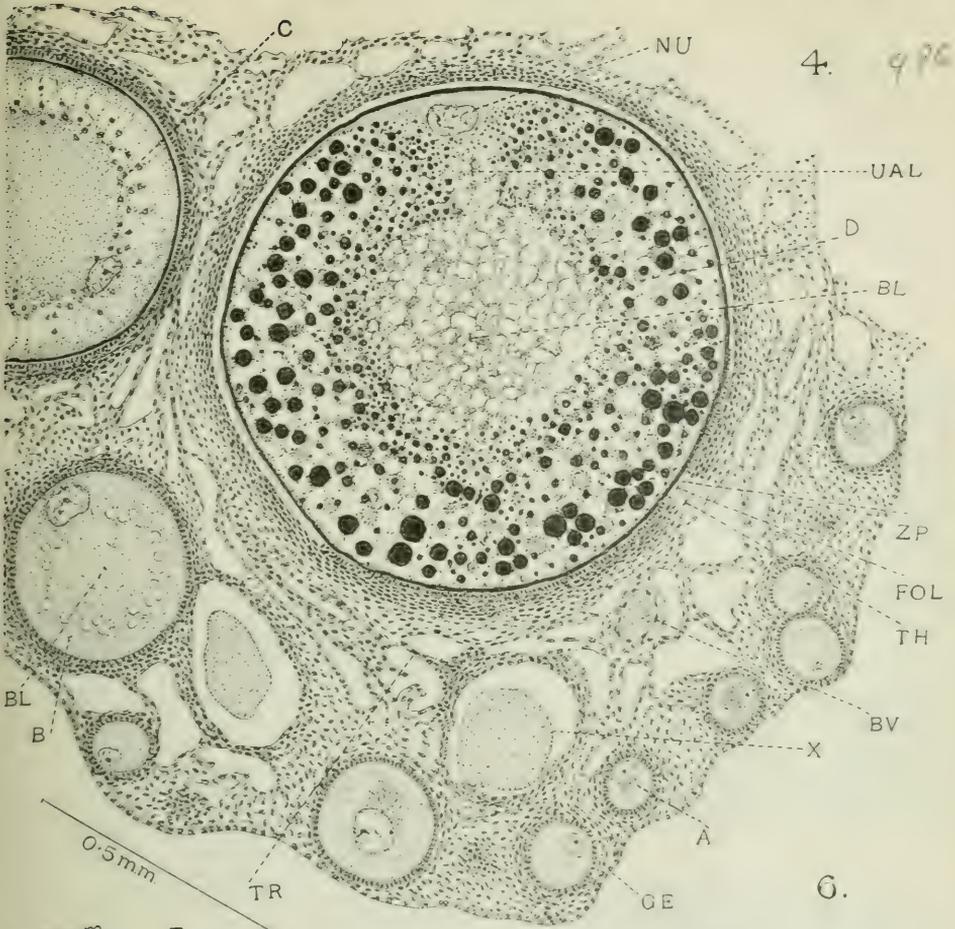
Fig. 12.—Follicle and part of egg at stage little later than in fig. 10, showing pre-zona substance apparently within follicle wall. Two layers of nuclei just forming in follicle.

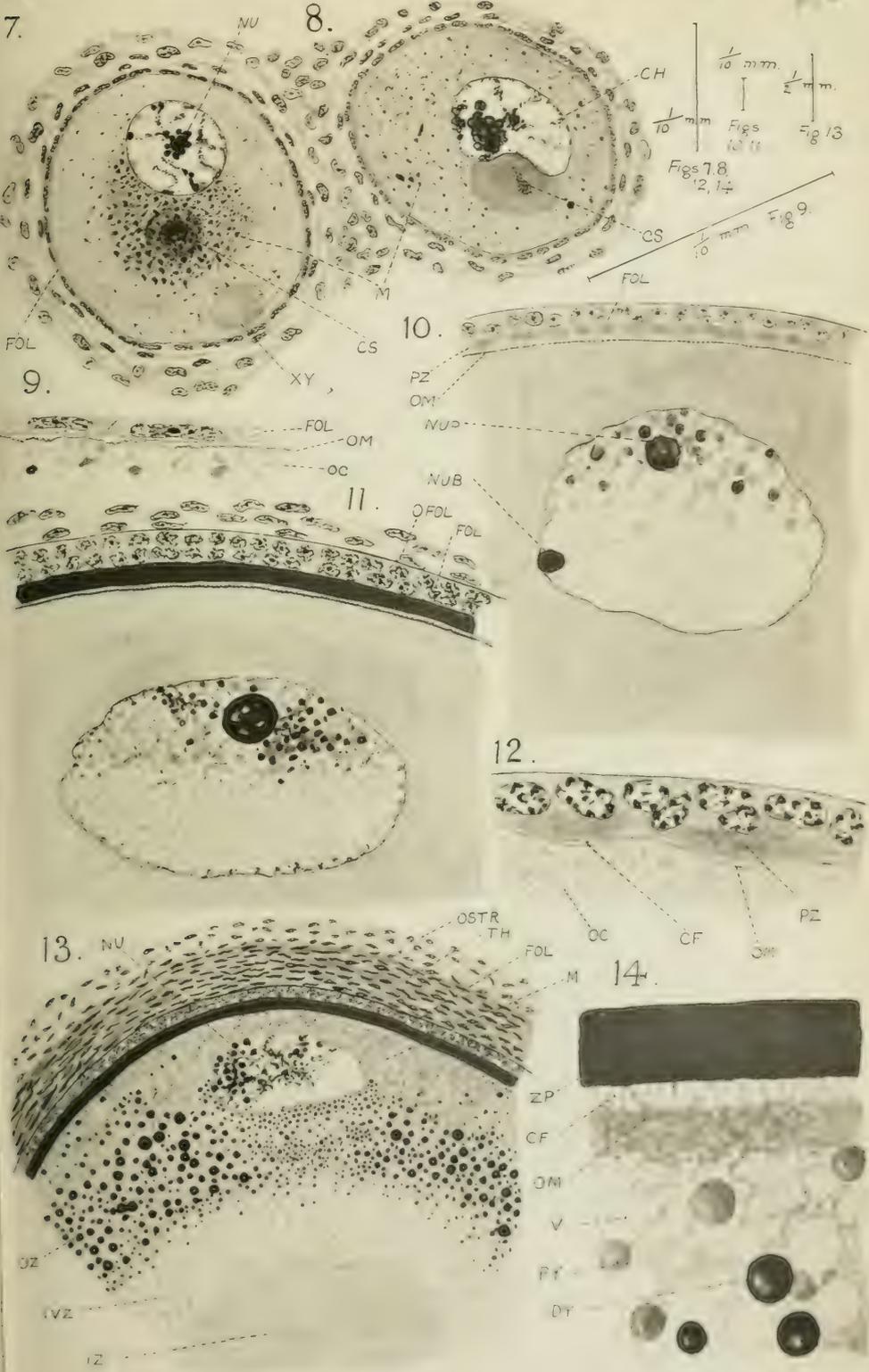
Fig. 13.—Detail of later egg, showing membranes. Mitochondria at m.

Fig. 14.—Cortex of later egg showing arrangement of layer beneath the zona.



GATENBY, del.





Note on the Comparative Effects on Tissues of Isotonic Saline and Distilled Water when used as Solvents for Mercuric Chloride and Formol in Histological Fixation.

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INTRODUCTORY.

SHOULD a simple fixative such as mercuric chloride or formol be dissolved in normal (i. e. isotonic) saline or in water? Curiously enough, observations are lacking on this simple but fundamental point.

Gustav Mann (6) states that formol should be diluted with isotonic saline 'because watery solutions cause such tissues as blood corpuscles and the central nervous system to swell up in whatever strength formol may be used'. That, moreover, is the only reference based (apparently) on actual observation which has come to my notice.

Most authorities on microscopical technique (Langeron, 3; Mallory and Wright, 5) advise that solutions of mercuric chloride and formol be prepared in distilled water, while Lee (4) advocates the dilution of formol with tap water, the salts in this tending to neutralize the free formic acid always formed after formol has been kept for any length of time. Finally, among practising histologists and pathologists there does not seem to be any consensus of opinion on the subject. Some consider that it is immaterial whether mercuric chloride or formol are dissolved in saline or water; others hold that it is 'safer' to make up these substances in saline.

The aim of these observations was to note the tissue-changes

(if any) caused by making up mercuric chloride or formol in distilled water or in isotonic saline.

TECHNIQUE.

In these experiments every attempt was made to modify only the tonicity of the sodium chloride and that alone. To attain this end the following precautions were observed :

(1) The concentration of the substance employed as a fixing reagent was kept constant throughout the series, that of the mercuric chloride being 6 per cent., that of the formol 5 per cent. The solutions of the latter were all prepared from the same sample of commercial (40 per cent.) formol, since the strength of this substance is very liable to variation, especially after keeping. By 5 per cent. formol is meant a 5 per cent. solution of formaldehyde, i. e. a solution prepared by diluting one volume of 40 per cent. formol with seven volumes of water.

(2) The same volume of fixative (50 cc.) was always used.

(3) Care was taken in cutting out the pieces of tissue for fixation to keep them, so far as was possible, of the same size.

(4) The conditions of embedding and staining were kept constant. The tissues were dehydrated in ascending grades of alcohol—beginning with 50 per cent. alcohol—cleared, and embedded in paraffin all in exactly the same manner. To reduce cell-shrinkage during these processes to a minimum, the tissues were passed from absolute alcohol to a mixture of equal parts of absolute alcohol and xylol. They were then transferred to pure xylol, next to xylol-paraffin, and finally to pure paraffin. Further, all tissues were cut at the same thickness ($8\ \mu$), and, as a final precaution, the sections used for microscopic observation were taken after 50 cm. of the ribbon containing the sections had been cut on the microtome. In this way the sections were all taken from approximately the same depth beneath the surface of the piece of tissue—a point of some importance in view of the tendency of many fixatives to shrink the periphery more than the centre of tissues. Sections were stained on the slide with Ehrlich's haematoxylin and

eosin as a standard method, supplemented by Heidenhain's iron-haematoxylin followed by Lichtgrun.

(5) Finally, the question of personal bias (far more important than is generally supposed) was dealt with in the following manner: the identification numbers on the slides were covered with unmarked labels. The degree of tissue-change was then carefully noted for every slide in each series. Only then were the labels taken off the slides. I am convinced that methods such as these are necessary if minute differences in tissues either in response to variations in the fixative or, indeed, to any other factors, are to be accurately recorded.

It is usually impossible to check histological observations by quantitative methods. Only in certain specialized spheres, e. g. the counting of cells in body and other fluids, can this be done. It is therefore imperative in qualitative observations—such as those forming the subject of this note—to adopt every means whereby the conditions of experimentation can be standardized, and the personal factor reduced to a minimum.

The Comparative Effects of Using Normal Saline and Distilled Water as Solvents for Mercuric Chloride and Formol.

Amphibian and mammalian tissues were used for these observations. The following organs were studied:

In the Frog.—Liver and small intestine.

In the Cat.—Liver, duodenum, and kidney.

Liver was chosen because the relatively large size of the hepatic cells renders observation of their size and shape comparatively easy. The abundant blood in the sinusoids of this organ also enables the behaviour of the red blood-corpuscles to be noted.

Small Intestine was selected because it makes possible the study of two different tissues—epithelium and non-striated muscle—in the same section.

Kidney was studied partly because of the histological differences in the different portions of the urinary tubule, partly on account of the sensitivity of renal tissue to the action

of fixatives. For in this connexion it is a matter of common knowledge that fixed specimens of kidney frequently show swelling or (more usually) shrinkage of the renal epithelium.

The frogs were killed by pithing, the cats by a blow on the head. The tissues were fixed immediately after death.

Amphibian (Frog) Tissues.—The concentration of the normal (isotonic) saline was 0.6 per cent. Two frogs were used, i. e. the experiments were once repeated so as to observe whether the effects were constant.

(1) 6 per cent. Mercuric Chloride in 0.6 per cent. NaCl.—The preservation of both liver and intestine is normal. There is no evidence of either shrinkage or swelling of the cells. The shape of the red blood-corpuseles is normal.

(2) 6 per cent. Mercuric Chloride in Distilled Water.—Tissues fixed in this solution are indistinguishable from those fixed in no. 1.

(3) 5 per cent. Formol in 0.6 per cent. NaCl.—The fixation is normal and comparable to that obtained with mercuric chloride dissolved in the corresponding grade of saline (no. 1).

(4) 5 per cent. Formol in Distilled Water.—**Liver.**—The cells are normal in size and shape. Their cytoplasm is vacuolated, and the whole appearance suggestive of some change—probably of the nature of a partial solution of the cell-contents—brought about by fixation. There is no distortion of the red blood-corpuseles in the sinusoids. **Intestine.**—In some specimens the epithelium is normally preserved, in others the columnar epithelial cells are vacuolated and somewhat swollen. The muscular coats of the intestine are well preserved.

MAMMALIAN (CAT) TISSUES.—The concentration of the normal saline employed in this series was 0.9 per cent. The observations were once repeated, as for the frog series.

(1) 6 per cent. Mercuric Chloride in 0.9 per cent. NaCl.—**Liver.**—The fixation is normal. The red blood-corpuseles are not distorted. **Duodenum.**—Both intestinal epithelium and muscle are well fixed. **Kidney.**—The fixation

varies in the different segments of the renal tubules; the glomeruli are somewhat shrunken, while there is absence of shrinkage in the other portions of the renal tubules, i. e. the fixation is fair in the first and second convoluted tubules, in the ascending and descending portions of the loops of Henle, and in the collecting tubules.

(2) 6 per cent. Mercuric Chloride in Distilled Water.—Liver.—Fixation normal. There is no distortion of the red blood-corpuscles in the sinusoids. Duodenum.—Both epithelium and muscle are well preserved. Kidney.—Some glomerular shrinkage; the other elements of the renal tubules are normally fixed.

(3) 5 per cent. Formol in 0.9 per cent. NaCl.—Liver.—Cells normal in size and shape. No distortion of the red blood-corpuscles. Duodenum.—Epithelium well preserved; some shrinkage of the fibres in the muscle-layers. Kidney.—No distortion of the glomeruli; there are small areas in the medullary rays showing swelling of the tubule cells—especially in the loops of Henle. Sometimes the swelling of the renal epithelium is so marked that the lumina of the tubules are almost obliterated.

(4) 5 per cent. Formol in Distilled Water.—Liver.—There is neither shrinkage nor swelling of the cells. Examination of iron haematoxylin sections with the high power reveals faulty fixation of the ground cytoplasm, in that the latter has the appearance of having been partially dissolved by the fixative. The red blood-corpuscles in the sinusoids are not distorted. Duodenum.—Epithelium well preserved; slight shrinkage of the fibres in the muscle-layer. Kidney.—Both glomeruli and tubules are slightly shrunken.

Note on the Effect of using Hypertonic Saline as a Solvent for Mercuric Chloride and Formol.

To ascertain whether increasing the concentration of the NaCl would produce cell-shrinkage, observations were made on amphibian and mammalian tissues. The fixatives already

employed (and in the same proportion to the volume of the solvent) were made up in saline solutions of double the normal concentration, i. e. of 1.8 per cent. and 1.2 per cent. for the cat and frog respectively.

The results for both cat and frog tissues may be summarized as follows :

6 per cent. mercuric chloride and 5 per cent. formol dissolved in saline of double the normal concentration caused shrinkage of the tissues examined. The degree of shrinkage was notably greater in the formol than in the mercuric chloride series. Further, while intestinal epithelium is relatively tolerant to this increase in the tonicity of the NaCl, intestinal muscle and liver are less so. Kidney showed both glomerular and tubule shrinkage—especially in the formol series. The red blood-corpuseles of the cat were crenated and distorted, while those of the frog retained their normal shape. Finally, the mitochondria of the hepatic cells, after staining with iron haematoxylin, were found to remain unchanged no matter whether the fixative (mercuric chloride or formol) were made up in distilled water, isotonic saline, or hypertonic saline of double the normal concentration.

CONCLUSIONS.

It appears to be of no histological importance whether saturated (6 per cent.) solutions of mercuric chloride be dissolved in normal saline or in distilled water. No differences could be detected in specimens of liver, small intestine, and kidney fixed in either way, nor would there be any reason to expect such differences on a priori grounds. For the relatively high molecular concentration of the HgCl_2 is only very slightly altered by dissolving it in either isotonic saline or in hypertonic saline of double the normal concentration. In fact, the only effect of making up a concentrated solution of mercuric chloride in normal saline is slightly to increase the tonicity of the mixture.

In the case of a 5 per cent. solution of formol the evidence is that this reagent fixes tissues more faithfully when made

up in normal saline than in distilled water. When dissolved in the latter the ground cytoplasm is often vacuolated, and, sometimes, partly destroyed.

Subject to revision in the light of further observations, I suggest the following as an explanation of the distortion of tissues caused by fixation in 5 per cent. formol made up in distilled water. As already pointed out, the only effect of dissolving mercuric chloride in normal saline is *slightly to increase* the molecular concentration of the mixture. But in the case of formol this is different, for the low molecular concentration of 5 per cent. solution of formol (as compared to 6 per cent. HgCl_2) is *appreciably increased* by making it up in normal saline instead of distilled water. This means that while a given concentration of formol in normal saline may be isotonic with tissues, the same concentration of formol in distilled water may be sufficiently hypotonic to cause distortion and swelling of cells.

Solutions of mercuric chloride and formol when dissolved in hypertonic solutions of saline of double the normal strength give rise to tissue-shrinkage. This shrinkage is more marked in the formol than in the mercuric chloride series. Further, the degree of shrinkage induced by the fixative varies greatly—as is well known—with different tissues.

These remarks concerning the dilution of mercuric chloride and formol in distilled water and in isotonic saline only apply to these two fixing reagents used in the concentrations already mentioned, these being, moreover, the concentrations at which they are the most commonly employed. The question of the tonicity of compound fixatives is omitted, for here, as claimed by Gatenby (1 and 2), the strength of the fixative is regulated by diluting it, if necessary, with distilled water. Thus, if fixation in a chrome-osmium mixture produces cell-shrinkage, the fixative should be diluted with a known volume of distilled water. This trial and error method is repeated until the dilution of the fixative is such that it does not cause shrinkage by a too rapid exosmosis from the cells.

The practical outcome of this note, then, is that while it is immaterial whether a concentrated (6 per cent.) solution of mercuric chloride be dissolved in isotonic saline or in distilled water, formol of 5 per cent. should be made up in isotonic saline and NOT in distilled water.

In conclusion, I have to thank Professor Sir Charles Sherrington for his interest in my work and for according me every facility in his laboratory.

March, 1922.

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On the Structure of the Alimentary Canal and its Ferments in the Bee (*Apis mellifera* L.).

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With 3 Curves and Plates 15, 16, and 17.

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THIS work was conducted by us with the same division of labour as the research on the intestine, its appendages and ferments in the scorpion, published previously. E. Pavlovsky undertook the zoological part of the work—the dissection of live bees, the preparation of the intestine, and the preparation of extracts from its parts. The chemical investigation of the ferments of these extracts was subsequently done by E. Zarin.

ANATOMICAL PART.

The intestine of the bee formed the subject of investigation for many scientists (for literature cf. Zander, Snodgrass), therefore its general anatomical relations may be considered to be sufficiently elucidated.

We shall limit ourselves to the description of the general organization of the intestine and point out some peculiarities in its microscopical structure, whilst the literature on the question will be omitted.

The intestine of the bee consists of the fore-, mid-, and hind-guts (Pl. 15, figs. 1–3). The fore-gut begins with the pharynx, which passes to the oesophagus dilating into the honey-stomach, crop, or ingluvies (Pl. 15, figs. 1–3; Pl. 16, fig. 4, *i*). The latter passes by means of the cardiac valve into the ventriculus (mid-gut or stomach, *-r*). The hind-gut is divided into the anterior portion—the small intestine (*it*), and the posterior—the large intestine (*-r*) with the rectal glands (*rg*).

The Malpighian vessels (*mp*) open on the border of the ventriculus and the small intestine.

Fore-gut.

The Fore-gut (pharynx, oesophagus, ingluvies) is lined within by a chitinous cuticle, to the exterior of which lies a layer of non-glandular epithelium (Pl. 16, fig. 5, *ep*) resting on membrana basilaris. The latter is covered by a network of transversally striated muscle-fibres lying in two layers—circular and longitudinal (Pl. 16, fig. 5, *m*₄, *m*₅).

The valve of the ingluvies is represented by a capitulated

eminence of the bottom of the ingluvies. The capitulum consists of four valves between which there is a cruciate slit (Pl. 16, fig. 5, *pc*). The valve is provided with three systems of muscular fibres—two longitudinal (Pl. 16, fig. 5, m_2 , m_3) and one circular (Pl. 16, fig. 5, m_1) between them. The former serve to open the valve, the latter to close it. The capitulum of the valve is set on a trunk connecting it with the stomach. From the circumference of the ventricular opening into the intestine hangs an intestiniform cardiac valvule preventing the contents of the stomach from returning into the crop. All these data were already established by previous investigators.

Mid-gut (Stomach).

The stomach of the bee consists of a fairly thick cylindrical tube with numerous circular constrictions on it corresponding to which the epithelium of the stomach protrudes into its cavity in the form of folds. The epithelium consists of cylindrical cells which assume the shape of clubs on the ridges of the folds. At the bottom of the depressions between them are situated round groups of cells called cryptae. Exteriorly to the membrana basilaris is disposed the connective tissue in the form of small groups of cells. The muscular membrane of the stomach is formed by two layers of transversally striated muscle-fibres—interior circular, and exterior longitudinal.

(a) The epithelium of the stomach consists of cells with an alveolar protoplasm of basophil character (Pl. 16, fig. 7, *ep*; figs. 9, 11, 13, *ep*). The oval nucleus with sparse chromatin granules, or a dense network of them, lies in the middle of the cell. In its protoplasm are produced oxyphil granules of secretion which are numerous in the superficial portion of the cells. In some sections the cells appear to be set on thin peduncles and to have truncated apices. This picture, as well as the formation of evaginated swellings on the surface of the cells, is in most cases artificial (Pl. 16, fig. 12, *bl*).

The superficial layer of protoplasm of the epithelium is transformed into a fairly broad band vertically striated and bearing the aspect of a brush of cilia (Pl. 16, figs. 9, 11, 12, *wp*).

This hairy layer of protoplasm stains with iron haematoxylin in a grey colour, whereas the protoplasm remains black. The hairy band is covered above by a cuticle (not chitinous); together with the latter it is cast off into the cavity of the stomach in the form of a peritrophic layer (Pl. 16, figs. 6, 7, *p*; figs. 9, 10, *p*). This casting off is repeated many times, on account of which in the stomach the membranes are disposed in concentric layers, sometimes in very great numbers (Pl. 16, fig. 6, *p*).

The peritrophic membrane presents a structure known for a long time in the articulated animals. With regard to the bee Petersen has demonstrated that the said membrane of this insect contains a proteolytic ferment. The significance of the peritrophic membrane is interpreted in different ways. Some investigators believe it to serve for the defence of the tender stomach epithelium against mechanical injury by vegetable food, especially by the flower pollen in the bee.

Such an interpretation cannot be extended to all Arthropods, since an analogous formation is also present in blood-sucking forms (*Culex*, *Anopheles*, according to Schaudinn), the liquid food of which cannot do any harm to the walls of the stomach. Probably those investigators are right who regard the peritrophic membrane as a cuticle formed by the secretion of the stomach epithelium. Originating by transformation of the surface protoplasm of the cells, the membrane itself presents a hard secretion. In the depth of the hairy layer, as in a sponge (Pl. 16, fig. 9, *p*), is retained the liquid secretion of the stomach, on account of which the same quantity of ferment is capable of acting for a longer period upon the food contained in the cavity of the mid-gut. On account of the relative shortness of the intestine this mode of action of the ferments is of special significance, especially in herbivorous insects, since the food does not pass through the intestine so rapidly, being detained in the folds soaked with the digestive juices of the peritrophic membranes. Thus, in our opinion, they compensate the relatively small length of the intestine in insects.

(*b*) At the bottom of the folds of the stomach epithelium

are situated groups of smaller cells forming crypts which are weakly developed in the bee.

The cells of the latter are disposed in two or three layers (Pl. 16, fig. 8; fig. 9, *k*). The deepest row situated on the basal membrane is represented by the smallest cells (in the section three or four of them are visible), covered above and laterally by larger cells bordering directly on the epithelium of the folds of the stomach (Pl. 16, fig. 11, *k*). In general the protoplasm of the cells of the crypts are more basophil than the stomach epithelium (Pl. 16, fig. 11, *k, d*). The nuclei of the cryptic cells are large and poor in chromatin. As described by Petersen we did not succeed in observing their karyokinesis. Nasonov (1898), however, observed the process of division of the nuclei in these cells.

The cryptal cells present the sources from which the stomach epithelium is newly formed. Besides, their cells seem to produce a secretion themselves as well. The following facts confirm this supposition. The most superficial cells of the cryptal are not adjacent to each other with their apices, so that there remains an ovoid lumen between them in the shape of a vacuole filled up with a drop of homogeneous secretion staining pink with Giemsa's stain (Pl. 16, fig. 7, *k*; fig. 11, *vc*). Besides, there is also observed an accumulation of secretion above the crypta which is revealed by displacement to the sides of the 'hairs' of the superficial band of the stomach epithelium (Pl. 16, fig. 9, *k*).

In general the secretory processes in the stomach of the bee take the following course :

(1) Separation of the peritrophic membrane (Pl. 16, fig. 6, *p*; fig. 9, *p*), (2) production of secretion by the surface of the glandular cells, (3) severance of the superficial portions of the epithelial cells (Pl. 16, fig. 8, *d*), and (4) separation of a homogeneous secretion by the cryptic cells (Pl. 16, fig. 11, *vc*).

(*c*) The epithelium of the stomach lies on a basal membrane clothed exteriorly by a transversely striated muscular membrane, the muscle-fibres of which are very rich in sarcoplasm. The muscle-fibrils are disposed in bundles occupying the greater

part of the surface of the transverse section of the fibre (Pl. 17, fig. 15, *mf*) at the point where the sarcoplasm and nuclei are scarce, and half the diameter of the fibre where the sarcoplasm and nuclei are strongly developed. The nuclei always lie in the sarcoplasm nearer to the periphery of the muscle-columns (Pl. 16, fig. 14; Pl. 17, fig. 15, 16, *sp*), and not between the latter as in the analogous membrane of the small intestine (Pl. 17, fig. 18).

Hind-gut.

The hind-gut is divided into two parts—both in its anatomical and histological structure—the anterior—small intestine (Pl. 15, fig. 3; Pl. 16, fig. 4, *it*), and posterior—large intestine (Pl. 16, fig. 4, *r*).

Small Intestine.

The structure of the small intestine has already been established by previous investigators. We may add to these some details in the microscopical structure of its single-layered cylindrical epithelium. The cells of the latter are covered on their interior surface by a thick chitinous cuticle. The protoplasm of the cells is divided into two portions, the exterior—granular (Pl. 17, fig. 17, *d*), and interior—characterized by a rod-line striation (Pl. 17, fig. 17, *ds*).

The fairly large rounded nucleus (*n*) lies nearer to the base of the cell. Interiorly to it in the layer of granular plasm are found large vacuoles with granules of secretion (*rs*). Both the protoplasm and secretion of the epithelium of the small intestine are oxyphil.

The basilar membrane of the intestine (Pl. 17, fig. 17, *mb*) is surrounded by circular muscle-fibres anastomazing with each other. They are thick and their nuclei are disposed along the axis of the fibres surrounded from all sides by bundles of myofibrils (Pl. 17, fig. 18, *cmf*).

In general the small intestine of the bee is characterized by the glandular character of its epithelium. The structure of the intestine described may serve as evidence either of its glandular function or of processes of absorption taking place

in it, or, lastly, of its excretory rôle. We have hitherto only established one fact for certain—the complete absence of ferments in extracts from the small intestine of the bee.

Large Intestine.

The large intestine, similarly to the crop of the bee, presents a thin-walled sac which is capable of expanding to enormous dimensions, as seen by comparison of figs. 1 and 3 of Pl. 15. During the whole winter the bees do not evacuate their excrements, but continue taking food, on account of which their large intestine becomes overfilled with faeces and swells into a voluminous bladder.

The scheme of structure of the large intestine is the same as in the crop. In the anterior third of its wall are situated six elongated cylindrical rectal glands (Pl. 17, fig. 19, *rg*), the microscopical structure of which was in general features correctly described by Snodgrass and Petersen.

We have also succeeded in establishing certain interesting details elucidating the structure of these glands. From the part of the cavity of the rectum each gland is covered by a chitinous cuticle forming on the periphery of the organ a marginal fillet. Within the gland there is an axial cavity (Pl. 17, fig. 21, *h*) dividing it into two parts—an exterior thin wall (*wa*) and interior thick one (*sn*). The latter is formed by tall wedge-shaped cells polyhedral in transverse section.

The exterior wall is formed by two layers of minute polygonal cells (Pl. 17, fig. 21, *wa*). At the point where both walls join together there lies a syncytial layer of cells containing pigment inclusions (Pl. 17, fig. 21, *sn*).

The exterior wall of the rectal gland (Pl. 17, fig. 20; fig. 22, *wa*) is perforated in some places by tracheae (*tr*) which pass into the cavity of the organ and penetrate with their branches into its inner wall.

To these data, which are to be found in the literature, we may add the following:

The ramifications of the tracheae passing to the thick inner wall of the rectal gland pass along the edge of the poly-

hedral epithelial cells (Pl. 17, figs. 23, 25, *tr*). The layers of protoplasm of the latter adjacent to the tracheae consist of a substance staining deep black with Heidenhain's iron haematoxylin (Pl. 17, figs. 22, 25, *z*).

These bordering layers differ from the alveolar-granular protoplasm of the cells in their dentate aspect; in some individuals they resemble coarse intercellular bridges; in others they are more weakly expressed; their striation, however, is always visible in a greater or less degree.

It is possible to trace the course of the tracheae to four-fifths of the height of the cells. At this level the tracheae which have hitherto pursued a radial course give off lateral branches forming beneath the inner surface of the gland a network rich in anastomoses (Pl. 17, fig. 24, *tr*). We did not observe anything like the opening of the tracheae directly into the cavity of the intestine in the rectum of the bee, as was described by Vallé in Diptera.

The protoplasm of the large cells is in general granular in some individuals with a fairly distinctly expressed alveolar structure. The protoplasm is oxyphil. To the chitinous cuticle is adjacent a layer of protoplasm staining less and bearing the aspect of vesicles lying close to each other. The nuclei of the cells described are of an irregular round shape. They are disposed either in the middle part, or basally, depending upon the degree to which the protoplasm is filled up with granular inclusions. The nuclei are poor in chromatin.

The variation in the contents of the cells described probably is in connexion with the seasons of the year. In the hibernating bee the large intestine of which had for several months been filled up with faeces, the protoplasm of the large cells of the rectal glands contains numerous globular inclusions and minute granules (Pl. 17, fig. 21, *gr*). Both are oxyphil, with the exception of some of the larger granules. In some of them are visible roundish portions not stained black with iron haematoxylin. All these formations occupy the middle two-thirds of the transverse section of the cell; whilst in the basal quarter of it lies the displaced nucleus (Pl. 17, fig. 21).

The protoplasm of the rectal glands of bees taken in ordinary condition, although granular, is devoid of the inclusions described above (Pl. 17, fig. 22, *d*).

In bringing together these facts, we may speak of the absorptive rôle of the rectal glands, which appears to be correct *a priori*, on account of the long period during which the faeces remain in the rectum in bees hibernating in our latitudes. The microscopical structure of the tall epithelium of the gland points to a possibility of true glandular processes taking place in it. Below we shall discuss the conclusion according to which the rectal glands present the source of seasonal production of catalase, and the point of development of energetic oxidizing processes which is evinced by the intimate connexion between these organs and the tracheae.

PHYSIOLOGICAL PART.

There are few data in literature regarding the ferments found in the organism of the bee. The first works in this direction were conducted by Erlenmeyer and Planta in 1877.

The authors named dissected 152 worker-bees separating head, thorax, and abdomen, and infused them separately in glycerine. It was found that all the three extracts converted starch to dextrin and sugar, and saccharose to inverted sugar, the extracts from the head and abdomen being much more active than that from the thorax. The extracts from the head and abdomen also contained a ferment dissolving fibrin of the blood, the latter extract being stronger than the former, whilst that from the thorax produced no effect.

The methods applied by Erlenmeyer and Planta for the preparation of extracts is of no use at all, since the exterior division of the body of the bee into head, thorax, and abdomen does not correspond at all to the division of the intestine into its characteristic portions.

In 1912 Petersen, whilst studying a question on the digestion in the bee, also made experiments on the determination of ferments in the digestive organs of the bee. In glycerine extracts from the stomachs of bees the author discovered the

following ferments: diastase, invertase, and a proteolytic ferment dissolving fibrin and splitting peptone. These are essentially all the data to be had in literature on the question discussed.

Methods used in Preparing the Material for Chemical Investigation of the Ferments.

The only fit material is presented by live bees, live for anatomical purposes. They are chloroformed and dissected in physiological solution (0.75 per cent.) of common salt for the preparation of the intestine. The removed intestine is washed in a Petri dish with the physiological solution and separated into the following parts: crop, stomach, small and large intestines.

Each portion is further dissected in order to remove its contents, washed in a fresh portion of the same solution, and placed in a small evaporating glass with a small quantity of desiccated sand and several drops of glycerine. After the portions of the intestine of all the bees have been placed in glasses, they are GROUND with a glass mortar until a uniformly opaque emulsion is produced. Then to each glass is added the necessary quantity of glycerine or some other liquid, the whole is rapidly mixed and poured out into a jar with a hermetically closing glass stopper.

Extracts were prepared with (a) glycerine, (b) distilled water, (c) a mixture of equal quantities of the liquids named, and (d) physiological solution of common salt. As an antiseptic a few (5-10) drops of toluol were added. The jars were several times shaken thoroughly and left to stand in the darkness at the temperature of the room for different periods.

The density of the extracts varied. We started from strong extracts, ninety bees per 30 c.c. of liquid, i.e. from each portion of the intestine taken from ninety bees an extract was prepared in 30 c.c. By degrees as the experiments progressed it proved to be more practicable to take weaker extracts, until finally we stopped at the proportion of 25 bees per 50 c.c. of liquid.

Altogether thirty analyses were performed, a table relating to the periods of which is adduced below.

TABLE I.

<i>No. of Experiment.</i>	<i>Date of Preparation of Extract.</i>	<i>No. of Bees from which Intestines were prepared.</i>	<i>Volume of fluid in c.c.</i>	<i>Base on which Extract was prepared.</i>	<i>Amount of Toluol in Drops.</i>
1	23 X 1916	90 workers	30	Glycerine	10
2	7 IV 1917	60 "	25	"	10
3	22 VI 1917	60 "	25	Water	10
4	"	30 "	12.5	Glycerine	5
5	20 VII 1917	50 drones	25	"	10
6	21 IX 1917	40 workers	20	"	8
7	15 XI 1917	30 "	30	15 c.c. glyc.+ 15 c.c. water	10
8	31 III 1918	30 "	30	"	10
9	"	30 "	30	Water	10
10	14 IV 1918	15 "	15	7.5 c.c. glyc.+ 7.5 c.c. water	5
11	15 IV 1918	15 "	15	"	5
12	21 IV 1918	15 "	15	"	5
13	23 IV 1918	15 "	15	"	5
14	17 VI 1918	15 "	15	"	5
15	"	15 "	15	"	5
16	"	15 "	15	"	5
17	2 VII 1918	25 "	50	"	10
18	3 VII 1918	15 drones	15	"	5
19	"	25 workers	50	"	10
20	6 VIII 1918	25 "	50	"	10
21	"	15 "	15	Water	5
22	"	15 "	15	7.5 c.c. glyc.+ 7.5 c.c. water	5
23	"	15 "	15	Glycerine	5
24	20 VIII 1918	25 "	50	25 c.c. glyc.+ 25 c.c. water	10
25	"	15 "	15	Water	5
26	"	15 "	15	Glycerine	5
27	"	15 "	15	Physiol. sol. NaCl	5
28	"	15 "	15	7.5 c.c. glyc.+ 7.5 c.c. water	5
29	24 X 1918	25 "	50	25 c.c. glyc.+ 25 c.c. water	10
30	"	25 "	50	Water	10
Total . .		795 bees			

As the primary aim of the present work we regarded the qualitative determination of the ferments in the different portions of the ventriculo-intestinal tract of the bee, omitting, meanwhile, the investigation of the ferments of their salivary glands.

In the intestine were established catalase, inulase, lactase, invertase, lipase, pepsin, trypsin, chimosin, and emulsin. Of these ferments we have investigated in fuller detail the catalase and invertase.

Catalase.

As is known, catalase is a ferment widely distributed in the animal and vegetable kingdom. Regarding its presence in the body of the bee no data are known in the literature.

For the determination of catalase we employed a special apparatus constructed by one of us (Zarin).

The process consisted in mixing 2 c.c. of corresponding extracts of ferments with 8 c.c. of water; to the filtered mixture were added 10 c.c. of freshly prepared 1 per cent. solution of hydrogen peroxide; the number of c.c. of oxygen evolved being marked after the expiration of twenty-four hours. It need not be mentioned that all the analyses were accompanied by control experiments. The results obtained in the investigations are shown in Table II.

TABLE II. CATALASE IN THE INTESTINE OF THE BEE.

No.	Date of Experiment.	Concentration and Composition of Extract.	Quantity of Oxygen evolved in c.c.			
			Crop.	Stomach.	Small Intestine.	Large Intestine.
1	8 IV 1917	60 bees : 25 c.c. glyc.	0	6.5	0	2.0
2	23 VI 1917	30 bees : 12.5 c.c. glyc.	0	1.5	0	0
3	16 XI 1917	30 bees : 15 c.c. glyc.+ 15 c.c. water	0	1.3	0	0
4	1 IV 1918	" "	0	11.5	0	3.5
5	"	30 bees : 30 c.c. water	0	9.5	0	2.0
6	15 IV 1918	15 bees : 7.5 c.c. glyc.+ 7.5 c.c. water	0	7.3	0	3.3
7	"	" "	0	13.2	0	2.8
8	22 IV 1918	" "	0	5.3	0	0.3 ¹
9	24 IV 1918	" "	0	7.0	0	0
10	19 VI 1918	" "	0	9.2	0	0
11	2 VII 1918	25 bees : 25 c.c. glyc.+ 25 c.c. water	0	3.0	0	0
12	4 VII 1918	" "	0	2.0	0	0
13	"	15 drones : 7.5 c.c. glyc.+ 7.5 c.c. water	0	2.5	0	0
14	7 VIII 1918	25 bees : 25 c.c. glyc.+ 25 c.c. water	0	8.5	0	0
15	21 VIII 1918	" "	0	4.0	0	0

¹ In experiment no. 8 the bees were taken three hours after the hives were removed from the hibernating quarters, when the contents of the rectum were discharged by them.

It is seen from the table that catalase is a specific secretion of the stomach in the bee and drone. This portion of the

intestine produces it continuously during the whole year, whereas in the rectum it was observed in our experiments only in spring.

The latter circumstance is explainable by the fact that the bee remaining in the hive during the six winter months does not discharge its excrements and retains all the faeces till the first spring flight. The discharge of catalase in the rectum depends upon the accumulation of the faeces in it, and evidently serves as a regulation of the different oxidizing processes and destroys the surplus of peroxides in the intercellular metabolism. On the day when the bees issue forth from the hive after hibernation, after three hours of flight during which they become evacuated, the catalase is contained in the large intestine only in a small quantity, and after two days it disappears altogether, as is seen from experiments no. 8 and no. 9 in Table II.

It would be interesting and important from the practical point of view to ascertain the relations presented by catalase in the southern races of bees, which hibernate for a very short period in comparison with our northern bees.

It may be supposed *a priori* that in the rectum of the southern bees less faeces are accumulated than in northern bees, and that the oxidative processes in the former proceed at a different rate from those in the latter. It is possible that respective investigations would provide an explanation of the failure to acclimatize southern bees in the north. The latter on arriving in the north encounter unusual conditions of a long winter, and have therefore to accumulate excessive masses of faeces in their rectum. It is natural that these bees, unaccustomed to such conditions, are liable to different diseases, amongst which pernicious diarrhoea plays the first rôle.

In order to ascertain whether the secretions from different parts of the intestine stimulate each other when mixed together, catalase was determined in all possible combinations of extracts from parts of the intestines; in each separate case 2 c.c. of the extracts named below were taken:

	<i>Spring</i> 14, IV, 1918.	<i>Summer</i> 4, VII, 1918.
A. Crop + small intestine + large intestine	4.0 c.c.	0 c.c.
B. Crop + small intestine	0.0 „	0 „
C. Crop + large intestine	4.0 „	0 „
D. Small + large intestine	3.9 „	0 „

The experiments prove that in spring, besides the stomach, catalase is produced only by the large intestine (experiments A, C, D). The remaining two portions of the intestine (crop and small intestine) do not produce catalase either separately or combined with others (B); in summer during normal nutrition of the bees this ferment is produced only by the stomach.

In order to study the influence of the time during which the extract from the intestine is infused upon the activity of the catalase, the following experiments were conducted. In one portion the separate parts of the intestine were carefully rubbed together with desiccated sea-sand in the presence of several drops of glycerine; the emulsion produced was diluted with a mixture of equal parts of glycerine and water with the addition of 10 drops of totuol; in another portion quite identical extracts, with the only difference that pieces of intestine were directly placed in a mixture of glycerine and water without being rubbed.

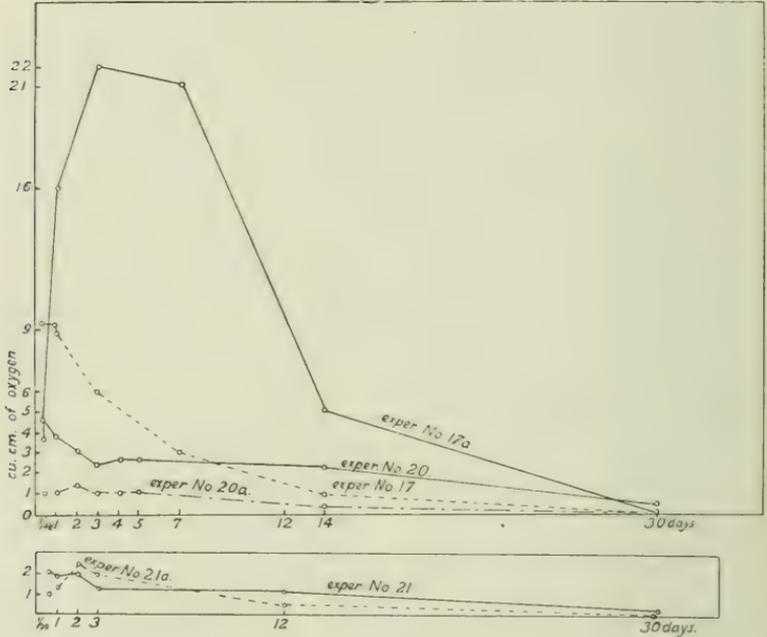
The catalase was determined for periods shown in Table III. in which the results obtained are shown.

An examination of the data represented in Table III reveals the following. Catalase was as usual produced only by the stomach of the bee. The quantity of ferment proved to differ sharply in extracts prepared from rubbed and intact stomachs. In the first extract (no. 17) after half an hour standing there proved to be nearly three times as much ferment as in the second (no. 17 a).

The activity of the ferment in the succeeding days differed in both extracts. In the extract from rubbed stomachs the activity of catalase invariably decreased right to its complete disappearance, which was established by us on the thirtieth day. In the parallel experiment with intact stomachs the

activity of the same ferment, on the contrary, sharply increased with the duration of infusion of the extract. Thus, already after one day, the active power of catalase increases nearly four times, and on the third day nearly six times; only beginning from the seventh day the activity began to decrease sharply.

CURVE I.



Curve representing the variation of the activity of catalase, according to the length of infusion of the intestine and in connexion with the method of preparation of the extract (series of experiments nos. 17, 20, 21. See Table III).

Such a difference in the activity of catalase in the two parallel experiments described is due, in our opinion, to the mode of preparation of the extracts. When the stomach is finely minced and rubbed, and the cells of its tissues destroyed, a maximum quantity of catalase is freed which gradually becomes inactivated while the extract stands. In extracts from intact pieces of the stomach in which but a small number

of cells were destroyed, when it was cut into parts a smaller quantity of ferment passes into the solution at once, its activity sharply increasing. This increase may be explained in two ways. Either the catalase continues to be produced by the intact cells of the intestine or its amount remains the same, but it only gradually passes from the tissue into the solution according to the time the extract stands.

In the following two parallel experiments (nos. 20, 20 *a*, and nos. 21, 21 *a*) conducted for the control of the data just discussed the picture was somewhat different. In extracts from rubbed stomachs the immediate discharge of catalase in large quantities, as well as its gradual decrease, was corroborated. However, the increase of activity of the ferment in extracts from intact stomachs was exhibited only in experiment no. 21 *a*, in which the activity of the catalase doubled in two days. In experiment no. 20 *a*, however, the activity of the ferment in the extract did not increase from standing, remaining on the same level during five days.

The experiments adduced point to the great variety in the action of catalase which depends on a series of conditions, amongst which the individual character of metabolism in the bee probably occupies the first place.

The solution of these questions should be the subject for special research.

Amylase.

The presence of amylase in the organism of the bee was discovered by Erlenmeyer and Planta and Petersen.

Erlenmeyer and Planta divided the bees into head, thorax, and abdomen, rubbed these parts of their bodies with sand, infused them in glycerine, and established in all three extracts the presence of amylase which converted starch into dextrin and sugar.

Petersen prepared fifty stomachs of bees, rubbed them with sand, infused in 10 c.c. of a mixture of equal parts of glycerine and 1 per cent. of sodium fluoride.

On acting with the extract obtained on starch solution

Petersen arrived at the conclusion that the splitting of starch proceeds to the formation of dextrins. In our experiments to 2 c.c. of corresponding extracts we added 0.1 c.c. of 0.5 per cent. solution of soluble starch, 8 c.c. water, and 2 drops of toluol; the test-tubes with the mixture were then placed for one hour in a water-bath at 45° C. At the expiration of this period the contents of the test-tubes were cooled, and to them iodine solution in potassium iodide was added by drops.

In all the experiments the extracts obtained from the stomachs produced a positive result: after the addition of iodine it always assumed a light-yellow coloration, whereas the extracts from the remaining three portions of the intestine, namely crop, mid-, and hind-guts, contained no amylase and assumed a blue colour after addition of iodine.

Thus our experiments proved that amylase is present only in the stomach of the bee, whereas the remaining portions of the intestine do not produce this ferment. In this case the splitting of starch proceeds not only to the formation of dextrins, as Petersen's experiments have shown, but to the formation of maltose and dextrose.

Owing to the presence of amylase in the digestive stomach the bee can digest starchy food.

The fact that Erlenmeyer and Planta discovered amylase in all the three extracts (from the head, thorax, and abdomen) is explainable on the basis of our findings in the following manner: the amylase in the extract from the abdomen doubtless is derived from the stomach of the bee: the same ferment in extracts from the head and thorax are probably produced by the salivary glands, since the fore-gut (oesophagus) passing through the named part of the body does not produce this ferment.

Inulase.

As is known, inulase converts the polysaccharid-inulin into levulose. Regarding the presence of this ferment in the intestine of the bee there are no data in the literature. The results obtained in our experiments allow us to conclude that the intestine of the bee does not produce inulase.

Lactase.

Lactase is a ferment splitting the disaccharid—milk sugar—into monosaccharids—glucose and galactose. There are no data in literature concerning the production of lactase in the intestine of the bee. In our experiments the presence of lactase was not established.

Invertase.

The presence of invertase in the organism of the bee was first discovered by Erlenmeyer and Planta in glycerine extracts from the heads, thoraces, and abdomens of bees. The extracts from the heads and abdomens prove to be more active than those from the thoraces.

Axenfeld divided the intestine of the bee into three parts—crop, stomach, and hind-gut. On acting with the named portion of the intestine on the solution of saccharase, the greatest activity was exhibited by the stomach, whereas the crop and hind-gut inverted sugar very weakly. The author named supposes that invertase is produced only by the stomach, but a small quantity of it is transferred mechanically to the hind-gut.

In our experiments we added to 5 c.c. of 10 per cent. solution of cane-sugar the tested extracts from the intestine of the bee and drone in quantities shown in the table, the liquid being placed—after addition of 1 c.c. of toluol as a conserving medium—in the thermostat at 36–40° C. for twenty-four to forty-eight hours; then after cooling the liquid to the temperature of the room and adding 1 drop of ammonia, in order to avoid birotation, the rotation of the plane of polarization was determined in a tube of 200 mm.

The results obtained are adduced in Table IV.

On examining the data adduced in the table it is seen that in regard to invertase the first two experiments differ essentially from the remaining, notwithstanding the similar methods employed in the analysis. Whereas in all the experiments, except the first two, the extracts from the stomach show an essential decrease of the right rotation of the polarization plane.

TABLE IV.

INVERTASE IN THE VENTRICULO-INTESTINAL TRACT OF THE WORKER-BEE AND DRONE.

No. of Experiment.	Date of Experiment.	Concentration and Composition of Extracts.	Concentration of Sugar Solution.	Quantity of Sugar Solution.	Quantity of Ferment Extract.	Time of Action hrs.	Control.	No. 1. Honey Stomach Crop.	No. 2. Mid-gut.	No. 3. Small Intestine.	No. 4. Large Intestine.	Nos. 1 + 3 + 4.
1	24 X 1916	90 bees per 30 c.c. of glycerine	%	c.c.	c.c.	48	+ 13-25	+ 13-22	+ 13-18	+ 13-23	+ 13-24	—
2	8 IV 1917	60 bees per 25 c.c. of glycerine	5	100	2	48	+ 6-13	+ 6-12	+ 6-10	+ 6-13	+ 6-14	—
3	23 VI 1917	30 bees per 12.5 c.c. of glycerine	5	100	5	24	+ 6-25	+ 6-23	+ 2-88	+ 6-26	+ 6-23	—
4	23 VI 1917	60 bees per 25 c.c. of water	5	100	5	24	+ 6-28	+ 6-24	+ 1-30	+ 6-27	+ 6-28	—
5	21 VII 1917	50 drones per 25 c.c. of glycerine	5	100	5	24	+ 6-08	+ 6-08	+ 5-78	+ 6-06	+ 6-06	+ 11-28
6	22 IX 1917	40 bees per 25 c.c. of glycerine	10	100	6	48	+ 11-45	—	+ 8-90	—	—	+ 5-25
7	16 XI 1917	30 bees per 15 c.c. of glycerine + 15 c.c. of water	5	50	5	48	+ 5-65	—	+ 2-12	—	—	+ 5-15
8	1 IV 1918	30 bees per 30 c.c. of water	5	50	5	48	+ 6-12	—	+ 2-30	—	—	—
9	"	15 bees per 7.5 c.c. of water	5	50	5	48	+ 6-00	+ 5-89	- 0-82	+ 5-84	+ 5-90	—
10	15 IV 1918	15 bees per 7.5 c.c. of glycerine + 7.5 c.c. of water	5	50	5	48	+ 6-00	—	+ 0-10	—	—	—
11	16 IV 1918	"	5	50	5	24	+ 6-13	+ 6-12	+ 0-66	+ 6-13	+ 6-12	—
12	22 IV 1918	"	5	50	5	24	+ 6-13	—	+ 2-55	—	—	—
13	24 IV 1918	"	5	15	3	24	+ 6-32	—	+ 2-30	—	—	—
14	"	"	5	100	2	24	+ 5-87	—	+ 3-55	—	—	—
15	18 VI 1918	"	5	50	5	24	+ 5-88	+ 5-87	+ 2-67	+ 5-88	+ 5-89	—
16	"	"	5	50	5	48	+ 5-88	—	+ 2-69	—	—	—
17	"	"	5	50	1	24	+ 6-15	—	+ 4-65	—	—	—
18	"	"	5	50	1	48	+ 6-15	+ 6-15	+ 4-60	—	—	—
19	2 VII 1918	25 bees per 25 c.c. of glycerine + 25 c.c. of water	5	50	6	24	+ 5-90	—	+ 2-18	—	—	+ 5-62
20	"	"	5	50	4	24	+ 6-08	—	+ 2-39	—	—	—
21	"	"	5	50	2	24	+ 6-35	—	+ 3-52	—	—	—
22	4 VII 1918	15 drones per 7.5 c.c. of glycerine + 7.5 c.c. of water	5	50	5	24	+ 5-17	—	+ 2-68	—	—	—
23	7 VIII 1918	15 bees per 15 c.c. of water	5	50	5	24	+ 5-94	—	+ 3-08	—	—	+ 5-71
24	"	15 bees per 7.5 c.c. of glycerine + 7.5 c.c. of water	5	50	5	24	+ 5-92	—	+ 2-50	—	—	—
25	"	15 bees per 15 c.c. of glycerine	5	50	5	24	+ 5-98	—	+ 3-94	—	—	—

Degrees of Rotation of the Plane of Polarization.

in experiments no. 1 and no. 2 the difference between the control and experimental solutions in this respect is so insignificant (0.07° and 0.03°) that it may be ascribed to error in analysis, and it is impossible to draw any definite conclusions from it. However, the great activity of invertase in the remaining experiments points to the absence of this ferment in the extracts tested in the experiments discussed. It is characteristic that in regard to the remaining ferments (amylase, inulase, lactase, lipase, pepsin, trypsin, and chimosin) there is no essential difference between these two and the remaining experiments.

As is seen from the table the two experiments named were conducted late in autumn and early in spring when the bees were in the stage of winter rest and fed on honey which, as is known, consists chiefly of inverted sugar and contains no saccharose at all, or contains it in insignificant quantities. The significance of invertase both in the animal and plant kingdoms lies in its capacity of converting saccharose into inverted sugar directly assimilated by the protoplasm.

Since the ferment is produced by the cells chiefly when the organism requires it, the absence of invertase in the first two experiments are provisionally explained by the fact that the bees feeding in winter on inverted sugar are not in need of it, and do not therefore produce this ferment.

Cases in which the same organism is capable, according to conditions, of different ferment-productive activity are not rare; sometimes the presence of a definite substance specific to the given ferment is quite sufficient to activate it.

Thus, according to Oppenheimer, some mucorines produce no ferments when cultivated in media containing substances assimilated by them directly. However, on addition of proteins to the medium the same mucorine produces proteolytic ferments on addition of starch-amylase, &c.

The investigations of Brown and Moris have shown that the germ of malt does not produce amylase if the grains are cultivated in media containing sugars capable of assimilation.

Therefore, it is possible that the organism of the bee is also

capable of not producing invertase in definite conditions. At any rate, as is seen from the table, the tested extracts of the same concentration manifest different activity.

If circumstances allow we shall dedicate special experiments to the solution of the question discussed.

A further examination of the data adduced in the table shows that only extracts from the stomach possess considerable power of preventing the rotation of the polarization plane in the sugar solution, whereas those from the crop, small and large intestines, are either altogether inactive or act very weakly. Evidently these latter portions of the intestine do not produce invertase, the latter penetrating there from the stomach together with the food.

In experiments nos. 6, 7, 8, 19, and 22, invertase was determined in extracts prepared immediately from the crop, small and large intestines, the alteration in the rotation of the plane of polarization being insignificant.

Thus we have arrived at conclusions coinciding with Axenfeld's opinion regarding the place in which invertase is produced, i.e. that it is produced in the stomach of the bee.

The data of Erlenmeyer and Planta, according to which greater activity was manifested by extracts from the head and abdomen of the bees than from their thorax, may be explained as follows.

The origin of invertase from the abdomen should be referred to the stomach of the bee. The greater activity of the extract from the head of the bee, as compared with that from the thorax, is explained by the fact that the salivary glands lying in the head produce invertase, whilst those lying in the thorax do not produce this ferment. Such an explanation is, of course, only probable, and must be verified by special investigations.

After having ascertained the general relations exhibited by invertase in the ventriculo-intestinal tract of the bee, we have also conducted several preliminary experiments with the view of a special study of the nature of this ferment.

Thus it was interesting to determine the influence of the method of preparation of the ferment on the activity of inver-

tase. Similarly to the analogous experiments with catalase (see Table III) we prepared extracts from finely rubbed stomachs, as well as from separate pieces of it.

The results obtained from investigation of these extracts are adduced in Table V.

The table shows that the rubbing of the tissues of the intestine does not manifest any visible influence on the activity of invertase, contrary to catalase. The slight difference between the rotation of the polarization planes in extracts from rubbed and intact stomachs is, probably, due to the individuality of the bees.

Further, we were interested in the influence of the quantity of extract on the course of inversion of sugar. For the elucidation of this question we have conducted a series of experiments in which to equal quantities of 5 per cent. solution of cane-sugar were added different amounts of the same extract from stomachs of bees. After standing a day at $+36-8^{\circ}$ C. the rotation of the polarization plane was determined in the mixture. The results obtained in these experiments are adduced in Table VI.

On comparing the results of the experiments in Table VI it is seen that by degrees as the amount of extracts increases the quantity of inverted sugar in the solutions tested also increases. However, there is no strict proportionality between these increases. In general, as all the analyses have shown, the ferment manifests a greater activity in smaller quantities, whereas with the increase of quantity its activity decreases. For instance, in experiments nos. 33 and 33 *a* 1 c.c. of extract evoked a diminution of the angle of rotation on 1.63° and 5 c.c. of the same extract only on 3.19° . That the rate of inversion is not strictly proportional to the amount of invertase is also corroborated by Kikkan,¹ who worked on invertase obtained by him from yeast.

A very interesting picture is produced by calculating the number of stomachs corresponding to the respective amount

¹ D. A. Kikkan, 'On the question regarding the process of inversion under the influence of invertin'. Dissertation for the degree of Mag. Pharm. Uriev., 1903, p. 53.

TABLE V.

THE INFLUENCE OF THE DEGREE TO WHICH THE STOMACHS OF THE BEES WERE TRITURATED
ON THE ACTIVITY OF INVERTASE.

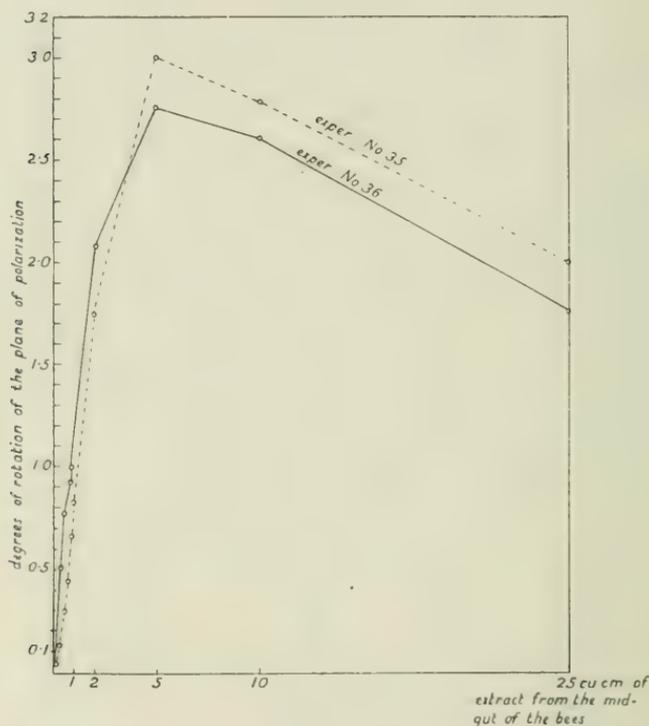
No.	Date of Experiment.	Method of Preparation of Extract.	Concentration of Sugar Solution in per cent.	Quantity of Sugar Solution in c.c.	Quantity of Ferment Extracts in c.c.	Time of Action of Ferment at 36-8° C.	Degrees of Rotation of Polarization Plane.			Difference in Degrees of Rotation of either Extract, showing relation to Rubbed.
							Control.	Rubbed Stomachs.	Mixture of Sugar Solution with Extract from:	
26	15 iv 1918	15 bees per 7.5 c.c. of glycerine+ 7.5 c.c. of water	5	50	5	24	+ 6.00	+ 0.10	- 0.22	+ 0.32
27	18 iv 1918	" "	5	50	5	24	+ 5.87	+ 2.68	+ 3.36	- 0.66
28	" "	" "	5	50	1	24	+ 6.38	+ 4.65	+ 5.19	- 0.57
29	2 vii 1918	25 bees per 25 c.c. of glycerine+ 25 c.c. of water	5	50	6	24	+ 5.90	+ 2.18	+ 2.35	- 0.17
30	" "	" "	5	50	4	24	+ 6.08	+ 2.39	+ 2.53	- 0.14
31	" "	" "	5	50	2	24	+ 6.35	+ 3.52	+ 3.90	- 0.38

THE INFLUENCE OF THE AMOUNT OF EXTRACTS ON THE DEGREE OF INVERSION.

No.	Time of Experiment.	Concentration and Method of Preparation of Extract.	Concentration of Sugar Solution in per cent.	Quantity of Sugar Solution in c.c.	Quantity of Extract in c.c.	Number of Stomachs of Bees per corresponding amount of Extract.	Time of Action of Ferments in hours.	Control Solution of Sugar.	Mixture of Sugar Solution with Extract from Stomachs.	Degrees of Rotation of Polarization Plane.	Size of Angle of Rotation.
32	15 IV 1918	15 bees per 7.5 c.c. of glycerine + .15 c.c. of water	5	100	5	5	24	+ 6.13	+ 0.66	+ 6.79	5.47
32 ^a	"	"	5	100	3	2	24	+ 6.32	+ 1.25	+ 7.57	5.07
33	18 VI 1918	"	5	50	5	5	24	+ 5.87	+ 2.68	+ 8.55	3.19
33 ^a	"	"	5	50	1	1	24	+ 6.38	+ 4.65	+ 11.03	1.63
34	2 VII 1918	25 bees per 25 c.c. of glycerine + 25 c.c. of water	5	50	2	1	24	+ 6.35	+ 3.52	+ 9.87	2.83
34 ^a	"	"	5	50	4	2	24	+ 6.08	+ 2.39	+ 8.47	3.69
34 ^b	"	"	5	50	6	3	24	+ 5.90	+ 2.18	+ 8.08	3.72
35	"	"	5	50	0.1	1/10	24	+ 6.50	+ 6.39	+ 12.89	0.11
35 ^a	7 VIII 1918	"	5	50	0.3	1/3	24	+ 6.50	+ 6.15	+ 12.65	0.35
35 ^b	"	"	5	50	0.5	1/2	24	+ 6.45	+ 6.02	+ 12.47	0.43
35 ^c	"	"	5	50	0.8	2/3	24	+ 6.40	+ 5.73	+ 12.13	0.67
35 ^d	"	"	5	50	1	1	24	+ 6.36	+ 5.54	+ 11.90	0.82
35 ^e	"	"	5	50	2	1	24	+ 6.30	+ 4.55	+ 10.85	1.75
35 ^f	"	"	5	50	5	2.5	24	+ 6.02	+ 3.02	+ 9.04	3.00
35 ^g	"	"	5	50	10	5	24	+ 5.43	+ 2.64	+ 8.07	2.79
35 ^h	"	"	5	50	25	12.5	24	+ 4.30	+ 2.30	+ 6.60	2.00
36	21 VIII 1918	"	5	50	0.1	1/10	24	+ 6.45	+ 6.40	+ 12.85	0.05
36 ^a	"	"	5	50	0.3	1/3	24	+ 6.45	+ 5.95	+ 12.40	0.50
36 ^b	"	"	5	50	0.5	1/2	24	+ 6.44	+ 5.65	+ 12.09	0.79
36 ^c	"	"	5	50	0.8	2/3	24	+ 6.30	+ 5.38	+ 11.68	0.92
36 ^d	"	"	5	50	1	1	24	+ 6.25	+ 5.25	+ 11.50	1.00
36 ^e	"	"	5	50	2	1	24	+ 6.08	+ 4.00	+ 10.08	2.08
36 ^f	"	"	5	50	5	2.5	24	+ 5.89	+ 3.12	+ 9.01	2.77
36 ^g	"	"	5	50	10	5	24	+ 5.29	+ 2.69	+ 7.98	2.60
36 ^h	"	"	5	50	25	12.5	24	+ 4.28	+ 2.50	+ 6.78	1.78

of extract. For instance, in experiment no. 35, 0.1 c.c. of extract corresponds to one-twentieth part of the stomach of one bee, and the quantity of ferment contained in this small particle already evokes an alteration of the polarization plane to

CURVE 2.



Curve representing the influence of the quantity of invertase upon the degree of inversion, according to the data obtained from the series of experiments nos. 35 and 36 (see Table VI).

0.11°. This small experiment clearly exhibits the power of activity of the invertase of the stomach of the bee.

In the more perfect experiments, nos. 35, 36, it was established that extracts added to sugar syrup in large quantities evoke not an increase of inversion, but, on the contrary, its decrease (nos. 35 f, 35 g, and 36 f, 36 g).

This apparently uncommon phenomenon may be due either

to the quantity of glycerine or the concentration of the sugar solution, as we added to 50 c.c. of the latter an amount of extracts varying between 0.1 and 25 c.c. In order to ascertain the real cause of this phenomenon, we conducted special experiments on the following plan. Extracts were prepared simultaneously from the stomachs of bees of equal concentration in water and in a mixture of equal parts of glycerine and water. To 25 c.c. of 10 per cent. sugar solution was added a certain amount of extract (see Table VII), and the mixture resulting made to reach 50 c.c. by addition of a corresponding amount of water or its mixture with glycerine. As a result, in all the tests analysed, the concentration of cane-sugar reached the same level, differing from each other only in the quantitative content of ferment. Thus we have conducted two parallel series of experiments in water and in a mixture of water and glycerine; the tests of one series of experiments differed from the other only in the presence of glycerine in it. The results obtained are given in Table VII.

On comparing the results of analyses adduced in Table VII we can state without doubt that in extracts of glycerine with water inversion increases only to 10 per cent. of the content of glycerine estimated in relation to the total volume of the liquid analysed. On further addition of glycerine the activity of invertase falls perceptibly. In parallel experiments with pure water extracts the degree of inversion rises according to the increase in the quantity of extract, that is to say, of invertase.

Thus, in certain quantities, glycerine has a repressive influence on the invertase as represented in Curve 3.

In order to determine the influence of the solvent upon the activity of invertase extracts from the stomachs of fifteen bees per 15 c.c. of water, 15 c.c. of glycerine, and, lastly, per 15 c.c. of physiological solution of common salt, were prepared in similar conditions; to each extract were added 5 drops of toluol for conserving purposes. On the following day to 50 c.c. of 5 per cent. solution of saccharose were added 5 c.c. of the extracts named and 10 drops of toluol; after twenty-

TABLE VII.

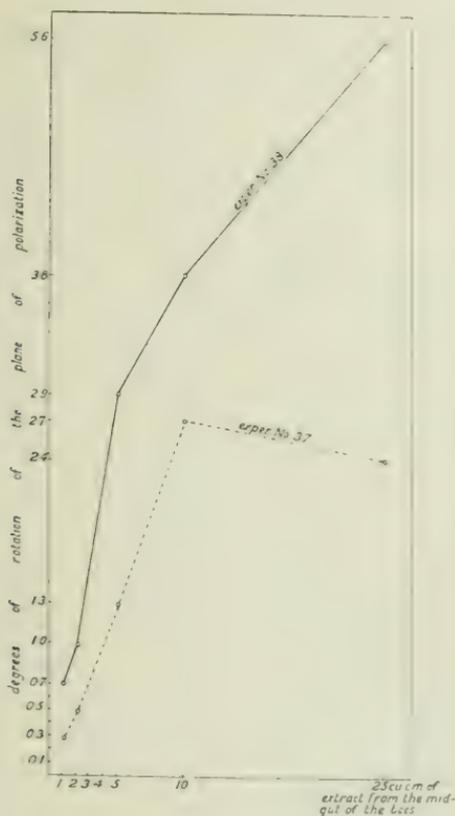
THE INFLUENCE OF GLYCERINE ON THE ACTIVITY OF INVERTASE.

No.	Method of Preparation of Extract.	Concentration of Sugar Solution in per cent.		Quantity of Sugar Solution in c.c.		Quantity of Ferment Extract in c.c.		Number of Stomachs of Bees per respective amount of Extract.		Volume of Mixture of both.		Central Sugar Solution.		Degrees of Rotation of Polarization Pole in Mixture of Sugar Solution with Extract from Stomachs of Bees at the Expiration of:		Size of Angle of Rotation.
		10	25	1	25	1	25	0.5	50	+ 6.56	+ 6.29	24 hours.	48 hours.	0.34	6:20	
37	25 bees per 50 c.c. of mixture of equal parts of glycerine and water	10	25	1	25	1	25	0.5	50	+ 6.56	+ 6.29	0.34	+ 6:20	0.36		
37a	" "	10	25	2	25	2	25	1	50	+ 6.56	+ 6.03	0.53	+ 6:06	0.50		
37b	" "	10	25	5	25	5	25	2.5	50	+ 6.56	+ 5.25	1.31	+ 5:19	1.37		
37c	" "	10	25	10	25	10	25	5	50	+ 6.56	+ 3.88	2.08	+ 3:90	2.70		
37d	" "	10	25	25	25	25	25	12.5	50	+ 6.56	+ 4.12	2.44	+ 4:10	2.46		
38	25 bees per 50 c.c. of water	10	25	1	25	1	25	0.5	50	+ 6.54	+ 5.86	0.68	+ 5:90	0.61		
38a	" "	10	25	2	25	2	25	1	50	+ 6.54	+ 5.55	0.99	+ 5:57	0.97		
38b	" "	10	25	5	25	5	25	2.5	50	+ 6.54	+ 3.67	2.87	+ 3:65	2.89		
38c	" "	10	25	10	25	10	25	5	50	+ 6.54	+ 2.76	3.78	+ 2:70	3.84		
38d	" "	10	25	25	25	25	25	12.5	50	+ 6.54	+ 0.90	5.64	+ 0:92	5.62		

four and forty-eight hours standing at 36–40° C. the rotation of the polarization plane was determined in the mixtures.

The results obtained are represented in Table VIII.

CURVE 3.



Curve demonstrating the depressive effect of glycerine upon the activity of invertase, based on the data of the series of experiments nos. 37 and 38 (see Table VII).

It is seen from Table VIII that invertase is extracted both by water and a mixture of water and glycerine, by glycerine alone, as well as by the physiological solution of common salt.

Evidently the most active extracts are produced by water, less by glycerine. Water, however, can be used effectively

TABLE VIII. THE INFLUENCE OF THE SOLVENT UPON THE ACTIVITY OF INVERTASE.

No.	Date of Experiment.	Method of Preparation of Extracts.	Concentration of Sugar Solution in per cent.		Quantity of Ferment Extracts in c.c.		Degrees of Rotation of Polarization Plane at the Expiration of:			
			Solution in per cent.	Quantity of Sugar Solution in c.c.	Control	Experiment.	24 hours.	48 hours.		
39	7 VIII 1918	15 bees per 15 c.c. of water	5	50	5	5	+5.94	+2.08	+5.93	+2.05
40	"	15 bees per 7.5 c.c. of glycerine + 7.5 c.c. of water	5	50	5	5	+5.92	+2.50	+5.95	+2.50
41	"	15 bees per 15 c.c. of glycerine	5	50	5	5	+5.98	+2.92	+5.98	+3.01
42	21 VIII 1918	15 bees per 15 c.c. of water	5	50	5	5	+5.90	+0.53	+5.92	+0.51
43	"	15 bees per 7.5 c.c. of glycerine + 7.5 c.c. of water	5	50	5	5	+5.92	+2.40	+6.03	+2.45
44	"	15 bees per 15 c.c. of glycerine	5	50	5	5	+6.13	+3.25	+6.15	+3.19
45	"	15 bees per 15 c.c. of physiol. Solut. NaCl	5	50	5	5	+5.75	-0.25	+5.83	-0.38

TABLE IX. THE ACTIVITY OF INVERTASE IN CONSERVED SOLUTIONS.

No.	Date of Experiment.	Method of Preparation of Extract.	Date of Analysis.	Concentration of Sugar Solution in per cent.		Quantity of Sugar Solution in per cent.		Quantity of Extracts in c.c.		Degrees of Rotation of the Plane of Polarization.		
				Solution in per cent.	Quantity of Sugar Solution in per cent.	Control Sugar Solution.	Solution of Sugar and Extract.	Control Sugar Solution.	Solution of Sugar and Extract.	Size of Angle of Rotation.		
46	15 XI 1917	30 bees per 30 c.c. of a mixture of glycerine and water (aa) and of toluol	16 XI 1917, after day	5	50	5	5	5	5	+5.65	+2.12	3.53
46a	"	"	10 IV 1918, 5 months	5	50	5	5	5	5	+5.80	+2.30	3.50
46b	"	"	15 X 1918, 11 months	5	50	5	5	5	5	+5.92	+2.48	3.44

only in cases which do not require long conservation of the material analysed, since the extracts rapidly decompose. A very suitable solvent for invertase, as regards resistance, is presented by a mixture of glycerine and water. Our experiments have shown that extracts prepared in this mixture retain their original activity at least during eleven months (Table IX). Glycerine, on the other hand, suffers from another defect: it acts depressively on the course of inversion, as is clearly visible from the series of experiments nos. 35, 36, 37 of Tables VI and VII and Curves 2 and 3. As in the preceding experiments invertase manifests its activity in different solvents also only during the first days.

In order to determine the durability of invertase after prolonged conservation of its solutions the following experiment was conducted.

On November 16, 1917, an extract from the stomachs of thirty bees per 30 c.c. of a mixture of glycerine and water, with the addition of 10 drops of toluol, was prepared. This extract, preserved in a dark place at the temperature of the room, was analysed in the usual way on November 17, 1917, April 10, 1918, and October 15, 1918. The results obtained from the analysis are represented in Table IX.

The series of analyses given in Table IX shows that the activity of invertase in solutions prepared in a mixture of glycerine and water does not alter after being preserved for at least eleventh months, in which respect invertase differs markedly from catalase.

Lipase.

As is known, lipase belongs to ferments splitting fats into fatty acids and glycerine.

The source of fatty food for the bee is presented by propolis which contains, according to the analysis of one of us (Zarin), about 6 per cent. of fat.

The question whether the fatty substances of propolis are assimilated by the organism of the bee cannot be regarded as

settled, since there are hitherto no data in literature referring to lipase in the bee.

Petersen, on failing to discover fat in the epithelial cells of the digestive stomach of the bee with the help of osmic acid and the stain-sudan III, writes: 'Als sicher darf ich wohl hinstellen, dass das meiste Fett, auch der normalen Nahrung, den Darm passiert, ohne gespalten oder resorbiert zu werden.'

For the detection of lipase we employed 1 per cent. solution of monobutyrim and the emulsion of Provence oil.

In all the experiments conducted the same results were obtained in general. An increase in acidity both of the monobutyrim solution and the Provence oil emulsion was observed only in those test-tubes that contained extracts from the stomachs; this increase of acidity varied in the experiments conducted between 0.3-0.6 c.c. $\frac{N}{10}$ NaOH, the ferment acting more intensely on the monobutyrim than on Provence oil. The extracts from the remaining portions of the intestine produced no distinct influence upon the acidity of the medium.

Thus, our experiments show that the stomach of the bee and drone produces, together with other ferments, lipase as well: the bee is therefore capable of assimilating fatty substances.

Pepsin.

For the determination of pepsin we employed:

(1) Sterile 1 per cent. gelatine acidulated with hydrochloric acid to a distinctly acid reaction, and poured 2 c.c. of this into thin test-tubes. To this quantity of gelatine were added 2 c.c. of extracts of ferments tested and 3 drops of toluol; the mixture was placed in the thermostat at 35-8° C., and every twenty-four hours the degree of its coagulation was observed on being cooled.

(2) The fibrin from blood. For our purposes we placed a piece of fibrin into a mixture consisting of 8 c.c. of sterile water acidulated with hydrochloric acid to a distinctly acid reaction, 2 c.c. of extracts of ferments tested and 3 drops of toluol; the test-tube being placed in the thermostat at 35-8° C.

(3) 1 per cent. solution of casein containing in 1 litre 16 c.c. of concentrated hydrochloric acid of specific gravity 1.124.

Test-tubes containing 10 c.c. of this solution and 2 c.c. of extracts of the ferments tested were placed for one hour in the water-bath at a temperature of 38-40° C., after which to their contents a concentrated solution of sodium citrate was carefully added by drops.

The methods described produced in all cases similar results, the presence of pepsin being established in the stomachs of the bee and drone, whilst the other portions of the intestine were devoid of it.

(1) Gelatine with extracts from the stomachs of the bee and drone lost the coagulating property after one to three days, whereas extracts from the remaining portions of the stomach produced no visible influence upon the gelatine in this respect during twenty days, after which the experiment was discontinued.¹

(2) In test-tubes with extracts from the stomachs fibrin dissolved during one to three days, and in the remaining it did not dissolve after twenty days, after which the experiment was discontinued.¹

(3) The casein test described also manifested a positive reaction only with extracts from the stomachs.

The results obtained from our experiments allow us to conclude that the stomach of the bee and drone contains a peptic ferment acting in an acid medium, whereas the crop, small intestine, and part of the large intestine with the rectal glands do not produce this ferment.

Trypsin.

For the determination of trypsin we employed :

(1) 10 per cent. alkaline gelatine, as in the case of pepsin ; liquefaction of the gelatine followed after one to three days only in extracts from the stomachs.

¹ In order to test the sterility of liquefied gelatinous mixture and the fibrin solution sowing was made in agar, but during three days at 37° C. no growth was observed.

(2) Alkaline solution of casein. To 10 c.c. of 1 per cent. solution of casein containing 1 in 200 c.c. 10 drops of 10 per cent. solution of soda were added 2 c.c. of extracts of ferments tested; the test-tubes with the mixture were placed for one hour in water at a temperature of 38–40° C., after which a 0.5 per cent. solution of citric acid was added to their contents by drops evoking a turbidity in the solution of undigested casein.

By applying the methods described we obtained the same results in all cases, trypsin being established only in the stomach of the bee and drone, whilst the remaining parts of the intestine were devoid of it.

Chymosin.

Regarding the presence of chymosin in the organism of the bee there are no data in literature.

For its determination we used a mixture of 10 c.c. of milk + 90 c.c. of water + 1 c.c. of 10 per cent. calcium chloride.

To 10 c.c. of this mixture was added 1 c.c. of extracts of the ferment tested, and the liquid was placed in a water-bath at 40° C.

The extracts from the stomach always caused milk to coagulate; the formation of the coagulum in the experiments conducted being observed not earlier than after two, and not later than after fifteen minutes. In the test-tubes containing extracts from the remaining portions of the intestine the milk did not coagulate during three hours, after which the experiment was stopped.

Thus our experiments show that the stomach of the bee and drone contains abomasum ferment of considerable activity.

Emulsin.

For the determination of the ferments splitting the glucosides we used 1 per cent. solutions of amygdalin, salicin, and arbutin. The results obtained were negative. It was impossible at that time to purchase andromedotoxin, which produces the toxic properties of honey as well as any other glucosides.

TABLE X. SUMMARY TABLE OF FERMENTS ESTABLISHED IN DIFFERENT PORTIONS OF THE INTESTINE OF THE WORKER-BEE AND DRONE.

Name of ferment.	Crop.	Stomach.	Small intestine.	Large intestine.	Notes.
Catalase	—	+	—	±	Found in the large intestine only during the second half of hibernation.
Amylase	—	+	—	—	
Inulase	—	+	—	—	
Lactase	—	+	—	—	
Invertase	—	+	—	—	
Lipase	—	+	—	—	
Lepsin	—	+	—	—	
Trypsin	—	+	—	—	
Chymosin	—	+	—	—	
Emulsin	—	—	—	—	

+ denotes constant presence of ferment.

± denotes indefinite periods of ferment.

— denotes absence of ferment.

CONCLUSION.

At the conclusion we shall examine the distribution of ferments in the different parts of the ventriculo-intestinal tract of the bee, as adduced in the table above (Table X).

It is quite natural that digestive ferments should be found only in the stomach of the bee. The latter presents the mid-gut of this insect, i. e. that part of the digestive tract which is developed from the entoderm. Contrary to the fore- (=crop =honey-stomach) and hind-guts (small and large intestines) the stomach of the bee is devoid of an inner chitinous lining: it is true it is provided with a peripheral membrane (Pl. 16. fig. 9, *p*) clothing the food masses in the lumen of the stomach, but this membrane is secreted by the entodermal epithelium of the latter and differs in its properties from the chitinous cuticle, being soluble in hydrochloric acid. It is permeable to ferments, and, according to Petersen's experiments, even contains them in its own substance. The fact that ferments are produced only in the mid-gut of the bee is fully in accordance with the phenomena observed in other insects. In all arthropods the chief digestive processes take place in the mid-

gut and its derivatives (digestive glands—hepatopancreas) when such are present.

On account of the difference in the mode of life and nutrition between the worker-bee and drones, we examined and compared the intestinal ferments of both in the hope of tracing some points of difference between them. However, in the conditions of the experiments conducted, when the ferments were determined only qualitatively we failed in our attempts. It was impossible to discover any visible difference between the ferments.

An examination of the comparative table of ferments in the intestine of the bee adduced above reveals the fact of the inconstant presence of catalase in the extracts from the large intestine. This circumstance seems to produce a certain dissonance in the results of the work and disagrees with the generally accepted facts. However, on closer examination it is explained quite definitely and convincingly. As a matter of fact catalase was discovered in extracts from the rectum not accidentally but at a certain time of the year, viz. in spring, previously to the hives being removed from their hibernating quarters.

At this period catalase is abundant in the rectum, but already after the first flights of the bees its quantity decreases sharply and two days later it is already impossible to trace any of this ferment in the rectum. These facts can be naturally connected with the work of the intestine in winter. Bees feed all the time, but during the period of seclusion in the hive they do not excrete at all. The faeces accumulate in the rectum and distend it to extraordinary dimensions, as is represented in Plate 15 of figures. On comparing the intestine (drawn at the same magnification) of a bee that has hibernated previously to its discharge (fig. 3), with a bee dissected in summer in the usual conditions of its existence and activity, we may form a clear idea of the degree to which the intestine is over-filled. The stomach becomes shorter and thicker, especially large dimensions are attained by the rectum which assumes the aspect of an enormous ovoid bladder. Doubtless the accumulation and long retention of faeces in the rectum reflects

in one or another degree upon the process of metabolism in the bee. Such an accommodation is presented by the production of catalase in the rectum at a period when the bee is incapable of excretion. The increased production of this ferment stands in connexion with the demands of the organism for more energetic oxidative processes, in which there is not so much need in summer when the intestine of the bee discharges its excrements normally.

After we have convinced ourselves of the logical necessity of the presence of catalase in the rectum, we must solve the question regarding the place where this ferment is produced. Two possibilities may be discussed in this connexion: (*a*) either catalase is produced by the walls of the rectum in the bee (local origin of the ferment), or (*b*) catalase is transported to the rectum together with the food from the anterior portions of the intestine, namely, from the stomach.

We shall first discuss the latter possibility. In preparing extracts from the rectum the large intestine of the bees was cut; the faeces falling out themselves, the wall of the intestine washed repeatedly in a fresh physiological solution of common salt. Only after being thus cleaned of its contents the intestine was rubbed up with sand in glycerine. The measure of precaution described guarantee to a certain degree the purity of the extracts prepared, and therefore allow one to ascribe the property of catalase production to the walls of the rectum.

The conclusion set forth is indirectly corroborated by another circumstance. If catalase were transported into the rectum from the stomach with the digested food, we should expect this ferment to be present not only in the rectum (the hindmost portion of the intestine) but in the small intestine uniting the rectum with the stomach as well.

However, catalase was absent in extracts from the small intestine in all cases, notwithstanding the fact that they had been less carefully prepared. This part of the intestine is usually not removed from it, and the extracts were prepared from whole pieces of the small intestine with all its contents. Since the extract from the anterior portion of the intestine

and from its contents contained no catalase, whilst the extract from the wall of the posterior part of the digestive tract alone contains this ferment, the inevitable conclusion is that catalase is produced by the rectum itself in the bee, and is not brought there from other parts.

Now the question arises, in what part of the rectum is this ferment produced? In the first part of the work the structure of the rectum was described in detail, and it was mentioned that the latter differs in structure from the crop of the bee in the presence of eight elongated rectal glands. The wall of both rectum (Pl. 17, fig. 20, *ep*) and crop (Pl. 16, fig. 5, *ep*) is formed by flat epithelium bearing no characters peculiar to glandular tissue. Therefore it is difficult to ascribe to it the property of producing ferments; and, indeed, in the crop they are never produced. It may therefore be naturally concluded that the place where catalase is secreted is presented by the rectal glands in the plump epithelial cells of which are found granules of zymogen (Pl. 17, fig. 21, *ep*). The correctness of such a conclusion stands somewhat in contradiction to the fact that catalase may also be produced by non-glandular tissue. Thus this ferment is present in the nerve-tissue of some animals. In the near future we shall endeavour to solve the question discussed more precisely. In the dilated rectum of the bee it is possible to separate the anterior part with the rectal glands from the posterior consisting only of flat epithelium.

An investigation of the extracts from these parts of the rectum will, possibly, be able to give a definite answer to the question regarding the rôle of the rectal glands which have hitherto been mysterious organs in insects. Concerning their rôle only suppositions have hitherto been expressed. Berlese supposes that these glands serve to absorb the remains of food, and there may be also present some kind of valve for the retention of the contents of the intestine previously to the final formation of the faeces. N. A. Cholodkovsky (1912) thinks that it is possible to speak only of a sort of excretive function of the rectal glands, under the cuticle of which in Lepidoptera and the cricket (*Gryllus domesticus*) he observed an accumu-

lation of some kind of excretion. Vallé (1900) supposes that 'les papilles rectales des Diptères jouent deux rôles : le rôle respiratoire et le rôle sécréteur. Rôle respiratoire par les gros troncs trachéens et les petites ouvertures qui servent de débouchés aux ramifications trachéennes ; rôle sécréteur par les cellules géantes et les pores terminaux leur donnant ouverture dans la cavité rectale' (loc. cit., p. 60).

Thus, Vallé unites the views regarding the glandular rôle of the rectal glands expressed by Lowne (1869) and regarding their analogy to the rectal gills of the dragon-fly larvae (Leydig, Chun, 1876).

It is quite possible that these organs discovered by Swammerdam in the bee play different rôles in insects. It is remarkable that the rectal glands are absent in beetles.

In other insects they appear only at the end of the pupal stage, and only the dragon-flies (*Libellulidae*) are provided with these glands in the larval stage as well (Faussek, 1887). Evidently these organs, the function of which is mysterious, stand in some connexion with the metamorphosis of insects.

On comparing these considerations with facts observed in bees its rectal glands may with a considerable degree of probability be regarded as glands one of the functions of which is the seasonal production of catalase.

Whereas the rectum of the bee is capable of producing a ferment albeit periodically (catalase), its crop lacks this property absolutely.

No ferments were ever established in extracts from the honey-stomach. This circumstance allows us to take a step nearer toward the solution of the question regarding the process of honey formation. The bee takes in the nectar into the crop from which it deposits it into the honeycombs. Does the crop present a passive reservoir adapted only to temporary conservation of the nectar, or does some other biochemical process, besides the splitting up of cane-sugar, take place in it? What takes place in the nectar deposited in the honeycombs during the ripening of the honey? In order to solve this question one of us (E. Zarin) had previously (1917) conducted

experiments by feeding bees chiefly on cane-sugar syrup which was successively passed through their organism twice. In the first experiment the bees received twenty-five pounds of syrup: when they deposited it in the honeycombs two days later the honey taken out of them was again offered the bees; the honey deposited for the second time was left in the hive till the moment of sealing up, after which it was again offered to the bees; the honey deposited for the third time underwent chemical analysis similarly to the sugar, syrup, and honey of the first and second deposits.

When the honey ripens the cane-sugar is inverted; when this occurs a certain quantity of dextrin-like substances which do not reduce Fehling's solution are produced. 'Thus in natural honey, besides the non-sugars of plant origin, are also contained such that are produced by the organism of the bee, probably with the help of a special ferment.'

The sugar syrup offered to the bees was quite devoid of ferments, whereas in the deposited portions the presence of invertase and diastase was discovered; therefore the ferments named could have found their way into the honey only from the body of the bee. Catalase was absent in such artificial honey, whereas it is always present in natural sorts. Evidently catalase is brought into the honey from the nectar.

These data throw light on the nature of the ferments in the samples of honey investigated—some of them (catalase) are of plant, others (invertase and diastase) both of animal and plant origin.

If invertase and diastase are brought into the honey by the bee, the question arises—where are these ferments produced in its organism? The investigation conducted by us throws some light upon this question. The most simple supposition is that the ferments of honey are produced by the walls of the reservoir into which the bee collects the nectar. That is, however, not the case, since the walls of the crop are not endowed with glandular properties. It was impossible in any circumstances to establish any ferment in the extracts from the crop.

Evidently the ferments of honey penetrate into it from other portions of the digestive apparatus; such may be either the stomach of the bee in which invertase and diastase are actually produced, or the salivary glands. If we assume that the ferments of honey are derived from the stomach of the bee, allowance must be made for the possibility of a kind of exurgitation, or their passage from the stomach into the crop, i. e. in a direction opposite to the normal course of food.

The anatomical data do not allow of forming such a supposition, as the bordering valve of the crop is provided with a long tubular valvule which prevents the usual contents, and therefore the ferments of the stomach as well, from penetrating back into the honey-stomach. If the existence of exurgitation of ferments were possible, then not only invertase and diastase would pass into the crop, but also catalase, which is always secreted by the sides of the stomach. In this case it would have been also discovered in the analysed portions of the artificial honey out of sugar syrup described above. The fact that this was not observed serves to confirm the conclusion that the catalase of honey is of vegetable origin, there being no basis for admitting the possibility of an elective exurgitation of ferments from the bee's stomach.

In this connexion it should be remembered that regarding the possibility of exurgitation in bees the investigators differ in opinion. Some of them, as Schönfeld, believe that the brood-food of bees is discharged by the latter from the stomach, whilst others regard it as a secretion of the large salivary glands occupying the greater part of the volume of the head in the bee (Pl. 15, fig. 4, dr_1). Regarding the ferments of honey a supposition analogous to the latter can be made. The ferments are produced by the salivary glands of the bee swallowed together with the nectar into the crop and removed from there on deposition of the honey into the honeycombs. Such a conclusion appears to be the most correct, although it also bears the character of probability, since the digestive properties of the salivary glands are unknown. In other insects very strong

digestive ferments (proteolytic ferment) have been found in the saliva; they are certainly present in the bee as well, otherwise the presence of a complex system of salivary gland would be incomprehensible. The task at hand is to study their ferments, if only it will be possible to apply to them the method of preparation for the extraction of ferments, which presents great difficulties in the given case.

The data obtained by our work allow of an attempt to a partial solution of the question regarding the food régime of the bee and regarding the assimilation of different sorts of food by it. We shall meanwhile adduce a particular case. Petersen fed bees on oil emulsion in sugar solution and arrived at the conclusion that 'das meiste Fett, auch der normalen Nahrung, den Darm passiert, ohne gespalten oder resorbiert zu werden. Der feste Aggregatzustand des Kofettes und seine Löslichkeit in Alkohol sind merkwürdig und lassen an einen reichlichen Gehalt von Fettsäuren denken' (loc. cit., p. 148).

The fact that we have constantly found lipase in the extracts from the stomach of bees refutes Petersen's view just quoted that fat passes through the stomach of the bee without splitting. Since the stomach contains a special ferment, it is obvious that it is produced for a respective purpose, i. e. in the given cases for the digestion of fat.

The next stage in the study of the digestive processes in bees should be experiments on preferential feeding of these insects with different nutritive substances conducted on a wide scale and the determination of the character of excretion and action of ferments in artificial conditions. The results of such an investigation are important not only from the theoretical but from the practical point of view as well. Bee-keepers have always to deal with the difficult task of artificial feeding of bees in winter, which frequently results in the appearance of dangerous diseases among these insects. When the conditions in which the intestine of the bee works are known, and a clear idea regarding the metabolism of its substances during the different seasons of the year is arrived at, it will be possible to solve the question regarding the methods of feeding it

artificially instead of taking false steps and applying empirical methods, as hitherto practised.

One of us (E. Zarin, 1917) has already made some progress towards the solution by purely chemical methods of the question regarding the necessity of acidulating the winter food of bees, which is of practical importance and is applied by many practical bee-keepers.

It was proved that acid (0.1 per cent. citric) produces no useful action upon the inversion of cane-sugar and the ripening of honey, whereas acid 'added to the food in the amount of 0.3 per cent. produces a depression not only in the process of sugar inversion, but in all the other processes taking place in the honey-stomach of the worker-bee, as well as in the hive during ripening of the honey'. In the final conclusions these data 'disagree with the view prevalent among bee-keepers regarding the necessity of adding acid to the food'.

At the first opportunity we shall endeavour to continue our work in the direction mentioned, which is especially advantageous, since in it deep scientific problems are conveniently combined with the requirements of practical life.

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EXPLANATION OF FIGURES.

GENERAL MEANING OF LETTERS.

ab, abdomen. *an*, anal aperture. *b*, bacteria. *bg*, connective tissue. *bl*, vesicular evaginations from the surface of cells of the stomach epithelium. *c*, chitinous cuticle. *cb*, suprpharyngeal nodules of the nerve-chain. *cd*, chitinous cuticle of the surface of the capitulum of the stomach valve. *cmf*, muscle (Leidig's) columns. *cn*, marginal chitinous fillet of rectal gland. *cp*, head. *d*, protoplasm. *da*, alveolar layer of weakly staining protoplasm under the chitinous cuticle of the rectal gland. *dr*₁, pharyngeal salivary glands of the bee. *dr*₂, salivary glands of the head (Vorderkieferdrüsen). *dr*₃, salivary glands of the thorax (Hinterkieferdrüsen). *ds*, bacilliform striated layer of protoplasm. *ep*, epithelium. *g*, granular contents of the stomach. *gr*, granules and inclusions in the protoplasm. *h*, cavity of rectal gland. *i*, crop. *ia*, fore-gut. *ic*, cavity of the crop. *im*, mid-gut. *ip*, hind-gut. *it*, small intestine. *k*, crypts of the stomach. *m*, muscles. *m*₁, transverse (circular) muscles. *m*₂, longitudinal muscles. *m*₃, longitudinal exterior muscles of stomach valve. *m*₄, circular muscles of the crop. *m*₅, longitudinal muscles of the crop. *mb*, basiliary membrane. *mf*, microfibrils. *mp*, Malpighian vessels. *n*, nucleus. *nv*, nerve. *o*, oesophagus. *p*, peritrophic membrane. *pc*, lumen of stomach valve. *ph*, pharynx. *pm*, perimysium of muscles. *r*, rectum (large intestine). *rg*, rectal gland. *sn*, syncytium in rectal gland. *sp*, sarcoplasm. *th*, thorax. *tr*, tracheae. *v*, stomach. *vc*, its vacuole with secretion. *wa*, exterior wall of rectal gland. *wp*, ‘hairy’ margin of the epithelium of the stomach. *z*, margins of the exterior wall of the rectal gland with tracheae between them.

EXPLANATION OF PLATES 15, 16, AND 17.

PLATE 15.

Fig. 1.—Ventriculo-intestinal canal of the worker-bee in summer. Large intestine of small size.

Fig. 2.—Same in drone. Stomach much longer than in the worker-bee.

Fig. 3.—Intestine of hibernating bee. Large intestine filled with faeces and therefore presenting the largest portion of the intestine in dimensions. The arrows denote the points at which the intestine was cut for the preparation of extracts from it. All the three figures were made with Zeiss' binocular microscope, ob. I 55, oc. 1.

PLATE 16.

Fig. 4.—Schematic longitudinal section of the body of the worker-bee with its organs of digestion. Combined, from two figures of Zander's monograph. The arrows on the left show the subdivision of the body into head, thorax, and abdomen, whereas the ones on the right denote the subdivision of the intestine into the fore-, mid-, and hind-guts. The subdivisions mentioned do not correspond one with another, as the fore-gut (*o, i*) passes through the head, thorax, and part of the abdomen.

Fig. 5.—Transverse section of the crop on the level of the stomach valve. *cd*, cuticle of the surface of the valvular capitulum; *ic*, cavity of the crop. Haematoxylin; eosin. Zeiss; ob. AA, oc. 4.

Fig. 6.—Slightly oblique section of the stomach of the bee. Its cavity is filled with a very great number of peritrophic membranes (*p*) disposed in concentric layers one on another. Zenker formol. Heidenhain's iron haematoxylin. Zeiss; ob. AA, oc. 1.

Fig. 7.—Part of transverse section of the stomach of the bee. A crypt in the depth of the epithelial fold is visible. Above the crypt is a vacuole with secretion. The hibernating bee was fixed in April. Duboseq's fluid. Mann-Holland stain. Zeiss; $\frac{1}{2}$ hom. imm., oc. 0.

Fig. 8.—Part of the wall of the stomach in the bee, dissected in May. The section has passed obliquely, on account of which the cells of the folds appear to be set on slender peduncles and interrupted. Zeiss; ob. $\frac{1}{2}$ hom. imm., oc. 1. Duboseq's fluid; Giemsa stain.

Fig. 9.—Transverse section of the wall of the stomach to show the formation and the rubbing off of the peritrophic membranes (*p*) developing at the expense of the 'hairy' layer of the epithelial plasm (*wp*). Above the crypt (*k*) the vacuole displacing the 'hairy' layer of the plasm is situated. Zenker formol: Heidenhain's iron haematoxylin. Zeiss; ob. $\frac{1}{2}$ hom. imm., oc. 1.

Fig. 10.—Peritrophic membranes in the cavity of the stomach of the

bee. Between them are disposed the granular contents (*g*) and two blood (?) corpuscles. Duboseq's fluid; Mann-Holland's stain. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 4.

Fig. 11.—Part of the epithelial wall of the stomach in the bee dissected in May. Between the glandular cells is arranged the crypt, the cells of which also discharge a secretion accumulating in the globular vacuole (*vc*). The epithelial cells bear on their surface the 'hairy' layer of protoplasm (*wp*). Duboseq's fluid; Giemsa stain. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 4.

Fig. 12.—Part of the wall of the stomach of the same bee. In the 'hairy' layer of the epithelial cells are visible swellings of the superficial layer of protoplasm corresponding to the vesicular evaginations of the cells observed in the mid-gut of the larvae of Ptychoptera by Van Gehuchten. Mann-Holland stain. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 2.

Fig. 13.—Tangential section of the wall of the stomach in the bee. The rounded evaginations of the epithelium with cryptae at their bottom are visible in section. Duboseq's fluid; Giemsa's stain. Zeiss; ob. DD. oc. 4.

Fig. 14.—Longitudinal section of circular fibres of the muscular membrane of the stomach. *sp*, sarcoplasm. Giemsa's stain. Zeiss; $\frac{1}{12}$, oc. 4.

PLATE 17.

Fig. 15.—Longitudinal section of a fibre from the muscular membrane of the stomach of the bee; the abundance of sarcoplasm (*sp*) with nuclei in it (*n*) is visible (see fig. 16, Pl. 17). Haematoxylin, eosin. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 4.

Fig. 16.—Transverse section of muscle-fibres from the circular membrane of the stomach in the bee. The fibres are rich in sarcoplasm. The nucleus is disposed beyond the area occupied by the myofibrils. Haematoxylin; eosin. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 4.

Fig. 17.—Part of transverse section of epithelium of the small intestine of the bee. In the cells are visible vacuoles with secretion and the striated superficial layer of plasm (*ds*) covered with a chitinous cuticle (*c*). Zenker formol; Mann-Holland's stain. Zeiss; ob. $\frac{1}{12}$, oc. 4.

Fig. 18.—Transverse section of the fibre of the muscular membrane of the small intestine in the bee. The fibre is thick; nuclei are disposed along the axis of the fibre surrounded exteriorly by tufts of myofibrils. The fibre is enveloped in the perimysium. Duboseq's fluid; Heidenhain's iron haematoxylin. Zeiss; ob. $\frac{1}{12}$ hom. imm., oc. 4.

Fig. 19.—Rectal glands in the wall of rectum. Total surface preparation. Alcohol; borax carmine. Ob. Winkler 1, oc. 0.

Fig. 20.—Schematic structure of rectal gland. The drawing represents the wall of the rectum with half of the gland inserted in its wall. Rectum represented exteriorly.

Fig. 21.—Transverse section of rectal gland of a bee that has hibernated. In the cells of its epithelium are visible numerous granular inclusions and granules of secretion. Duboscq's fluid; Heidenhain's iron haematoxylin. Zeiss; $\frac{1}{2}$ hom. imm., oc. 0.

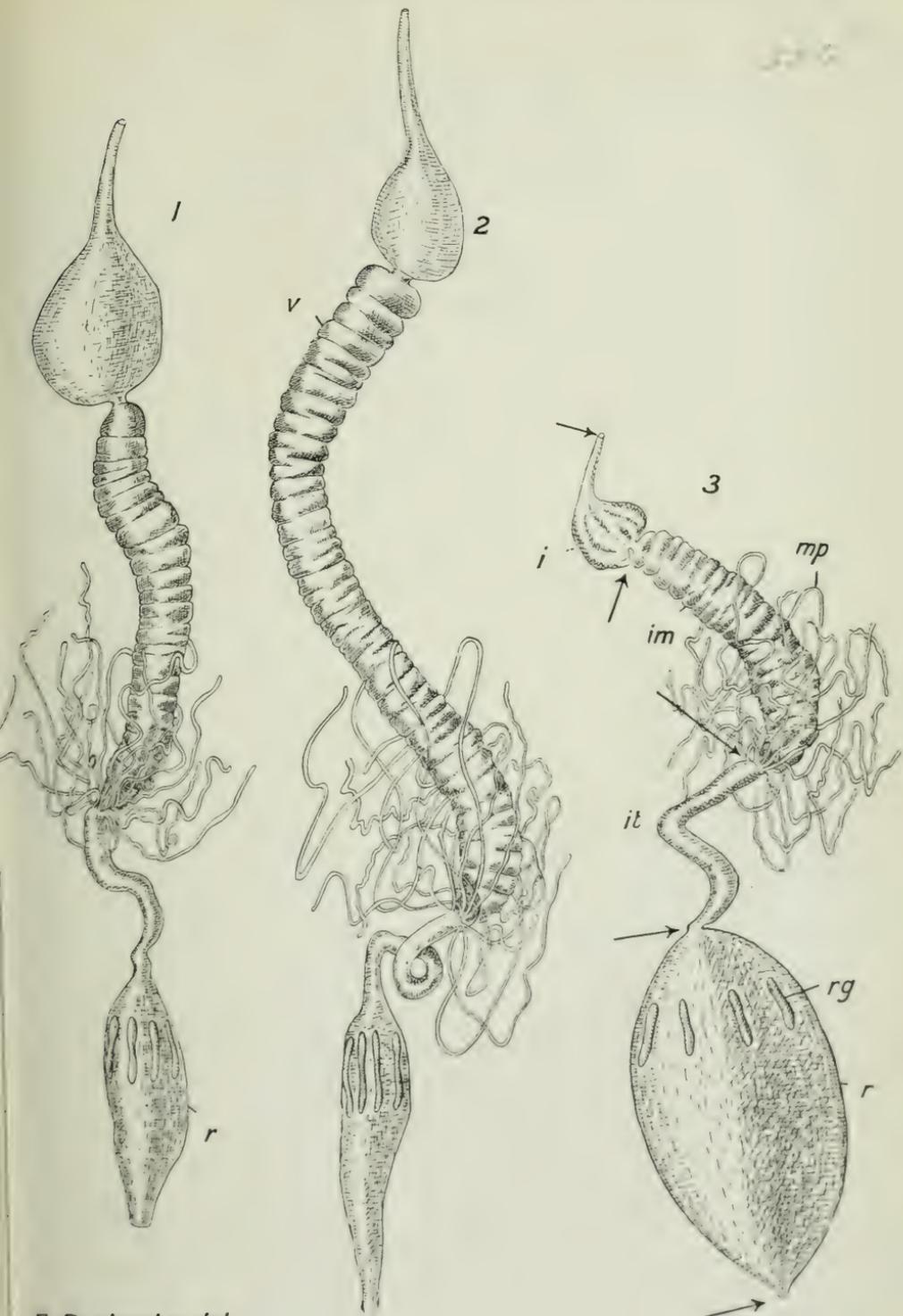
Fig. 22.—Oblique transverse section of rectal gland of a summer bee. The network of deeply stained cell borders in which tracheae pass (fig. 25, Pl. 17) are visible. In the protoplasm of the epithelial cells there are no inclusions. Zenker formol. Same stain and magnification as in fig. 21.

Fig. 23.—Part of longitudinal section of the rectal gland. The entrance of the trachea between the cells of its external layer and the formation of lateral transverse branches of the trachea under the surface of the gland are visible. Zenker formol; iron haematoxylin. Zeiss; $\frac{1}{2}$ hom. imm., oc. 4.

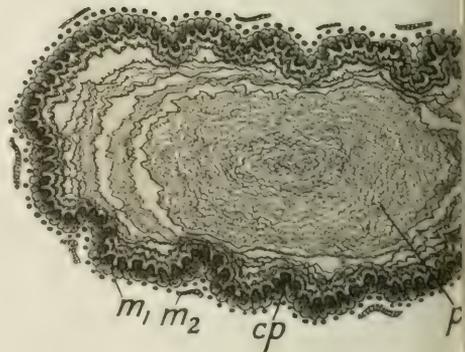
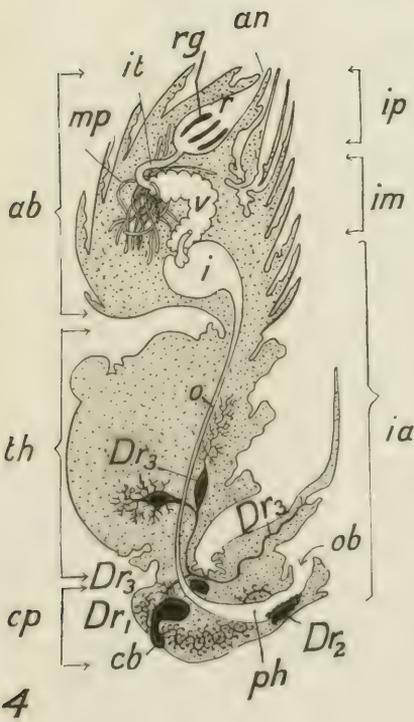
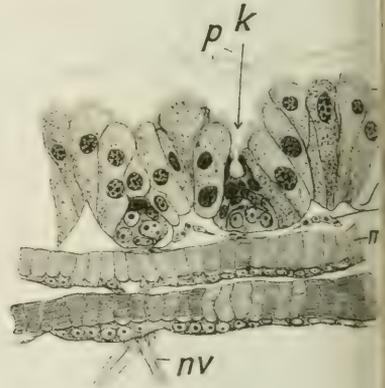
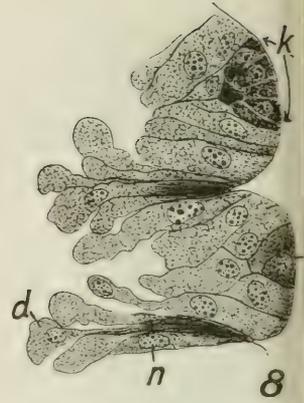
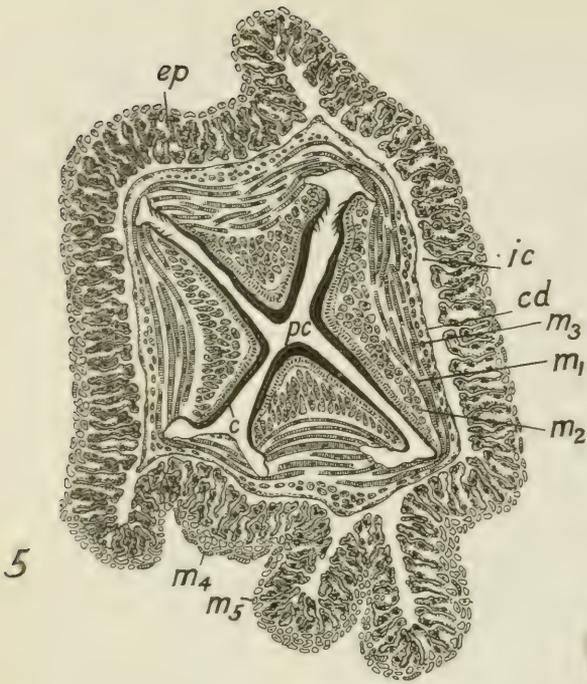
Fig. 24.—Network of tracheae under the surface of the inner wall of the rectal gland. Same treatment. Zeiss; $\frac{1}{2}$ hom. imm., oc. 1.

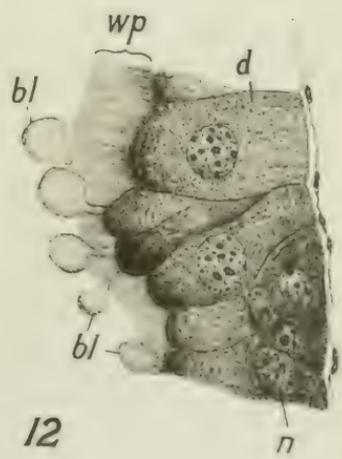
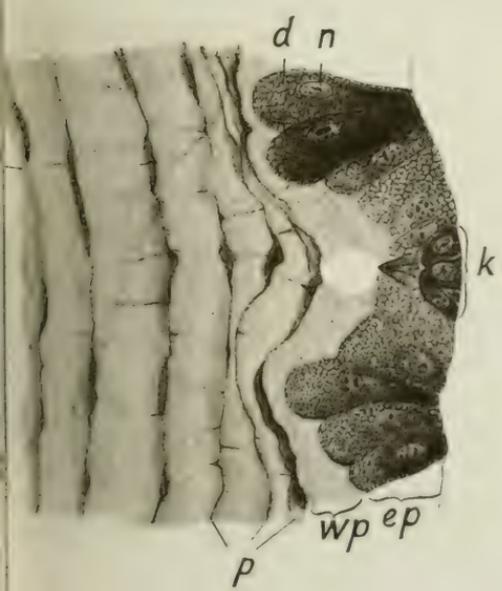
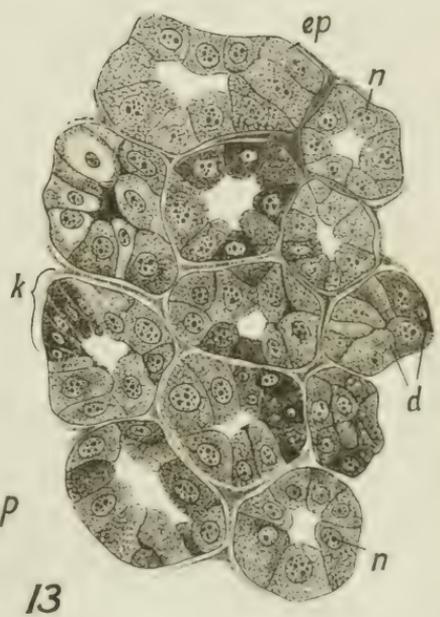
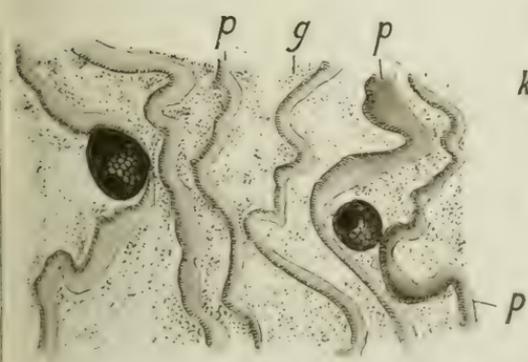
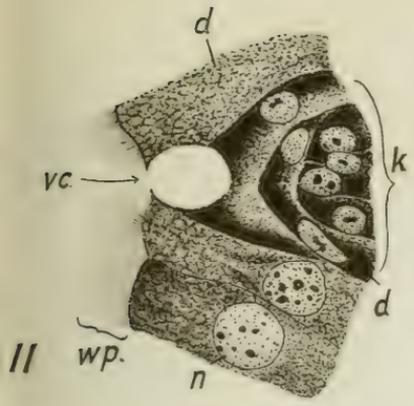
Fig. 25.—Part of surface section of the exterior wall of the rectal gland. Tracheae running between the cells are visible. The surfaces of the latter adjacent to them stain sharply black. Same treatment and magnification as in preparation no. 23.

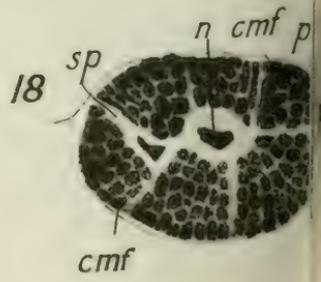
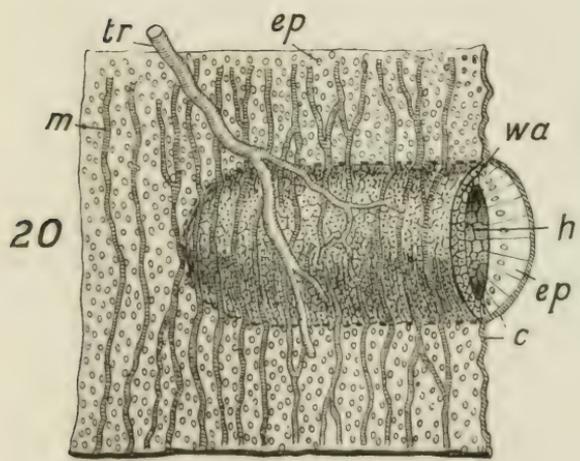
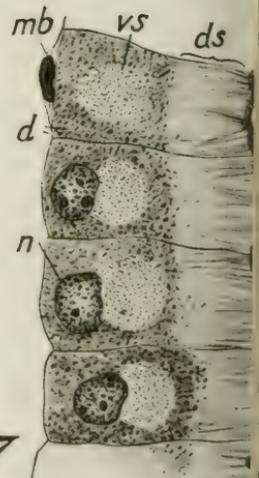
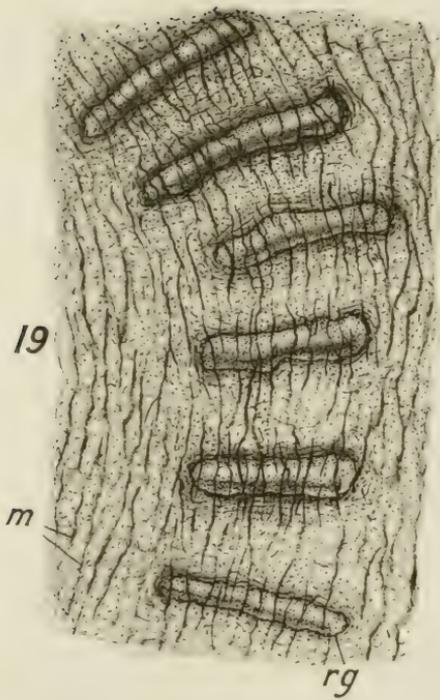
Fig. 26.—Cuticle of the epithelium of the small intestine with numerous bacteria on its surface. Zenker formol; Giemsa. Zeiss; ob. $\frac{1}{2}$, oc. 4.

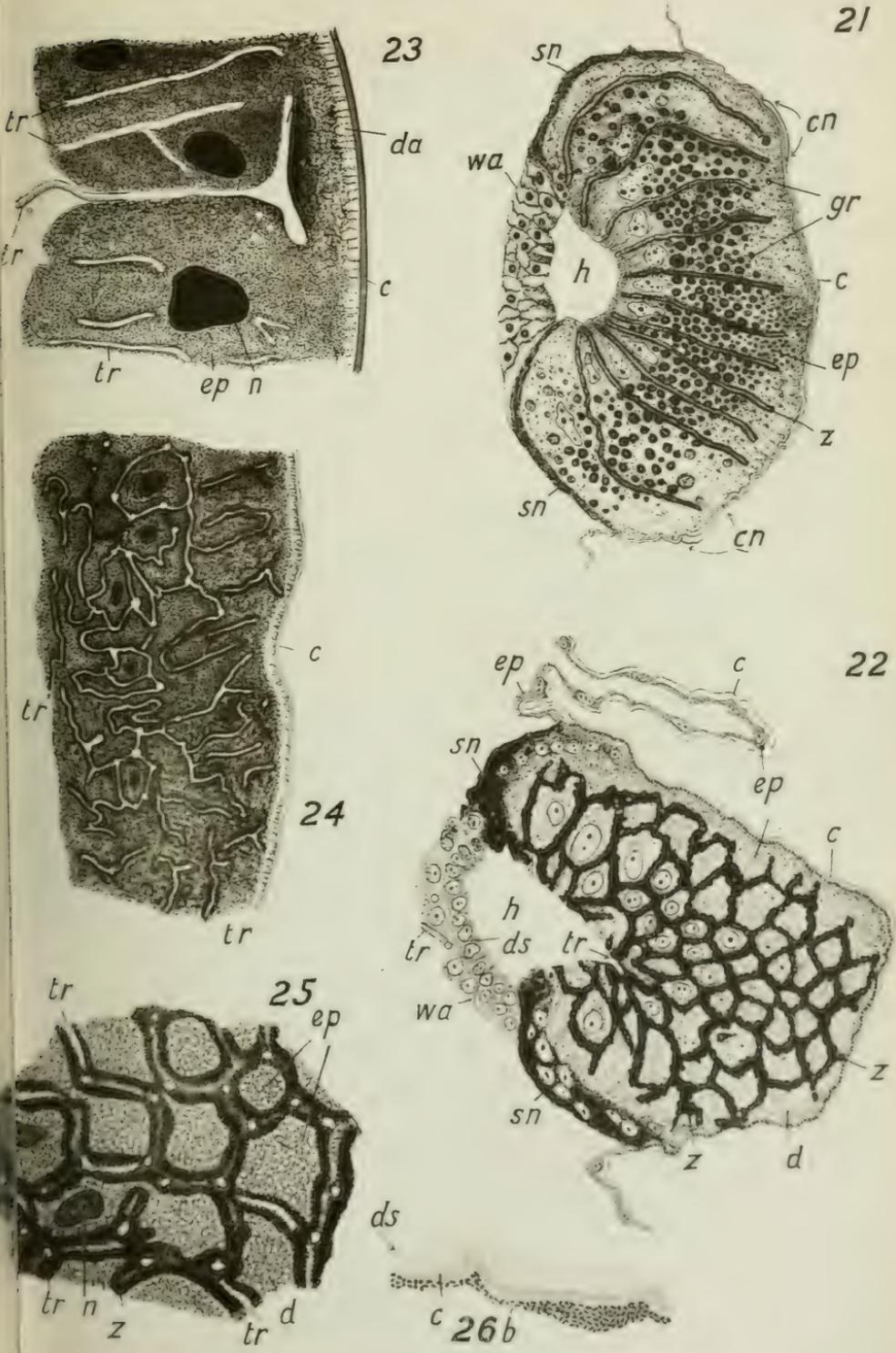


E. Pavlovsky, del.









The Nature of certain Ovum-like Bodies found in the Seminiferous Tubules.

By

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Edinburgh.)

With Plates 18-23.

INTRODUCTION.

DURING the systematic examination of a goat with an abnormal reproductive system sent to this Department by Mr. T. H. Gillespie, Director-Secretary of the Scottish Zoological Park, certain peculiar ovum-like bodies were found within the seminiferous tubules of the ectopic testes.¹ In order to decide as to the exact nature of these, it was necessary to extend the investigation so as to include an examination of other abnormal gonads. To Mr. M. S. Pease, of the School of Agriculture, Cambridge, we are indebted for an undescended testis from a rabbit in which he had found bodies exactly similar to those found by us in the case of the goat, and also for permission to include a description of his series in this paper. An undescended testis from the human was sent to this Department by Sir Edward Sharpey Schafer, and one from a cat by Mr. A. Cameron, of the Royal (Dick) Veterinary College. Without this material our work must have remained very incomplete, and in acknowledging our debt we wish to render our grateful thanks.

We are also much indebted to the Staff of the Pathology Department of the University of Edinburgh, and to Mr. E. G. Glass for much practical assistance in the course of our study.

¹ 'Veterinary Journal', April 1922.

METHODS.

With the exception of the goat the material was already fixed when received by us. The testis of the goat was fixed in Zenker's fluid, that of the rabbit in Bouin's fluid, and that of the frog and of the cat in 10 per cent. formalin.

The specimens were embedded in paraffin wax and sections were cut at 5-6 μ . We employed the following stains: eosin and Delafield's haematoxylin, Haidenhain's iron haematoxylin, Mallory's and Van Gieson's. A good method for displaying the interstitial cells was to stain with iron haematoxylin in the usual way and counter-stain with Van Gieson's.

The sections of the frog's gonad, which had been used in previous research, were stained with Delafield's haematoxylin and Bismarek brown.

The coloured drawings were made with the aid of a camera lucida. With the exception of fig. 17, for which a Koristka 1/12 oil immersion lens was employed, they were all drawn to the same scale with a Zeiss no. 7 objective.

DESCRIPTION OF THE UNDESCENDED TESTIS OF A GOAT.

The specimen was obtained from an adult goat whose sexual behaviour was sometimes of the male and at other times of the female type. The internal and external genitalia were also intersexual in character. The testes were discovered in the region of the external inguinal ring embedded in the subcutaneous tissue of the ventral body-wall and surrounded by a thick investment of fat.

GENERAL STRUCTURE OF THE GONAD.

Seminiferous Tubules.—The seminiferous tubules show greater degeneration than any other tissue in the gonad. Degeneration has not proceeded at a uniform rate throughout the testis, and the tubules may be described in three classes according to the stage which the atrophic changes have reached.

Class A.—Comparatively normal tubules. The cross-

section is circular or very slightly compressed. The nuclei of the peripheral cells are distinct and in some cases show a reticulate structure. Degenerative changes, however, are beginning to appear in the more central cells, which show no nuclear structure. The laminated basement membrane and its cells are normal.

Class B.—The more central epithelial cells are very degenerate. A single layer of epithelium remains adherent to the basement membrane. The cells of this layer are usually spaced at fairly regular intervals round the wall of the tubule. The cytoplasm is fragmentary and degenerate, but the nuclei are distinct. The chromatic material in the nucleus is in the form of irregular deeply-stained granules. The rest of the epithelium has become loosened and is spreading into the lumen as an irregular syncytium. The nuclei of the component cells are in many cases indistinguishable, and the cytoplasm is reduced to anastomosing threads.

Class C.—Even the single layer of cells adhering to the basement membrane is indistinguishable, and the basement membrane itself is degenerate and is breaking down altogether in places. The outlines of the tubules in cross-sections are most irregular. The lumina are filled with cell detritus which is often aggregated at one or more points into somewhat deeply-staining irregular masses. These masses frequently contain globules which adhere to one another giving the appearance of a minute drop of emulsion surrounded by cellular matter.

The most noteworthy features of the seminiferous tubules occur in Classes B and C. These are remarkable deeply-staining bodies which are often present in the lumina of the tubules. They are always closely invested by a layer of degenerate epithelial cells which might easily, at first sight, be taken for a corona radiata. A more detailed account of these structures and a discussion as to their nature will be given later.

Rete Testis.—The rete testis appears normal. The walls of the vessels are composed of a single layer of cubical epithelial cells resting on the surrounding fibrous tissue. The cell-limits

are well marked and the large oval nuclei show no indication of degeneration.

Intertubular Tissue.—Large quantities of intertubular tissue are present, the connective tissue being considerably in excess of the interstitial. The connective tissue appears normal. The interstitial cells have an oval nucleus showing a granular structure and very clear cytoplasm the periphery of which is difficult to make out. In many cases the cells appear as mere naked nuclei, some of which stain more densely than others. It would seem that the interstitial cells are either normal or in a very early stage of degeneration.

Tunica Albuginea.—The testis is invested by the usual fibrous capsule or tunica albuginea, the histology of which is normal. The only point of interest with reference to this structure is the presence in places of a layer of adipose tissue, some three cells deep, which splits the tunica albuginea into a peripheral and a central layer.

Vasa Efferentia.—These, like the rete testis, show no sign of degeneration. They are lined by normal columnar ciliated epithelial cells, the large oval nuclei of which show a somewhat reticulate structure. The bunches of cilia appear as protoplasmic tags extending into the lumen.

Epididymis.—The structure of the epididymis is in every way typical. It is composed of a layer of ciliated columnar epithelium resting on a basal layer. The basal cells contain large oval nuclei whose long axes are set transversely to the radius of the tube. Cross-sections of the epididymis differ from those of the vasa differentia in the presence of the basal layer, the relatively larger nuclei, and greater thickness of the columnar epithelium.

Blood-vessels.—Closely associated with the gonad in the region of the epididymis is a considerable venous plexus, the interstices of which are largely filled with adipose tissue. The substance of the gonad is richly supplied by a capillary system. In many instances the walls of the capillaries have ruptured owing to the degeneration of the walls of the semin-

iferous tubules and haemorrhage into the lumina of the tubules has resulted.

Account of the Ovum-like Structures in the
Seminiferous Tubules and a Discussion as to
their Nature.

We have already referred to the remarkable ovum-like bodies invested by epithelial cells which occur in the lumina of many of the more degenerate seminiferous tubules. Two of the more typical cases will now be described.

A. (Pl. 19, fig. 7) Two of these structures are present in the lumen of a single tubule. The outline is very sharply marked by a dark ring well seen in the lower specimen, showing that the refractive index is higher than that of the surrounding tissue. Both bodies are composed of concentric layers sharply demarcated and apparently homogeneous. In the lower specimen there are three such layers. The innermost is the most deeply stained and surrounds a paler central area. Each primary ring is subdivided into faintly marked secondary rings. Only two primary rings are visible in the second body. In both bodies the central area is paler and less homogeneous than the rings.

The investing cells are very degenerate. They are indistinctly delimited and the nuclei appear as deeply-stained structureless masses of vague outline. Syncytial strands connect the investing cells with those lining the tubule.

The epithelium of the tubule is somewhat less degenerate than is usually the case. The peripheral cells, presumably spermatogonia, display distinct though structureless nuclei. The cytoplasm, however, shows no definite outline, but coalesces in neighbouring cells and tends to spread out into the lumen. Most of the more central cells have disappeared except at those points where a syncytium connects the investing cells with the wall of the tubule. This is significant. It should also be noted that as a rule the more central the position of the cell the greater is its degeneration.

B. Only one body is present in the tubule. This specimen

is somewhat larger than either of those described above. The section was stained with Mallory's stain. The body is ovoid in shape and does not display the usual concentric structure. The main feature is the presence of two broad superimposed rings of small granules which stain a purplish blue, those of the lower ring having a more reddish tinge than those of the superficial circle. The general coloration of the body is difficult to describe accurately and a coloured drawing has therefore been made (Pl. 18, fig. 6). It will be seen that the centre has an orange tint whereas the periphery is bluish.

The investing cells are extremely degenerate. As seen in section they are reduced in places to a narrow band. The nuclei are almost indistinguishable. A single protoplasmic strand connects the investing cells with the epithelium of the tubule. The epithelial cells are reduced to a single degenerate layer adhering to the basement membrane. A comparatively large clear space intervenes between the wall of the tubule and the tissue surrounding the central body.

The distinctly female appearance of the external genitalia led us to think that possibly ovarian tissue would be found in the gonads. On first examining our preparations it seemed to us that such was the case. The spherical structures, two of which have just been described, had much the appearance of degenerate ova in typical, if degenerate, Graafian follicles. We regarded the pale somewhat granular central area so commonly present as the nucleus, the dark line bounding the body as a zona pellucida, and the investing cells as the *corona radiata*. The intervening spaces between the investing cells and the epithelium lining the tubule could be interpreted as cavities for the liquor folliculi, and the epithelium adhering to the basement membrane would, of course, correspond with the epithelium of the Graafian follicle. More extensive examination, however, revealed the presence of obviously homologous bodies with the concentric structures so marked as to remind the observer of a starch granule. These obviously were not of ovarian nature, as was also shown by the fact that in some instances two or more occurred in the same tubule.

As is well known, it is rare to find more than one ovum in a single Graafian follicle. It was significant also that a careful search did not reveal one of these bodies in the interstitial tissue. On considering these facts, and knowing that the seminiferous tubules were in process of atrophy, we came to the conclusion that the bodies were degeneration products. Further investigation provided what seems a series of stages in their formation. These will now be described.

(1) (Pl. 19, fig. 8) A quantity of cell detritus appears in the lumen attached by protoplasmic threads to the epithelial cells adhering to the basement membrane. No cell structure is visible. On the left of the mass is an aggregation of deeply-staining globules, each with a somewhat lighter central area. The epithelium of the wall of the tubule is very degenerate, only the nuclei of the peripheral cells being recognizable. A small group of erythrocytes is seen in the lumen, the presence of which is probably due to the rupture of a capillary in the tubule wall.

(2) (Pl. 19, fig. 9) In the upper right-hand corner of the photograph is figured a tubule containing an aggregation of cellular material which to the right forms a deeply-staining mass similar to that described above. It is composed of four large globules which appear to be coalescing. These are surrounded by detritus the cellular nature of which is still apparent. The cells lining the tubules are reduced to a narrow darkly-stained layer fused to the basement membrane.

(3) In the same illustration appears a single ovoid body in a very degenerate seminiferous tubule. The body contains a number of highly refractile granules and shows no concentric structure. It is surrounded by protoplasmic debris displaying little or no cell structure, except at one point where it is connected with the wall of the tubule. The tubule itself is of very irregular outline and the basement membrane appears to be breaking down in places.

(4) (Pl. 19, fig. 10) The tubule in this instance is less degenerate than in the cases previously described. As usual, however, only the peripheral layer of the epithelial cells is present.

These show distinct nuclei, and the outlines of the cytoplasm, though irregular, are distinguishable.

The centre of the lumen is occupied by an aggregation of cells surrounding a circular body composed of two concentric rings and a darker central area. The investing cells show two distinct layers: an outer layer in which the nuclei are visible, and an inner layer presenting no cell structure whatever. This inner layer might be regarded as part of the central body which it closely resembles both in general structure and staining reaction. Though of an asymmetrical contour which corresponds with that of the outer layer, it displays none of the angularities of the latter. The appearance suggests that during life surface tension was causing it gradually to assume a spherical shape.

(5) (Pl. 18, fig. 4) This section was stained with Mallory's stain and a coloured drawing has been made to show the somewhat remarkable staining reaction. A single circular body of rather small size is present in a seminiferous tubule. The body consists of a whitish central area surrounded by a bright blue ring. A circle of more darkly-stained granules is present. The body is surrounded by a large mass of débris which on one side spreads out into a syncytium connecting the central structure with the wall of the tubule, and on the other side is condensing into a narrower more deeply-stained layer. There is a sharp line of demarcation between the body and the surrounding protoplasm which stains an orange brown. The epithelial cells are arranged in the usual single degenerate layer applied to the basement membrane.

Discussion.—From a study of the series of specimens which have been described above it would appear that the process of formation of these remarkable bodies is somewhat as follows:

In the seminiferous tubules we have seen that degeneration spreads from the centre towards the periphery. The chief factor in this degeneration seems to be a gradual softening of the protoplasm of the epithelial cells. As this softening increases, the innermost cells can no longer adhere to the

layer beneath, but drift into the lumen of the tubule where they form the syncytial masses we have described. As more cells are added, these masses condense into more deeply-staining aggregates. The process of liquefaction continues until the more central cells lose all cytological structure and finally give rise to colloid globules which, adhering together, give the emulsoid appearance seen in (1) (Pl. 19, fig. 8) and in (2) (Pl. 19, fig. 9). If the desquamation and liquefaction of the different layers of cells are rapid, a single large colloid globule is produced, as in B (Pl. 18, fig. 6). Usually, however, the process is more gradual. Calcification appears to follow the colloid degeneration and, if desquamation and liquefaction take place slowly, may wholly or partially metamorphose the colloid globule formed by the first layer of cells, before liquefaction of the next layer is completed. In this way a body having a concentric structure is produced, each of the rings seen in the cross-section representing a layer of cells. It would appear that these objects resemble *corpora amylacea* in significance and in mode of formation. In (5) (Pl. 18, fig. 4) we have an example of gradual degeneration. Though the central structure is comparatively small, calcification is already complete. The preparation is stained with Mallory's stain, and the central structure has taken on the Aniline Blue, whilst the investing cells have stained with Orange G. In B (Pl. 18, fig. 6) we have, as has already been stated, an example of rapid colloid degeneration. The body which is here figured occurred in the same section as (5) (Pl. 18, fig. 4) but has stained with Orange G. like the epithelial cells of the tubule. Calcification, however, has at length set in as is shown by the blue coloration at the periphery.

On studying sections stained with Van Gieson's stain, it was found that the small globules and the most degenerate of the epithelial cells give the orange coloration characteristic of colloid degeneration, whereas the larger, concretion-like structures stain a deep magenta (Pl. 18, fig. 1). This also lends support to the view that the spherical structures have been produced by colloid degeneration followed by calcification.

DESCRIPTION OF AN UNDESCENDED TESTIS OF A RABBIT.

The rabbit from which this testis was obtained was killed at the age of one year and seven months. The other testis was scrotal and normal. On section the testis was found to be degenerate and to contain a number of ovum-like bodies similar to those described in the goat.

General Structure of the Gonad.

Seminiferous Tubules.—The seminiferous tubules are small and degenerate.

Epithelium.—The condition of the epithelium presents considerable variation in different tubules. In some the cells are comparatively normal and completely fill the lumen. In other tubules the cell-limits are indistinguishable except in the peripheral layer, and the lumen contains a loose syncytium of protoplasmic débris (Pl. 20, fig. 11). Spermatogenesis appears to have reached the spermatocyte stage and to have stopped at that point. Most of the peripheral cells are typical spermatogonia. The more central cells are mostly large clear spermatocytes in various stages of synapsis.

As in the goat the more central nuclei on the whole display most degeneration. The nuclei of the peripheral spermatogonia are large, oval, and of a finely granular structure, whilst those lying farther in the lumen are of irregular shape, smaller size, and are more deeply staining. In many cases the epithelial cells are in active mitosis, but in the more central spermatocytes the process does not appear ever to be completed. The nucleus swells up to twice its original size and appears as a large clear vesicle containing darkly-staining chromosomes or nuclear skein. The chromatin material subsequently breaks down into smaller granules, and a further increase in the volume of the nucleus is noted. Such degenerate nuclei have been found surrounded by a mass of semi-fluid cell detritus, and in such cases would appear to serve as centres around which intratubular bodies are being formed (Pl. 20, fig. 12). Several instances of pluripolar mitosis were seen in the epithelium—a sign of degeneration.

Basement Membrane.—The basement membrane of the tubules presents a somewhat remarkable appearance (Pl. 20, fig. 13). Between the basement membrane cells and the epithelium is a layer, the substance of which is in some cases almost amorphous and in others distinctly fibrous. Elongated nuclei occur either within or central to this layer. Rudiments of this structure are present in all the tubules. In some it has become so thickened that the epithelial cells are compressed into a small mass in the centre of the tubule, and in a few instances are completely obliterated. The layer appears to be white fibrous tissue in various stages of development and with Van Gieson's and Mallory's stains takes on the same coloration as does the intertubular fibrous tissue. It is presumably formed by the cells of the basement membrane.

The same phenomenon was observed in the human testis (Pl. 20, fig. 14). In this specimen the majority of the seminiferous tubules are completely filled with a lightly-staining substance of gelatinous appearance bounded by the cells of the basement membrane. Occasionally a small central space containing cell debris remains. With Van Gieson's stain this material takes on the acid fuchsin as does the white fibrous tissue in other parts of the gonad. In some tubules fine fibres can be distinguished in the amorphous matter. A similar gelatinous layer invests groups of interstitial cells and the tubules of the rete testis. In these latter cases the substance is apparently produced by the neighbouring connective tissue cells.

It would appear that increase in fibrous tissue is correlated with colloid degeneration of the germinal epithelium. Large quantities of fibrous tissue are conspicuous both in the goat and in the rabbit, but not in the undescended testis of a cat (to be described later) in which degeneration had only just begun, nor in the testis of a pig in which spermatie tissue has undergone fatty degeneration. Our explanation of this correlation is that the colloid produced by the degeneration of the germ cells has two possible fates:

(1) It may form large colloid globules in the centre of the lumen.

(2) It may soak between the peripheral epithelial cells into the intertubular tissue and stimulate the connective-tissue cells to an increased production of white fibrous tissue. Such a condition is commonly met with in cancer and other pathological states in which colloid or mucoid degeneration is in progress. The white fibrous tissue is usually laid down around some solid structure such as a tubule or an aggregation of cells. This is the condition in the human.

The colloid may follow both courses in the same testis as in the case of the goat and the rabbit.

Stone, in his paper on a pseudo-hermaphrodite goat,¹ mentions a 'layer of hyaline material' within the basement membrane of the tubules. Doubtless this is of the same nature as the structures we have described.

Intertubular Tissue.—As in the goat, the intertubular tissue is present in large quantities. It is composed chiefly of white fibrous tissue and interstitial cells. The bundles of fibrous tissue divide the gonad into a number of small compartments in which lie the interstitial cells.

The interstitial cells are well developed and very numerous. The cell-limits are distinct. The cytoplasm is granular and contains a large circular nucleus of finely granular structure. A nucleolus is present. The interstitial cells appear to be in rapid proliferation in certain areas in which numerous mitotic figures are seen (Pl. 21, fig. 15). In places the interstitial cells seem to be undergoing a remarkable metamorphosis (Pl. 21, fig. 16). The cytoplasm increases in volume and becomes more granular, while the nucleus takes up an eccentric position. The cytoplasm finally increases to about twice its original size, and the nucleus is situated at the extreme periphery—it may even cause a slight bulging of the cell wall. Such cells are usually formed in patches which are clearly demarcated from the surrounding tissue. They may, however, occur in small groups in which the various stages in the transformation can be followed. It would appear that the granular cells are fat-

¹ R. S. Stone, "Atypical Male Sex-ensemble in the Domestic Goat", 'China Medical Journ.', v. 34, November 1920.

forming.¹ In the very degenerate abdominal testis of a pig which has been examined in this Department we meet with a precisely similar type of cell.² In this case, however, the proliferation has been completed and enormous quantities of interstitial tissue are present. The fibrous tissue has almost disappeared. All the interstitial cells have assumed the fat-forming character we have described, but are at a slightly more advanced stage than is usually the case in the rabbit in that the cytoplasm shows small circular vacuoles from which the fat has been dissolved during fixation. In the human undescended testis to which we have already referred a similar condition is found. The immense increase in interstitial tissue, so striking a feature in the pig and rabbit, was not seen, but all the interstitial cells have taken on the character of fat-forming cells. The cytoplasmic vacuoles are much larger than in the pig, and in some cases occupy most of the cell.

Tunica Albuginea.—The tunica albuginea is considerably thickened at one point but otherwise appears normal.

Epididymis.—The epididymis (Pl. 21, fig. 17) is closely invested at all points by a thick layer of adipose tissue which separates it from the gonad. The cytology of the epithelial cells appears almost normal, although in certain of the cells the cytoplasm has a slightly vacuolated appearance. Cilia are visible. The lumen is filled with a lightly-staining coagulum in which appear circular vacuoles. Deeply-staining cell detritus is also present.

Intratubular Bodies.—Ovum-like bodies are present in large numbers. They closely resemble those found in the goat but have not reached such an advanced stage of formation.

Structure.—Calcification appears in comparatively few; most give the vivid orange coloration with Van Gieson's stain, characteristic of colloid degeneration (Pl. 18, fig. 3). When

¹ Since writing the above this question has been investigated further. It would appear that the cells are not 'fat-forming' but are enlarged owing to accumulation in the cytoplasm of various nutritive materials. A paper dealing fully with this subject is in the press.

² 'Veterinary Journal', March 1922.

stained with Mallory's stain they are affected by Orange G. only, whatever the period of staining. A few display the blue centre and bluish superficial film which we take to represent incipient calcification. Pairs of serial sections stained alternately with Mallory's and Van Gieson's stains show that those parts of a body which stain blue with Mallory's stain, stain magenta or remain colourless with Van Gieson's stain. In many of the tubules the bodies are surrounded by an aggregation of cell material, but in others they lie free in the lumen. The concentric structure characteristic of the bodies described in the goat is most marked but the number of rings is smaller (Pl. 21, fig. 18). Indeed, in most cases only one ring is present surrounding a more lightly-staining somewhat granular central area. The primary ring is usually marked by secondary rings. Sometimes two or more primary rings occur.

Formation.—The method of formation of the intratubular bodies appears to be the same as in the goat, viz. by the liquefaction of aggregates of epithelial cells. This liquefaction may take place round a degenerate epithelial nucleus, as described above (Pl. 20, fig. 12), or may proceed directly with the formation of one or more colloid globules surrounded by a mass of desquamated cells. The centre is formed by a degenerate nucleus, the ovum-like appearance is very striking. As has already been stated, calcification has begun in relatively few instances.

Description of the Undescended Testis of a Cat.

The testis was taken from a six months old animal and was found at the external aperture of the inguinal canal. The other testis had been removed from the scrotum a month previously and there was then no sign of a second testis. The cat was otherwise normal.

A microscopical examination reveals arrested development and early degenerative changes.

Seminiferous Tubules.—The seminiferous tubules are of normal size and shape but show only the first stage of sperma-

togenesis. Mitotic figures are rare. The lumina are occupied by a protoplasmic syncytium. The nuclei of the epithelial cells are mostly arranged in a single peripheral layer, although many lie more centrally. In the majority of cells the outline of the cytoplasm is indistinguishable as these cells tend to coalesce laterally. Others, however, are clearly defined.

The epithelial cells appear to be of three types as follows :

(1) Typical spermatogonia. These contain a reticulate nucleus with well-marked nucleolus. The limits of the cytoplasm in neighbouring cells cannot be made out.

(2) Large cells lying immediately central to the basal layer. They may be twice or three times as large as the basal cells. The cytoplasm is very hyaline and the cells in consequence are sharply demarcated from the more darkly-staining surrounding tissue. The nucleus varies in size and staining capacity. Typically it is finely reticulate and contains a nucleolus. It may, however, be small and dense, or so large as to fill two-thirds of the cell. In the latter case the chromatin material is reduced to a few small strands and granules surrounding a very large nucleolus (Pl. 18, fig. 2).

These large clear cells are probably late spermatogonia. Many appear degenerate, and in a few the nucleus is altogether disintegrating. It is noticeable that the larger the cell the greater is the relative size of the nucleolus. This fact will be referred to later.

(3) The third type of cell has a rather small granular ovoid nucleus containing a nucleolus. The cytoplasm is pyramidal in form. It is attached at the base to the basement membrane and is often drawn out into a protoplasmic filament. These we regard as cells of Sertoli. The lumina of the tubules often contain a deeply-staining amorphous substance which appears to be a mixture of protoplasmic detritus and coagulated fluid. Degenerate nuclei are usually present in such masses (Pl. 22, fig. 19). Small deeply-staining globular bodies (Pl. 22, fig. 20) occasionally occur in the central syncytium of the tubules and more rarely among the peripheral cells. They are found to stain brightly with the Orange G. of Mallory's stain. It would

appear that they are colloid globules of the same nature as those described in the goat and the rabbit. A coloured drawing has been made (Pl. 18, fig. 5) from one of the more typical of these. The figure was taken from a section stained with Mallory's stain. The body is seen to consist of a darker central area surrounded by a lighter ring. It is invested by a protoplasmic syncytium. The tubule is lined with epithelial cells most of which are attached to the basement membrane.

The formation of the colloid body seems to be initiated by the nucleolus of one of the more centrally situated nuclei. The process appears to be as follows: The nucleolus increases to several times its original size, apparently absorbing the rest of the nuclear material. The nucleus becomes more and more vacuolated and is finally reduced to a large clear vesicle containing an enormous nucleolus. All stages intermediate between a normal nucleolus in a reticulated nucleus and a well-developed colloid globule are seen (Pl. 18, fig. 2).

Intertubular Tissue (Pl. 22, fig. 21).—The Interstitial Cells show reduction instead of hyperplasia. They occur in columns and groups which are usually closely applied to the basement membrane of the tubule.

A large elongate group is found intercalated between two layers of the tunica albuginea. The nuclei are spherical and have a very distinct nucleolus. They are usually eccentrically situated. The cytoplasm is very vacuolated, especially in those cells which occur in the tunica albuginea. In many of the latter the cell is almost an empty vesicle. Fibrous tissue is present in small quantities.

Epididymis.—The epididymis appears normal and typical. It is lined with the usual columnar ciliated epithelium resting on a basal layer. The lumen in places contains masses of débris. The connective-tissue stroma is normal.

Vasa Efferentia.—These consist of the usual layer of columnar ciliated cells somewhat shallower than that of the epididymis.

Tunica albuginea.—The tunica albuginea is typical and normal.

DESCRIPTION OF THE DISPLACED TESTIS OF A FROG.

A frog, *Rana temporaria*, was killed in April 1916. It was an adult, measuring 5.2 cm. from the tip of its snout to the symphysis of the pubis, while its accessory sexual apparatus and its secondary sexual characters were entirely and typically male. During the dissection it was noticed that while the left gonad was a testis, normal in every respect, the right gonad was missing. But there was a tumour 10 mm. long and 6 mm. broad, encased in a fibrous tissue capsule and firmly attached to the muscle-sheaths of the *m. rectus abdominis* and *m. triceps femoris* and lying upon and distal to the right groin. Naturally it was assumed that this tumour was the missing testis and search was made for vasa efferentia but none could be found, for the tumour was not connected with any part of the genital system. Further dissection showed that there was a hernia, the sac of which was provided by the abdominal peritoneum and which was covered by the stretched fibres of the *m. obliquus externus*. The neck of the sac was almost completely obliterated by fibrous over-growth and was adherent to the adjacent surfaces of the *m. rectus abdominis* and *m. triceps femoris* at the site of their origin, while the dorsal surface of the tumour was firmly adherent to the ventral surface of the latter muscle. It is of interest to note that the location of this tumour is very nearly that of a testis in the scrotum: it lay close to where the external ring of the inguinal canal would be.

On section the left gonad has the structure of the normal testis. The tumour proved to be the missing testis, but its structure is peculiar in that while the tissues near the periphery have the normal structure of a testis, the central parts of the gonad consists of a loose matrix of indefinite tissue amongst which many large bodies closely resembling ova are found. The gonad has the structure of the intermediate gonad as defined by Schmitt-Marcel¹ and of the testis of indirect development as described by Witschi.² The testis is encased

¹ 'Arch. f. mikr. Anat.', vol. 72, p. 516, 1908. ² Ibid., vol. 85, 1914.

in a thick capsule of fibrous tissue and muscle-fibres and branches of the femoral artery provide a blood-supply by which the gonad is nourished. No vasa efferentia are present.

The condition of the seminiferous tubules shows that there had been active spermatogenesis prior to the displacement of the gonad. Many of the tubules are still comparatively normal and are thickly lined with spermatozoa which are arranged with the heads attached to the basement membrane and the tails extending into the lumen (Pl. 23, fig. 22). In other tubules, however, we find evidence of degeneration. As in the cases of goat, rabbit, and cat, degeneration takes place from within outwards. The tails lose their outline and fuse to form a lightly-staining granular mass in the centre of the tubule. The heads may remain distinct, but in more advanced stages of degeneration they also tend to coalesce.

As we have already stated, large ovum-like structures completely filling the lumen are present in some of the tubules (Pl. 23, fig. 23). These consist of a deeply-staining peripheral layer which is sharply demarcated from a more lightly-staining somewhat granular central area. So far as we can judge from the limited number of serial sections at our disposal, it seems probable that the bodies are tubular and follow the convolution of the tubule in which they are contained. We are, however, not certain on this point, although it is significant that the bodies almost invariably occur in groups of two or three, as if several convolutions of a single tubule had been sectioned at once.

We regard these structures not as ovarian in character but as a result of a more advanced stage in the degeneration of the seminiferous tubules. It would appear that they represent an early phase of that colloid degeneration which has been described in the goat, rabbit, and cat. Actual colloid has not yet been formed, but liquefaction has advanced so far that the heads and tails have coalesced into single protoplasmic masses. The dark peripheral layer appears to be formed by the heads, the light central area by the tail, and the seminal fluid occluded in the tubule.

SUMMARY.

In the study of the ectopic testes of a goat, rabbit, and frog, bodies were found bearing a strong resemblance to ova within Graafian follicles. However, these proved to be not ovarian but the degeneration products of the germinal epithelium of the seminiferous tubules. The bodies appear to be produced by the gradual liquefaction of masses of desquamated cells, whereby large colloid globules are formed which subsequently undergo calcification. The gonads of the frog, rabbit, and goat present a progressive series of these degenerative changes.

1. In the frog the bodies are still protoplasmic and are formed by the liquefaction of spermatozoa. The tails of the sperms give rise to a central lightly-staining area which is surrounded by a broad dark ring formed by the lateral coalescence of the heads.

2. In the rabbit degeneration has in most cases reached the colloid stage. Large spherical bodies, which with Van Gieson's stain give the orange coloration characteristic of colloid, are found in the lumina of many of the seminiferous tubules. A few are showing signs of calcification. They are formed (*a*) by the liquefaction of protoplasmic detritus around the nucleus of a degenerate spermatocyte; (*b*) by the coalescence of several small colloid globules formed in the centre of a mass of cell débris.

3. In the goat the large colloid globules are undergoing calcification, as is shown by a study of sections stained with Mallory's and Van Gieson's stains. Only a few of the smaller globules give the colloid coloration. The formation of the bodies appears to be the same as in the gonad of the rabbit.

The undescended testis of a young cat was also examined microscopically. This shows a very early stage of degeneration. Spermatogenesis has ceased and the syncytium in some of the tubules is degenerate. Minute colloid globules which seem to be derived from the hypertrophied nucleoli of spermatocytes occur in the syncytial protoplasm of some of the tubules.

Points of interest in connexion with this study are as follows :

1. It seems probable that many of the structures in abnormal testes which have been regarded as ova are in reality of a degenerative character, similar to that of the bodies we have described.

2. The interstitial cells of the rabbit's testis are in certain areas being metamorphosed into what appear to be fat-forming cells.

3. The colloid degeneration of the spermatic epithelium is correlated with an increased production of white fibrous tissue. This phenomenon may be explained on the assumption that some, or, as in the human, the whole of the colloid percolates through the peripheral layer of cells and stimulates the cells of the basement membrane and of intertubular connective tissue to greater activity.

EXPLANATION OF PLATES 18-23.

PLATE 18.

Fig. 1.—Goat. Seminiferous tubule. $\times 470$. Stained with Van Gieson. All the epithelium is desquamated into the lumen to form a syncytial mass in which several ovum-like bodies are in the process of formation. Two complete bodies are seen, the lower one is completely calcified but the centre of the upper one still gives the orange coloration characteristic of colloid. A number of colloid globules are seen in other parts of the lumen.

Fig. 2.—Cat. Spermatocytes within seminiferous tubules. $\times 900$. Stained Mallory. Shows every stage in the formation of colloid globules by hypertrophy of the nucleolus. In (a) and (c) the cells are centrally situated, in (b) they are peripheral.

Fig. 3.—Rabbit. Seminiferous tubule. $\times 470$. Stained Van Gieson. Two ovum-like bodies are present surrounded by desquamated cells. They show the vivid orange coloration characteristic of colloid. Calcification has not yet set in. The thickened basement membrane is also seen.

Fig. 4.—Goat. Seminiferous tubule. $\times 470$. Stained Mallory. A single circular body lies within the tubule and consists of a central whitish area surrounded by a bright blue ring. A circle of more darkly-staining granules is also present and the body is invested by a large mass of debris which at one side spreads out into a syncytium connecting the central body with the wall of the tubule.



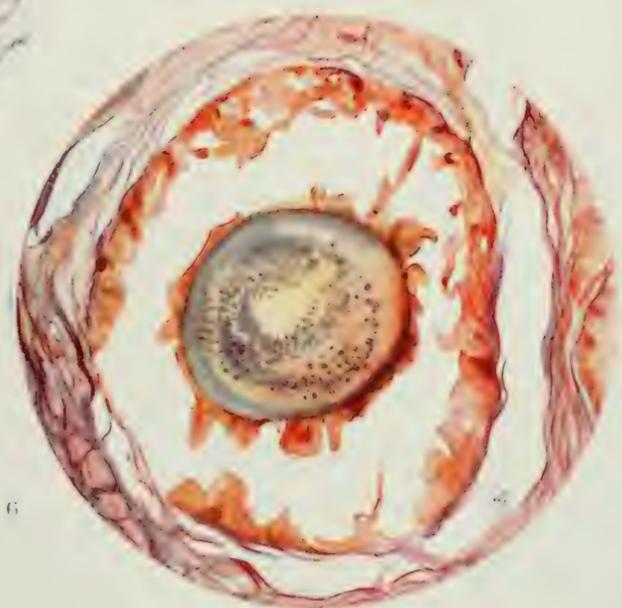
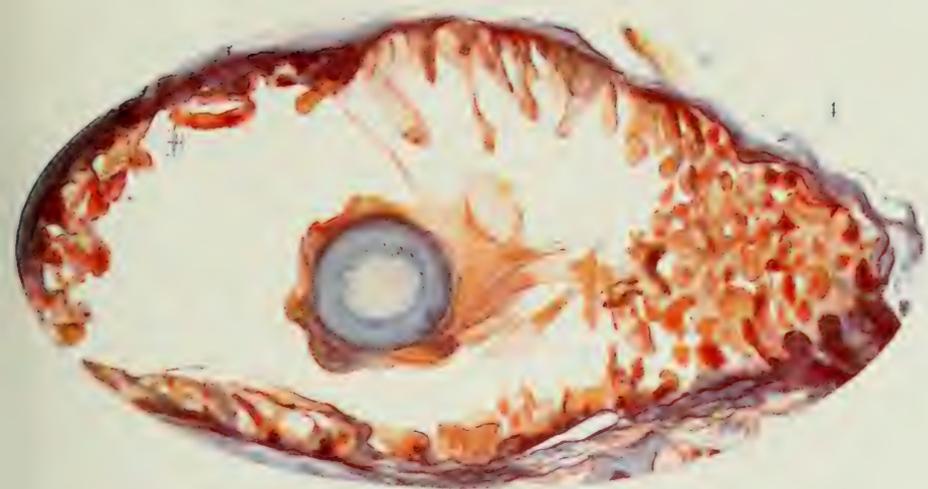




FIG. 7 $\times 320$



FIG. 8 $\times 250$



FIG. 9 $\times 125$

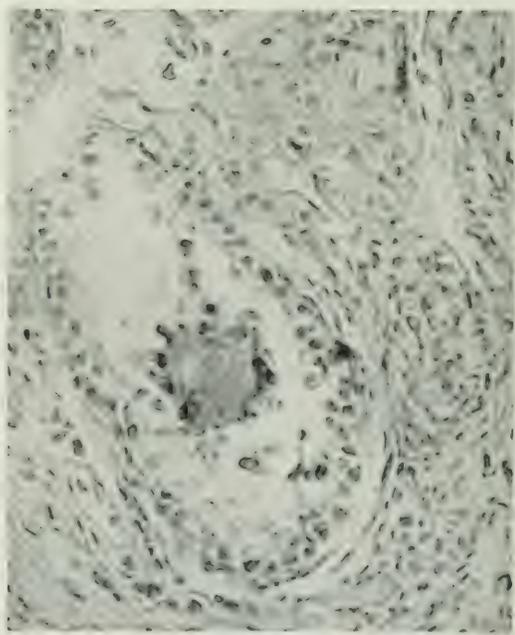


FIG. 10 $\times 320$

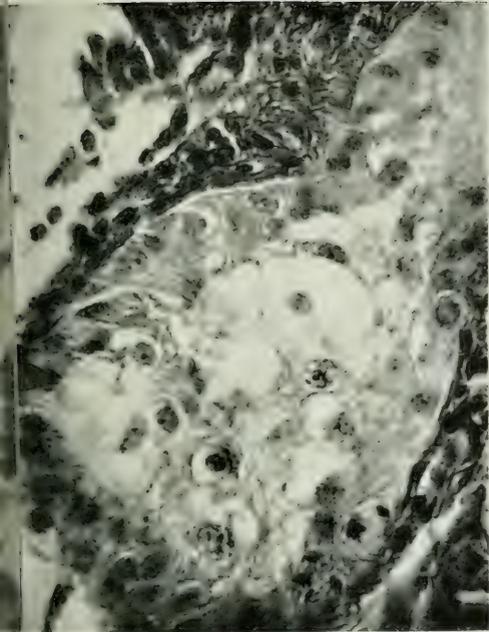


FIG. 11

× 400

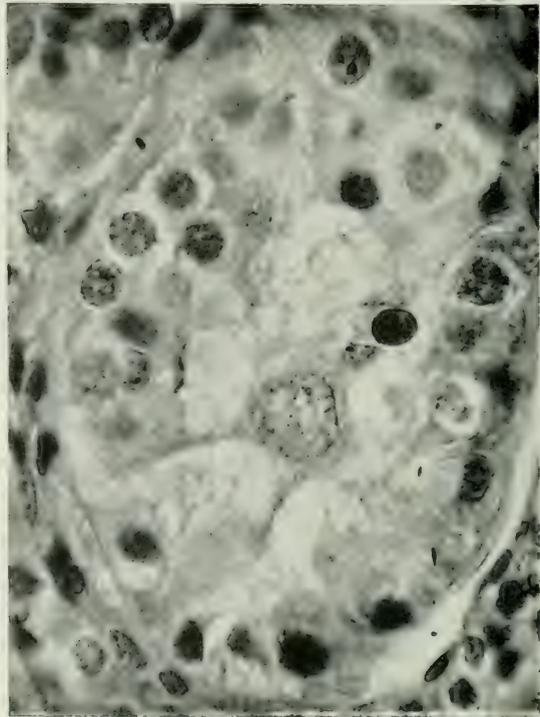


FIG. 12

× 770

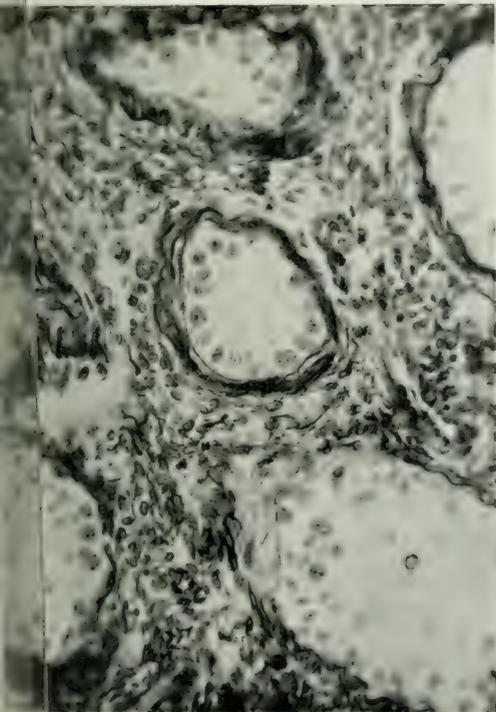


FIG. 13

× 285

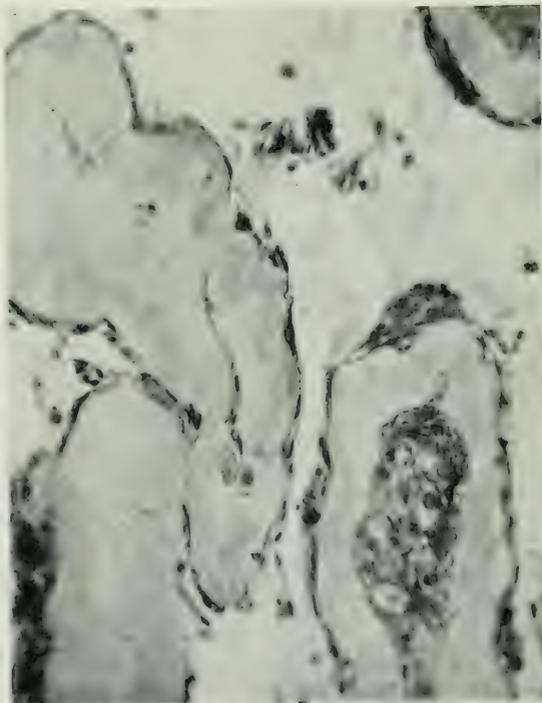


FIG. 14

× 285



FIG. 15 $\times 950$

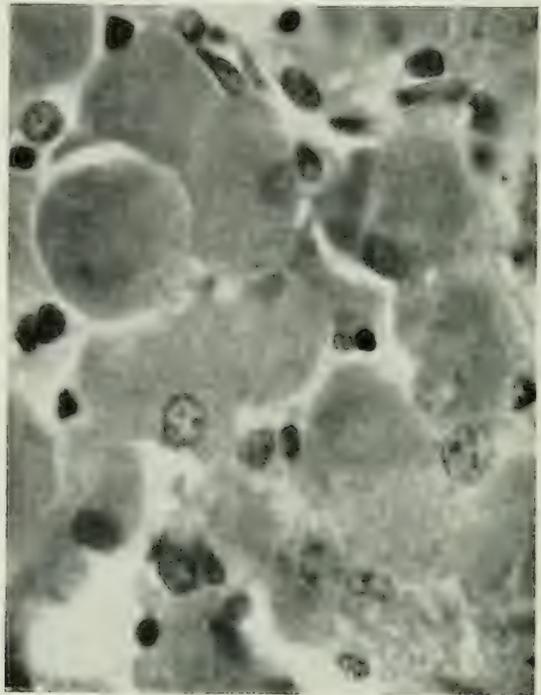


FIG. 16 $\times 950$



FIG. 17 $\times 60$



FIG. 18 $\times 300$

3765

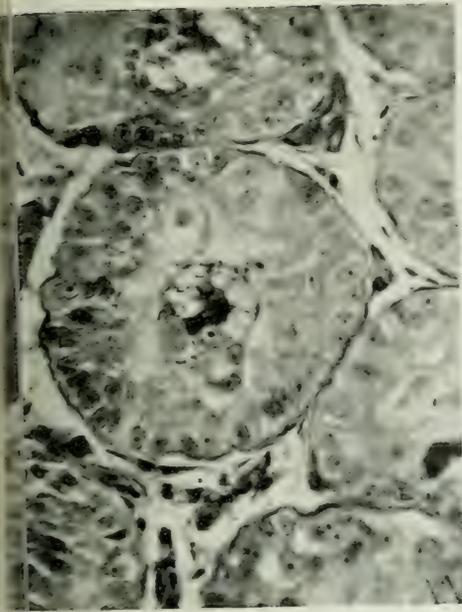


FIG. 19

× 350

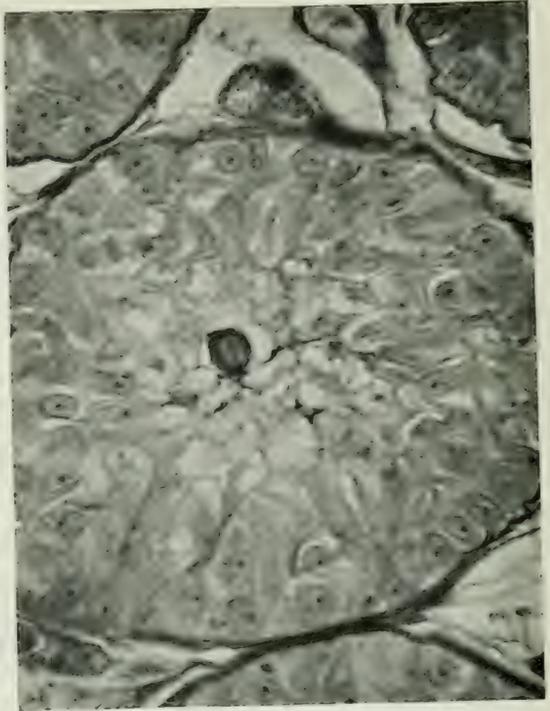


FIG. 20

600

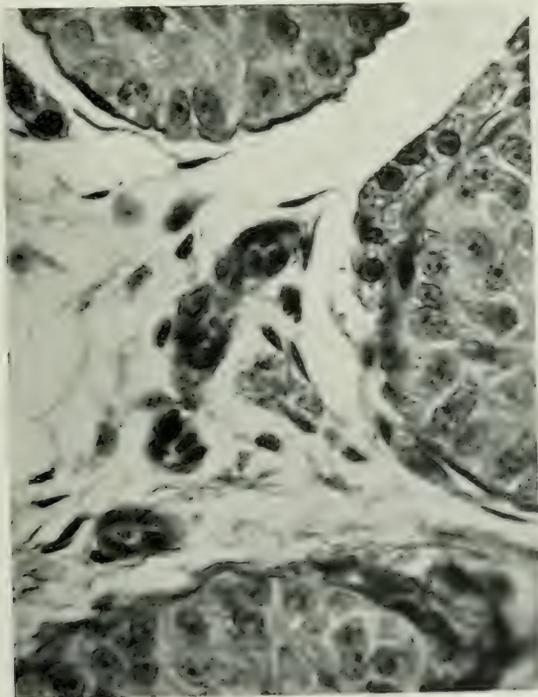


FIG. 21

× 600



FIG. 22

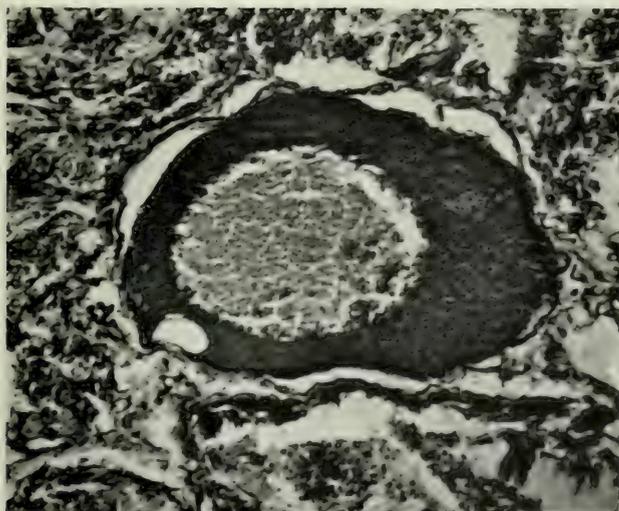


FIG. 23

Fig. 5.—Cat. Seminiferous tubule. $\times 470$. Stained Mallory. The centre of the lumen is filled by structureless protoplasm in which is seen a spherical body brilliantly stained with Orange G. The peripheral cells, though showing no mitosis, are comparatively normal.

Fig. 6.—Goat. Ovum-like body within a seminiferous tubule. $\times 470$. Stained Mallory. The peripheral parts stain blue indicating commencing calcification. The germinal epithelium is more degenerate than in fig. 7.

PLATE 19.

Fig. 7.—Goat. Seminiferous tubule containing two of the ovum-like bodies. $\times 320$. Each body is composed of concentric layers sharply demarcated and apparently homogeneous. The central area is paler and the investing cells are very degenerate. The cytoplasm of the germinal epithelium shows no cell-limits.

Fig. 8.—Goat. Seminiferous tubule. $\times 200$. The mass of cell detritus within the tubule is undergoing a colloid degeneration. Globules of colloid are seen at the top right-hand part of the mass.

Fig. 9.—Goat. Seminiferous tubules. $\times 125$. In the upper right-hand corner is a tubule containing an aggregation of cellular material showing a further stage of colloid degeneration than in fig. 8. A single ovoid body is also seen within a very degenerate tubule. In this there are no concentric layers, but the mass contains a number of highly refractile granules.

Fig. 10.—Goat. Seminiferous tubule. $\times 320$. The tubule is less degenerate than the preceding. Only the peripheral layer of epithelial cells is present. In the centre of the lumen is an aggregation of cells surrounding a circular body built up of two concentric rings and a darker central area. The investing cells show two distinct layers, an outer in which the nuclei are visible and an inner in which no cellular structure can be distinguished.

PLATE 20.

Fig. 11.—Rabbit. Seminiferous tubule. $\times 400$. The peripheral cells (spermatogonia) are still comparatively normal. Two examples of pluripolar mitosis are seen in the bottom right-hand corner of the lumen. The centre of the tubule is filled with a loose syncytium in which lie a few large clear spermatocytes and some degenerate nuclei.

Fig. 12.—Rabbit. Seminiferous tubule. $\times 770$. The centre of the tubule is occupied by a hypertrophied nucleus surrounded by a stellate mass of protoplasm. This probably represents the first stage in the formation of these intratubular bodies.

Fig. 13. Rabbit. Seminiferous tubule. $\times 285$. Showing the thick fibrous layer surrounding the tubules. This is apparently formed by the cells of the basement membrane in response to stimulation by the colloid, some of which probably percolates through the peripheral epithelial cells.

Fig. 14.—Human. Seminiferous tubules. $\times 285$. Shows the enormous

development of fibrous tissue which in the case of the left-hand tubule has entirely obliterated the lumen. The fibres in this case are very fine.

PLATE 21.

Fig. 15.—Rabbit. Intertubular tissue. $\times 750$. Shows a large number of interstitial cells, one of which is in mitosis. The nuclei are large, circular, and not very deeply staining.

Fig. 16.—Rabbit. Intertubular tissue. $\times 750$. Shows metamorphosed interstitial cells. The nuclei are of the same type as in fig. 15, but the cytoplasm is much increased in quantity and is very granular.

Fig. 17.—Rabbit. Epididymis. $\times 60$. Almost normal in structure but closely invested by adipose tissue.

Fig. 18.—Rabbit. Seminiferous tubule. $\times 380$. A single large body enveloped by degenerate epithelial cells is seen. The concentric structure is most marked. A single primary ring is present surrounding a light central area. Both the primary ring and the central area are marked by secondary rings.

PLATE 22.

Fig. 19.—Cat. Seminiferous tubules. $\times 350$. The epithelial cells are fairly normal, containing an oval nucleus with a well-marked nucleolus. The centre of the lumen is occupied by a dark coagulated mass containing degenerate nuclei.

Fig. 20.—Cat. Seminiferous tubule. $\times 600$. The tubule shows little sign of degeneration save absence of mitosis and the presence of a deeply stained ovoid body in the centre of the syncytial protoplasm. This probably represents an early stage in the formation of bodies such as those found in the goat and rabbit.

Fig. 21.—Cat. Intertubular tissue. $\times 600$. A few groups of interstitial cells are seen, one of which is applied to the basement membrane of the tubule on the right-hand side of the figure. The nuclei are darkly stained, circular, and contain a nucleolus. The cytoplasm is much vacuolated. Only a small quantity of intertubular tissue is present.

PLATE 23.

Fig. 22.—Frog. Complete section of the displaced gonad. In the peripheral tubules the structure is fairly normal but the tubules lying more centrally are shrunken and degenerate. Two ovum-like bodies are seen in the lower half of the section.

Fig. 23.—Frog. Ovum-like body within seminiferous tubule. $\times 320$. The body consists of a dark outer layer formed by the coalesced heads of the spermatozoa and a light granular central area composed of coagulated seminal fluid and liquefied sperm flagella.

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**Glossobalanus marginatus, a new
species of Enteropneusta from the North Sea.**

By

Alexander Meek.

With 14 Text-figures.

THE only species of Enteropneusta which have been recorded from the area of the British Isles are two species of the genus *Dolichoglossus*, one from the west coast of Ireland (Ballynakill Harbour—Tattersall, 1905), the other from the west coast of Scotland (Sound of Mull—Assheton, 1908). These presumably, like other species of the genus, have an embryonic development. The occasional capture of *Tornaria* off the coast of Ireland, in the Irish Sea, in the Channel, and at St. Andrews have indicated that *Balanoglossus* and its allies occur also in the area, but hitherto such have evaded capture. The specimen about to be described was captured off the Farne Islands and thus extends the range of the group to the east coast and the North Sea, and it has the further importance of belonging to a genus and a family the members of which more than probably pass through a larval phase. The specimen was obtained during a survey made off the coast of Northumberland by the Ministry of Agriculture and Fisheries at a depth of about 52 fathoms off the Longstone, the instrument in use being a small Petersen grab. The position of the boat at the time (9 a.m. on August 22, 1921) was $55^{\circ} 33' N.$, $1^{\circ} 7\frac{1}{2}' W.$ I have to thank the scientific staff for kindly submitting the specimen to me for description.

1. THE ADULT.

External Characters.—The specimen is the anterior end of a mature male and measured longitudinally, proboscis

7 mm., collar 5 mm., and the trunk, which was cut off at the end of the genital region, about 50 mm. At the posterior end the beginning of the region of the liver caeca was fortunately included. The specimen, as Text-fig. 1 shows, was bent at right angles in the post-branchial region.

The colour after preservation in spirits is brown, due as will be seen to dark pigment-cells lodged internally to the

TEXT-FIGS. 1, 2, AND 3.

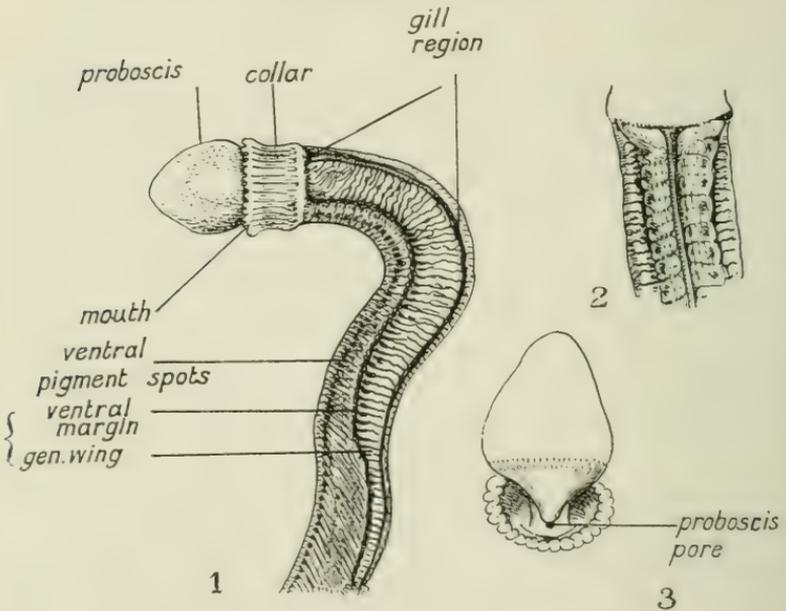


Fig. 1.—View from left side of anterior end.

Fig. 2.—Ventral view of the anterior end of the trunk to display folds of oesophageal region which is limited on either side by the ventral genital groove.

Fig. 3.—Dorsal view of proboscis showing proboscis pore.

longitudinal muscles of the body-wall. The proboscis is almost white and the gonads yellow. The brown colour marks the collar, on the trunk it differentiates the ventral body-wall from the genital wings. The yellow-coloured gonads stand out prominently on the wings, and the brown colour which intervenes between them and the body-wall

helps to define them. Ventrally on the trunk the brown pigment is concentrated to form a paired series of dark spots which lie one on each side of the ventral median line.

The proboscis is short and rounded, laterally compressed in front and expanded on each side near the collar. At this point it measures transversely 5 mm. It narrows again rapidly to form a neck of attachment to the collar, which provides a recess for its reception. The neck dorsally presents a single median proboscis pore. Behind the pore an opening on the anterior wall of the collar may be artificial, but it is in the position of the neuropore (Text-fig. 3). The proboscis was cut in two transversely before the specimen reached me. The interior is a wide cavity which, as has been said, communicates with the exterior by a median proboscis pore. Basally it presents a simple diverticulum or so-called notochord, and there is no vermiform process.

The collar is markedly muscular. It forms a folded edge anteriorly, and it is occupied around the middle of its length by a groove. It is rounded in section and the groove measures about 5 mm. in all diameters. The collar is also defined posteriorly by an edge, but passes after a slight depression into the trunk.

The trunk is resolved externally into the ventral body-wall and the genital wings, and these are separated by a lateral groove which, like the genital wing at first gradually and posteriorly more rapidly, is carried towards the dorsal aspect of the body (Text-fig. 1). The wings are occupied by the gonads in the form of irregularly folded but on the whole transverse ridges which project from the surface. They are large and in the specimen approximated over the dorsal region of the body so as to occlude the latter from the exterior.

The genital wing of the left side is larger transversely than that of the right, and this mark of asymmetry is emphasized by the sinuous path of longitudinal features of structure like the dorsal groove between the genital wings, the ventral nerve-cord, and the paired series of pigment spots. The

ventral line is carried to the left, then to the right, and again to the left. At the posterior end of the body, which is laterally flattened, the ventral angle is not that of the ventral nerve but to the right of that line (Text-fig. 4).

A still more noteworthy feature of the exterior is the presence of folds of the body-wall, the glandular folds of Spengel. In front they are irregular but mainly transverse (Text-fig. 2); behind the gill region they become markedly

TEXT-FIGS. 4 AND 5.

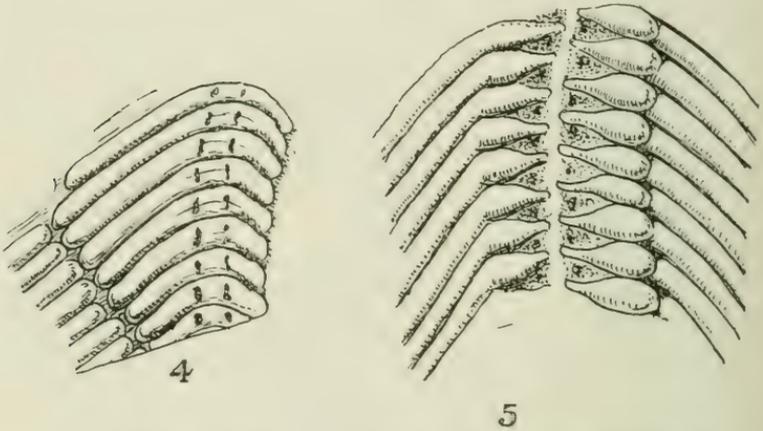


Fig. 4.—Internal surface of alimentary canal at the posterior cut end of the specimen—the region of the liver caeca—to show the disposition of the folds and the real median line to one side of the actual median line. The upper end is anterior.

Fig. 5.—External view of the body-wall at the posterior end of the genital region to show the condition of the folds on either side of the ventral nerve-cord.

transverse on that part of the body-wall below the genital wings, the area occupied increasing as that of the genital wings diminishes. In this posterior portion of the specimen (Text-fig. 5) they emerge from the mid-ventral line as narrow folds which expand into a club shape and narrow again, either ending abruptly or passing into oblique folds of the body-wall. If they end they do so in a series which defines a longitudinal groove, and similar but usually oblique grooves

are formed by the folds ending in succession along the same line. Posteriorly the lateral walls are occupied by grooves and folds which pass in this manner from the ventral aspect upwards and backwards. The condition ventrally of the folds is remarkably similar to that described by Spengel in the case of *Glossobalanus elongatus* from the Gulf of Naples. The only difference is that in the North Sea specimen the folds are fairly regular in size, whereas in the Naples form they were alternately large and small. Spengel pointed out with regard to them: 'Mir ist eine solche Anordnung, die sicher bei *Gl. sarnienseis* nicht vorkommt, bis jetzt von keiner Enteropneustenform bekannt.' It may be remarked that the Naples specimen is asymmetrical much as the North Sea one is asymmetrical.

The right series of gonads end slightly in front of the left and give place to the liver caeca in the form of diverticula of the gut and the body-wall on either side of the mid-dorsal groove which emerges from between the genital wings. At this posterior end of the specimen it is seen that the external folds are paralleled by folds of the wall of the alimentary canal (Text-fig. 4).

It is impossible from the strongly approximated condition of the genital wings to state the length of the branchial region, and it will be as well here to anticipate the further description and to complete our review of the external characters by saying that it is about 15 mm. This region is covered externally by the large genital wings so that the usual triangular space behind the collar is reduced to a slit and even basally next the collar the space is not very wide (Text-fig. 1). It is further to be observed, not merely by the presence of a groove which delimits the genital wings from the ventral body-wall of this front region of the trunk, that the branchial region is defined from a widely expanded oesophageal region.

These features, the distinct oesophageal chamber below the branchial region, the absence of a vermiform process to the diverticulum, the presence of liver caeca, the single median

proboscis pore, all lead to the impression that the specimen belongs to the family Ptychoderidae. This family includes the genera Ptychodera, Balanoglossus, and Glossobalanus, and it has already been seen that the specimen bears some undoubted claims to affinity with Glossobalanus.

Now there is no better region of the body which may be appealed to for the purpose of settling which genus is the right one than the post-branchial region. In Ptychodera the gills open by wide lateral slits, in the others by small pores. In Ptychodera the gonads open by many openings on the inner aspect of the genital wings, in Balanoglossus by a single series of pores near to the gill-pores, in Glossobalanus by a single series of pores on or near the margin of the genital wings.

I felt justified, therefore, in excising and cutting into sections a small part of this region. Unfortunately the specimen was indifferently preserved, and had to be subjected to several changes of alcohol. Good sections were therefore not to be expected and they were not obtained. The internal epithelium suffered most, as Spengel found in the case of *Glossobalanus elongatus*. But the general morphological features were quite well displayed, and in the case of the gonads in particular even histological detail.

Before proceeding to describe the more important features displayed by the sections it is obvious that the presence of synapticula shows that we were right with regard to family, and the opening of the gonads near the margin and the disposition of the lateral septum indicate that the genus to which the specimen belongs is *Glossobalanus*. The specimen agrees also with the majority of the species in possessing a comparatively short branchial region.

Post-branchial Region.—A portion of about 6 mm. of this region was removed and cut into transverse sections and Text-figs. 7-11 are from slides 1, 11, 19, 22, 23, and 24. Text-fig. 12 is taken from slide 16, and Text-fig. 13 from slide 4. The sections show that in spite of the large genital wings the specimen is structurally a *Glossobalanus*. The asym-

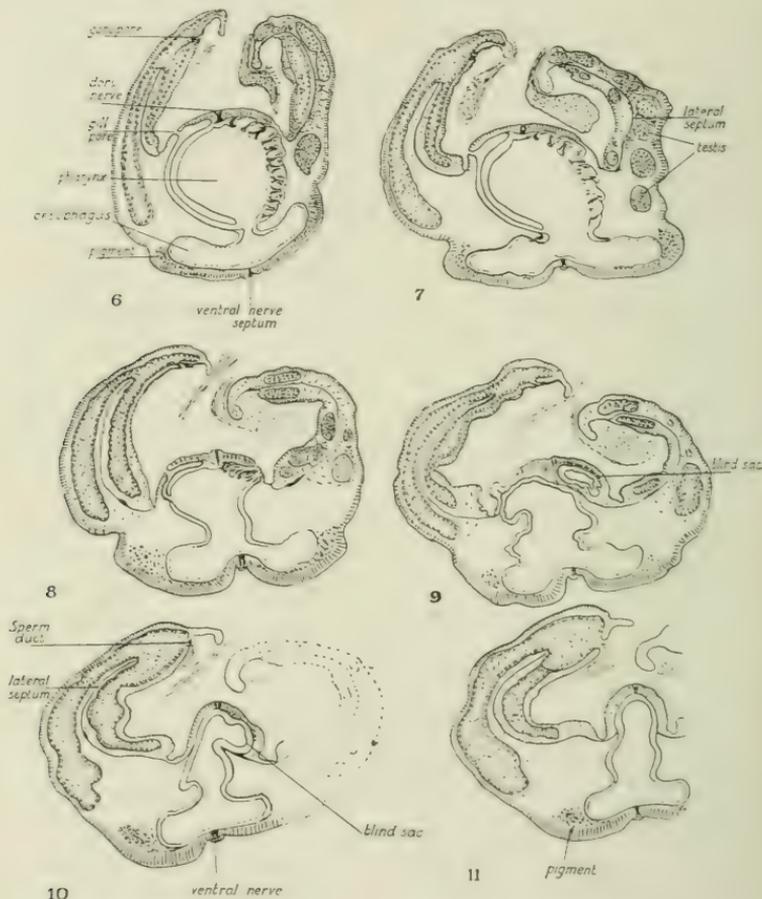
metry is apparent, and, as has been seen, it is not produced altogether by distortion.

The body is enclosed in a dorsal and ventral wall and on each side is expanded into the genital wings, and the body-wall at the origin of the wing on each side presents distinct grooves—dorsal and ventral genital grooves. The ventral groove has already been observed externally and extends, as has been noted, from the collar to the end of the genital region. The dorsal genital groove likewise extends along not merely the branchial region but the whole of the genital region. The alimentary canal is resolved into a large dorsal pharynx and a wide oesophagus. The walls of the pharynx are perforated by gills opening into gill-pockets which are provided with openings in succession along a line medially situated to the dorsal genital groove on the dorsal wall of the body. The gills have the typical enteropneustan structure. The gill septa or primary bars present ridges externally and are supported by skeletal plates, which are conjoined and forked internally and ventrally. Intervening between them are the tongues or secondary bars, which are likewise supported by a pair of plates, but these are not fused. They lie on either side of a narrow diverticulum of the body-cavity, thus not very far apart. They end below at the free end of the tongue. They are connected at intervals with the primary bars by synapticula and the slits are thereby converted into a fenestrated succession of openings in each case (Text-fig. 13). I have not been able to find out exactly the number of synapticula, but successive sections indicate the number to be about ten. Nor can I state definitely the number of gills, but measurements made from the sections and from the specimen show that the number is about forty to forty-five on each side.

The medial line is marked above and below by the dorsal and ventral mesenteries with the dorsal and ventral nerves and vessels. The circular layer of muscles is very thin. The longitudinal muscles reach their highest development on the ventral wall of the body. They are still well developed

along the outer wall of the genital wing, but they are small on the inner wall of the wing and rise again distinctly on the

TEXT-FIGS. 6-11.



Figs. 6-11.—Transverse sections of the post-branchial region. The left side is on the reader's left. The reconstructed relationship of the gonad and gill is shown on the left side, the section as cut on the right.

medial side of the gill-pores. As usual the space immediately at the side of the dorsal and ventral mesenteries is devoid of muscle, and along the line of the gonopores there

is certainly an interruption, along the line, that is to say, which Spengel defines as the sub-median line.

The genital wings are large and bend over the dorsal part of the body to arch over a spacious atrial cavity. The gonads occupy the wings and are resolved each into outer and inner lobes, both much folded, but they amalgamate near the summit of the wing to open by a pore not at the margin but near it on the inner face of the wing. The margin of the wing beyond the series of gonopores is a thin flap which is reflected or may be reflected over the openings and serves to occlude that part of the atrial cavity. The outer lobe of the gonad is continued into this margin so that a short third branch is present. In this respect the specimen indicates an approach to the condition of *Balanoglossus*, and is distinct from all the described species of *Glossobalanus*. An indication of the displacement of the gonopore on the inner side of the genital wing is shown in Spengel's drawing of *Glossobalanus minutus*.

In the anterior end of the branchial region it is evident that the gill-pores and the gonopores come into closer relationship, but with the expansion of the genital wings they are carried apart reaching the condition figured. An inspection of the specimen shows, moreover, that posteriorly, that is to say behind the branchial region, the gonopores still occupy a high position on the wings. Several authors have attempted to show that a relationship exists segmentally between the gonopores and the gill-pores, and in this specimen this appears to be the fact, for the succession of the gill-pores is accompanied by a succession of gonopores. In other words the region cut into sections bears the same number of each. There are about five gonopores to 2 mm. in the post-branchial region, and if the same distance between them is retained over the length of the genital region there ought to be some 120 pairs of gonads and gonopores.

The outer and inner lobes of the gonad are separated by a lateral septum which extends from the region of the line of the gonopores to the dorsal genital groove, and it is

displayed in all the sections figured. This is the disposition of the septum in the branchial region, but with the disappearance of the gills the septum is extended medially to be attached to the upper part of the post-branchial alimentary canal. It is obvious, therefore, that in the branchial region of the body the septum forms a cavity for the inner branch of the gonad, and that this cavity excludes this branch of the gonad from the space between the dorsal wall of the body and the branchial region of the alimentary canal; or, to put it another way, from the space between the dorsal

TEXT-FIGS. 12 AND 13.

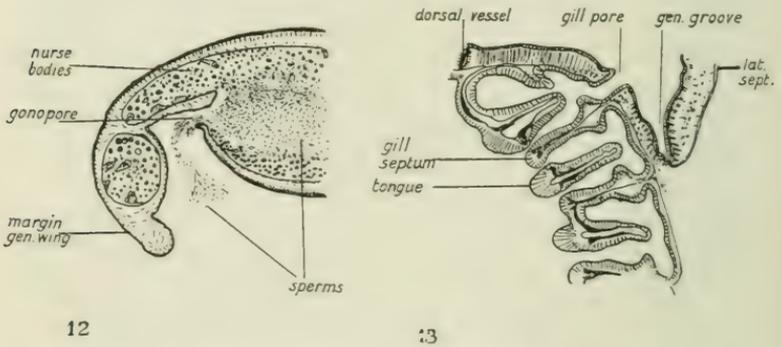


Fig. 12.—A more detailed view of a section of the margin of the genital wing through the gonopore.

Fig. 13.—Section through a gill-pore of the right side to show disposition, the gill skeleton in section with synapticula, and the insertion of the lateral septum to the genital groove.

genital groove and the dorsal mesentery. The gill-pores are, therefore, not merely medial to the gonopores, they are medial to the gonad. The outer lobe could certainly penetrate this region but it does not do so. It is only behind the branchial region that the displacement of the septum to the wall of the gut permits of the inner branch of the gonad invading this region of the body cavity. This is a feature of importance, for it is the disposition of the septum and the gonad in the genus *Glossobalanus*, and it has been discussed by Spengel in the monograph and in his paper on *Glossobalanus*.

balanus elongatus (1904). The septum is a double one and transmits the lateral vessels to the wing. It is also connected by strands to both the inner and outer walls of the wings and here also the pigment cells occur. The pigment cells are found also under the muscles of the dorsal wall of the body, between the oesophageal region of the alimentary canal and the muscles of the ventral body-wall, and on either side the pigment expands into conspicuous masses which rise and fall with the folds of the body-wall, as has been noted.

In the above respects the specimen is very like other species of *Glossobalanus*. There is a close resemblance also with reference to another feature, with at the same time a distinction of importance and morphological interest (Text-figs. 8-11). At the posterior end of the branchial region the gills become short and relegated to the dorsal side of the region. The shortening of the gills is accompanied by a gain of the narrow part of the alimentary canal intervening between the pharynx and the oesophagus. As soon as the gills end this upper part of the tube suddenly expands dorsally and projects as a short diverticulum over the posterior gills of the right side, and in this region the dorsal mesentery is lengthened on the inner side of the diverticulum. The other species of *Glossobalanus*, as Spengel has shown, present such a diverticulum, but in all evidently it is a median one, or nearly so, in front of which a similar lengthening of the dorsal mesentery has been remarked. The condition of the North Sea specimen is therefore like that of the others, but it differs in the out-growth being asymmetrical. It is scarcely necessary to say that the blind sac recalls in its position and its origin the hepatic diverticulum of *Amphioxus*.

The North Sea specimen is a male in the act of spawning. Most of the gonads in the region of the sections are empty or nearly so, and the sperms are collected in two masses which occupy the sides of the atrial cavity. The lateral masses are due not only to their issuing from the gonads of each side but to the branchial current which tends to separate

them. But they are enclosed in a mucous-like secretion probably derived from the ectodermal gland-cells of the atrial cavity. The secretion, however, is present in the neighbourhood of the pore and plays its part in directing the sperms laterally. The mass is tucked in within the space formed by the margin of the genital wing and spreads outwards as a thin sheet outside the marginal flap. The anterior gonads appear to discharge their contents before the posterior and the left before the right. In the sections the sperms may be easily seen issuing from the gonopores and joining the mass below, a mass containing countless numbers, each having the usually flagellate shape with a rounded head.

Besides the sperms the gonadial sacs are occupied by bodies which are highly refringent and eosinophile, and in the latter respect in striking contrast to the sperms. Like the sperms they arise in marginal cells and become detached. They vary greatly in size and appear to have a protoplasmic envelope, and the bodies, though usually apparently homogeneous, have sometimes an appearance as if they were made up of a mass of smaller bodies. They are not as far as can be seen nucleated. In the undischarged or partially discharged folds of the gonad they surround the mass of sperms which occupies the centre, but small groups of sperms are found amongst them peripherally. In the discharged parts of the gonads they press into the interior, but whether this is due to collapse of the tube or to actual multiplication could not be said. A few escape with the sperms and are seen in the atrial mass and there appear to lose gradually their eosinophile character. In the gonads also here and there are large cells of the margin which project into the cavity each provided with a large nucleus containing a large nucleolus. The refringent bodies have been observed in other specimens of *Enteropneusta*, and so far as can be said at present they may be regarded as nurse-bodies.

It is possible that the peculiar condition of the atrial cavity is due to the fact of the discharge of the sperms. In the male the apposition of the genital wings may be

necessary at that period to prevent the escape of the sperms while the creature occupies the burrow. But even so it must be acknowledged that the marginal outgrowth is a morphological feature, and this has been indicated in the specific name chosen for the species, which is further characterized by (1) the brown pigment cells and their concentration into a double series of ventral pigment spots: (2) the asymmetrical condition of the body, the blind sac, and the genital wings; (3) the large size of the genital wings: (4) the short third branch of the gonad. The depth at which the specimen was obtained, 52 fathoms or 95 metres, may also be a peculiarity.

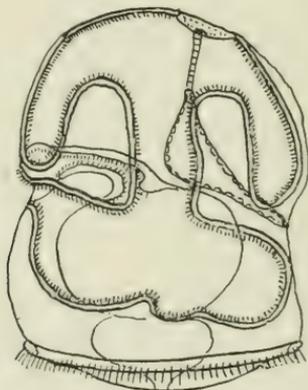
2. THE LARVA.

The adult is not common and probably occurs in isolated communities, one of which lies off the coast of Northumberland. An enteropneustan larva has been got rarely in the North Sea, and it is worth while inquiring whether it is likely related to *Glossobalanus*.

On August 6, 1890, a *Tornaria* was captured at the surface in St. Andrews Bay, and I made a drawing of it in the living condition in the Marine Laboratory there. This drawing I reproduce (Text-fig. 14). It will be seen to be very like a figure published by Bourne (1890) of a *Tornaria* captured over deep water in the Channel. His figure 13 and the one I now give are so similar as to lead to the opinion that they belong not merely to the same genus but to the same species. The anterior region is broad, the apical plate somewhat dorsal to the mid-longitudinal line and occupied by a pair of optic pits, the pre-oral and post-oral ciliated bands are simple not presenting lateral folds or processes, there is a slight characteristic bending of the transverse part of the post-oral band, the stomach and intestine are wide. A *Tornaria* of this type, and agreeing completely with the St. Andrews example, was captured on July 27, 1921, off the Longstone, during a plankton trip of the *Evadne*, in the mid-water net. Bourne regarded his specimen as a fully-developed larva. A similar

larva is got at Heligoland, and at St. Andrews Professor McIntosh says it is obtained fairly regularly in August and September. In the Channel another kind of larva is also procured, *Tornaria krohnii*. *Tornaria krohnii* is distinguished by a more conical shape anteriorly, by the longitudinal ciliated bands being thrown into folds, and by the stomach and intestine being narrow comparatively. This larva is common in the Mediterranean, and it is common inshore at Plymouth. Dr. Lebour was good enough to send

TEXT-FIG. 14.



Tornaria captured at St. Andrews, August 6, 1890, which it is suggested may be the larva of *Glossobalanus*.

me examples from the Plymouth plankton and they all are *Tornaria krohnii*, and Bourne had already stated that the inshore larvae were *Tornaria krohnii*. Since Heider (1909) obtained so fortunately early stages of *Balanoglossus clavigerus* which he found to agree with early stages of *Tornaria krohnii*, Stiasny (1913) has been able to follow the history in greater detail, and there appears to be little doubt that *Tornaria krohnii* is the larva of *Balanoglossus clavigerus*. This species is common on the south side of the Channel and on the north-west coast of France.

At the west end of the Channel it appears then that there

are two types of larvae, one of which is more than likely the larva of *Balanoglossus clavigerus*, and the other somewhat but not widely different. There are also found on the south side of the same region two nearly related genera, *Balanoglossus* and *Glossobalanus*, the latter with the species *G. minutus* and *G. sarnienseis*. It is possible that the second type of larva is connected with *Glossobalanus*, and if the evidence be extremely slender it gains a little in weight by the fact that the same type of larva and adult are present in the North Sea. Bourne's larva may thus be related to either *G. minutus* or to *G. sarnienseis*, or it may refer to a species which is not obtained inshore but at some depth and not yet discovered.

The North Sea type of larva is not unlike that described by Agassiz and by Morgan from the Atlantic coast of the North Atlantic, and a similar larva also occurs on the coast of California (Ritter and Davis, 1904).

SUMMARY.

The specimen described was captured off the Northumberland coast on August 22, 1921, and its discovery extends the range of the Enteropneusta to the North Sea and to the east coast of the British Isles. It also adds a new genus to the British list. It belongs to the family Ptychoderidae and to the genus *Glossobalanus*, but it presents features which indicate that it is a new species which has been called *Glossobalanus marginatus*. It has been suggested that it may be related to a larva which has also been found in the North Sea.

It is a pleasure to express grateful thanks to Sir S. F. Harmer and Mr. Kirkpatrick of the British Museum of Natural History—to the former for valuable guidance in literature, and to the latter for an opportunity of examining the museum's collections of Enteropneusta.

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Studies on Insect Spermatogenesis.

V. On the Formation of the Sperm in Lepidoptera.

By

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With Plates 24-26.

In a paper published several years ago by Gatenby (1917 *a*) an account of sperm formation in Lepidoptera was given, certain features of which departed rather widely from the results of previous workers on similar material. Particularly interesting from my own point of view were the descriptions of the origin of the acrosome and the history of the mitochondria ('macromitosome' or 'nebenkern') in the spermatid, these matters having proved especially difficult to elucidate in the Hemiptera upon which I had resumed work early in 1919. As this work progressed, it became increasingly evident that the facts in the Hemiptera (and in other forms which I have studied subsequently) did not agree with certain important features of Gatenby's account, and aroused the suspicion that perhaps the facts in the Lepidoptera might be open to a somewhat different interpretation. I decided accordingly to put up some lepidopteran material for purposes of comparison, and during the last three years this has been done whenever opportunity offered. Considerable difficulty was encountered, due to the impossibility of accurately determining the age of pupae from their external appearance, and a proper range of preparations was therefore not easy to obtain. Certain stages are in fact still incomplete, but the general features are now

sufficiently clear to permit comparisons with other insects and it did not seem worth while to pursue the matter further at present. This paper will deal, therefore, only with the mitochondria and Golgi apparatus ('acroblasts'), and with them particularly in the older spermatids.

I must confess that when I first saw Gatenby's figures they aroused considerable scepticism. However, having now examined the material for myself, I find that we are actually in close agreement as to general appearances (so far as Gatenby's account extends), but that my interpretation differs materially from his in regard to several important points.

MATERIAL AND METHODS.

For material I have made use particularly of moths belonging to the family Saturniidae, the cocoons of which were readily collected during the winter and early spring in various localities adjacent to New York City. Of these moths this paper deals almost exclusively with *Callosamia promethea*. The cells in this form are rather small, but this disadvantage is offset to some extent by the large size of the testes. *Callosamia* (and other saturnids) has been studied by Cook (1910), to whose account reference may be made for the structural features of the testes, &c.¹ For purposes of comparison a study was also made of *Pygaera bucephala*, the form upon which the original work of Platner (1889) and the later classical studies of Meves (1900 and 1903) were based. Material was obtained from Mr. L. W. Newman of Bexley, Kent, *Pygaera* not being native to the United States.

Various methods of fixation were tried, but I found, in agreement with Gatenby, that the best results were obtained with Champy or Flemming without acetic acid (diluted in both cases with water). Gatenby is undoubtedly correct in his insistence on the necessity of eliminating acetic acid in the case of lepidopteran material, for even in its absence the mitochondria in the spermatocytes are very difficult to fix

¹ See also Dederer (1907) on the closely related form, *Philosamia cynthia*.

properly. The F.w.a. mixture gave the best general results and was particularly valuable for studying the Golgi bodies and acrosome ; while for the mitochondria in spermatocyte and early spermatid stages only Champy was satisfactory. Fe-haematoxylin, sometimes with light green as a counter-stain, was employed exclusively for staining.

THE PHENOMENON OF POLYMEGALY IN LEPIDOPTERA.

In his first paper on *Pygaera*, Meves (1900) called attention to the fact that the spermatocytes are of two sizes, the larger of which produces normal spermatids and sperms, while the smaller undergoes abnormal maturation divisions and produces abnormal (apyrene) sperms. The problem stated in this simple form by Meves has been complicated by the account of Munson (1906), who finds in *Papilio* two sizes of spermatocytes and spermatids both of which develop in a perfectly normal way. He believes that the small generation is to be considered 'normal' in size, since the large generation only makes its appearance late in the life of the butterfly. Munson unfortunately mixed up normal and abnormal stages in his account of sperm formation, and his statements are accordingly difficult to evaluate. Without at first recalling either of these cases, I noted independently that the spermatids in *Callosamia* are of at least two (possibly more) well-marked sizes. Of these, the larger generation certainly gives rise to normal sperms. On the other hand, the smaller spermatids apparently give rise as a rule to abnormal sperms, but nevertheless they are often found in an advanced stage of normal sperm formation, and considered separately would certainly not be thought abnormal. However, Gatenby (1917*b*) states that the abnormal condition which results in the formation of apyrene sperms may exert its influence at different times, and it seems probable, therefore, that these small spermatids in *Callosamia* would undergo degeneration at a later stage ; and cysts of small spermatids in later stages have in fact been found in course of changes possibly degenerative in nature. This being the case, my results would coincide

in a general way with those of Meves. The chromosome numbers in the large and small generations seem not to have been examined carefully, but if they are the same, as seems probable, the Lepidoptera might be considered as another example of the 'polymegaly' which Montgomery (1910) first described fully in Hemiptera.

I have recently (Bowen, 1922*c*) given a full account of this particular type of spermiatic polymorphism in the family Pentatomidae (Hemiptera), to which reference may be made for the details of this phenomenon. It may be pointed out, however, that in one important respect the conditions in the Hemiptera and Lepidoptera differ markedly; for while in the former all the cells, regardless of size, give rise to normally formed sperms, in the latter the small generation seems to give rise only to abnormal sperms, although the small spermatids may first proceed for some time on an apparently normal course, as noted above. Furthermore, in the Hemiptera all the spermatocytes and spermatids in a given testicular lobe are involved, while in the Lepidoptera only part of the cysts in each lobe are affected and these apparently without any noticeable plan. It may also be noted that in the Lepidoptera the appearance of the polymorphic cells seems to be an accompaniment of testicular old age, while such a relation is entirely lacking in the Hemiptera.

NOTES ON THE SPERMATOCYTES AND THE SPERMATOCYTE DIVISIONS.

The notes in this section deal only with *Callosamia*.

In *Callosamia* the mitochondria are present in spermatocytes of the growth period in the form of very numerous vesicular spheres or ovoids (fig. 42), approaching most nearly in general appearance those figured by Gatenby (1917*a*) in *Smerinthus populi* and *Pieris brassicae*. They tend to be accumulated particularly in one region of the cytoplasm, and in my preparations are usually so closely packed that they give the impression of a mass of soap bubbles. I wish

especially to corroborate Gatenby's statement¹ of the duplex structure of these mitochondrial spheres, since upon this point depends a proper understanding of the formation of the nebenkern. Each sphere consists primarily of a droplet of some substance which has little or no affinity for the usual stains, and to which the name of chromophobic material has been appropriately applied. This material is enclosed in a delicate envelope of some substance which takes haematoxylin rather sharply, and is accordingly termed the chromophilic substance. I wish to call special attention to the fact that in a surface view of one of these mitochondrial vesicles this chromophilic layer is so delicate that as a rule it does not appreciably affect the transparency of the vesicle as a whole. Only around the periphery of the sphere, where the thickness of chromophilic material is sensibly increased by the effects of curvature, does it become clearly visible. In other words, a single (or double) thickness of chromophilic material would not be noticeable in a properly differentiated preparation. This point should be clearly understood, since upon the optical principle involved depends a proper interpretation of the 'spireme' in the nebenkern.

In the maturation divisions the mitochondrial vesicles seem rarely to retain their spherical shape, but, as Gatenby has also noted, are usually more or less drawn out in a direction parallel to the long axis of the spindle. This is sometimes so pronounced that in a cross-section through the region of the spindle poles the vesicles, closely packed and decidedly elongate, are seen to radiate outward from the neighbourhood of the centrioles, reminding one very strongly of the conditions which I have described in the Hemiptera (Bowen, 1920). Something of this same appearance is shown by Gatenby (1917*a*) in his fig. 48, which is especially interesting because the nucleus is still in the prophase, with its membrane intact. As the groups of daughter chromosomes separate during the anaphase, the mitochondrial vesicles become drawn out along the spindle,

¹ This structure of the mitochondrial vesicles was first correctly described by Meves (1900).

and are finally separated into two equal masses by the constriction of the cell wall. The vesicles then draw away from the region of the mid-body and, gradually rounding up, regain their former shape. This is shown particularly in the second maturation division, at the close of which the nebenkern is constructed (fig. 43).

The 'acroblasts' of Gatenby are obviously the representatives of the Golgi apparatus, as he has also indicated in a later publication. I wish only to call attention to the intimate structure of the individual Golgi bodies, since on this point I am not entirely in agreement with Gatenby's account. According to my observations each Golgi body is made up on a plan essentially similar to that which I have described in Hemiptera (Bowen, 1920), except that they are somewhat smaller, and hence less easy to analyse. Each Golgi rodlet is accompanied by a small, plate-like mass of material, which stains relatively less than the Golgi substance itself, and which, as I have shown elsewhere, is to be looked upon as a portion of the fragmented idiosome. The vesicular portion which Gatenby sometimes finds is obviously the equivalent of this idiosomic portion of each Golgi body; but I have never happened to see in my preparations any case in which it presented such a vesicular appearance.

In the maturation divisions the collection of the Golgi material around the spindle poles has been correctly described by Gatenby, but I cannot at present corroborate his statement that the 'acroblasts' are sorted out entire. The possibility of their undergoing more or less fragmentation, such as seems to occur so extensively in the Hemiptera (Bowen, 1920), ought, I think, to receive much more thorough study before a final decision is reached. In any event we seem to be agreed that the Golgi bodies are ultimately present in the spermatids in substantially the same form as in the spermatocytes.

Concerning the centrioles I have nothing new to contribute, except that Cook's (1910) statement concerning the loss of the tail filament in the first spermatocyte division of *Callisamia* is apparently incorrect, and was based in all probability on faulty technique.

THE FORMATION OF THE SPERM.

The Structure and Fate of the Mitochondrial Body or Nebenkern.

The condensation of the mitochondrial vesicles to form the nebenkern (macromitosome of Gatenby) in *Callosamia* follows immediately upon the completion of the second spermatocyte division (fig. 43). For a detailed study of the method of this condensation the Lepidoptera offer the best material which has yet been found, the individual chondriosomes being so large and their constituent parts so clearly differentiated that the progress of events is not obscured by the stain, as in the case of the Hemiptera (cf. Bowen, 1922*b*). Gatenby (1917*a*) has given a series of figures showing the various steps in the process, and up to the stage shown in his figs. 15 and 38 the appearances in *Callosamia* are so nearly identical that additional figures seem unnecessary. According to this worker the process of condensation consists of a flowing together of the mitochondrial bodies, 'forming at first elongated structures, then loops, and finally filaments, the latter joining up gradually to form a tangled anastomosing figure', and finally, 'a perfectly coiled spireme'. With this interpretation of the process of condensation I am inclined to disagree, and would like to suggest an alternative explanation which, I believe, is also more in harmony with the later condition of the nebenkern.

I agree with Gatenby that the essential feature in the condensation phenomena is the flowing together or fusion of the mitochondrial vesicles. But, as I interpret it, this results not in forming loops or threads but merely larger aggregates of chromophobic material, the chromophilic material running together to form more or less complete partitions between the chromophobic droplets. Simultaneously the chromophilic material is withdrawn from the periphery of the mass as a whole, so that finally a spheroid of chromophobic material remains, subdivided in an irregular manner by chromophilic partitions. One might, indeed, liken the whole nebenkern to

a mass of soap bubbles. Although Gatenby's figures do not show the point satisfactorily, I have found that in *Callosamia* the surface of the nebenkern is often deeply indented at the points where the chromophilic partitions reach its periphery, emphasizing the impression of a vesicular mass. Doncaster and Cannon (1920) also show this very clearly in the nebenkern of the louse (see their figs. 18 and 19).

In contrasting these two interpretations it must be frankly admitted that the appearance of a thread-work is exceedingly deceptive. Indeed, it is possible that neither view can be conclusively proved without taking into consideration the later stages, in which the facts are very clear. Nevertheless two points against the thread-work interpretation may be urged. In the first place, in none of Gatenby's figures or in my own preparation is there any indication of the cut ends of a thread such as are obvious in sections of the chromatin spireme of dividing nuclei. It is exceedingly difficult to understand how such loose ends could be constantly avoided in sections. If, however, the structure of the chromophilic substance is that of a plate-work, the absence of ends is easily explicable. A second objection is based on the optical arrangements upon which the demonstration of the chromophilic substance in the spermatocyte chondriosomes was shown to depend (see preceding section). It will be clear from a consideration of these conditions that the visibility of the chromophilic material in the nebenkern may well depend on its disposition between closely adjacent chromophobic masses, the chromophilic septa being visible when seen on surfaces of sharp curvature, but invisible when seen in plane view, just as in the case of the individual chondriosome vesicles in the spermatocytes. Indeed, every feature of the condensation process becomes readily explicable if we think of it merely as a reduction in the number and arrangement of droplets of chromophobic material by the concentration of their chromophilic envelopes into more extensive separating membranes. Such a conception also helps us very much to understand the nature of the same process in other insects—the Hemiptera.

for example—in which the ultimate structure is clearly a combination of plate-work and vesicles, the intermediate steps being obscured by the less favourable structural features of the chondriosomes.

The next step in the condensation of the nebenkern is the withdrawal of the chromophilic substance from all contact with the periphery of the chromophobic mass as a whole, resulting in the formation of a clear zone enclosing the now centrally located chromophilic substance. This condition was described by Platner (1889), and will be recognized as a constant feature of the nebenkern in insects of all kinds. In accordance with the view elsewhere developed (Bowen, 1922*b*), that all this early activity in the nebenkern is merely indicative of a centripetal condensation of the chromophilic material, I would interpret the complete withdrawal of the chromophilic material from the outer boundary of the nebenkern as merely the last step in the progressive withdrawal of this material, first from the outer periphery of the chromophobic mass as a whole, and then from the connecting pathways which at first traverse the outer chromophobic zone.

The stages in this process of withdrawal have been omitted by Gatenby so far as I can judge from his figures, and in my own preparations I have been unable to get completely satisfactory illustrative material. The process is undoubtedly difficult of analysis because it is during this interval that the rearrangements are completed which lead to the final organization of the chromophilic material into a more regular plate-work. A frequent appearance of this stage has been figured by Meves (1900, fig. 67) in *Pygaera*, and is shown still more clearly by Doncaster and Cannon (1920, figs. 20 and 21) in the louse. The central area of the nebenkern tends to stain more or less completely (as a result of slight imperfections in technique), concealing the detailed arrangements of the chromophilic material, while from this central accumulation delicate connexions pass out to the periphery of the nebenkern. These connexions are gradually withdrawn and the disposition of the chromophilic material now becomes progressively clearer.

In the larger spermatids of *Callosamia*, the general appearance is still exceedingly complex, perhaps justifying the representation which Gatenby gives in his figs. 40 and 42. for example. But in the smaller spermatids, where the chromophilic material is much less extensive, the condensation early reaches a point where arrangements are sufficiently simple for a practically complete analysis. Such a small spermatid of *Callosamia* is shown in fig. 44. The chromophilic material occupies the central area of the nebenkern, enclosed in a cortical zone of chromophobic substance, and arranged in the familiar 'onion' pattern which has been repeatedly figured by many workers on insect sperm formation. (Compare my figures from the Hemiptera (Bowen, 1922*b*).)

The further condensation of the chromophilic material now goes on rapidly, in a manner very similar to that which I have described in Hemiptera. In the Lepidoptera, however, the nebenkern begins to elongate soon after the cortical chromophobic area is established, this area remaining, in the immediately subsequent stages, as a characteristic feature of the nebenkern structure. With the elongation of the nebenkern the condensation of the chromophilic material has soon progressed to a point where the details of its arrangement become sufficiently simplified for satisfactory analysis. I have studied these later stages in both *Callosamia* and *Pygaera*, the latter being particularly good on account of the large size of the spermatids. An early stage in the elongation of the nebenkern of *Pygaera* is shown in fig. 1, and in fig. 3 a cross-section through a nebenkern of the same stage. Figs. 4 and 5 are similar views at a slightly later stage in the elongation, and fig. 6 is a cross-section of the nebenkern in a still older spermatid. In fig. 45 is shown a cross-section of a nebenkern in *Callosamia* when its elongation is well begun, and fig. 46 is a total view of a spermatid at a stage intermediate between that of figs. 4 and 8. Figs. 8, 10, 14, and 13 are progressively later steps in the condensation of the chromophilic substance.

A comparative study of this series of longitudinal and cross-

sections will, I think, make clear the nature of the processes at work, and their extraordinary similarity to the conditions which I have described fully in the hemipteran nebenkern. In the first place, it is abundantly clear that a 'spireme' is in these stages an impossible interpretation. There is no conceivable arrangement of a thread in the nebenkern which will produce a regular bounding line in both long and cross-sections of the chromophilic material. Such an appearance can only be produced by a continuous surface, which, in accordance with the optical principles previously referred to, would, if of proper thickness, produce the effect of a simple line or thread when seen in optical section. In other words the chromophilic substance is arranged in a plate-work, exactly as it is in the Hemiptera. This is further proved by the fact that the chromophilic material now stains with sufficient intensity to be visible in surface views (fig. 4, for example), a result impossible with an open thread formation. The cross-sections particularly show that this plate-work is arranged as a series of concentric shells in which, however, more or less extensive irregularities occur. The longitudinal sections are not so satisfactory, since the section is rarely exactly parallel to the long axis of the nebenkern, and this, coupled with the irregularities in the plate-work and the difficulty of differentiating the various layers with equal clearness, makes the picture particularly confusing in the earlier phases of elongation.

This series of figures shows further that the chromophilic substance is constantly diminishing in volume, with an increasing simplification of its structural arrangements. Indeed, in the later stages of condensation the cross-sections are especially simple (figs. 47 A and 9 A and B), and exhibit in every particular an exact parallelism with the same condensation steps in Hemiptera. The final result of this process of condensation is the complete disappearance of the chromophilic material, the last stages in this process being shown in figs. 8, 14, and 13, the last two being from the same cyst. The ultimate fate of the chromophilic substance, and, indeed, all of the stages which directly precede its complete disappearance are thus exactly

comparable to those which I have described in *Brochymena* (Bowen, 1922*b*). These later stages seem for the most part to have been overlooked by Gatenby—at all events his account of the fate of the chromophilic substance ('spireme') seems to be entirely incorrect. The source of his statement as to the breaking up of the 'spireme', as shown in his Text-fig. 3, will be considered in a later paragraph.

It will be convenient at this point to refer briefly to the parallel course of events in the chromophobic material. As the nebenkern draws out along the axial filament of the tail, both chromophilic and chromophobic substances are at first involved (fig. 1). Very soon, however, the chromophilic substance ceases to elongate (figs. 4 and 46), and begins gradually to shorten up as its dissolution advances. The chromophobic material, on the other hand, continues to elongate very rapidly (fig. 8), and tends gradually to become spun out towards both ends with a median swelling in the region occupied by the remains of the chromophilic plate-work (fig. 46). The proximal end of the nebenkern (not to be made out in fig. 46) seems to be anchored in the vicinity of the insertion of the tail filament, as is the case in other insect sperms. The continued spinning out of the nebenkern results in the production of a mitochondrial sheath for the tail filament, exactly as in the Hemiptera.

It has long been known that the nebenkern becomes divided into two equal masses in many insect spermatids (Hemiptera and Orthoptera), prior to the spinning-out process, while in Lepidoptera, according to the current descriptions, this division is entirely omitted. In my study of the nebenkern in Hemiptera (Bowen, 1922*b*), I noted for the first time the relation between the final disappearance of the chromophilic matter and the complete division of the nebenkern into two equal parts. This relation was found to hold true in the Orthoptera and Coleoptera also (Bowen, 1922*d*), and I ventured the guess that in the Lepidoptera, 'a division of the nebenkern will be found to occur once the chromophilic substance has been disposed of' (Bowen, 1922*b*, p. 69). This point has been

very carefully examined, especially in serial cross-sections of the nebenkern, and it is now clear that this guess was correct in every particular. As in the Hemiptera the division of the nebenkern is foreshadowed by the symmetrical disposition of the chromophilic material (figs. 3, 5, and 45), and the division itself is accomplished in the regions unoccupied by chromophilic material soon after elongation begins. In the region of the chromophilic material itself, however, the division is not (usually) completed until after the final act of dissolution, a point in which the Lepidoptera agree with the Coleoptera in which the splitting of the nebenkern is delayed in a somewhat similar manner. In the Lepidoptera, however, there seem often to be more or less local irregularities in the division process, and it thus happens not infrequently that the final remnant of the chromophilic material is left to complete its dissolution in one of the nebenkern halves, while the division plane is completed (fig. 9 B). The general features of the division process as outlined above are well shown in figs. 9, 12, and 47. In fig. 9, which represents a nebenkern at the stage of fig. 8, the division above (and below) the chromophilic substance is completed (fig. 9 c), but in the region of the plate-work it is still incomplete (fig. 9 A), with the exception of cases like fig. 9 B already noted. In fig. 47 cross-sections of a nebenkern like fig. 46 are shown. The more spun-out portions at the ends of the nebenkern masses are shown in fig. 47 c, while the parts nearer the middle are shown in fig. 47 B, and the region of the plate-work itself in fig. 47 A. Comparison of figs. 3, 5, and 6, with figs. 9 A and B, and fig. 45 with fig. 47 A, shows clearly how the structure of the plate-work becomes progressively simplified as the chromophilic material condenses. Just before its final dissolution the plate-work is reduced to a simple ovoid shell (figs. 9 A and 14), such as I have described in Hemiptera and Coleoptera. (Compare also the figures of Doncaster and Cannon (1920) in the louse.) In these late condensation stages the plate-work, as against the thread-like structure of the chromophilic material, seems to me unquestionable. As the figures show, the axial filament lies in the

groove between the two halves of the divided nebenkern, just as in other insects.

After the division of the nebenkern (probably) the chromophobic material begins to develop constrictions, at first in the more distal region of the sheath, which divide it into a series of bead-like masses. This process is shown particularly well in figs. 7 and 10, although the development of these bleb-like swellings is often (usually ?) deferred until after the disappearance of the chromophilic material. The last-mentioned figure is from a cyst of abnormally large sperms, and this may account for the unusually early development of the swellings. These bead-like masses are rapidly separated from each other by the spinning out of the intervening chromophobic material. As a rule these delicate connecting strands are not well seen, and the tails look like a series of clear vesicles often without any apparent connectives (fig. 11). This is particularly true in the later stages of sperm formation, when the vesicles seem merely to be scattered loose along the tail filament (figs. 17, 25, 35, 37, 40, and 52 for instance). However, in material fixed in Flemming without acetic acid and strongly stained in Fe-haematoxylin the chromophobic material can sometimes be coloured very sharply, and it is possible in favourable cysts to make out the actual structure of the nebenkern derivatives with the greatest clearness. From such preparations it is evident that the original halves of the nebenkern have become spun out into delicate threads which run parallel to the tail filament and at intervals bear the bleb-like swellings, now present in larger number but individually much reduced in size. The general appearance is exactly like that in *Euschistus* (see Bowen, 1922*b*, fig. 27). It is clear that these swellings are homologous with the 'tail vesicles', the formation of which was described fully in the Hemiptera (Bowen, 1922*b*).

Finally, the central substance which I have described in detail in the Hemiptera (Bowen, 1922*b*), and less completely in Orthoptera (Bowen, 1922*d*), remains to be considered. As in the Hemiptera this material first becomes visible in the chromophobic area of the nebenkern during the middle

stages in the dissolution of the chromophilic substance (figs. 4, 5, and 6). It does not stain very sharply with Fe-haematoxylin and its exact morphology is difficult to make out. It seems, however, to consist of small droplets which tend to run together to form more or less irregular threads traversing the chromophobic material in a direction parallel to the long axis of the nebenkern (fig. 7, 8, and 13). As the chromophilic material disappears, the central substance becomes more conspicuous (fig. 10), and in cross-sections of the nebenkern appears exactly as it does in the Hemiptera (figs. 9 c and 12 b). In fig. 47 b it has become condensed into a single strand in each half of the nebenkern. (Compare with Holmgren's (1902) account in *Silpha*, fig. 9 m.) As in *Ceuthophilus* (Bowen, 1922 d), the central substance is present in the tail vesicles in much the same form in which it appears in the unconstricted nebenkern (figs. 10, 11, and 48). A more detailed account of the central substance may be omitted here, since I have discussed the subject in another paper (Bowen, 1922 b), to which the reader is referred for comparative details, especially in the Hemiptera.

It will be observed that Gatenby has failed to recognize the central substance in the lepidopteran nebenkern. In studying his figures I have come to the conclusion that the thread-like formations shown in the nebenkern in his figs. 47 and 20 are to be interpreted as central substance. He describes these threads as resulting from the breakdown of the 'spireme', a conclusion which he seems to have reached on the basis of the supposed structure of the early nebenkern, without having traced out the necessary connecting links between the two. My observations leave no doubt that the chromophilic and central substances are morphologically distinct, and that whatever the structure of the chromophilic material may be, that of the central substance is in no way dependent upon it.

So far as my observations go they indicate that the threads, spun out from the halves of the nebenkern, ultimately form a sheath for the tail filament of the mature sperm, as described in other insects by various workers. The fate of the tail vesicles is not known.

NOTES ON THE CENTRIOLES AND THE TRANSFORMATIONS
OF THE SPERMATID NUCLEUS.

I have found the centrioles in the lepidopteran spermatid exceedingly difficult to demonstrate with any degree of satisfaction. I am, therefore, unable either to confirm or deny the extraordinary account given by Gatenby. His statement that one of the centrioles is cast off ought certainly to receive the most careful examination. It is usually an easy matter in the insect spermatid to demonstrate the centrioles in some form or other at the point of insertion of the tail filament, but in the moths which I have studied even this has usually proved impossible. Figs. 28-30 show at the end of the axial filament a small granule, which is presumably the centriole(s), and Gatenby's fig. 51 seems to show something similar. I wish only to point out here that in the Lepidoptera, as noted by many workers, the head of the spermatid is bent very sharply at the point of insertion of the axial filament, so that the original insertion seems to be near the anterior side of the nucleus rather than at its base, as is customary. Subsequent stages indicate that this may actually be the case, the centriole perhaps shifting its position to the base of the nucleus when the latter elongates to form the sperm head.

The breaking up of the chromosomes at the close of the second maturation division offers no points of special interest. The chromatic material becomes eventually spread out in a thin and slightly uneven layer on the inner wall of the nucleus (figs. 1, 4, 8, and 13), somewhat as in the Hemiptera (Bowen, 1922 *a*). During the later stages in the spinning out of the nebenkern halves, a rearrangement of the chromatic material is accomplished. This results in the appearances shown in figs. 15 and 16, in which one gets the impression that a portion of the nucleus is being cleared up by the withdrawal of the chromatin. This seems actually to be the nature of the process, for subsequently the nucleus appears divided rather sharply into two areas, one of which is perfectly clear and transparent, while the other retains the chromatic material probably still

in the form of a thin layer on the nuclear wall (figs. 18 to 20). The exact appearance depends, of course, on the orientation of the nucleus with respect to the observer. This rearrangement of the chromatin is again reminiscent of the hemipteran spermatid (Bowen, 1922*a*), with the difference that in the Lepidoptera the clear area seems to be opposite the insertion of the tail filament, rather than around it, as in Hemiptera. This arrangement of the chromatin is very clear in *Pygæra*, but made out with great difficulty, if at all, in my preparations of *Callosamia*. Only in rare cases did the chromatin stain with characteristic intensity in any of my preparations, the fixation in Flemming without acetic acid being apparently responsible for this. I have noted the same result in the testes of other animals. It is an exceedingly fortunate failure, for it allows of many observations which could not possibly be made if the sperm head were intensely coloured. Occasionally, especially in later stages, some of the heads in a cyst will stain intensely (compare figs. 40, 41, and 60), a result which makes easy the determination of the exact limits of the head itself.

The division of the head into the staining and non-staining areas noted above, seems to have been made out by Platner (1889), but his figures do not give a very adequate idea of the actual conditions. During the early stages in the elongation of the acrosome, the clear area gradually disappears (figs. 21, 22, and 26), and the head then stains uniformly (figs. 28, 29, &c.). During these latter changes the head seems to undergo a diminution in size, a phenomenon which is met with not uncommonly (always?) in insect sperm formation. The nucleus, at first spherical, gradually elongates (figs. 35 to 41 and 57 to 60), as in other insect spermatids, and eventually becomes a long, delicate rod, not unlike the sperm head in Hemiptera (Bowen, 1922*a*).

Aside from the differentiations already noted in the spermatid nucleus, I have also constantly found within it a small darkly-stained body, of spherical shape, which is perhaps of nucleolar origin, possibly related to the intra-nuclear body which I have described in the hemipteran spermatid (Bowen, 1922*a*). In

Callosamia (where it was observed by Cook (1910)) this body seems to appear very early (fig. 44), but in *Pygaera* it becomes conspicuous only in later stages. In the latter it can be recognized as a minute granule at the time when the elongation of the nebenkern is well started (figs. 4, 8, and 13), and subsequently (figs. 15 to 18) it becomes larger and much more prominent. In *Callosamia* it may divide into two parts (often unequal) at a fairly early period (fig. 46). In *Pygaera* the division is delayed until the clear area (in which it tends to be located (figs. 20 and 22)) in the head is differentiated, and when it does occur, it tends to take place in all the heads of a cyst (figs. 19 and 20). In later stages this body seems to become less conspicuous (figs. 31 and 35), and, I believe, eventually disappears entirely, being presumably dissolved in the nuclear sap. Not infrequently this body is in line with the tail filament, and it might easily be mistaken for a centriole (figs. 52 and 57). As far as I can make out, however, it has no real connexion with any extra-nuclear structure.

Finally, I would like to mention in passing a phenomenon which seems to have been overlooked by previous workers on Lepidoptera, and which I myself do not fully understand. An examination of cysts of sperms in later stages of transformation (figs. 36 and 58, for example) shows the elongated aerosomes to be embedded in a mass of large, clear vacuoles which have the appearance of a large number of soap bubbles crowded together. I supposed at first that these vacuoles represented an elaboration of the protoplasm of the so-called nurse-cell in which the sperm heads of insects are characteristically embedded. Further study indicated that this view was not tenable, for at a slightly earlier stage more or less separated vacuoles could be found among the heads without any apparent connexion with cells of the cyst wall. I would like to suggest, as a possible explanation of their origin, that these vacuoles represent material extruded from the nucleus probably at the time of its diminution in size, and comparable to the similar extrusions which seem to occur in the Hemiptera (see Bowen, 1922*a*) and other animals. This view is borne out by the

fact that occasionally (in *Callosamia*) cysts are found at about the age of fig. 56 (or later), in which each sperm head is enclosed in a clear vacuole—presumably the vacuoles noted above which have perhaps failed to be formed in a normal manner. Something of this appearance is shown by Cook (1910) in her figs. 132 and 134 of *Automeris*. This opinion is further strengthened by the fact that in degenerate (apyrene) sperms of *Callosamia* I have found that each head (now moving back in the tail region) is accompanied by a droplet of non-staining material (see Munson, 1906, fig. 49), over which the acrosome passes. If these vacuoles are a product of the nucleus we should expect just such a disposition of them in the apyrene sperms; but their connexion with the nucleus is not easily accounted for on any other explanation of their origin. In normal cysts, as the sperms grow older, these vacuoles seem gradually to disappear, but their exact fate has not been traced.

THE GOLGI APPARATUS AND ACROSOME.

My chief interest in examining spermiogenesis in Lepidoptera was centred on the origin and development of the acrosome, especially in view of the account given by Gatenby (1917*a*) of the rôle of the Golgi bodies (his acroblasts) in this process. According to Gatenby, all the Golgi bodies become swollen into vesicular spheres during the early spermatid stages, and these spheres fuse to form the basis of the acrosome. In each of these spheres there is differentiated a small, darkly-staining granule, which is also involved in the construction of the acrosome itself. In a subsequent statement Gatenby and Woodger (1921) say, 'Our recent observations . . . on several other moths (e.g. *Biston*) have shown that in these insects much of the apparatus finally passes as isolated crescents, spheres, or dictyosomes into the elongating tails of the spermatozoa.' The problem is thus left in a very unsettled condition. In my previous papers on spermatogenesis I have endeavoured to show that the acrosome is a product of the Golgi apparatus plus idiosome, but that neither of the latter structures is made

directly into the acrosome. After the acrosome is formed the Golgi complex as a whole is cast off and has no further connexion of any kind with the acrosome. I have recently developed my views on this subject in a more general form (Bowen, 1922 *e*), and it will be the purpose of this section to show how the observations of Gatenby can be harmonized with my previous results.

The early steps in the formation of the acrosome cannot be analysed with any satisfaction in *Callosamia* on account of the very small size of the acrosome in this form. In *Pygaera* my material begins at a point where the acrosome is already nearing completion. I will accordingly refer to Gatenby's figures for the earliest stages, the essential features of which are also shown clearly in the older spermatids of my *Pygaera* preparations.

My observations confirm the statement of Gatenby and Woodger (1921) concerning the casting off of the Golgi bodies, but I would go further and state that not merely 'much', but all of the Golgi apparatus is thus disposed of. The Golgi bodies can be seen in any of the older spermatids at varying distances from the nucleus (figs. 8, 10, 13, 46, and 48), and in much later stages they can be found scattered in groups at various points along the sperm tail. Furthermore, they show no evidence of a vesicular structure, but they do show, in favourable cases, the differentiation into Golgi rodlet and idiosomic substance which I have found to be so characteristic of them in the primary spermatocyte. How then does the acrosome arise? Gatenby's figs. 36 and 37 indicate, I believe, the essential features of the answer to this question. In these figures each of the acrosomic vesicles has attached to one side a Golgi rodlet, the idiosomic substance not being shown. I have been similarly unable to make out the idiosomic material in *Pygaera*, but the conditions in *Callosamia* leave no doubt that it is present, but temporarily obscured, after ordinary staining, by the development of the vesicles. In other words I would interpret the vesicles as differentiation—rather than direct transformation—products of

the Golgi bodies. These spherical vesicles are then deposited on the nuclear wall, and gradually fuse to form the acrosome, the granules differentiated within them fusing at the same time to form a single large acrosomal granule. As the acrosomal vesicles are deposited the Golgi bodies are cast off, as in the formation of the acrosome in other animals, and move off down the tail. It is clear in my *Pygaera* preparations that the formation of the vesicles by the Golgi bodies is not completed simultaneously in all of them, but rather that there is a gradual production and deposition of the vesicles extending over a considerable period, and concluded only at a relatively late stage (figs. 13, 15, 16). Figs. 1 and 2 show the latter part of the acrosomal formation in progress. The acrosomal granule in *Pygaera* stains very intensely and is of extraordinary size, often concealing the vesicular portion, especially if the latter is not well differentiated by the staining. Several Golgi bodies are grouped around the acrosome, and particularly in fig. 2 one gets the impression that one or two of them are in the act of depositing the small acrosomal vesicle which each has elaborated. Not infrequently the acrosome is multiple, as in fig. 2, one portion being much the smaller, but later on these parts always merge into a single acrosome. Gatenby shows this process in his Text-fig. 4, and with the general plan of this figure I am in entire agreement. The Golgi bodies seem to clear away from the acrosome as they deposit their quotas, and thus they tend to be scattered along the tail rather than to be collected in a single group. In the later stages of deposition the acrosome in *Pygaera* can be very clearly separated into its two fundamental constituents—the intensely-stained acrosomal granule and the clear, unstained acrosomal vesicle (figs. 13 and 15).¹ The contour of the vesicular portion is at first rather irregular, which I take to be indicative of its multiple origin; but the irregularities are gradually

¹ I have found the material of the acrosomal vesicle very difficult to differentiate sharply in the earlier stages. It is, furthermore, often obscured by the enormous acrosomal granule, so that in my figures of young spermatids the vesicular part of the acrosome may not appear at all.

smoothed out (figs. 15 and 16), and all traces of its original composition are lost.

In *Callosamia* the whole acrosome tends to stain darkly during the period of formation, a phenomenon which I have also noted in Hemiptera and in *Ceuthophilus* when the staining is not perfect. This is presumably the source of the similar condition in *Callosamia*, for when the Golgi bodies have all cleared away the acrosome is clearly constructed on the same plan as in *Pygaera*. The difference in size is, however, astonishing, for in *Callosamia* the whole acrosome is exceedingly small and inconspicuous (figs. 50 and 51). Nevertheless, it is differentiated into a vesicular and a granular part exactly as in *Pygaera*. The granule tends to be slightly elongate rather than rounded in the stage at which I have first succeeded in differentiating it. Earlier stages (figs. 46 and 48) show very clearly the relation of the Golgi bodies to the forming acrosome, but I have not been able to make out their individual contributions, which are presumably very minute. Fig. 49 shows an interesting case in which the last Golgi body is just on the point of separating from the acrosome. In this case the Golgi body appears to be a fusion product of several smaller Golgi bodies.

The interpretation which has here been given furnishes, it seems to me, a complete explanation of Gatenby's results, and brings the lepidopteran acrosome into harmony with the conditions as we now know them to exist in many other animals. According to the idea which I have developed each Golgi body in a lepidopteran spermatid would be an acroblast on a small scale, and the formation of the acrosome is thus a multiple process. In its essential outlines it is, however, clearer than in the case of the grasshopper which I have described in another place (Bowen, 1922 *d*). For the relation of this type of acrosome formation to more familiar cases in other animals the reader is referred to my paper on the acrosome, in which I have tried to bring the whole series of known facts under a common view-point (Bowen, 1922 *e*).

Gatenby's account leaves the further history of the acrosome

practically untouched; but as its later development offers a number of interesting features I have thought it worth while to work out the subsequent events in *Callosamia* and *Pygaera* from a comparative standpoint. The acrosome in *Pygaera* is particularly favourable for study because of its large size.

In *Pygaera* the acrosome when finally deposited consists, as noted above, of a clear vesicular portion, the acrosomal vesicle, and a very large, darkly-stained granule, the acrosomal granule. The latter is presumably contained within the former, but the large size of the granule gives one the impression rather of a bipartite mass (figs. 15, 16, and 17). The vesicular material seems now to undergo further concentration, its outline becoming very clearly marked. Meanwhile the granule becomes slightly drawn out into a spindle shape, with the vesicular material applied along one surface (figs. 18 and 19). In fig. 19 various aspects (oblique, cross, and longitudinal optical sections) of the acrosome at this stage are shown. It will be noted that the acrosome tends to be located on the nuclear membrane at the edge of the chromatic lining.

It soon becomes evident that the assumption of the spindle form by the acrosome is merely the initial stage in a process of elongation which now progresses rapidly (fig. 20 et seq.). In this elongation one end of the acrosome is temporarily fixed near the anterior pole of the sperm head, the acrosome thus growing backward over the nuclear wall until it projects considerably behind the nucleus (figs. 20, 22, 24 to 27). Having reached the stage shown in fig. 27 the acrosome becomes detached anteriorly, and slides bodily forward until the originally posterior free end becomes applied to the anterior nuclear wall. Steps in this remarkable migration of the acrosome are shown in figs. 27 to 31. Eventually the posterior tip of the acrosome seems to be attached to the nucleus at the point of insertion of the axial filament (figs. 31 et seq.). The cytoplasm in the head region is at first carried forward with the acrosome (figs. 28 to 33), but in later stages it gradually moves backward (figs. 35 to 38) until the entire head region

is free from cytoplasm (figs. 39 to 41), exactly as in other insect sperms.

After moving into its definitive position the acrosome continues to elongate, and eventually becomes spun out into a remarkably long, delicate apical piece (figs. 33 to 41). Fig. 41 is the latest stage in which I have seen the acrosome in anything like its entirety, but the head of the sperm itself is still in an intermediate stage of elongation. The extraordinary size relations of the acrosome in the mature sperms can be inferred from Meves' (1903) fig. 152. (See also his descriptive account.)

To go back now to the early elongation stages, the structural features of the acrosome itself deserve further attention. Once the acrosome has become markedly elongate I have found it as a rule impossible to differentiate the vesicular material which is obscured by the heavily-stained 'granule'. However, in cross-sections, especially of intermediate stages in elongation, the two materials can be readily distinguished (fig. 34), and there can be no doubt that the two original constituents of the acrosome remain distinct at least for a long period. A comparison of figs. 32 and 33, and 36 and 37, with fig. 34, suffices to explain the appearances presented by the acrosome when viewed from different aspects. It will be evident that in figs. 33, 37, and 38 the acrosome is turned so that the acrosomal granule is seen in plane view, while in figs. 32, 36, and 39 the acrosome is seen in what one might call a side view (compare fig. 34). As the figures show, the side of the acrosome on which the vesicular material is disposed is usually turned towards the major axis of the sperm.

Within the acrosome itself various changes take place, the first of which to be noted is the production, at the temporarily attached end, of a clear area, which seems to be the first part of the acrosome to free itself from the nuclear membrane prior to migration (figs. 26 to 30). This clear zone is subsequently lost, and another one develops at the permanently attached end of the acrosome, while at the very point of attachment a darkly-stained body appears (figs. 31 to 39). Whether this represents a differentiation of the acrosome,

or is possibly of centriolar origin, I have not been able to determine. At any rate this seems to be a common point of attachment for both acrosome and tail filament. There are indications that subsequently (fig. 40 and later) the centriolar apparatus, or a portion of it, becomes shifted to the base of the sperm head, but I have not found my material satisfactory for a detailed study of these phenomena.

In *Callosamia* the general progress of events is exactly parallel to that in *Pygaera*, with the possible exception of the method of orientation of the acrosome. The structure of the acrosome in this moth does not permit detailed study of a possible forward migration, and it is possible that the migration may occur in a different way. In the early figures, it will be seen that the acrosome is deposited at a point some distance removed from the insertion of the tail filament. As the acrosome begins to elongate, however, it apparently migrates anteriorly (fig. 52), and becomes attached by one end near the insertion point of the axial filament (figs. 52 et seq.).¹ From this point on the exact course of events is uncertain, and whether or not, with the elongation of the acrosome, there is a further change of orientation, as in *Pygaera*, has not been ascertained. However, appearances like that of fig. 54 suggests that perhaps the acrosome at first grows posteriorly, as in *Pygaera*, and shifts later into its definitive position. As already noted in *Callosamia*, the acrosome, as originally deposited, is relatively much smaller than in *Pygaera* (figs. 50 and 51), and we should therefore expect its later stages to be much more delicate in structure. As a matter of fact this expectation is exactly realized, and almost from the beginning of elongation the acrosome has a thread-like form, in which it is impossible to distinguish the two acrosomal constituents (figs. 51 to 59).

The various steps in the forward growth of the acrosome are shown clearly in figs. 56 to 59, and present no points of special interest. The length of the acrosome in the mature sperm as compared to the length of the sperm head is much less

¹ It is possible that a similar migration occurs in *Pygaera*, where it would be masked by the large size of the acrosome.

in *Callosamia* than in *Pygaera*, correlated apparently with the very much smaller amount of available acrosomal material. The thread-like nature of the acrosome and its attachment so near the insertion of the axial filament offer the possibility of a natural error in interpretation which should be guarded against in the study of other sperms. If one studied only the later stages of sperm formation, or observed the earlier ones inaccurately, a most obvious conclusion would be that the acrosome was really a forward growth from the spermatid centrioles, comparable in its method of formation to the tail filament. It is possible that some of the accounts which have been given of the rôle of centrioles in acrosome formation may actually be traceable to errors of this nature. In any event it is clear that great care should be exercised in the future in interpreting thread-like formations in the sperm head, since it is now evident that topographical relationship to the centrioles may not be in the least degree indicative of organic relationship. It is interesting to note in this place that Goldsmith (1919) has described a thread-like formation in the sperm head of *Cicindela*, without, however, offering any explanation of its homologies. I think it probable that the facts made out in *Callosamia* will furnish a clue to the enigmatic structures described by Goldsmith.

THE APYRENE SPERMS.

In the saturnid moths large numbers of apyrene sperms are formed after the normal sperm formation has been largely completed. The nucleus in every case is reorganized in the spermatid in a normal manner, as Gateby (1917*b*) found in *Pieris brassicae*. However, at an early stage the spermatid becomes visibly abnormal by reason of the improper orientation of the nucleus and nebenkern, their relative positions being exactly reversed in the abnormal spermatids. The result is quite striking when an entire cyst is observed, the nebenkerns being adjacent to the cyst wall instead of the nuclei, as is so characteristic in insect testes.

As the nebenkern elongates the nucleus moves back along

the tail, as Gatenby shows for *Pieris brassicae*. The acrosome behaves at first in a normal manner, so far as can be judged from stages corresponding to that of fig. 55, for it can be readily found in the form of a delicate, elongate rod or thread attached to the nucleus. Gatenby (1917*b*) is apparently dealing with an acrosome of this kind in *Smerinthus populi*, as indicated in his fig. 9, which shows a condition very similar to that in *Callosamia*. As I have shown, the early elongation of the acrosome is in no way dependent on the elongation of the nucleus, and Gatenby's conclusions (pp. 474-5 of his paper), from the figure mentioned above, seem to me entirely unwarranted. On the whole, the degeneration phenomena in the lepidopteran testis seem still to offer many problems which call particularly for an intensive study of the whole germ-cell cycle for their adequate solution.

CONCLUSION.

As I have indicated elsewhere the primary purpose of this study was to compare the fundamental differences in the sperm formation of Lepidoptera and Hemiptera as brought out by the work of Gatenby and myself. As a result of my observations on the Lepidoptera it appears that such differences as actually occur are primarily ones of detail, and that in all essential respects these two insect groups have a remarkably similar spermiogenesis. Indeed, a further comparison with the Orthoptera and Coleoptera which I have studied gives unmistakable indication of the fundamental similarity in the processes of sperm formation in all insects, and discounts in a most decided manner the bizarre accounts of insect spermiogenesis with which the older literature is full.

This seems to be particularly true of the nebenkern, the history of which I have treated in another paper (Bowen, 1922*b*). In that paper I made extensive use of Gatenby's studies of the Lepidoptera because in this group conditions seem unusually favourable for an exact analysis of the early condensation of the nebenkern. I then accepted the account of the mitochondrial 'spireme', and suggested that the neben-

kern 'patterns' might follow either a 'spireme' type or a 'plate-work' type. As a result of the studies here recorded, however, I no longer find myself able to accept the reality of the 'spireme', and I now believe that the evidence is indicative of a plate-work in the lepidopteran nebenkern essentially like that which I found in the Pentatomidae. Indeed, it appears quite likely that the 'spiremes' sporadically figured by other workers and referred to in my previous paper are all to be accounted for on similar grounds and to be equally explicable on the basis of their plate-work structure. A plate-work seems to be the fundamental structure of the chromophilic substance in the nebenkerns of all insects.

In this connexion the results of Doncaster and Cannon (1920) on the louse are of special interest. These workers have employed modern technique, and have reached a conclusion which is in accord with my own interpretation of the vesicular nature of the early nebenkern. Their account is characterized by the remarkable conclusion that the nebenkern is formed and goes through the earlier condensation stages in the primary spermatocyte. In the single and abnormal maturation division it passes bodily into the functional spermatid, and is thus already well along in its evolution when sperm formation proper is just beginning.

Finally, one point in Platner's (1889) old description of *Pygera* suggests interesting matter for comparison. I have long been puzzled by this author's small mitosome, which was thus designated because of its supposed relation to a part of the spindle fibres. It is clear now that what Platner really saw was the acrosomal granule, and his account of its elongation, occurring at first in a posterior direction, is remarkably suggestive of my own observations. It is evident from his figures, that the small mitosome was not intensely stained by his technique, and his failure to make out the final fate of the 'small mitosome' (acrosome) is probably to be ascribed to the increasing difficulty of demonstrating it as its form became less compact. Gatenby's revival of the term as the micromitosome, for a cytoplasmic granule somewhat

recalling the chromatoid body of other workers, seems thus to be a rather unfortunate one, as it is quite improbable that this body has anything whatever in common with the acrosome.

SUMMARY.

1. The lepidopteran nebenkern passes through a series of condensation phenomena which are essentially similar to those previously described in Hemiptera.

2. The structure of the chromophilic material is probably that of a plate-work rather than a spireme.

3. The central substance is developed in the nebenkern exactly as in other insects.

4. The Golgi bodies in all probability give rise each to a small vesicle, these vesicles fusing gradually to form the acrosome.

5. As in other insects, the Golgi bodies are not directly transformed in the building up of the acrosome, but, after giving rise each to its miniature acrosomal vesicle, they pass back along the tail and are probably cast out of the sperm along with other detritus in the concluding stages of spermiogenesis.

6. The essential parallelism existing between the formation of the sperm in Lepidoptera and in other insects, especially the Hemiptera, is particularly emphasized.

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EXPLANATION OF PLATES 24-26.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. At so great an enlargement it has of course been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly, those of Pls. 24 and 25 to an enlargement of approximately 2,530 diameters, those of Pl. 26 to approximately 3,000 diameters. All of the figures are from material fixed in Flemming without acetic acid, except figs. 42, 43, and 44, which are from material fixed in Champy.

REFERENCE LETTERS.

A, acrosome. *B*, nuclear body of doubtful nature. *C*, centriole(s). *f*, tail filament. *G*, Golgi apparatus = acroblasts (where the acroblasts are much scattered, only a few representative ones are specifically labelled). *K*, nucleus. *M*, mitochondria. *N*, nebenkern. *S*, central substance. *V*, vesicles developed on the mitochondrial tail sheaths.

PLATE 24.

EXPLANATION OF FIGURES.

All the figures are from *Pygaera bucephala*. Figs. 1, 2, and 3 are from the same cyst of developing sperms; likewise figs. 4 and 5, and figs. 8 and 9; figs. 10, 11, and 12 are from a cyst of abnormally large spermatids.

Figs. 1, 4, 8, 10, and 13.—Progressive stages in the elongation of the nebenkern. The acrosomal granule is stained intensely black.

Fig. 2.—Section through a spermatid nucleus, showing the acrosomal granule in two parts, and numerous Golgi bodies, some of which seem to be engaged in depositing their quota of acrosomal material.

Figs. 3, 5, and 6.—Cross-sections through the nebenkern in successive stages of its elongation to show the structure of the chromophilic material.

Fig. 7.—Portion of the distal end of the nebenkern showing the constriction of the chromophobic material to form the tail vesicles.

Fig. 9.—Cross-sections of the nebenkern at the stage of fig. 8: *A* and *B*, through the region of the chromophilic material; *C*, through the chromophobic material above the plane of *A* and *B*.

Fig. 11.—Frequent appearance of tail vesicles when fully formed.

Fig. 12.—Cross-sections of the nebenkern in a condition slightly younger than that of fig. 10; *A*, through the chromophilic material; *B*, above (or below) the chromophilic material. From adjacent sections of the same nebenkern.

Fig. 14.—Small portion of a nebenkern showing the last remnant of chromophilic material just prior to its complete dissolution.

PLATE 25.

EXPLANATION OF FIGURES.

All the figures are from *Pygaera bucephala*. Figs. 28, 29, and 30 are from the same cyst.

Figs. 15–41.—Progressive stages in the construction and fixation of the acrosome. The material of the acrosomal granule is stained intensely black in every case. The acrosomal vesicle can be clearly differentiated in figs. 15 to 19 and fig. 21. In fig. 34 are shown cross-sections of the acrosome at the stage (approximately) of figs. 32 and 33. The total length of the acrosome in the later stages of its elongation is approximated as nearly as conditions will permit. As a rule it could not be exactly determined owing to the extreme tenuity of the tip.

PLATE 26.

EXPLANATION OF FIGURES.

All the figures are from *Callosamia promethea*. Fig. 44 is from the small generation of spermatids.

Fig. 42.—Primary spermatocyte, growth period, to show the chondriosomes.

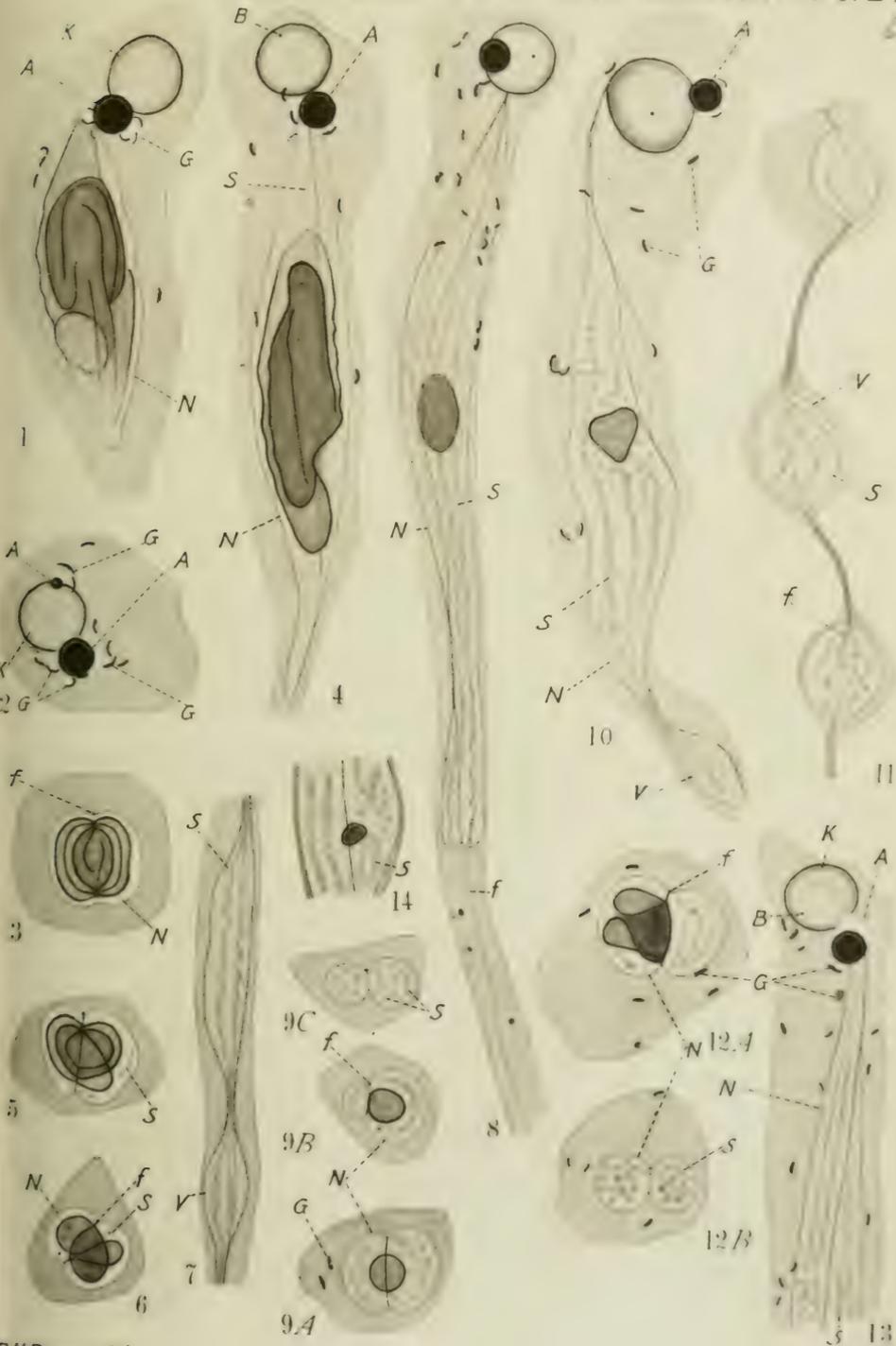
Fig. 43.—Final telophase of the second spermatocyte division, showing first step in the condensation of the nebenkern.

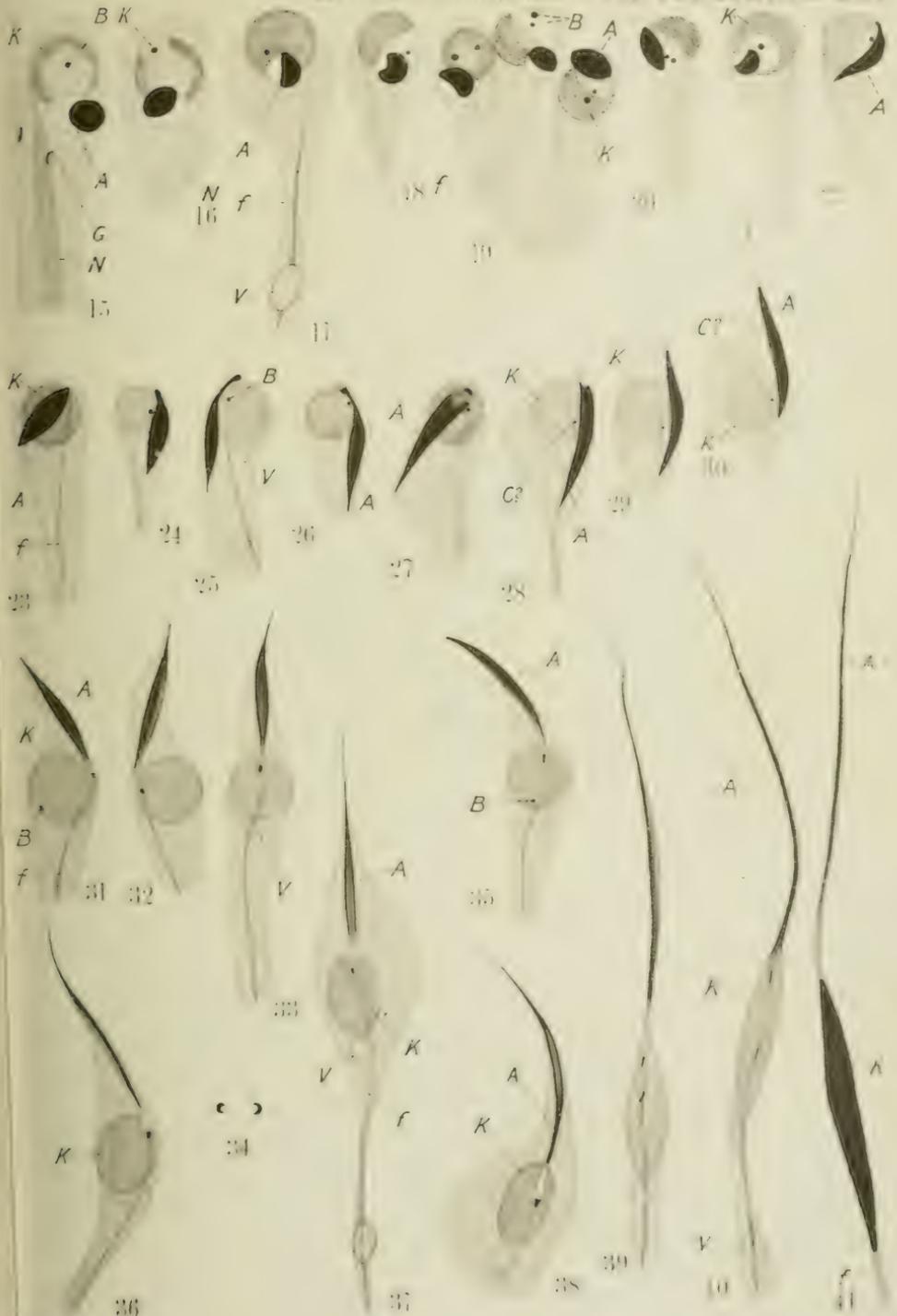
Figs. 44, 46, 48, and 49.—Progressive stages in the transformation of the nebenkern and the deposition of the acrosome. The acrosome (in figs. 46, 48, and 49) appears as a more or less darkly-stained spherical body in contact with the nuclear membrane, and closely related to adjacent Golgi bodies.

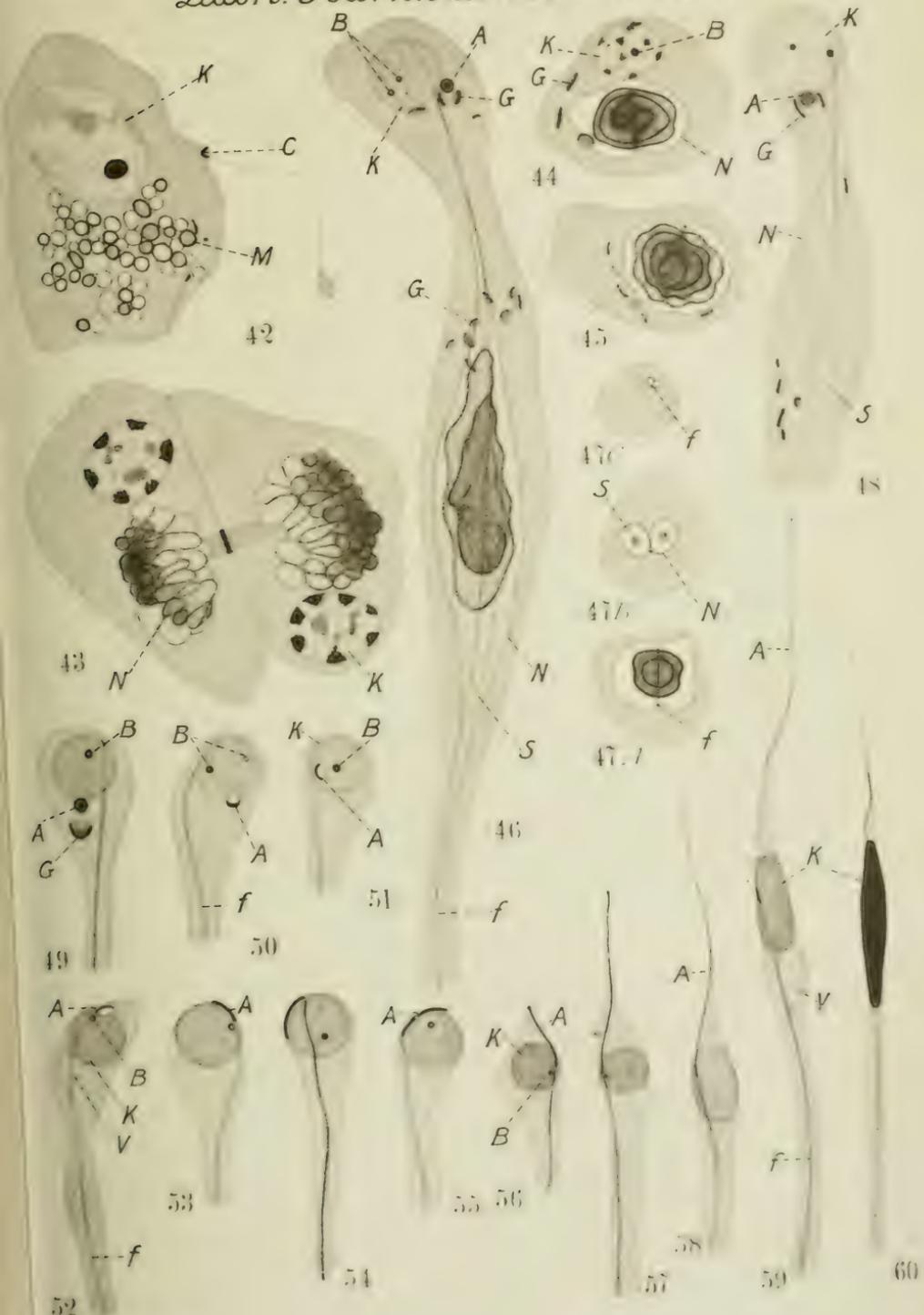
Fig. 45.—Cross-section of the nebenkern in an early stage of elongation.

Fig. 47.—Cross-sections of the nebenkern at the stage of fig. 46: *A*, through the region of the chromophilic material; *B*, immediately above or below the chromophilic material; *C*, near the much elongated, free (or attached) end of the nebenkern.

Figs. 50–60.—Progressive stages in the formation of the definitive acrosome. The length of the acrosome has been approximated as closely as possible in figs. 58 and 59. In fig. 60 only a part of the acrosome is shown.







On the Biology and Structure of the Larvae of *Hydrophilus caraboides* L.

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With Plate 27 and 16 Text-figures.

IN May 1918 I captured in the vicinity of Petrograd some cocoons of a hydrophilus beetle, one of which I kept for breeding purposes. On June 13 there emerged about fifty small larvae very similar to *Hydrophilus caraboides*. These latter are characterized by the presence of a pair of lateral (pleural) appendages covered with a dense brush of hairs on each of the seven abdominal segments (Text-fig. 13, *pla*).

In my larvae (Text-fig. 1) these pleural hairy appendages were also present, but with the difference that each appendage bore on its summit a long thin hair. This peculiarity caused me to look for other differences between my larvae and the description of the larvae of *Hydrophilus caraboides*, as given by Schiödte in his paper, 'De metamorphosi eleutheratorum observationes; bidrag til insekternes undviklingshistorie'. 1861.

These differences may best be pointed out by a parallel comparison of the text of Schiödte's diagnosis and the description of the newly emerged larvae as observed by me, as follows :

Hydrophilus caraboides Schiödte
Caput obovatum.

Hydrophilus caraboides
—a larva at first stage. Head irregularly tetragonal, shaped rather like a trapezium turned with its base forward, and with broken sides.

Antennae articulo primo longissimo, tenui, ciliato (Text-figs. 6, 7, *b*), secundo et tertio tenuibus, pusillis, subaequalibus.

Antennae scapo longissimo ciliato, natatoriae.

Mandibulae elongatae, falcatae, acutissimae, subaequales, dente medio valido, duplici, acumine, posteriore acuto, priore lanceolato, maiore (Text-fig. 6, *md*).

Stipites maxillares gracillimi, longissimi, palpos labiales excedentes. Palpi maxillares graciles; stipes gracilis, recurvus, articulo primo triplo longior.

Mentum (Text-fig. 4, *m*) amplum, fornicatum, e basi sensim, decrescens latitudine, angulis dentiformibus, lateribus serrulatis. Stipes palporum labialium elongato-quadratus, basin versus angustior. Palpi labiales (*pl*) articulo priore brevissimo. Ligula elongata, acuminata (*lg*).

First joint of antennae long, slender and flat, inner margin with seven very distinct teeth (Text-fig. 5, 7, *a*), giving the joint a saw-like aspect. Second and third joints almost of equal length, each being about three times shorter than the first. Second in the middle of its outer margin with an elevation bearing a sense-organ in the shape of a chitinous ringlet with hairs in the centre.

Antennae scapo longissimo, serrato, masticatoriae.

Mandibles shaped as a sharp, bent, and strong sickle with secondary teeth on its concave surface, the first largest, lancet like; the middle tooth tetragonal with a concave free surface and sharp prominent edges; hind tooth in the shape of a small tubercle at the base of the middle tooth.

Mentum (Text-fig. 3, *m*) bell-shaped, both its fore angles protruding in the form of sharp teeth. Side margins smooth, slightly S-shaped. Stipes compared with the mentum much more developed than in the mature larva of *H. caraboides*, and in the form of an elongate rectangle which in its fore part is wider than behind. The base of the ligula occupies almost half of the width of the anterior margin of the mentum (in the mature larva of *H. caraboides* only one-fourth). Tip of the ligula is somewhat bilobate (*lg*).

Scuta prothoracica concreta, completa, integra. Scuta mesothoracica discreta, tergum medium occupantia, triangula, apicem truncatum versus constructo-attenuata. Scuta metathoracica discreta, incompleta, apice incurva.

Praeterga abdominis integra. Terga obscurius plicata, scutis verruciformibus, cylindricis. Appendices pleurarum abdominis praeter octavum par elongatae, inaequales, ciliis gracillimis vestitae, nata toriae; appendices octavi paris deorsum flexae, acuminatae, sinuosae, suspensoriae.

The prothoracic tergite has the shape of a continuous shield of an almost regular quadrangle, the posterior corners being obliquely cut or rounded (the tergite of the mature larva of *H. caraboides* is conspicuously narrowed in front and in outline much like a truncated cone). The meso- and metathoraces bear each on their tergites two triangular shields, the apex of which is not bent.

Abdomen densely hairy (Text-fig. 1). Tergites with four warty tubercles which are also covered with hair, and with a long seta on the summit. Before the middle pair of tubercles there is a pair of brown shields of strong chitin, also bearing long setae.

Pleural appendages in shape of cylindrical prominences, and densely covered with hair, resembling a lamp-brush. From the summit of each appendage there emerges a long and thin seta (Text-fig. 1).

Above the base of each lateral pleural appendage there is a small warty tubercle with a long seta.

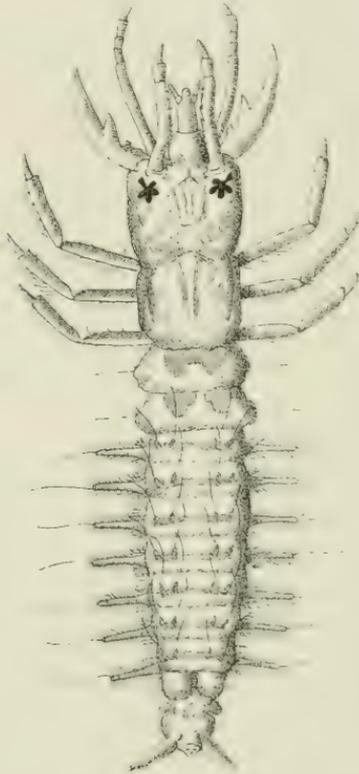
The examination of more advanced larvae of *Hydrophilus caraboides*, i.e. such that had already moulted, convinced me of the accuracy of Schiödte's description. The antennae of such larvae are in fact hairy on their inner edges, and the base of the lamella of the under-lip has a dentate edge. The long setae on the tips of the pleural appendages are partially present but not conspicuous.

On the whole one might say that from the egg of *Hydrophilus caraboides* emerges a larva which in its successive

ecdyses changes some structural characters, especially in the antennae, the under-lip, and partly the pleural appendages.

In the literature the excellent drawing of the mature larva given by Schiödte is generally adopted ; but I have not been

TEXT-FIG. 1.



Hydrophilus caraboides. A larva, first stage.

able to find any figure of a young larva, therefore I here adduce its figure, drawn unfortunately from a specimen preserved in spirit, on account of which the abdomen seems shorter than it is in reality (Text-fig. 1). It is true that it easily extends, a fact which causes some variation in its length even under normal conditions.

These facts found by me caused me to investigate the larvae

of *Hydrophilus caraboides* more thoroughly. The larvae were brought alive into Duboseq's mixture (15 c.c. of 1 per cent. solution of picric acid in 90 per cent. alcohol + 6 c.c. of formalin and 1.5 c.c. of ac. aceticum glaciale), and after they had ceased to move they were cut in two with scissors.

The fixation lasted from twelve to sixteen hours. After this the larvae were brought through several portions of 85 per cent. alcohol to wash out the picric acid. Further, they were embedded in paraffin. The sections were stained with iron haematoxylin, Giemsa stain, and Unna's polychrome methylene-blue.

ON THE STRUCTURE OF THE DIGESTIVE APPARATUS.

The larvae of *H. caraboides* are distinguished from all other aquatic insects by their unusual way of taking food. Observations on this point have already been made by several naturalists, and Brocher (1913) even figures (semi-diagrammatically) a larva of *H. caraboides* taking food (fig. 68). According to a more detailed description of Portier (1911) this process takes place as follows.

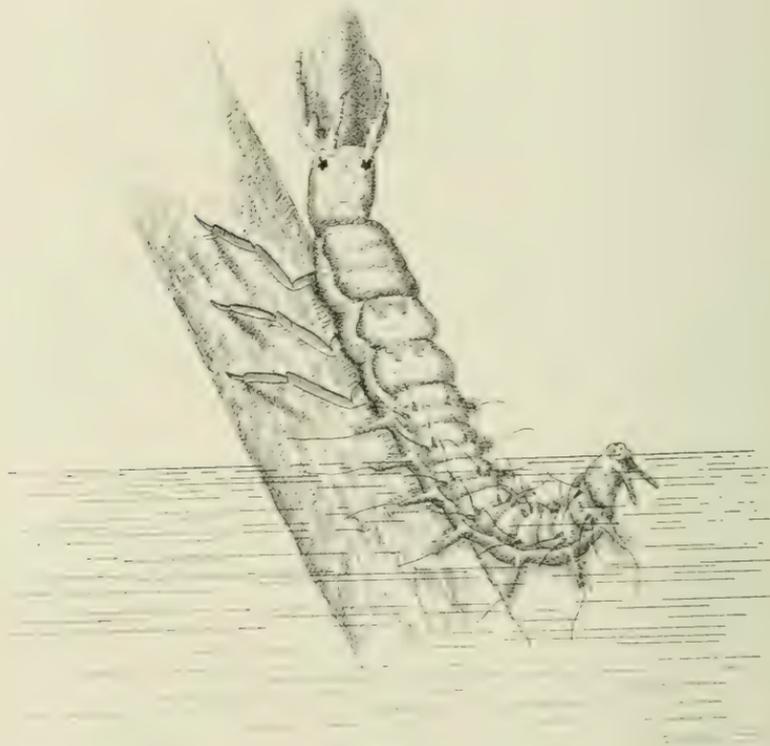
Having caught with its powerful and sharp mandibles its prey (for instance a small crustacean, an insect, &c.), the larva of *Hydrophilus* 'se dirige vers une plante aquatique ou contre la paroi du vase qui la renferme, si aucun objet ne flotte à la surface de l'eau. Au moyen de ses pattes antérieures, elle s'accroche à une aspérité quelconque située au-dessus de la surface, puis elle renverse sa tête en arrière sur son dos. On comprend que, dans cette situation, sa tête émerge complètement. C'est, en effet, le résultat auquel la larve semble tendre. . . . La proie ayant été ainsi élevée au-dessus de la surface, on voit l'appareil masticateur entrer en jeu, la chitine est perforée par les tubercules chitineux qui garnissent les mandibules et le sang rouge de la larve de *Chironomus*¹ se met à couler.

'A ce moment, un flot de liquide noir envahit les organes

¹ Portier describes the feeding of a larva of *H. caraboides* on a larva *Chironomus*.

buccaux ; c'est le liquide digestif qui a été injecté à travers l'œsophage par l'intestin moyen. Ce liquide noir adhère par capillarité à la proie ; il est contenu dans une espèce de corbeille formée par les différentes pièces de l'armature buccale

TEXT-FIG. 2.



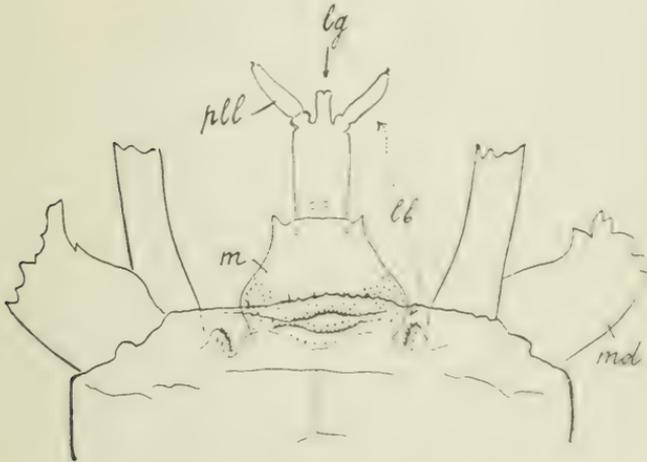
Larva of *Hydrophilus caraboides* devouring a cypris.

si développée chez ces larves. La proie est ainsi complètement baignée dans la liqueur digestive. Après quelques instants de contact, le liquide chargé maintenant des produits de la digestion est réabsorbé ; il passe par l'œsophage, puis dans l'intestin moyen dont il gagne immédiatement la partie postérieure, ainsi qu'on peut s'en assurer sur les jeunes larves, ou sur celles qui viennent de changer de peau, toutes deux possé-

nant des téguments d'une transparence parfaite' (Portier, 1911, pp. 175-6).

The inconvenience of the attitude during the feeding process (Text-fig. 2) is increased for the larva of *H. caraboides* by the necessity of breathing. For this purpose the larva bends its body in such a way that the posterior end of the abdomen

TEXT-FIG. 3.



Anterior margin of head and lower lip of newly-hatched larva. Base of lower lip (*lb*) with simple lateral margins (*m*). On the sides spined folds of the connective chitinous cuticula (*t*). *md*, mandible; *lg*, ligula; *pll*, palpi labiales. Zeiss, ob. AA, oc. 0.

bearing the breathing aperture touches the surface of the water (Brocher).

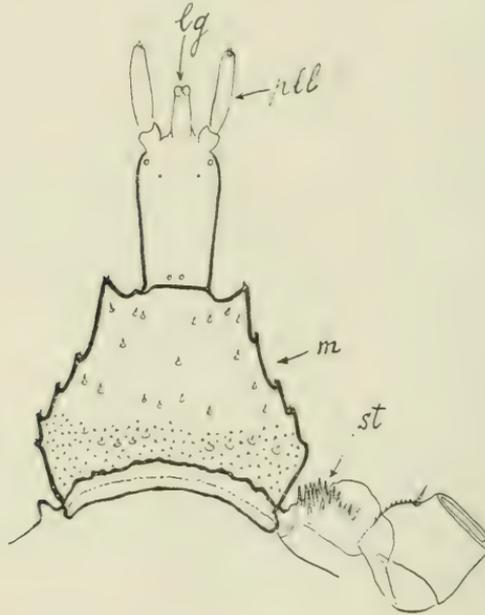
In connexion with these peculiarities we shall look into some of the details in the structure of the digestive apparatus of the larva of *H. caraboides*.

The well-developed and powerful mouth-parts of the larva serve to seize the prey and to treat it mechanically. Having seized some suitable animal, the larva of *H. caraboides* pierces it with the teeth of its sickle-shaped mandibles (Text-fig. 6, *md*), tearing open the integument of its prey. After this the larva assumes the habitual feeding attitude and begins to work energetically with all its mouth-parts, closing and

reopening them. Gradually the prey crumples and is thrust in deeper and closer to the oral opening.

In the grinding and kneading not only the mandibles take part but also the antennae, which in the larva at the first stage have a special contrivance for this purpose in the form of a row

TEXT-FIG. 4.



Lower lip of mature larva. Dentation of lateral margins and sensory cones of upper surface of base of lip (*m*). On the sides spined folds of the connective chitinous cuticula (*st*). *lg*, ligula; *pll*, palpi labiales. Zeiss, ob. AA, oc. 0.

of sharp teeth on the inner edge (Text-figs. 5, 7, *a*). These teeth are chaetoids (after the terminology of Nasonov, 1901), i. e. they are evaginations of the integument of the larva and the body-cavity is continued into these appendages. The antennae of the mature larva do not take any energetic part in the mastication of the prey, having on the inner edge about three rows of long flexible setae (Text-figs. 6, *a*, 7, *b*), which are therefore inconvenient for tearing or kneading the prey.

The under-lip (Text-figs. 3, 4, *lb*) serves as a plate on which the preparation of the food is effected. During the feeding process the labial palpi (Text-figs. 3, 4, *pll*) are all the time in motion. Owing to the work of the mouth-parts the body of the captured animal is kneaded, with the result that the liquid

TEXT-FIGS. 5, 6.

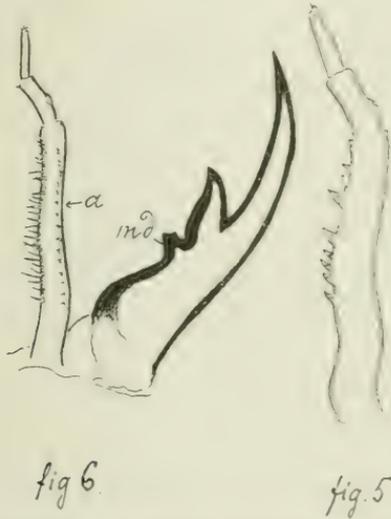


Fig. 5.—Right antenna of newly-hatched larva. The inner edge is serrated; outer edge simple. Winkler, ob. 1, oc. 2.
 Fig. 6.—Antenna and mandible of mature larva. Inner edge of antenna set with hairs, outer edge bears a row of short spines (*a*). *md*, mandible. Winkler, ob. 1, oc. 0.

parts or those being in a state of emulsion can be sucked up by the larva.

But into the alimentary system of the larva are introduced also the solid constituent parts of the prey after they have been treated with the ferments of a black digestive fluid which is exurgitated from the intestine.

The whole of the feeding of the larva of *H. caraboides* takes place outside the water, a fact that, as has already been said, depends on the structure of its mandibles, which, in contrast to those of the larvae of the *Dytiscidae*, are not pierced

by a canal (Text-fig. 8, *md*) serving for the pumping of the sucked-up liquid into the intestine.

The prey is in the air while the larva of *H. caraboides* is feeding, and in order that the liquid parts may be sucked up it is necessary that the prey should be tightly pressed to the mouth-aperture of the larva.

The oral slit is situated between the upper- and under-lips of the larva (Text-fig. 9, *ap*), and the hind third or quarter of the mentum is covered with minute chitinous spines (Text-figs. 3, 4, *m*). Larger spines are found sticking out as a bundle on the tuberculiform elevation on the connective chitinous (Text-fig. 4, *st*) covering at the base of the lower jaws.

Thanks to these peculiarities, the deeper parts of the periphery of the oral aperture adhere tightly to the adjacent parts of the seized prey, and when the mouth-parts are closed the slits between their bases are filled up by soft connective chitin. Thus is obtained a hermetic contact of the oral opening with the body, the liquid parts of which have to be sucked up.

The oral armature of the larva of *H. caraboides* does not play a mechanical part only.

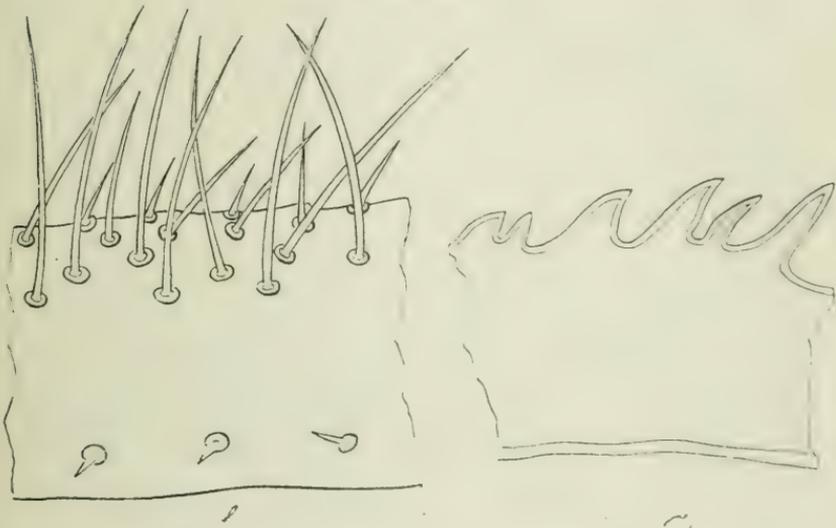
Very likely it serves also for receiving gustatory irritations, as on the lower lip there are scattered special sensory organs in shape of large pits in the chitinous cuticula. Ring-shaped sense-organs are found also on the outer edge of the third antennal joint; on the tips of the antennae and labial palpi there are situated bundles of special chitinous spines (sensory organs?); it may be remembered that on the upper surface of the mentum of the mature larva there are symmetrically scattered short and stout spines, bordered at their base; analogous formations are found also on the dentate lateral prominences of the mentum (Text-fig. 4, *m*).

The prey, prepared mechanically (by the mouth-parts) and chemically (by the ferments of the digestive fluid), enters in parts into the feeding larva. The work of the mouth-armature one is inclined to compare superficially with chewing. This comparison obtrudes itself upon one's mind when one sees how energetically the larva kneads the prey with its mandibles,

turns it round and round and squeezes it. The liquid parts of the food are sucked up at this time by means of a special sucking apparatus of which we give a description, based on the study of series of transverse and longitudinal sections of the head and thorax of the larva of *H. caraboides*.

The fore-gut of the larva described runs as a straight tube from the oral aperture to its point of mержence into the stomach,

TEXT-FIG. 7.



Parts of antennae of a newly-hatched (*a*) and a mature (*b*) larva at the same magnification. Zeiss, ob. DD, oc. 0.

which takes place in the thorax. In this fore-gut three divisions can be recognized, viz. the fore part reaching to the nervous ganglia; the middle part corresponding with the pharyngeal ring of the nervous system; and the hind or post-cerebral part, or the oesophagus proper.

The fore part is a dorso-ventrally flattened tube (Text-fig. 10) with sharp lateral edges. Its upper surface is covered with transverse muscular bundles (s_1), which are fastened to the sharp edges of the intestine. These muscles can, as I believe, be regarded as sphincters of a suctorial apparatus, for when

contracted they press the upper wall of the tube to the convex under-surface of the same. The antagonists of these muscles are the muscular bundles, extending from the upper surface

TEXT-FIGS. 8-11.

From a series of transverse sections of the head of a larva of the first stage. Zeiss, ob. AA, oc. 0.

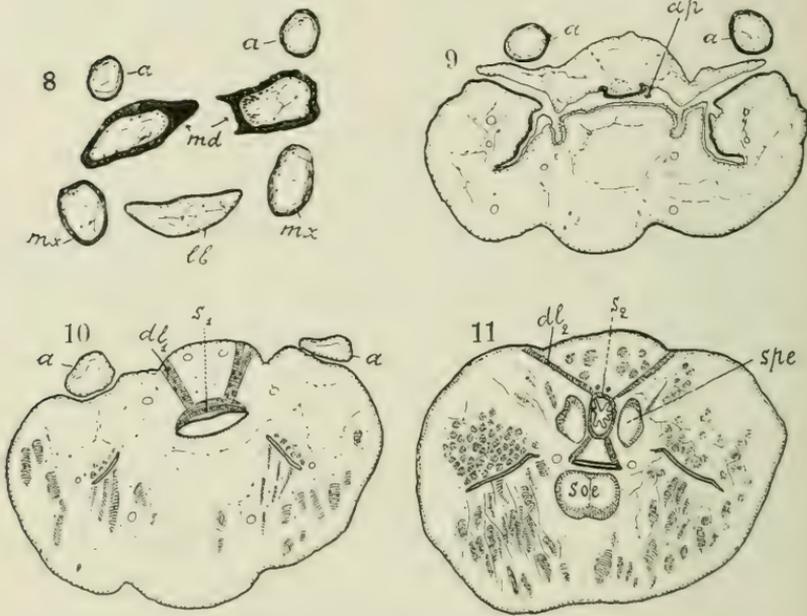


Fig. 8.—Transverse section through mouth-parts. Mandibles (*md*) without inner canals; *mx*, maxilla; *a*, antenna; *lb*, labium.

Fig. 9.—Transverse section at level of union of mandibles with head. The oral aperture (*ap*) with its spiny inner surface is visible.

Fig. 10.—Section near the region of antennae (*a*). Suctorial apparatus—sphincters (*s*₁) and dilatators (*dl*₁), attending fore part of intestine.

Fig. 11.—Section at level of cephalic nervous ganglia (*soe*, *spe*). Fore-gut with dilatators (*dl*₂) and sphincters (*s*₂) of pharynx.

of the intestine to the upper wall of the head (Text-fig. 10, *dl*₁). When contracted they lift the upper wall of the tube, and, therefore, increase its cavity; these levators might be called suctorial muscles. There are several pairs of them.

Farther backwards, the alimentary tube when compressed takes the shape of a groove, the upper edges of which are tied together by muscular bundles; the levators running in an oblique direction are located at the bottom of the groove, and between them, just in the middle, there are muscular bundles which, perhaps, serve for the contraction of the part of the intestine here described.

Nearer to the level of the brain-ganglia (Text-fig. 11, *spe, soe*) the structure of the intestine is different. The walls of the tube grow thinner. In section it looks like a strongly plicated ring surrounded by circular muscular filaments (second sphincter, Text-figs. 11, 12, s_2). They alternate with bundles of the radiating dilators of the middle part of the fore-gut. There are four groups of dilators—two upper (dl_2) and two lower ones; the upper groups are fastened to the upper wall of the head, the under ones go round the sub-oesophageal ganglion and, as it appears, partly terminate on the chitinous fold of the endoskeleton which lies above the fore end of the sub-oesophageal nervous ganglion. Behind the pharyngeal ring the intestine retains its form of a thin-walled tube strongly plicated longitudinally, clad in the usual muscular covering. The fore-gut opens directly into the mid-gut and does not form any valve or cardiac fold at the border of the stomach.

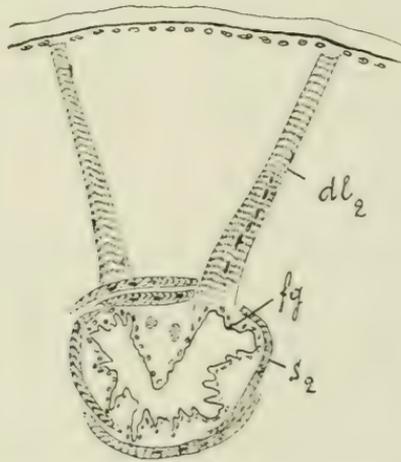
On the whole it may be said that the fore-gut of the larva of *H. caraboides* is provided with two muscular apparatuses: (1) the suctorial apparatus, which sets into action the first group of sphincters and levators (Text-fig. 10), and (2) the pharyngeal apparatus (the second suctorial apparatus), the sphincter and dilator of which act on to the middle part of the fore-gut (Text-fig. 11).

Having acquainted ourselves with the structure of the fore-gut of the larva of *H. caraboides* we can picture its functions as follows.

With the contraction of the second group of sphincters (Text-fig. 11, s_2) the middle portion of the fore-gut is tightly closed. Then the levators of the suctorial apparatus (Text-fig. 10, dl_1) contract and lift up the upper wall of the digestive

tube, at the same time there develops in the suctorial apparatus a negative pressure, which causes the liquid matter of the prey, which is squeezed out by the mandibles, to enter the apparatus and fill its cavity (the sucking act). The next moment of feeding is characterized by the relaxation of the second group of sphincters (Text-fig. 11, s_2) and the contraction of the dilators (Text-fig. 11, dl_2) of the pharyngeal apparatus

TEXT-FIG. 12.



Dilators (dl_2) and sphincters (s_2) of pharynx (fg). Zeiss, DD, oc. 6.

(of the middle portion of the fore-gut); at this moment the corresponding part of the intestine is strongly dilated. Simultaneously the levators of the suctorial apparatus (Text-fig. 10, dl_1) relax and its sphincters contract (s_1). As a result the apparatus is closed, and the food which it contained is pressed into the dilated oesophagus (the act of forcing).

Then sets in the third act of food-taking—the closing of the pharyngeal apparatus (the suctorial apparatus being closed): the relaxing walls of the intestine force the food into the oesophagus and the stomach. In this way the larva makes a gulp of food (the act of swallowing). The whole act of food-taking

consists of the successive action of the suctorial and pharyngeal apparatus in the order described above.

Generally speaking, one may say that in the head of *H. caraboides* the fore-gut is organized as a forcing pump; during the suction the part of sucker of the pump is played by the upper wall of the suctorial apparatus, which is lifted up by the levators: simultaneously the pharyngeal apparatus contracts its sphincters and acts as the valve of the pump, preventing the food already swallowed into the stomach from being sucked back again.

At the next act—the forcing—the action of the different parts of the apparatus change. The pharyngeal apparatus acts as a sucking contrivance, and the proper suctorial apparatus comes to a state of rest and, pressing on the liquid it contains, produces a forcing action. This liquid cannot re-enter the body of the prey, as the latter, being worked into a lump, is tightly pressed towards the oral aperture and is, moreover, firmly squeezed with the mandibles.

Consequently not only liquid parts of the prey are swallowed, but also solid portions of its body, as for instance bits of tracheae, bits of the chitinous cuticula, &c. I have found similar remnants in the rectal sac of the larva of *H. caraboides* (Pl. 27, fig. 1, *ch*), a fact which would be impossible in the case of typical suctorial insects, as for instance the larva of *Dytiscus*.

In the latter the food enters the suctorial apparatus by canals in the mandibles which remain perfectly immovable during the suction of food; as the latter is submitted to a chemical treatment by ferments only, a mechanical action on the food-stuff is absolutely excluded.

It is interesting that the general idea of a sucking and forcing apparatus (consisting of two contrivances—the suctorial apparatus proper and the pharyngeal apparatus) is repeated with insignificant modifications in different arthropods, for instance in scorpions (Pavlovsky, 1917; Pavlovsky and Zarin, 1918), in Arachnida (Schimkevitch, 1884), and insects (lice (Pavlovsky, 1906; Sikora, 1916), bugs (Voronkov, 1907).

aphids, gnats (Nuttall and Shipley, 1903), and many others). This likeness may be explained by a convergence, caused by such a typical action, from the physical point of view, as the suction of liquids.

The gastric fluid (black in colour), which is poured over the seized prey, is regurgitated from the stomach under the influence of an antiperistaltic movement of its walls. The regurgitation is favoured by the absence of any adjacent cardiac valves.

The mid-gut or stomach has, as has been pointed out by Portier, many short blind papillae, or cryptae, formed by the evagination of the epithelial wall of the intestine into the body-cavity. On my preparations closely set mitoses of the epithelium were also visible.

It is instructive to compare the size of the cells of the intestine in just emerged and mature larvae of *H. caraboides*. A better representation than any description can be given by figs. 3 and 4 (Pl. 27), made at the same magnification. On the longitudinal section of the crypt (Pl. 27, figs. 3, 4, *crp*) of a young larva the number of cells is counted by units, and in the mature larva by tens. The cells themselves are much larger in the latter; the association of these causes has an influence on the size of the cryptae. The nuclei of the cells of the cryptae and the intestine itself in larvae of different age differ in size from each other comparatively less than the size of the cells themselves. The latter fact depends upon the real growth of the cells as well as their secretive action.

The growth of the cells in dependence on the growth of the larva is best demonstrated in the muscular fibres of the cover of the intestine. In the larva of the first stage the muscular fibres are very thin (Pl. 27, fig. 3, *mt*); whereas in the mature larvae they are fifteen to twenty times stouter (Pl. 27, fig. 4, *mt*). This growth is explained by the increase of the myofibrillae, which are differentiated in the sarcoplasm of the muscular cells.

The lack of material did not allow me to study the question of the regeneration of the intestinal epithelium, which in the adult beetle is periodically cast off and replaced by a new one,

which develops by karyokinesis of the cells of the cryptae (Rengel, 1898).

Even in the young larva the intestine for all its shortness forms a loop in the hind part behind the entering point of the Malpighian tubules. In the mature larva the latter are characterized by the accumulation of a large number of pigment grains (Pl. 27, fig. 5, *pg*) which concentrate in the basal half of the cells, and surround their nuclei. The parts of the cells which are turned to the lumen of the tubule look in dissection like a broad pale border with a slight indication of a faint striation (the Stäbchensaum) without accumulations of pigment.

It is of interest to compare here the size of the cells of the Malpighian tubules in the young and mature larvae of *H. caraboides*, drawn under the same conditions (Pl. 27, figs. 5, 6). In the young larva the nuclei occupy nearly the whole of the cells, which do not yet contain accumulations of pigments.

The ileum enlarges nearer to the end of the body into a rectal sac the structure of the walls of the latter being not all alike. The anterior wall of the sac, which borders on the ileum, has the same structure as the latter. It consists of a cylindrical, rather high epithelium, the free surface of which shows a conspicuous striation (Pl. 27, fig. 1, *ep*): such a structure of the protoplasm is not an exceptional one; analogous relations are found for instance in the ileum of the bee (E. Pavlovsky and E. Zarim), the rounded nuclei with minute thickly-crowded grains of chromatin occupying the middle part of the cells or in some places moving nearer to the surface of the epithelium.

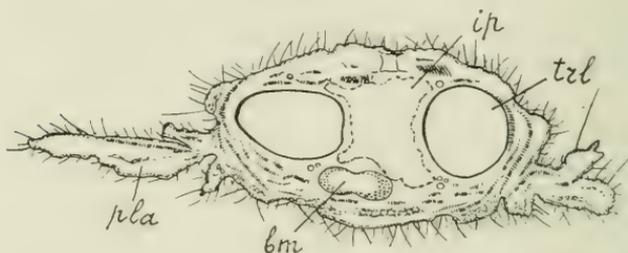
The latter forms on the back of the rectal bladder a thickened disc, the edges of which are strictly defined, as the cylindrical epithelium abruptly passes over into flat epithelium (Pl. 27, fig. 1, *ep_p*), which is of usual occurrence in the rectal part of the intestine of many insects.

In the young larva the cylindrical epithelium of the rectal sac (as well as the ileum) is correspondingly lower than in the adult larva (Pl. 27, fig. 2, *ep*), a fact which depends chiefly on the degree of growth of the cells, as no secretory processes, which could

influence the size of the cells, have been observed in the rectal sac of either the young or the mature larvae.

The greater part of the hind-gut is occupied by the rectal sac, which is located between the main tracheal side-trunks (Text-fig. 13, *trl*). The high epithelium of the rectal sac has not the significance of rectal glands, which are present also in the larvae of some insects with hemimetabolous development in the shape of longitudinal stripes of high cylindrical epithelium (for instance, the rectal glands of Orthoptera).

TEXT-FIG. 13.



Transverse section through one of hind segments of abdomen with pleural appendages (*pla*). Rectal sac (*ip*) between main tracheal trunks (*trl*). *bm*, brain. Zeiss, ob. AA, oc. 0.

The presence of a cylindrical epithelium in the rectal sac of *H. caraboides* is simply explained by the fact that in this instance the anatomical border between the parts of the hind-gut, which can be defined by superficial inspection, does not correspond to the histological border between the tissues composing it.

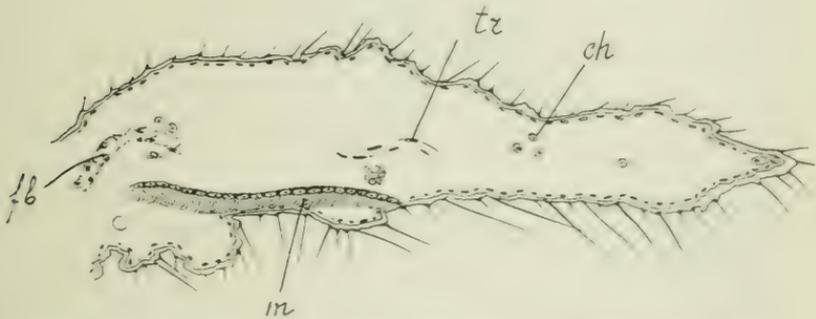
Analogous relations have been observed by me in the poison glands of Hymenoptera; its reservoir is similarly well differentiated, and histologically it is built of a wall which on one side bears a decidedly glandular character, and on the other of that flat epithelium peculiar to the duct of the acid gland (Pavlovsky, 1912).

RESPIRATORY ORGANS.

Two main longitudinal tracheal trunks enter a common spiracle chamber (atrium stigmatique, Text-fig. 16, *at*) situated

in the hind end of the body, which opens into a single breathing aperture (*a*). The details of the structure and function of this part of the respiratory system are given in Portier's paper, who says of the larvae of *Hydrophilus piceus*, *H. caraboides*, and *Hydrobius fuscipes*, the following: 'Il n'y a point de faux stigmates apparents sur les parois latérales du corps, et pas non plus naturellement de ramifications trachéennes qui se rendent à cette région des

TEXT-FIG. 14.



Longitudinal section of pleural appendage of abdomen. The tracheae (*tr*), muscles (*m*), fat-body (*fb*), and cells of haemolymph (*ch*) are visible. Zeiss, ob. DD, oc. 0.

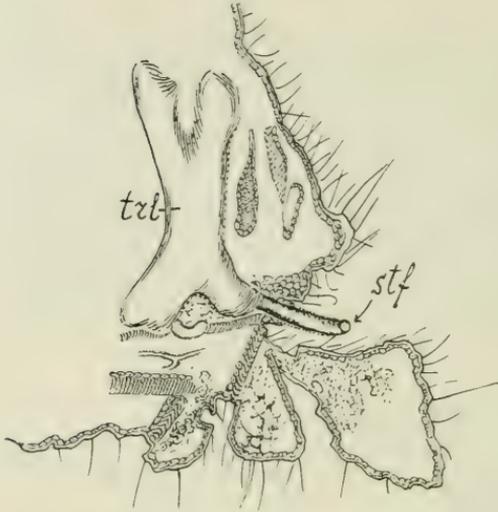
téguments comme on en voit chez les larves de *Dytiscides*' (loc. cit., p. 259).

This conclusion of Portier's is not precise, at least in the case of the larvae of *H. caraboides*, as the latter have nine pairs of lateral spiracles, two of which are on the thorax and seven on the abdomen.

The spiracles have an oval external outline, the latter showing two smaller ovals closely adjoining each other. The spiracles are located on the tops of conical evaginations of the integument, which in these places are covered with dark-brown chitin. These evaginations are situated on the sides of the body in the part of the pleural appendages of the larva (Text-fig. 15. *stf*). From the spiracles the initial narrow trunks of the tracheae start as a sort of vestibulum.

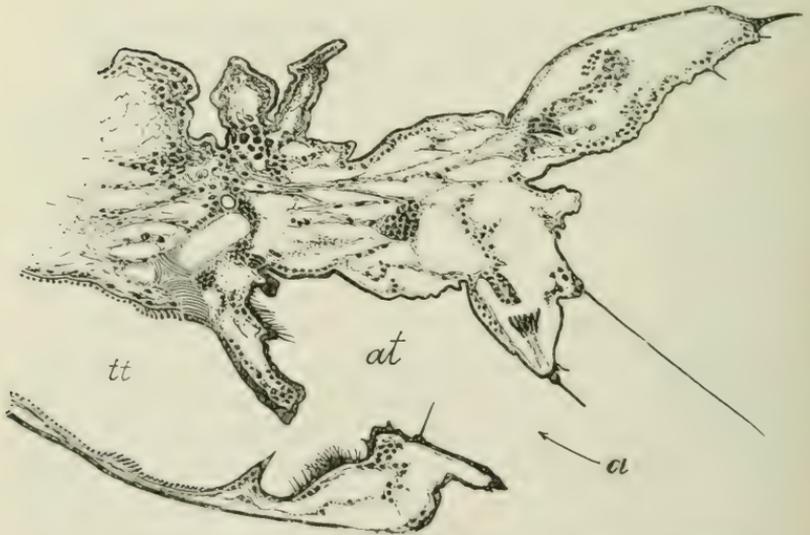
They are thick-walled and set inside with a dense brush of

TEXT-FIG. 15.



Transverse section of lateral wall of abdomen of larva of first stage through a closed spiracle (*stf*). *trl*, tracheae. Zeiss, ob. DD, oc. 4.

TEXT-FIG. 16.



Longitudinal section of hind end of abdomen with breathing apertures (*a*), atrium (*at*), and end of main longitudinal tracheal trunk (*tt*). Zeiss, ob. DD, oc. 0.

pillars, that is to say they have a structure similar in its idea to that of the spiracles and the beginning of the tracheae in other insects, as, for instance, in the caterpillars of *Lepidoptera* (*Bombyx mori*) (Verson and Guayat).

In the latter instance the inner brush-like structure of the vestibulum plays the part of a filter, serving to retain the dust from the air inhaled.

In the larva of *H. caraboides* the pillar-like cuticula of the initial parts of the tracheae evidently has no special application, since the spiracles themselves are closed by a chitinous membrane. That they are really closed, and therefore do not act as breathing apertures, can be well observed on a living larva immersed in a fixing fluid, as for instance Duboseq's mixture. The body is compressed by the action of the fixative, and the air comes forth from out of the spiracles in the form of silvery bubbles. Such bubbles appear in the larva of *H. caraboides* only on the hind end of the body, i. e. from the mouth of the tracheal chamber (atrium stigmatique). But the lateral spiracles join, as has been observed above, the main longitudinal tracheal trunks by means of narrow branches.

The tracheal trunks play also an accessory part—they serve as natatory air-bladders which facilitate the ascent of the larva and passive flotation near the surface of the water.

The larvae of *H. caraboides* are very sensible to lack of air. During the feeding process they must breathe; on account of this necessity the larva is obliged to assume a very peculiar and uncomfortable attitude when feeding, as is illustrated in Text-fig. 2.

If the larva is put into a small vial with water corked in such a manner that there is no bubble of air left inside the vial, and it cannot therefore find the surface of the water, it begins to make restless movements.

The larva starts up and meets with the glass wall of the vial. It struggles with the end of the abdomen, swims actively about, changes place in search of the surface of the water. Its restless movements become swiftor, it turns along the axis

of the body, draws in the end of the body and swiftly pulls it out again, as if trying to tear off something closing its breathing apertures. Probably such movements are made by the larva also under natural conditions after moulting, in order to throw off parts of the old skin or the chitinous lining of the tracheae, which might be left sticking to the hind end of the body and thus diminish the breathing aperture or even altogether shut it.

After fifteen to twenty minutes the periods of movement of the larva alternate with periods of rest. The movements of the legs and the mouth-parts become convulsive, and are repeated more and more rarely. The larva rises passively and lies under the surface of the glass in a bent attitude with its belly up. In this case the significance of the tracheae as a natatory apparatus is manifested best. The immovable larva is not yet dead and can regain life if transferred into an open vial; the time during which a larva can be kept without fatal results without air varies and depends on various circumstances, as temperature, individual peculiarities of the larva, &c. In some of my experiments the larvae came to life again after having been deprived of air for two and even seven hours.

The question arises whether the pleural appendages (Text-fig. 14) of the body and the caudal appendages play an accessory part of tracheal gills, but I have not been able to clear this up for want of material and other reasons.

In conclusion of these remarks on the respiratory organs we might discuss the finer structure of the tracheal trunks of *H. caraboides*. As in all insects the chitinous lining (intima) of its tracheae bears filiform thickenings, the taenidia, the windings of which give the tracheae a transversely striated aspect. In the larva of *H. caraboides* the taenidia have not necessarily the aspect of a spiral thread, as in some places there are free ends of them between two adjacent windings; thus presenting a picture of structure similar to that observed by Minot (1879) in *Hydrophilus* (*Hydrous*) *piceus* (cited after Berlese, 1909).

The degree of independence, or rather of precision, of the

taenidia is disputed by R. Schneider (1902), who writes on the tracheae of *Hydrophilus piceus* as follows: '...eine faserartige Verdickung der Intima existiert aber nicht, es kann also nur von einer Spiralfalte geredet werden. Genaue Untersuchung zeigt folgendes. Zu unterscheiden sind die Furchen, welche eine Falte begleiten, ferner die steil aufsteigenden Faltenwände und die flache Faltendecke, deren Breite im Durchschnitt der einer Furche entspricht. An geschwärztem Materiale treten entweder die Wände als schwarze Striche, die parallel nebeneinander verlaufen, oder die Faltendecken als schwarze Streifen scharf hervor, während die Furchen immer mässig dunkel erscheinen' (loc. cit., p. 505).

When stained with iron haematoxylin, or Giemsa's stain, the taenidia of the main tracheal trunks of the larva of *H. caraboides* stand out vividly on the colourless ground of the chitinous cuticula, from which they are more separated, than the structures in the shape of grooves or folds.

In the taenidia just described I succeeded in observing character of their structure which bears evidence in favour of their strong individualization from the lining of the tracheae, of which the former are of course a product. Thanks to the comparatively considerable thickness of the taenidia, their heterogeneous structure is plainly visible in sections (transverse, or better in tangential sections). The taenidium is a thin-walled capillary tube, as the darker wall and the lighter lumen are easily distinguished.

This structure has a certain meaning. The walls of the tracheae must possess a considerable elasticity, as for breathing purposes their lumen must be open for the passage of air. Besides, they perform another function of a more mechanical significance, i. e. they are the ligaments that hold the organs in their mutual position. Finally, the tracheae represent elastic pillows, which lie between different internal organs.

The latter function of the breathing tubes is clearly visible in the larva of *H. caraboides*, since the greater half of the cross-section of the abdomen is occupied by the tracheae. Between the latter passes the hind intestine, which, when over-filled with excrements, presses on the adjacent walls of the

tracheae. These latter resist the pressure owing to the elasticity of the included air (which acts only when the breathing apertures are closed), as well as in consequence of the structure of the tracheal walls themselves. The taenidia having the structure of pipes resist any mechanical influence, in particular pressure, more completely than would taenidia of the same diameter if they were solid. An analogous principle in the resistance of materials is applied in the engineering practice. The forms of taenidia are not the same in different insects. Passing over the details we might point out that the taenidia of hemipterous *Zaitha fluminea* have the form of a groove, which structure gives the trachea a mechanical advantage.

ON THE INTEGUMENT AND ITS APPENDAGES.

The body of the larva of *H. caraboides* is thickly set with hairs. These are of several kinds :

(1) Fine hairs ; the most numerous on the segments and the pleural appendages of the abdomen.

(2) The long terminal threads of the pleural appendages.

(3) The scarce setae.

(4) The setae on a pigmented and elevated base.

The latter are arranged in fours on the tergites of each abdominal segment, forming together four longitudinal rows.

In connexion with its dense hairiness the integument of the larva of *H. caraboides* possesses a high degree of sensibility. It is sufficient to touch one of the long hairs of the pleural appendages of the abdomen (Text-fig. 1) to make the larva instantaneously turn its head to the side where the irritation comes from and to seize the disturbing object with its mandibles.

These organs are useful in two respects : (1) They serve the larva for self-defence, as its whole body with the exception of the head and perhaps also of the thorax is very soft, easily vulnerable, and accessible to the attacks of different carnivorous inhabitants of fresh water ; receiving due notice of any possible danger, the larva gets time to put its defensive organs into action, i. e. its sharp and strong mandibles. (2) If a weaker or harmless creature happens by chance to touch one of the

long sensory hairs of the larva it becomes its prey, as it gets very little chance of escaping the murderous mandibles; in this latter case the sensory hairs attend indirectly the feeding wants of the larva.

The sensory hairs of the pleural appendages are of considerable length; in the young larva of the first stage the hair might be twice or thrice as long as the pleural appendage. In consequence the receiving surface of the body of the larva is considerably increased, and the latter is able to orientate itself better in the surrounding medium, both for taking measures of defence and for the capture of prey. The armature of the head of the larva is usually kept in readiness, i. e. all oral appendages are wide open and have only to contract at the suitable moment.

An analogous sensibility is found also in the hairs on the tergites of the body. When the irritation comes from above, the larva throws its head up and backwards with the same quickness and generally attains its end.

But the larvae of *H. caraboides* are not absolutely safe from peril. For instance, they are ready to devour each other if kept in close vials and fed unsatisfactorily. An examination of the skins of devoured larvae showed that they were all wounded in the tergites of the thorax only. This place is, so to say, the Achilles-tendon of our larvae.

The thoracic integuments are also not devoid of sensibility; but if the attacking animal succeeds in seizing it at once by the tergites of the thorax, the larva of *H. caraboides* finds itself in a defenceless position, because in this case it cannot throw back its head and put its mandibles into action.

In some of the larvae the pleural appendages and their terminal hairs were partly torn off. The aperture of the wounded places were shut by dark-coloured chitinous plugs of an evidently inflammatory origin. Doubtless the respective larvae had been in a position endangering their lives, and they had come out safely thanks to their sensory and tactile apparatus, a partial loss of which is not fatal.

After these biological remarks we shall discuss the structure

of the integumental appendages of the larvae of *H. caraboides*.

The greater part of the hairs are chaetoids, i. e. organs developed exclusively from the chitinous cuticula; the hypoderm under them does not show any peculiarities in its structure (Pl. 27, fig. 7).

The terminal (long) hairs of the pleural appendages are also chaetoids, but they take their origin from a differentiated terminal platform under which there are located large cells with large nuclei. These cells are probably of a neural character; but this could have been proved only by application of special methods of staining (with methylene-blue for instance), which did not enter into the task of my work.

The setae of the integument are distinguished by greater length, stoutness, and stiffness (Pl. 27, fig. 8). The base of the seta is lodged in a chitinous cup-like tubercle (theca, *ct*) in the interior of which is found a differentiated ring, which is stained black by iron haematoxylin. Under this chitinous armature lie two cells (fig. 8, *ctc*), of which the larger one is trichogenous and the smaller thecogenous. Together they form a kind of follicle which invaginates into the cavity of the body from a row of hypodermal cells.

The setae described perfectly correspond with the type of dermatochaetae, according to the classification of the integumental appendages given by N. Nassonov (1901). In particular they belong to the dermatochaetae plerothecatae (E. Pavlovsky, 1917), i. e. setae with a solid theca.

Finally, the largest setae (Pl. 27, fig. 9, on a pigmented base) are distinguished by the most complicated structure. The long chitinous rod (fig. 9, *tc*₁) has its base set into a barrel-like elevation of the integument. Into the upper part of this barrel a short cylindrical cartridge (fig. 9, *tc*₂) is inserted, with which, properly speaking, the seta is articulated.

Under this formation there lie two large cells (*tc*), one of which is distinguished by an enormous nucleus (the trichogenous cell); in the protoplasm the borders between the nuclei are not visible.

The hypodermal cells (Pl. 27, fig. 9, *hp*) are distinguished from the ones just described by a smaller size. In the hollow of the cartridges there is a trace of lighter protoplasm directly connected with the protoplasm of the trichogenous cell. In this place there are located cells which stain pale and have nuclei containing but little chromatin, and which are evidently the nuclei of the hypoderma of the cartridges. From beneath this structure emerges a rather stout sinuated fibre, which terminates in a darker widening with a slender terminal appendage (Pl. 27, fig. 9, *n*? a nervous fibre?); the nervous fibre reaches from the body-cavity to the trichogenous cell (Pl. 27, fig. 9, *ns*?).

These setae can be characterized as dermatochaetae dupli-thecatae, as their basal cup (the theca) is double.

In conclusion, a few more words must be said regarding the structure of the pleural appendages of *H. caraboides*. The hypoderm and the chitinous cuticula are not distinguished by any particular peculiarities from the usual integument of the remaining parts of the body. They are covered with chaetoids; the terminal long hairs are connected at their bases with nervous cells.

The muscles are fastened to the lateral wall (Text-fig. 14, *m*) of the pleural appendage and their function is to contract the appendage. Through the lumen of the latter pass slender and few tracheae and the fat-body; besides the indispensable cells of the haemolymph are present.

ON THE NERVOUS SYSTEM.

The central nerve-cord consists, excluding the cephalic ganglia, of three pairs of thoracic and eight abdominal ganglia, of which the last ones, i.e. the tenth and eleventh, almost touch each other, and the ninth is situated nearer to the tenth than to the eighth. The thoracic ganglia are larger than the abdominal ones; the first abdominal ganglion closely touches the last pair of thoracic ganglia. On the whole the ganglia are disposed fairly equally, and the whole cord shows a more or less regular structure.

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DESCRIPTION OF PLATE 27.

Fig. 1.—Transverse section through rectal sac of mature larva of *Hydrophilus caraboides* at the anterior end; high (*ep*) and low (*epp*) epithelial walls are visible. In the cavity of the sac are bits of torn and ground chitin (*ch*), swallowed together with liquid parts of food. Duboseq, iron haematoxylin. Zeiss, ob. AA, oc. 2.

Fig. 2.—Same, but in a larva of the first stage. Duboseq, iron haematoxylin. Zeiss, ob. AA, oc. 2.

Fig. 3.—Part of longitudinal section of middle intestine (stomach) with crypts (*crp*) of a larva of first stage. *mt*, circular muscles; *ml*, longitudinal muscles. Duboseq, Giemsa's stain. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 1.

Fig. 4.—Crypt of stomach of mature larva. Duboseq, iron haematoxylin. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 1.

Fig. 5.—Oblique section of Malpighian tubule of mature larva. In the protoplasm of cells conspicuous differentiation of layers and copious accumulation of pigment grains (*pg*). Duboseq, Unna's Polychr.-Methylenblau. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 0.

Fig. 6.—Same, in a larva of first stage. Duboseq, iron haematoxylin. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 0.

Fig. 7.—Hair (*cht*) of chaetoid type from back of larva. Under the hair is the usual hypodermic cell (*hp*). *ch*, chitine. Duboseq, iron haematoxylin. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 2.

Fig. 8.—Hair with thaecca (*tc*) and ampulla (*ctc*) consisting of two—the thaeccogenous and trichogenous—cells. *ch*, chitin; *hp*, hypodermic cell; *cht*, chaetoid. Duboseq, Giemsa's stain. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 2.

Fig. 9.—Hair (*tr*) with double thaecca (*tc*₁, *tc*₂) and attending nerve (*ns*? *n*?); *tc*, ampulla; *hp*, hypodermic cell; *cht*, chaetoid. Duboseq, iron haematoxylin. Zeiss, ob. $\frac{1}{12}$ hom. imm., oc. 4.

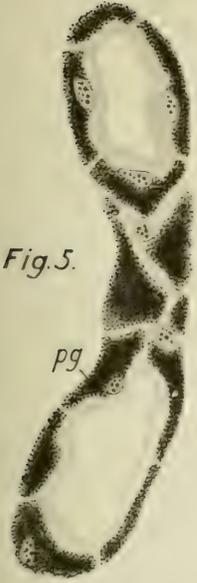


Fig. 5.

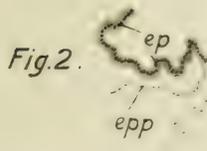


Fig. 2.



Fig. 1.



Fig. 6.

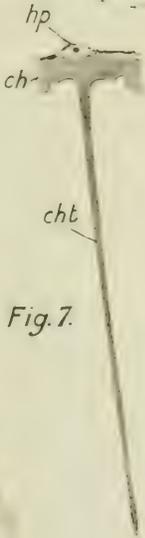


Fig. 7.

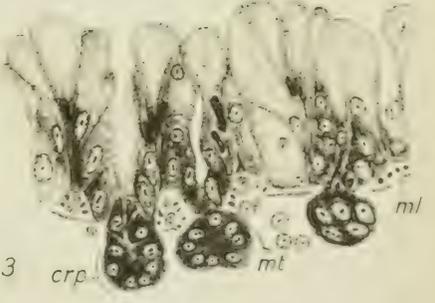


Fig. 3.

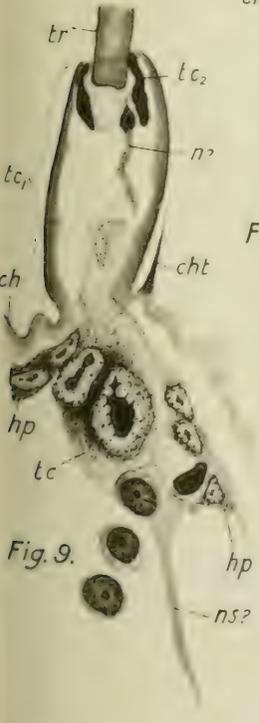


Fig. 9.

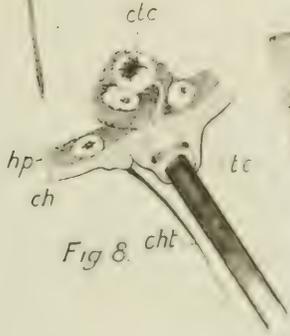


Fig. 8.

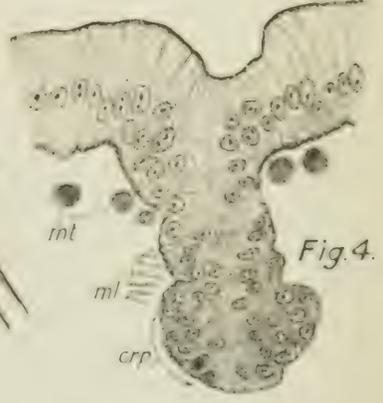


Fig. 4.

A further Account of the Spermatogenesis of Lice.

By

H. Graham Cannon.

With 1 Text-figure.

IN 1919 the late Professor Doncaster and the present author investigated the gametogenesis of the lice *Pediculus corporis* and *Pediculus capitis*. An account of this work was published during the following year (3). The cytology of the spermatogenesis was found to be so peculiar and in some respects unique, that it was decided to extend the work to other lice. At the time of his death Professor Doncaster was working on the gametogenesis of the horse-louse and the dog-louse. The material collected by him together with his notes was handed over to me by Professor J. Stanley Gardiner.

A perusal of the notes showed that Doncaster had only cursorily examined his material and had not arrived at any new conclusions. For this reason it was not considered advisable to publish any further account based on the new material. However, at the end of 1919 an account of the spermatogenesis of *Pediculus vestimenti* was published by Miss Foot (4) which differed so considerably from that published by Doncaster and myself that a thorough examination of Doncaster's new material seemed called for, and the present note is an account of that work.

The material examined consisted of testes of the dog-louse (*Lignognathus piliferus*) and of the horse-louse (*Haematopinus asini*). Some fixed material of the last-named species, obtained from a donkey, was kindly sent to me recently by Mrs. Bisbee of Liverpool University, who was assisting Professor Doncaster in this work. I also obtained

some specimens of *Haematopinus consobrinus* from a monkey that died in the Zoological Gardens.

As with *Pediculus*, the material was found to be extremely refractory with regard to fixation. The best fixation generally was obtained with Flemming and Flemming without acetic. For the various stages of the mitosome Mann's corrosive osmic fixative gave excellent results. Kopsch's method of prolonged fixation in osmic was found to be useless—the unstained sections showing nothing impregnated. For staining—as with *Pediculus*—the only stain of any considerable use was Heidenhain's iron haematoxylin. Altmann's methyl green-fuchsin was used for some of the material but did not give results of much value.

The most important points in the spermatogenesis of *Pediculus* as described by Doncaster and myself are as follows: (1) While the somatic chromosome number is twelve the spermatogonial figures show only six. This apparently haploid number of chromosomes in the spermatogonia we ascribed to premature pairing. (2) There is only one spermatocyte division, which is extremely unequal, leading to the separation of a minute 'polar body-like' cell which degenerates. (3) The centrosome of the spermatid is double and from each half an axial filament grows out so that the developing spermatozoa have two conspicuous axial filaments.

The account given by Miss Foot differs from our description in the following points: (1) The somatic number of chromosomes is stated to be ten, and in the few spermatogonial groups found it is also stated to be ten. (2) There is always an unequal bivalent in the first spermatocytes. (3) Although the division of the second spermatocytes was not observed, it is assumed that this division is similar to that in corresponding mitoses in 'other species of Hemiptera'.

With regard to the chromosome number, Miss Foot states that the chromosomes are so small and so frequently constricted that the estimated number can always be questioned. The chromosomes certainly are very small, but in equatorial plates of follicle cells and of cells that we called spermatogonia we

experienced no difficulty in making an accurate count of the chromosomes, and, as we stated, the frequency with which we were able to make these counts can leave no doubt as to the actual number of chromosomes. No evidence was found of an unequal pair. The sizes of the chromosomes varied somewhat and this, combined with their smallness, would have made it possible to postulate dimorphism in the chromosomes only if the difference in size were very marked.

The third difference between the two accounts, namely that referring to the unequal spermatocyte division, is the most important. Miss Foot maintains that the first spermatocyte chromosomes of *Pediculus* are very similar to those of *Euschistus*, and states that as the first spermatocyte chromosomes 'have the same morphological characteristics as the corresponding stages in other species of Hemiptera, it is logical to assume that the second spermatocyte chromosomes would be equally typical'. Unfortunately the paper is not illustrated by the usual excellent photographs which characterize so many of Miss Foot's works, and it is difficult to compare her *Pediculus* figures with her series of photographs of spermatocyte divisions of *Euschistus*. However, by comparing our preparations of *Pediculus* with Miss Foot's *Euschistus* photographs I have to confess that I cannot see any resemblance at all.

Further, Miss Foot's observations were made on smear preparations. In such preparations I think one may say that it is highly probable that spermatocytes dividing in such an unequal manner as we described would be so distorted, if not completely collapsed, as to be unrecognizable. The small polar body-like cell which is given off from the spermatocyte is, at the moment of its formation, a long finger-like process. In a smear preparation it is most probable that this attenuated process would be either torn away from the remainder of the dividing cell or else the whole cell on being freed from the surrounding cells would round itself off and appear as a cell in equal mitosis.

Sections of the testis of *Pediculus* show an orderly sequence of stages from the spermatogonia at the free end to the fully

formed spermatozoa at the thicker end nearer the vas deferens. The position of a cell is thus to some extent an indication of the stage of its maturity. In smear preparations this orderly arrangement is obliterated, and hence it appears to me it would be an easy matter to overlook the unequal spermatocyte divisions in such preparations, even if they were demonstrable, unless their presence were suspected.

Another aid in working out the spermatogenesis is the development of the cytoplasmic inclusions, especially the mitosome, *pari passu* with the maturation stages. It is significant that Miss Foot does not mention or figure the mitosome which is so conspicuous in the later stages of spermateleosis.

From the examination of the new louse material it is clear that the course of spermatogenesis in the three species observed is, in its main features, the same as that in *Pediculus corporis* and *Pediculus capitis* as described by Doncaster and myself, and does not agree with the process as described by Miss Foot. This fact is in itself strong indirect evidence in support of our original description.

The testes of the three species examined were all similar to those of *Pediculus*, those of *H. consobrinus* being somewhat more pear-shaped and less ovoid. The arrangement of the follicles of cells was the same in all cases, the spermatogonia being at the free end and the mature spermatozoa being found at the broader end at the entrance to the vas deferens. A conspicuous mitosome is formed in the spermatid by the coalescence of the mitochondria as in *Pediculus*. In the horse-lice all stages of the single spermatocyte divisions were found, and these were, in all essentials, closely similar to those in *Pediculus*. The metaphase spindle is always eccentric, the main part of the cell being occupied by the mitochondrial mass. During anaphase the spindle elongates very considerably, and one pole, with its centrosome, is carried outwards from the main body of the cell on a long finger-like projection extending outwards to a distance of the diameter of the cell. This process, with its contained chromosomes, breaks away from the body of the cell, forming a minute 'polar body-like' cell.

In the dog-louse and in *H. consobrinus* I have been unable to find the spermatocyte divisions, in the latter case perhaps because my material was scarce; but in the former case all mitoses were extremely rare. As, however, all the other stages of spermatogenesis in the two forms corresponded closely to those of the horse-louse and of *Pediculus*, it is logical to infer that the missing spermatocyte divisions will be of the same type.

In the horse-louse the number of chromosomes in spermatogonial metaphase plates is nine. Spermatocyte prophases indicate a similar number, but not so clearly. In anaphase the chromosomes are too clumped to count with certainty. In *H. consobrinus* the spermatocyte prophases show seven chromosomes. In the dog-louse no reliable count was obtained.

After the telophase of the spermatocyte division of the horse-louse the centrosome appears to double as in *Pediculus*, and from this double centrosome the double axial filament of the tail of the spermatozoa commences to grow out. At this stage the chromatin of the nucleus appears in a clumping which is very irregular but in which a conspicuous nucleolus is always present (Text-fig. 1). As the spermatid elongates the nucleolus becomes attached to the nuclear membrane so that it projects partly in and partly out of the nucleus (Text-fig. 1). It usually appears near the apex of the spermatid but its position is not definite. As spermateleosis proceeds all the staining material in the nucleus disappears, and with it the nucleolus becomes gradually smaller and also disappears (Text-fig. 1). This process does not take place in the dog-louse nor in *H. consobrinus*. In *Pediculus* we described a deeply-staining body which lies within the nucleus at the posterior end of the heads of fairly late spermatids. This does not occur in any of the new lice examined, and probably corresponds to the late appearance of the nucleolus described here in the horse-louse.

In *Pediculus* we described a body which we provisionally called the 'acroblast'. In referring to acrosome formation generally, Gatenby and Woodger (8) state 'according to the account given for *Smerinthus* by Gatenby (6) and for *Pediculus*

by Doncaster and Cannon (3) all the Golgi apparatus is taken up in the formation of the acrosome'. This statement might pass

TEXT-FIG. 1.

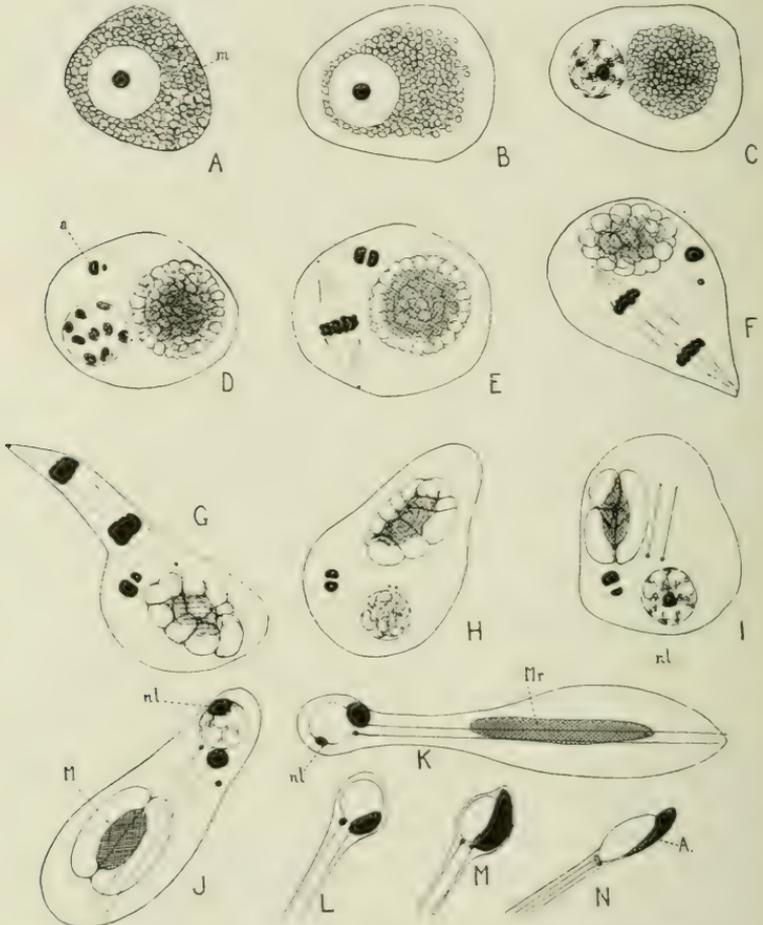


Diagram illustrating the spermatogenesis of lice. *a.*, acroblast. *A.*, acrosome. *m.*, mitochondria. *M.*, mitosome. *Mr.*, mitosome remnant. *nl.*, nucleolus.

without comment were it not that it is grossly inaccurate. We stated 'we have been unable to determine with certainty either the origin or the nature of the "acroblast", but on the analogy of the bodies in Lepidoptera, which Gatenby calls by

the same name, we suspect that it belongs to the Golgi apparatus. Attempts to prove this by Kopsch's method . . . have, however, failed to confirm this belief. . . . We took this view that the body with which we were dealing was probably the 'acroblast' on account of its behaviour in moving ultimately towards the tip of the developing spermatozoon, and we associated it with the Golgi apparatus because we were convinced that the 'acroblasts' of Gatenby were really the Golgi bodies of earlier writers—a conclusion at which Gatenby ultimately arrived—not in the paper on *Smerinthus* quoted by Gatenby and Woodger, in which no mention is made of the Golgi apparatus, but in a paper published two years later (7).

In the three species of lice examined there is, in each case, a conspicuous body which corresponds to the 'acroblast' of *Pediculus*. In the horse-louse its history could be made out most clearly. It first appears just prior to the prophase of the spermatocyte division (Text-fig. D). In *Pediculus* we stated that the 'acroblast' may sometimes be double at the time of its first appearance, but is always a single spherical body later. In the horse-louse it is usually, but not always, double, and remains so until the spermatid has formed and is elongated. Its appearance is very striking and its shape somewhat difficult to describe. Its two parts are sometimes equal (Text-fig. H), but more usually one is smaller than the other (Text-fig. D and J). The shape of each half may best be described as 'bun-shaped'. They are placed with their flat sides together but are never touching. They are always separated somehow by a transparent acromatic layer. Sometimes the two halves are seen to be comparatively far apart (Text-fig. F and J). If the difference in size between the two halves is great the smaller half is almost spherical. In the elongated spermatid just before the nucleolus finally disappears the acroblast is a single body (Text-fig. K) placed against the nucleus close to the double centrosome, as in *Pediculus*. It thus looks highly probable that during spermateliosis the double acroblast loses one of its halves, which passes away from the nucleus and

disappears. The stages in which the two halves are far apart (Text-fig. F and J) very probably illustrate this process taking place.

In a recent paper on the sperm of Hemiptera Bowen (1) enumerates fifteen cases by other authors besides his own description in which the acroblast of the spermatid in giving rise to the acrosome, which forms the tip of the spermatid, gives off a body termed by him 'the Golgi remnant', which is lost in the protoplasm of the tail region of the spermatid. Probably the case of the horse-lice must be added to this list.

In the dog-lice and in *H. consobrinus* the acroblast is single as in *Pediculus*.

Whether or not this body which we tentatively called the acroblast is really the homologue of the Golgi apparatus in other cells cannot be said with certainty until a more precise definition of the Golgi bodies is found. In the dog-lice in Mann-Kopsch preparations there are indications of the acroblast arising from two or three scattered granules which may be the true Golgi bodies. However, these are not impregnated by prolonged fixation with osmic acid. A character that is as specific of Golgi bodies as is their staining reactions is that they always show a definite relation to the centrosome during mitosis. It is significant that in all the lice examined the acroblast is peculiar in that it exists as such during the spermatocyte division, and also it does not show any definite spacial relations to the centrosomes of the dividing cell.

With regard to the mitochondria, preparations of dog-lice material fixed in Mann-Kopsch completely confirmed the account that we gave of the development of the mitosome in *Pediculus*. There was a slight difference in that the earliest spermatogonia in the dog-lice showed the cytoplasm completely filled with vacuolated mitochondria, whereas in *Pediculus* Professor Doncaster was of the opinion that some of the earliest spermatogonia showed granular mitochondria. However, the gradual fusion of these mitochondrial vacuoles to form a mitosome consisting of a central chromophilic mass surrounded by two large vacuoles took place exactly as in *Pediculus* and will not

be described further here. The process is figured in Text-figs. A-J.

We pointed out that the appearance of the mitochondrial mass that we described was ascribed by Gatenby to faulty fixation. This author favours the view rather, that the apparently vacuolated mitosome is the result of faulty fixation on the 'spireme' type of mitosome that he describes in *Lepidoptera* (5). In a recent paper of Bowen's (2), 'On the structure of the "Nebenkern" in the insect spermatid', there is a review of the work on this subject, and from this it is clear that the description given by practically all other workers, besides the very exact account given by Bowen himself, agrees closely with the development of the mitosome as we described it in *Pediculus*.

Apart from the fact that Gatenby's results have not been confirmed by any other worker; there are several points in his original description of the origin of the 'spireme' mitosome which make one cautious in accepting his views. He states 'that the spireme is formed from the chromophile rim (outer layer) of the mitochondrial body, while the substance, in which the spireme lies, is the coalesced inner substance (chromophobe part) of the mitochondrial layer'. He gives three diagrammatic figures illustrating the process by which this transformation is brought about and these figures are certainly very misleading. In the first one are drawn optical sections of spherical mitochondrial granules, in the other two these granules are shown elongating and fusing together and apparently thus forming, first of all loops, and finally a spireme. Now if two bodies with a chromophilic outer layer and a chromophobic inner substance coalesce, whether they are elongated or not, they merely form a larger body of chromophobic inner substance with a larger chromophilic outer layer. They do not form a thread of chromophilic substance in a mass of chromophobic substance, at least not by the mere act of coalescence, as Gatenby's figure indicates. What these figures really show is the lengthening of the optical sections of spheres, which are of course circles, to form loops, and their joining together to

form a continuous thread. This process may be possible; but what Gatenby has overlooked is the fact that the circles in his first figure, and presumably the drawn-out loops in his second figure, do not actually represent loops of thread at all but surfaces.

I do not wish to maintain that it is impossible to obtain from a system of mitochondrial bodies, such as Gatenby describes, a mitosomal spireme. If it is imagined that the chromophilic substance disappears from the interfaces between the adhering bodies and remains in the interspaces then some sort of a network of chromophilic substance would be obtained which might be described as a spireme, but this would not be a continuous single thread but a much-branching system of threads.

In viewing the mitosome, at any level of focus, one sees a coiled thread-like mitosome just as Gatenby figures, but on focusing up and down one is able to see that without any doubt it is actually a plate work formed by a system of vacuoles.

SUMMARY.

The main results of the examination of cytological preparations of the testes of the horse-louse (*Haematopinus asini*), of the dog-louse (*Lignognathus piliferus*), and of *Haematopinus consobrinus* may be summarized briefly.

1. In all main points the spermatogenesis of these three species of louse agrees with that described for *Pediculus corporis* and *Pediculus capitis* by the late Professor Doneaster and the present author. Miss Foot's account of the spermatogenesis of *Pediculus vestimenti* is criticized.

2. In the elongating spermatid of the horse-louse, the nucleolus appears for a short period as a chromatic mass adhering to the nuclear membrane, projecting partly in and partly out of the nucleus.

3. The acroblast of the horse-louse is usually a double body consisting of two 'bun-shaped' halves which are sometimes of equal size, and which are separated with flat sides together

by a transparent achromatic layer. The 'acroblast' exists as such during the single spermatocyte division, and finally forms the 'acrosome' of the spermatid.

4. The description of the mitosome given for *Pediculus corporis* is substantiated by an examination of that of the dog-louse. Gatenby's description of a 'spireme mitosome' is criticized.

IMPERIAL COLLEGE OF SCIENCE, SOUTH KENSINGTON.

May 6, 1922.

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Cannibalism in *Amoeba vespertilio* (Penard).

By

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With Plates 28, 29, and 3 Text-figures.

1. MATERIAL AND METHODS.

TOWARDS the end of July 1920 an old hay infusion, which had been made some ten years previously and had been left untouched in the laboratory since then, was examined to find out what organisms it still contained. Among a fairly abundant fauna, which included Ciliata and some Flagellata, a good supply of small amoebae was obtained from the bottom deposit.

By transferring portions of this bottom deposit to Petri dishes and adding tap-water, these amoebae were cultivated. Although some of the cultures failed, others thrived well, especially those in which the amoebae were feeding on the layer of small diatoms which quickly spread over the bottom of the dish and were present also in the clumps of vegetable débris.

From time to time aquarium water and boiled hay infusion were added to replace the loss of fluid by evaporation.

The amoebae were examined on slides with and without cover-glasses; but this method was soon abandoned in favour of hanging drops, made in the following way:

A glass ring, vaselined on both surfaces, was placed upon an ordinary slide. A cover-glass, upon which a drop of the culture fluid from the bottom of the culture had been placed, was inverted, lowered upon the glass ring and then pressed

down, so that the drop hung in a sealed chamber. A small drop of water was placed on the bottom of the chamber before it was closed, as an additional precaution against evaporation. These preparations were similar to those used for the study of *Helkestimastix* (Woodcock and Lapage, 31) and in them the amoebae could be observed for several days. It was found that, after a day or two, all the organisms in the drops, and especially *Ciliata* such as *Paramoecium bursaria*, became very sluggish; but they could be readily revived by lifting the cover-glass for a few minutes and replacing it again. The renewal of the air in the chamber, effected in this way, had a remarkably invigorating effect upon the organisms, the *Paramoecium bursaria*, for example, immediately resuming their normal lively activity. The method had the additional advantage that the organisms under observation could be fixed at any desired moment, by simply removing the cover-glass, spreading out the drop upon it, after removing the adherent vaseline, and then dropping the film on to the surface of a dish of fixative.

Permanent preparations were constantly made in this manner. In addition amoebae were daily taken from the cultures and fixed upon albumenized slides, the culture fluid being spread out in a thin film before the fixative was added.

The fixative used was that introduced by Dr. H. M. Woodcock and was made up of two parts of a saturated solution of corrosive sublimate in water to one part of absolute alcohol with glacial acetic acid in the proportion of 5 per cent. Most of the slides were stained by Dobell's alcoholic modification of Heidenhain's iron haematoxylin method (Dobell, 8). This method, though in some respects inferior to the watery iron haematoxylin method, gave very good results. It has the double advantage over the watery method of being quicker and of avoiding the treatment of the preparation with water or the lower grades of alcohol, in which many organisms, unless previously hardened overnight in 70 per cent. or 90 per cent. alcohol, are frequently washed off. It is undoubtedly a very useful and reliable method for staining all kinds of

Protozoa. Other stains used were Heidenhain's watery iron haematoxylin and Delafield's haematoxylin. A counterstain was not used, since it is quite unnecessary after these stains and, in the opinion of the writer, tends to obscure, rather than to improve, the results. Both Bausch and Lomb and Zeiss microscopes were used, the ordinary high power dry lens being sufficient for most of the observations on the living objects; but, when higher magnification was needed, a Zeiss apochromatic oil immersion was used.

2. CHARACTERS OF AMOEBIA VESPERTILIO.

Considerable difficulty was experienced in the identification of the amoebae present in the cultures. It is not my intention to enter here into this vexed question; but it is necessary to record the opinion that species of amoebae established upon descriptions of their external characters alone, without a prolonged study of them under all conditions and a knowledge of their nuclear apparatus and life-history, supported by the evidence of stained preparations, must be regarded as provisional only.

Until such detailed knowledge is available, however, the existing data must be utilized; and, when I say that the amoeba which forms the subject of this paper corresponds with that described by Doflein (9) and Penard (21) as *Amoeba vesperilio*, it should be understood that I do not necessarily regard *Amoeba vesperilio* as a true species.

The account and figures of this amoeba given by Doflein (9) are so excellent, and my own observations upon it confirm his so exactly, that it is unnecessary for me to give here more than a summary of its distinctive characters.

Amoeba vesperilio is a small amoeba, showing a well-marked contrast between clear ectoplasm and granular endoplasm, and is, when healthy, very active. Its pseudopodia are typically branched, with pointed ends, and are composed mostly of ectoplasm. When the amoeba is creeping along a substratum, it assumes a very characteristic shape, resembling that of a bat's wing or of a duck's foot. The form is,

however, very variable and, under certain chemical and physical conditions, star-shaped and other forms occur.

The nucleus is vesicular, with a well-marked endosome, which stains deeply and shows, in preparations which have been suitably differentiated, a well-marked meshwork structure (Pl. 28, fig. 1). This endosome is surrounded by a clear halo in which no structure can be made out, and this clear area does not appear to be separated from the endoplasm of the amoeba by a definite nuclear membrane. The area of endoplasm immediately surrounding the nucleus stains, however, more deeply than the rest of the endoplasm, an effect which is due to the heaping up, as it were, in this region, of the fine granules of deeply-staining matter which are distributed throughout the endoplasm on the strands of its meshwork.

The size of the amoeba varies considerably. Dofflein (9) gives the size of the motile creeping forms as being 220–250 μ long by 40–60 μ broad, whilst the star-shaped forms measured from 60–150 μ , according to the length of their pseudopodia. He says that the nucleus varies from 10–15 μ in diameter and the endosome from 7–10 μ . The amoebae in my cultures were rather smaller than this, the motile forms reaching 200 μ long and sometimes rather longer, when the pseudopodia were well extended; but the majority of both motile and star-shaped forms varied between 60–100 μ in diameter. The nucleus measured from 7–9 μ in diameter and the endosome from 4–7 μ . It should be mentioned, however, that these measurements were made upon stained preparations in which some shrinkage may have occurred.

The endoplasm usually contains numerous vacuoles as well as abundant granules. One or more contractile vacuoles are present. Penard (21) states that generally there is only one, but that two or three are often present, one of which seems to be the principal one, and that there is almost always a great number of vacuoles distributed here and there, which appear and disappear as if they played the part of contractile vacuoles. I have also found that the presence of several contractile vacuoles is a frequent feature of the amoebae in cultures, but

amoebae with only one contractile vacuole were at least as common.

Doflein (9) placed some of his amoebae in a culture which contained *Frontonia leucās* which were full of green zoochlorellae. The amoebae fed upon the 'remains' of the *Frontonias* and themselves became infected with zoochlorellae. A similar infection occurred in some of my cultures also, the zoochlorellae being acquired in this case apparently from *Paramoecium bursaria*. These zoochlorella-infected amoebae were not, however, used for any of the observations described below and there is no evidence that cannibalism occurred in them.

The cultures also contained other small amoebae, which were definitely different in external appearance from the *Amoeba vesperilio*, and which remained so. As far as I was able to judge from external characters only, these small amoebae were of the *Amoeba limax* or *Vahlkampfia* type. Their average size was 28-30 μ long by 6-8 μ broad; but their length varied from 20-50 μ , and their breadth from 4-12 μ . They possessed a vesicular nucleus, similar in structure to that of *Amoeba vesperilio*, its diameter being 5-6 μ , while the diameter of its endosome was 3 μ . These amoebae were present in large numbers in some of the cultures, especially in the later stages of the work.

A few amoebae, with a diaphanous appearance, rather larger than the *A. limax* and without the slug-like form which is characteristic of the latter, were not identified. They may have been either large *A. limax* forms or small examples of *Amoeba vesperilio*, or they may have belonged to another species altogether.

Other Protozoa present in the cultures included *Paramoecium bursaria*, *Pleuronema chrysalis*, and numbers of small Flagellata. No Thecamoebida were ever seen.

3. OBSERVATIONS ON THE SPHERES.

The amoebae had not been long under observation before attention was arrested by certain remarkable inclusions which many of them contained.

These were nucleated, spherical bodies, with a sharply-defined outline, whose protoplasm resembled that of the amoebae themselves. They would, indeed, have been almost indistinguishable from the amoebae containing them had they not been in some cases enclosed in a very obvious vacuole, the margin of which was very distinct. Between the enclosed body and the margin of the vacuole was a space, varying in extent in different cases (cf. Pl. 28, figs. 2 and 5; Pl. 29, figs. 9, 10, and 11), which was pinkish in colour and presumably contained fluid.

The diameter of the spheres varied between 8–46.5 μ , both these figures representing extreme sizes. The majority of them varied between 20–26 μ . They were very distinct and well-marked objects, much larger than the ordinary food vacuoles. In certain positions of the amoebae, however, when the endoplasm was packed with food or when the protoplasm, in the course of its streaming, became heaped up over the sphere, the latter became very indistinct. This was especially the case in the rare examples in which the vacuole round the sphere was narrow. It was then difficult to determine the exact line of demarcation between the enclosed sphere and the surrounding protoplasm. On such examples it is quite possible for an inexperienced worker to mistake the spheres, in spite of their large size, for the nucleus, a point to which we shall return later (cf. below, p. 690).

In stained preparations the spheres showed a typical vesicular nucleus, exactly similar in structure to the nucleus of the amoeba itself, consisting, that is to say, of a central endosome with a meshwork structure, surrounded by a clear halo, free from chromatin and apparently structureless. Here again, as in the amoeba, no definite nuclear membrane could be made out. The whole nucleus of the sphere, including the clear halo round the endosome, measured from 6–8 μ , and the endosome itself from 4–5 μ .

Since the measurements were made from stained preparations, in which some shrinkage may have occurred, the actual size may have been rather larger than this, although very little

difference was observed between the sizes of the nuclei and the endosomes of amoebae and spheres of the same size; but, of course, the bigger spheres showed bigger nuclei than the smaller ones. A comparison of these measurements of the nuclei of the spheres with those of the nuclei of the amoebae was very striking:

Diameter of the amoeba	60-130 μ	Diameter of sphere	20-26 μ
Diameter of nucleus of amoeba	7-9 μ	Diameter of nucleus of sphere	6-8 μ
Diameter of endosome of amoeba	4-7 μ	Diameter of endosome of sphere	4-5 μ

The correspondence was very remarkable, especially when it was noted that the sphere scarcely differed in any respect, except in shape, from the amoeba and was almost indistinguishable from the rounded-off forms of the latter.

While it was inside the vacuole, the sphere was never seen to move in any way by its own efforts. It was not ciliated nor flagellated, nor did it put out pseudopodia, but maintained, in most cases, a perfectly even spherical contour, although a few cases were seen in which its outline was oval or even irregular (Pl. 29, figs. 8 and 10). The spheres were sometimes rolled over and over in the vacuoles by the streaming movements of the protoplasm, in which case the whole vacuole probably rolled about as a whole. But, in one instance, when the streams of protoplasm were very active along the sides of the vacuole, the enclosed sphere was seen to rotate in the opposite direction.

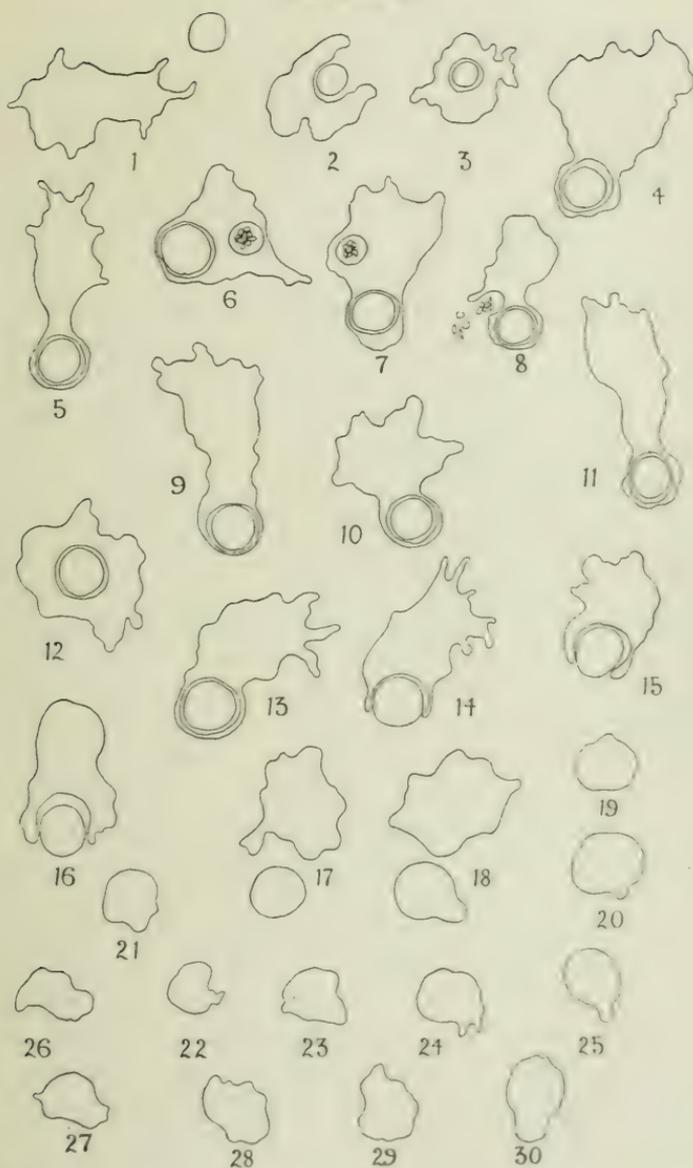
The spheres could be squeezed out of the amoebae by gentle pressure on the cover-glass, and then lay quite motionless and spherical in the water near by. Two such squeezed out on July 21, 1920, at 2.30 p.m., remained quite unchanged until 11 a.m. on the following day. It was also noticed then that numbers of such free, motionless spherical bodies, resembling rounded-off amoebae, could be found in the cultures. Doulein (9) states that, in old cultures of *Amoeba vesperilio* which had become foul and acid in reaction, the amoebae tended to round off and to die. Two questions therefore arose:

(1) Were these rounded bodies in my cultures individuals which had rounded off? and (2) Were these rounded bodies the same as the spheres which had been seen inside the amoebae, and had the amoebae been extruding them into the culture?

In order to throw some light on these questions a series of amoebae containing spheres was kept under observation, and the fact was established that the spheres were actually extruded by the amoeba very frequently. Text-fig. A gives the successive stages in the process. It is a composite figure drawn from numbers of sketches made during observations on the living object, and it shows that the extrusion of the spheres resembles ordinary defaecation of the undigested remains of food. It should be noted, however, that the ingested sphere was often carried about in the amoeba for a considerable time, and might often remain for some time in the posterior end of the amoeba, separated from the water only by a very thin layer of ectoplasm, giving the impression that it is about to be extruded. Frequently, however, the protoplasm flowed round it again, and it was taken once more into the central part of the endoplasm. Further, when a vacuole containing a sphere was lying near the surface of the amoeba, and an ordinary food vacuole was lying close against it, the two being separated only by a thin film of protoplasm, the food vacuole might discharge its contents, while the sphere remained unaffected and might be taken again into the depths of the endoplasm (Text-fig. A, 8).

There was, therefore, no external appearance which could be taken as an invariable sign that the ingested body was about to be extruded. A sphere might be carried about thus, on the verge, as it were, of extrusion, for a long time, and might then be taken in again; or it might be suddenly extruded: or it might, when deep in the endoplasm, rapidly approach the surface and be extruded almost immediately after it had arrived there. In one case the process lasted, from the first rupture of the enclosing membrane to the time when the sphere was quite free, about thirty seconds, from which it will be realized that, when once the extrusion had begun, it proceeded rapidly. Further, although the extrusion usually took place

TEXT-FIG. A.



Freehand sketches of living amoebae to illustrate the ingestion and subsequent extrusion of the sphere (rounded-off amoeba).

at or near the end of the amoeba which was posterior in progression, this was not invariably the case. The sphere might be extruded at the side or at any other point. Probably it is correct to say that, when the amoeba was progressing rapidly in one definite direction, extrusion usually occurred at or near the posterior end; but when the amoeba was putting out pseudopodia in all directions and was not changing its position much, extrusion might then occur at any point of its surface. This is probably true of ordinary defaecation also. When the ingested body was about to be extruded it appeared as in Text-fig. A, 13, and was usually, though not always, surrounded by a well-marked vacuole, pinkish in colour, and separated from the water only by a thin layer of protoplasm. This layer became thinner and thinner, until it was reduced to a mere membrane. Finally it was broken at one point. The ingested sphere then seemed to be forced out, slowly at first and then more rapidly, and at the same time the two halves of the enclosing membrane were withdrawn along its sides, so that the opening to the water was widened (Text-fig. A, 14, 15, and 16). A final effort of expulsion then quite suddenly forced the ingested body out and the cavity which it had occupied rapidly closed.

That an active effort of expulsion occurred is suggested by the fact that the ingested sphere did not merely slide out, but was projected by the force of the expulsion well away from the side of the amoeba. This may have been, however, merely the result of the explosion of the fluid vacuole in which it lay.

The important detail to be noted here is the fact that the vacuole containing the sphere sometimes contained diatoms or the partially digested remains of zoochlorellae, as well as the sphere, and that these were expelled with it and lay with it free in the water. This is a small point which suggests that the sphere had been ingested at the same time as the diatoms, that the vacuole in which it lay was a true food vacuole, and that the sphere was an ingested organism and not a body formed by the amoeba itself.

4. DESCRIPTION OF THE FREE SPHERE.

One of these recently extruded spheres, observed on July 21, 1920, was being rolled over and over by the movements of *Paramoecium bursaria* in the culture, and was seen to be perfectly spherical. The cytoplasm was clear, containing fine dark-looking grains together with some larger refractile granules, the nature of which I have been unable to determine. In stained preparations the cytoplasm showed a well-marked meshwork structure and the fine grains referred to above were distinguishable, being distributed over the strands of the meshwork and especially heaped up around the nucleus (cf. the description of the amoeba, p. 672).

In the living sphere the nucleus could not usually be distinguished, but in a few cases I was able to detect it. It is possible that the spheres in which it was visible were dead ones.

As has been noted above, in stained preparations the nucleus of the sphere shows the same structure as that of the amoeba itself. While some of the spheres contained no other structure, others, on the contrary, were full of diatoms and other bodies in food vacuoles (Pl. 28, figs. 2, 3, and 5). Sometimes, when the amoebae contained zoochloellae the spheres also contained them.

The outline of the spheres was very definite, appearing as a dark line, giving the impression that a definite limiting membrane was present. Examination of stained preparations showed, however, that no such limiting membrane is really there, the effect of a membrane being produced by the arrangement of the meshwork structure of the cytoplasm at the surface, an effect which is commonly seen also in rounded-off amoebae.

In spheres observed under the oil-immersion lens, it was noted that, while immediately after extrusion no contractile vacuoles were present, these appeared a short time after extrusion. In no case have contractile vacuoles been seen in the spheres while they are still in the amoebae. They were never present when the spheres were extruded, but they often appeared soon after extrusion. Since their appearance is at

least a sign of vitality, some attention was paid to the time of their appearance, their number, and their rate of pulsation.

A series of observations upon many extruded spheres established the fact that the contractile vacuoles appeared in them at irregular intervals after their extrusion. In one case, for example, a contractile vacuole appeared in the extruded sphere in less than a minute after its extrusion, and one minute after extrusion, two contractile vacuoles were present. In another case, however, no change occurred in the extruded sphere until twenty-two hours had elapsed, when two contractile vacuoles appeared. But in the majority of cases one contractile vacuole had appeared in anything up to twenty minutes after extrusion and two were present about half an hour later. The number thereupon generally increased to four, or in a few cases to six or even eight.

It would be natural to assume that the appearance of several contractile vacuoles in the sphere was an exceptional occurrence, perhaps indicating a pathological condition of the sphere itself or unfavourable physical conditions of the fluid in which it lay. The active amoebae in the same fluid also contained more than one contractile vacuole. Indeed, according to Penard (21), *Amoeba vespertilio* often possesses two or three. In my cultures some amoebae were certainly seen with only one and others with several, so that no accurate statement can be made as to what is the normal number. But, if the amoebae in the hanging drop contained more than one, it was not remarkable that the spheres should also develop several, when they were extruded into the same chemical and physical environment.

It was, however, noted that the numbers of contractile vacuoles in any particular sphere might change. In spheres which contained four or more this number was often reduced to two, especially in those spheres which, as we shall see below, developed pseudopodia and moved away. The observations on this point were not, however, sufficiently numerous to bear more than the suggestion that the development of numerous contractile vacuoles in the sphere was a temporary reaction to its sudden change of environment, which disappeared as

soon as the organism was able to adjust the physical state of its protoplasm to that of the fluid around it.

It may also be suggested that, if the vacuole in which the sphere had been enclosed were a food vacuole, the sphere, when set free, would be suffering from the effects of the attempt of the amoeba to digest it and would therefore naturally be in a pathological condition, and that the contractile vacuoles would be among the first of the organellae to betray this condition.

The contractile vacuoles arose deep in the protoplasm of the sphere and could be seen to move to the surface, when they were ready to burst. Often in doing so they glided between the granules in the protoplasm, and were then compressed into a dumb-bell shape when they passed between the granules, much as an air bubble is distorted when it is pressed under a cover-glass.

The pulsation rate was not very regular. It varied from as much as one contraction every quarter of a minute to one every six and a quarter minutes, the average being about every minute or rather less. Dofflein's experiments (9) showed that high concentration in the medium, such as would be likely to occur in a hanging drop, induces slow pulsation and a decrease in size of the contractile vacuole. Some such influence probably in part explains the irregularity observed here: but no definite evidence can be offered either in support of or against this view. The slowest pulsation seemed to occur in the spheres with several contractile vacuoles.

When several contractile vacuoles were present they often burst simultaneously, leaving the sphere free from them: but when only two were present they seemed to alternate, one bursting while the other one grew, so that the sphere always contained one. Further, when several were present, two half-grown ones often fused to form one larger one, which then moved to the surface and burst.

The contractile vacuoles did not appear constantly in any one position in the sphere, but, after bursting, might reappear anywhere. That this is not a false impression produced by rolling over of the sphere is shown by the fact that it was observed in perfectly motionless spheres, and also by the fact that when the bursting of one set of four was delayed the second

set of four might appear all in different positions from the first set, so that the sphere appeared to contain more than four contractile vacuoles.

The appearance of odd numbers of contractile vacuoles in this way, their occasional coalescence, their irregular pulsation rate, their multiple number, often subsequently reduced, together with the presence of several contractile vacuoles in the amoebae in the same preparations, suggested that abnormal phenomena were being witnessed. The physical conditions of the hanging drops were probably responsible for some of these irregularities. But the absence of contractile vacuoles from the spheres while they were still in the amoebae, and their appearance in them after they were set free, proved, at any rate, that the spheres were not mere dead defaecated matter, but were alive and were attempting to adapt themselves to the sudden change in their environment.

This view was confirmed by the occurrence in some, though not by any means in all, of the spheres, of tentative amoeboid movements, which, in a few cases, resulted in the sphere being transformed into an active small amoeba.

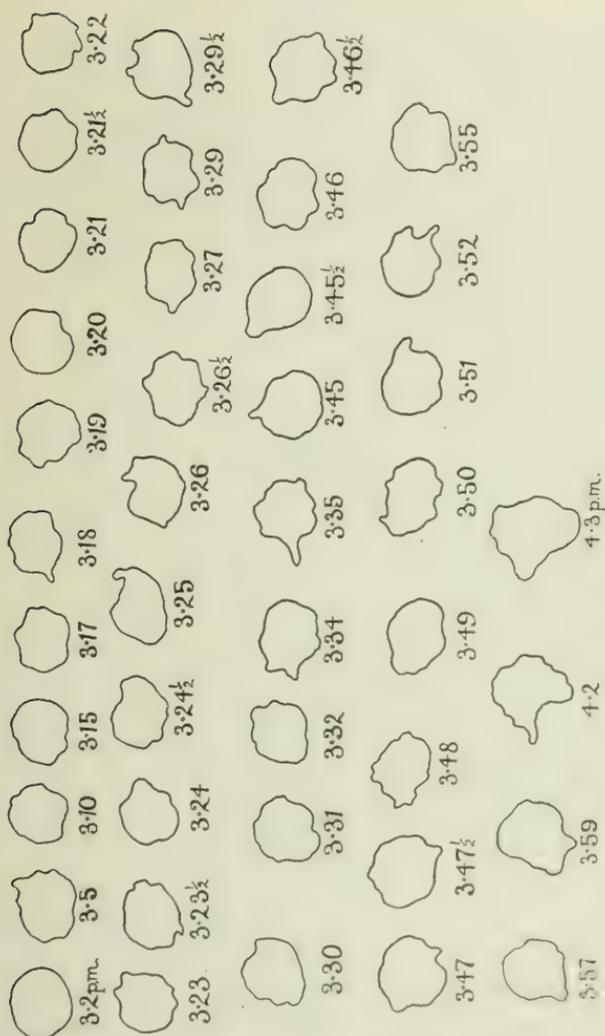
5. AMOEBOID MOVEMENTS IN THE FREE SPHERE.

In several cases spheres which were extruded under observation were kept under observation for several days, in the hope of some change being observed in them. In most of these cases the only change was the appearance of contractile vacuoles, the pulsation rate of which gradually became slower and slower, until they stopped and the spheres disintegrated.

In other cases, however, the spheres not only acquired contractile vacuoles, but also exhibited slight amoeboid movements. These were often no more than slight changes of form, but definite small pseudopodia were sometimes put out (Text-fig. B). In other rare cases the sphere became transformed into a small active amoeba, which moved out of the field of observation.

Text-fig. B represents drawings made with the camera lucida of the changes undergone by such a sphere, and in Text-fig. A are freehand drawings of another case. It is interesting to note that, although in the period between extrusion and the

TEXT-FIG. B.



Outlines of an extruded sphere (rounded-off amoeba), drawn with the camera lucida at the intervals of time stated in the figure, to show the amoeboid movements often performed by the sphere after it had been extruded.

appearance of the pseudopodia the number of contractile vacuoles might vary from one to eight, it had always been reduced to two at the most, by the time that the amoeboid activity of the sphere had been well established.

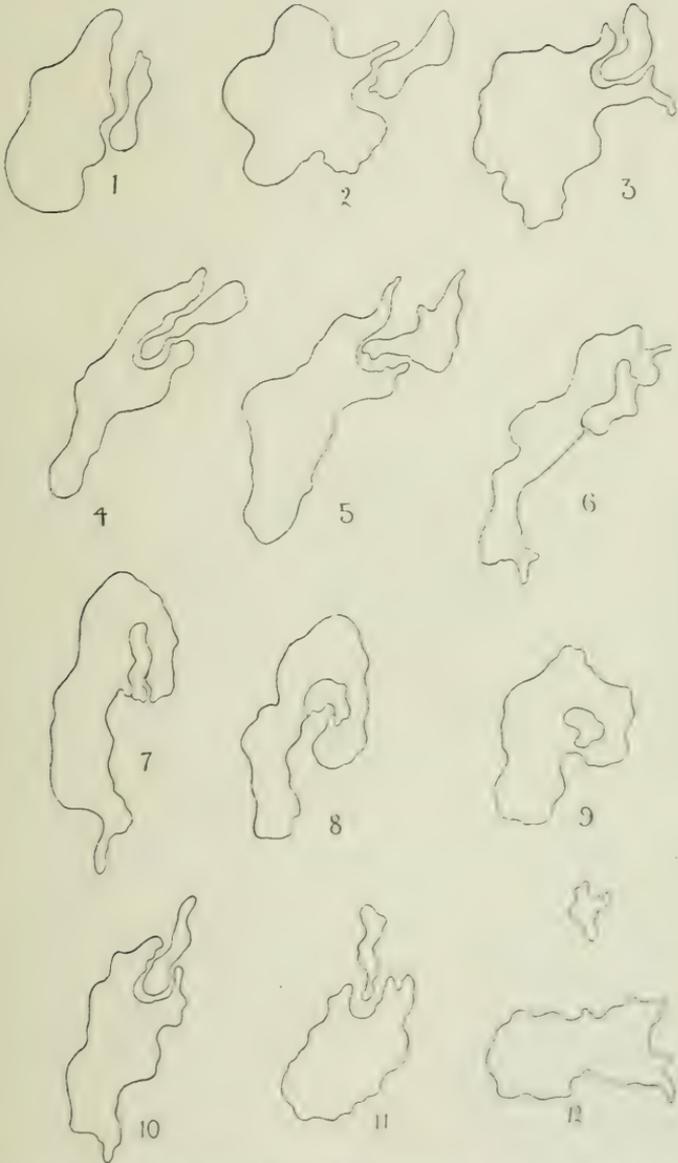
6. INGESTION OF THE SPHERE.

The extrusion of the spheres and the development of some of them into small amoebae had been seen before I was fortunate enough to observe an amoeba actually ingesting a free sphere. I had just watched the extrusion of this sphere, and the amoeba which had extruded it had hardly moved out of the field before another amoeba entered and immediately took up the sphere which the other had left behind. Further observation showed that this fate was suffered not only by the motionless spheres but also by those which had already become transformed into small active amoebae.

The process of ingestion was perfectly normal in every way. The big amoebae put out pseudopodia round the sphere and gradually enclosed it in a typical food vacuole. The result was an amoeba containing a sphere, exactly resembling the original amoeba, with a sphere inside it, which had awakened my interest at the beginning of the observations.

Not long afterwards I was able to follow and to sketch the dramatic chase of a small *Amoeba limax* by a large active *Amoeba vespertilio*. Text-fig. c gives the details of this drama. It will be seen that the large amoeba at first attempted to surround its prey (4, 5, and 6), and, after cutting off its retreat, nearly succeeded in enclosing it (7 and 8). At 9 the small amoeba is not inside the large one, but underneath it, the *Amoeba vespertilio* having streamed over the *Amoeba limax* so as to hold it between itself and the glass. I have often seen *Amoeba proteus* capture *Paramoecium* and other Ciliates in the same manner. The *Amoeba limax*, however, was too nimble in this instance, for it escaped again (10) and the large amoeba made no further attempt to capture it. A similar case has been described and figured by Jennings (16), in which the amoeba also failed to secure its prey. Jennings concluded that the behaviour of the captor to the victim could not be explained as the result of chemical or tactile stimuli only, but that there was a finely co-ordinated adaptation of the movements of the captor to those of the victim,

TEXT-FIG. C.



Freehand sketches of the chase of an amoeba of the 'limax' type by an *Amoeba vespertilio*, and the partial ingestion and subsequent escape of the former.

a conclusion with which I am entirely in agreement. The behaviour of *Amoeba proteus* in its capture of large Ciliata like *Paramoecium* and *Colpidium* in cultures strikingly supports the same view (cf. also Schaeffer, 23).

Penard (21, p. 700) has described another instance of the chasing of one amoeba by another which ended in the fusion of the two, and Leidy (18) has described and figured what is undoubtedly the successful capture and digestion of an *Amoeba verrucosa* by *Amoeba proteus*. This latter case is particularly interesting, since Leidy says that the *A. verrucosa* assumed, in the body of its captor, 'the appearance of a large sphere, still retaining its contractile vacuole unchanged'. Later on the 'victim had become pyriform and striate, and was then included in a large water vacuole. Still later the body of the *A. verrucosa* appeared to have become broken up into five spherical, granular balls. . . .' Leidy was unable to follow the ultimate fate of these 'granular balls', but he supposed that they were digested. A comparison of Leidy's figures with those illustrating this paper leaves no doubt that he was dealing with an isolated instance of a process which was occurring on a larger scale in my cultures.

The fact, however, that Leidy's is the only one of these cases in which anything resembling actual digestion was seen, and the fact that I have only in one instance (cf. below) seen in my cultures doubtful evidence of digestion of the spheres, suggest that the amoebae only rarely are able to digest other amoebae which they may capture. Further, it seems probable that amoebae only rarely even attempt to capture other amoebae, and usually fail to retain these when they are active, however frequently they may succeed in ingesting them when they are sluggish or resting in a rounded-off condition.

The case in which the doubtful evidence, referred to above, of digestion of a sphere was seen, was that of a sphere which was spherical when it was ingested, but which did not remain so. It underwent distinct form changes while it was still inside its captor. Pl. 29, figs. 8 and 10, represent other ingested spheres, drawn from stained preparations, which had assumed an irregular form while inside their captors. In the case just

referred to, which was kept under observation, the sphere returned to the spherical form after it had been inside the amoeba about five hours. Later it became less and less distinct, and seven hours after it had been ingested it could no longer be distinguished. Apparently it had been digested. This was the only instance in which anything like digestion of the spheres was seen. In all other cases which were kept under observation the spheres were sooner or later extruded by the amoebae which had digested them.

One other point remains to be mentioned before we discuss the nature of the spheres. It is illustrated by Pl. 28, figs. 1, 2, and 4, and Pl. 29, fig. 7, drawn from stained preparations, in which several examples of it were found at different dates. Pl. 28, figs. 1 and 2, show the phenomenon in its most typical form, and it will be seen, on reference to them, that there are here as many as four amoebae, enclosing one another, giving the impression of concentric fission. The figure looks, at first sight, like the dream of a pre-formationist, but we shall see that it has a much more prosaic explanation. It is so remarkable that I at first believed it to be an artefact, due, I supposed, to drying of the preparation, or to imperfect fixation. The other organisms on the slide were, however, well fixed and stained, and these remarkable structures did not occur on slides of one batch only but were present on slides made on widely different dates. Further, I saw what I interpreted as the same structures in the living organisms, although I was never able to convince myself of this. In any case the phenomenon admits, as we shall see, of a perfectly natural explanation if we adopt the only hypothesis which fits the whole of the facts.

There can be no doubt that there are actually several independent amoebae enclosing one another, because their nuclei are perfectly distinct and each amoeba possesses a vacuole for the reception of the others. The nuclei are, moreover, all exactly similar in structure to one another. Pl. 29, fig. 7, is perhaps the most remarkable and was the most difficult to interpret. There are here present seven nuclei, and the interpretation of the figure is best deferred to a later stage (cf. below, p. 700).

7. DISCUSSION.

Three main possibilities suggested themselves as explanations of the observations just described.

First, the spheres may have been parasites; secondly, they may have represented some form of reproduction, such as endogenous budding; thirdly, they may indeed have been food bodies, the amoebae having ingested other amoebae of the same or other species. On this last view, the phenomena were those of 'cannibalism'. As the title of the paper shows, I believe this last to be the correct interpretation.

In order to give my reasons for this conclusion, it will be necessary to discuss these three views in turn.

(1) The Parasite Hypothesis.

At first this view seemed very probable. The spheres resembled, at first sight, organisms like the Suctorian Sphaerophrya, which is so common a parasite of Ciliata in cultures. Closer examination of them quickly proved, however, that not only did the spheres never show any structure resembling tentacles but also that no Suctoria were ever present in the cultures. Further, the nucleus of Sphaerophrya is not vesicular. The spheres, in fact, did not show any single feature by which they could be classified as Suctoria.

Prandtl (22) has described a Thecamoebidan, *Allogromia*, which became parasitic upon *Amoeba proteus*, *Arcella*, *Nuclearia*, and *Paramoecium* in order to accomplish its sexual cycle in their interior. This organism, however, does not in any way resemble the spheres described above. Not only were no shelled Rhizopods ever seen in any of my cultures, but the structure of *Allogromia*, its possession of chromidia and the changes which it undergoes in its host, together with the fact that it is capable of reducing its host's vitality, definitely exclude any possibility that the spheres were parasites of this nature.

Buck (1) has described, under the name *Phonergates vorax*, another shelled Rhizopod, identical, according to

Bütschli (2), with *Lecythium hyalinum*, which also may become parasitic upon *Amoeba proteus*, Rotifera, Crustacea, &c., during its sexual cycle. Buck states that this organism may, when it is parasitic in *Amoeba* and other organisms, closely resemble *Sphaerophrya*. But I am convinced, after reading his paper and studying his figures, that *Phonergates* has no points of resemblance to the spheres here described, except, perhaps, that it is about the same size.

Penard (21 d) has described in an amoeba which he names *Amoeba alba*, a parasite similar to one seen by Buck in *Arcella* and later found by Dangeard (7c) in the *Heliozoa Nuclearia simplex* and *Heterophrys dispersa* and called by him *Sphaerita endogena*. The form seen by Penard belongs to the Chytridiaceae, and he thinks that it is similar to that described by Chatton and Brodsky (5) in *Amoeba limax*. The latter authors discuss the whole question of these and allied parasites, and it is obvious that none of these parasites resembles the spheres described above.

Another parasite, *Nucleophaga amoebœa*, allied to the above, has also been described by Dangeard (7b), Penard (21 d), and others. It attacks the nucleus of various amoebae. Doflein (9) has further described the formation of giant nuclei in *Amoeba vespertilio*, which is the amoeba with which we are dealing, due to a parasite which he regards as being closely allied to, if not identical with, the *Nucleophaga* of Dangeard. The spheres described above have, however, obviously nothing to do with this or any other nuclear parasites, since the nucleus of the amoeba containing the sphere was always intact and normal and the sphere itself had a nucleus of its own, which was very similar to that of the amoeba which contained it.

Leidy (18) has described and figured a number of interesting inclusions in *Amoeba proteus* and other species. His observations were, however, made upon the living object only, and it is unfortunately impossible to determine from his figures and descriptions what was the real nature of these inclusions. Some of his figures of them, described by him as nuclei, might

equally well be interpreted as parasites of the Chytridiacean type referred to above. On Pl. viii, figs. 12-16, of his book he figures a 'multinucleate' *Amoeba villosa*, and in fig. 15 he shows a process which he describes as the bursting of the nucleus and the expulsion of its coarsely-granular contents. He was almost certainly dealing here with a Chytridiacean parasite and not with a multinucleate amoeba at all. Doubt must, therefore, be entertained as to whether his other figures of the nuclei of the various amoebae described by him really represent the nucleus. It is doubtful, for example, in the case of the form of '*Amoeba proteus*' which he figures in Pl. viii, figs. 17-28, and describes on p. 53; and also of those shown in Pl. iv, fig. 25, also of '*Amoeba proteus*'. The same doubt applies to the nucleus of *Dinamoeba* (Pl. vii, figs. 5, 7, and 8) described on p. 91 as a 'large, pale granular nucleus, surrounded by a clear halo', an appearance which the true nucleus of *Amoeba proteus* rarely or never presents. It is much more likely that what he saw was either a parasite or some other granular organism which had been ingested. The excellence of Leidy's observations in general leads one, however, to accept most of his interpretations, and it is to be remembered that, without the control of stained preparations, mistakes of this kind are almost unavoidable.

Wallich (29) records a number of observations upon living *Amoeba villosa*, but in this case also it is practically impossible, in the absence of stained preparations, to determine exactly what he was dealing with. In the first place it is doubtful whether the bodies which he regarded as nuclei were in reality nuclei at all. If they were, it is probable that they were, as some of Leidy's undoubtedly were, nuclei infected with a *Nucleophaga*. And Carter (4, 4a) probably fell into the same error.

It became obvious, therefore, that the spheres showed no resemblance to any of the parasites of amoeba of which a full description was available. The following general considerations also contributed to the abandonment of the parasite hypothesis.

First, the sphere did no damage to the amoeba which con-

tained it. At any rate no damage was demonstrable, and the amoebae lived and multiplied normally while they contained spheres, and are, indeed, still living at the present time in the same cultures, although only rarely do they now contain spheres.

Secondly, if the spheres were parasites it is difficult to understand why they were so frequently extruded by the amoebae. When a parasite has gained entrance to its host it usually does not leave it, except for the purpose either of propagative reproduction or of mechanical distribution of its species. Such a parasite would, at some time or other during its sojourn in its host, be likely to show some evidence of its reproductive cycle. The spheres, however, never showed any signs of any reproductive capacity whatever, either when inside or outside the amoebae. They were taken in and passed out in the same manner as ordinary food matter would be ingested and extruded, behaving in a strictly passive manner.

It occurred to me that the amoebae and the spheres might be symbionts or commensals. Against this highly improbable theory was the fact that a vacuole, filled with fluid, was present round the sphere. In other cases of symbiosis among the Protozoa, as, for example, that of the zooxanthellae and zoochlorellae, the latter occurring under certain conditions in the very amoebae under consideration, no vacuole surrounds the algae.

(2) The Hypothesis of Endogenous Budding.

The second hypothesis, that the spheres were endogenous buds, was much more attractive and led me astray for some time. I should have liked to have been able to prove that they were buds, and very nearly succeeded in convincing myself that they were. But the finding of such structures as those shown in Pl. 28, figs. 1 and 2, and Pl. 29, fig. 7, where two, three, or four amoebae were enclosed within one another, seemed to stretch the theory of endogenous budding rather far. Before ascribing such remarkable structures as these to endogenous budding it

seemed wise to reconsider the data. When this was done it became obvious that the spheres were not endogenous buds.

Throughout my stained preparations I have never seen any signs of change in the nucleus, either in the amoeba or in the sphere, although I have very carefully searched for such cytological evidence of the formation of a bud. Whatever the size of the amoebae or of the spheres might be, the nuclei of both were always in the same condition, that is to say, in the 'resting' condition which has been figured; the nucleus of the sphere was always similar in structure to that of the amoeba.

I have tried hard to find evidence of the division of the nucleus of the amoeba to form the nucleus of the sphere, or evidence of the formation of the latter from chromidia extruded by the nucleus of the amoeba. Indeed, under the influence of the hypothesis of endogenous budding I have often thought I have seen chromidia, just as I have often thought I have seen in this and in other forms, centrosomes, centrioles, and other structures, when I have wanted to find them. But these structures have, on re-examination, proved to be, in every case, either figments of my own imagination based upon improperly differentiated slides, or artefacts. I am now convinced that there is no evidence, of any sort or kind, of changes in the nuclei either of the amoebae or of the spheres in my slides.

If endogenous budding had been going on to the extent that the abundance of the spheres would suggest, some evidence of the mode of formation of their nuclei from the nuclei of the parent amoebae would have been seen. It is true that even binary fission is seen only very rarely, as Doflein also points out (9). In my slides I have seen only two or three dividing amoebae, and in those the two daughter nuclei had already returned to the 'resting' condition. This is the only evidence that I have seen, either in the stained or in the living material of any reproductive processes whatever.

It is to be remembered, moreover, that when the endogenous buds are being formed in an organism like the Suctorian *Dendrocometes paradoxus*, the contractile vacuole

is present while the bud is still within the parent. It is, in fact, one of the first of the organellae to appear, and its presence can be taken as an indication that bud formation is in progress (Lapage and Wadsworth, 17). In the spheres, on the contrary, a contractile vacuole was never seen while the sphere was within the amoeba. It did not appear in the sphere until an appreciable interval had elapsed after the sphere had been expelled.

Further than this, endogenous buds, in other groups of Protozoa, do not usually vary much in size in any particular species producing them. They are cut out of the parent to a definite size which remains unaltered, and it is not true that they are smaller when they are first formed and that they grow to a mature size before their birth. The spheres, however, although they show a striking uniformity of structure, do vary a good deal in size, some being as small as $10\ \mu$ in diameter, others up to $46\ \mu$. This variation in size suggests that they are not endogenous buds. Further, in the smallest ones the nucleus is fully formed and typical, measuring $6\ \mu$ in diameter, the endosome measuring $3\ \mu$ in diameter. This is a significant fact, when we remember that the nucleus of the *A. limax* also present in the culture is $5\text{--}6\ \mu$ in diameter with an endosome of $3\ \mu$. The variation in size of the spheres is, therefore, more simply explained on the hypothesis that they represent amoebae of different sizes which have been ingested, than in any other way.

The fact that some of the spheres developed, after they were extruded, into typical small amoebae certainly suggested that they were reproductive bodies; but this was just as easily explained as the escape of an ingested amoeba after successful resistance to the digestive juices of its captor, and such an explanation was more in accordance with the other facts.

Another important fact against the view that the spheres were endogenous buds was the observation that the spheres, while inside the amoebae, often contained diatoms and other food matter in food vacuoles (Pl. 28, figs. 2 and 5, and Pl. 29, figs. 7, 8, and 9). This is highly significant in view of the fact

that the amoebae in the culture were all feeding principally upon diatoms. Endogenous buds, when they are formed in other groups of Protozoa, are invariably free from food vacuoles until after they are born, and it is indeed difficult to imagine how they could obtain any solid food until they are set free. Even if we adopted the fantastic view that, in the case under consideration, the spheres had obtained the diatoms from the amoebae in which they lay, it is impossible to explain how they did so, seeing that a vacuole filled with fluid lay between them and the protoplasm of the amoebae. The presence of that vacuole is, of course, in itself no argument against their being endogenous buds, since most endogenous buds develop inside a cavity or 'brood chamber' in the parent.

A still more significant detail is, however, the observation, made upon the living object, that, when the sphere was extruded, the remains of diatoms might be extruded with it from the same vacuole. This can only mean that the diatoms were taken up at the same time as the sphere, a fact which is easy to understand when we remember that the amoebae were feeding mostly in the clumps of diatoms and débris in the culture rather than in the open. The vacuoles in which the spheres lay were, therefore, true food vacuoles and not of the nature of 'brood chambers'. This does not prove, of course, that they were not buds, since the amoebae were seen to ingest the free spheres, and it might be argued that the spheres were no less true buds because their parents were eating them. But, taken in conjunction with the absence of any evidence of the mode of formation of buds and the presence of food vacuoles in their cytoplasm, it is a very significant piece of evidence.

Another observation pointing in the same direction is the fact that the spheres were not always perfectly spherical, but were often irregular in shape and, indeed, were, in some cases, seen to undergo form changes while inside the amoebae (cf. p. 686, supra). This strikingly suggests that they were amoebae which had been ingested.

The hypothesis of endogenous budding breaks, however, on

the same rock as did the parasite hypothesis. It fails to explain the occurrence of several amoebæ enclosing one another, as are shown in Pl. 28, figs. 1 and 2. This could, it is true, be interpreted as endogenous budding with pathological delay of the birth of each bud, so that an appearance of concentric fission resulted; but there seems to be no necessity for so fantastic a view, when the structure can be explained naturally and simply as the result of cannibalism.

Lastly, it is difficult to understand why endogenous budding, if it occurs in the Amoebæ, has not been fully described already, seeing that such a vast amount of work has been done on these organisms. It is true that Penard (21) has made several references to the occurrence of so-called 'embryos' in *Pelomyxa* and in various amoebæ. With regard to *Pelomyxa*, he says that 'in the month of October, 1900, the greater part of the individuals examined contained, in their bodies, true embryos. These embryos, apparently swimming in the plasma, . . . showed as little grey masses, spherical, ovoid or pyriform, in the interior of which one saw some little, brilliant grains, one or two vacuoles and a vague appearance of nuclei. Isolated by compression of the *Pelomyxa* the embryos pushed out slowly prolongations in the form of little waves or lobes and continually deformed themselves in their entirety.' He was also able to convince himself of the presence of a contractile vacuole, which 'only functioned in a lazy manner', and he was sure of the presence of a 'nucleus, round, with a nuclear membrane already formed and distinct, with nuclear sap and a central nucleolus and one or two other spherules, . . . which seemed to represent nuclei also'. He adds his opinion that 'the presence of these embryos, living in good health in the plasma of the *Pelomyxa* and usually multinucleate, seems to me to indicate that they are products of the animal itself and not parasites'.

This description suggests that he may have been dealing with either parasites or with amoebæ of the *Amoeba limax* type which had been ingested by the *Pelomyxa*; but doubt is thrown over the whole of the observations by his statement,

on another page of the same work, that he believes with Greef that the so-called 'Glanzkörper' of *Pelomyxa* develop into small amoebae similar to those which he saw pass out of the *Pelomyxa*. From his account it seems likely that he has confused various different structures, true 'Glanzkörper', fungal and Flagellate food, and ingested small amoebae. This is only another instance of the difficulties which arise, especially for other workers, when observations on living specimens are not controlled by properly made permanent preparations.

Penard, in the same work, makes other references to the occurrence of similar 'embryos' in the amoebae which he names *A. nitida*, *A. villosa*, *A. annulata*, *A. nobilis*, *A. terricola*; and in Rhizopods like *Diffugia*, *Diaphorodon*, and, above all, in *Nebelidae*, he found bodies which he thought may have been reproductive in nature. In most of these cases he gives figures which certainly suggest strongly that he was dealing with amoebae which were ingesting and extruding again other amoebae of the same or other species. In the 'embryos' of *A. nobilis* he saw 'little diatoms' and 'little grains which appear to proceed from digestion'; and those of *A. nitida* contained 'the appearance of little grains of starch or little diatoms, which themselves seemed to be in course of digestion'. But he does not seem to have thought it necessary to explain how these 'embryos', while inside their 'parents', had been able to ingest their diatoms. It seems very likely that these 'embryos' were similar in nature to the spheres in my amoebae and that Penard fell into the same error as that from which I was only saved by the study of permanent preparations.

Grosse-Allermann (13), in a study of *Amoeba terricola*, saw, in two instances only, a swollen amoeba full of small spheres of 30–40 μ in diameter, and he supposed that he was dealing with the end result of multiple fission. Penard (21 d) saw somewhat similar phenomena in the same amoeba, but regarded the spheres as parasites which had developed inside the *Amoeba terricola* and which were set free by its death.

Much more plausible, however, are the accounts of endogenous budding in amoebae given by Liston and Martin (19), Wherry (30), and Hogue (15). The last-named worker also describes the formation of 'exogenous' buds, by the streaming out of chromatin granules from the karyosome into the ectoplasm, where they collect to form the nuclei of the exogenous buds. Her figures and description, however, suggest that the so-called chromatin granules were either artefacts or parasites like the Chytridiaceae referred to above.

Hogue's figures of the endogenous buds, like those of Wherry, are much more convincing and show a striking resemblance to the figures illustrating this paper. Neither of these workers, however, has given a detailed description of the so-called 'buds', nor was the development of the 'buds' followed. Had this been done in all probability a different conclusion as to their real nature would have been reached. It should be noted, also, that in both these cases the amoebae were studied in agar media, which cannot be regarded as a sound method of cultivating these organisms. Further, the cultures were crowded with amoebae, a state of affairs which would tend to encourage the ingestion of the amoebae by one another.

Liston and Martin (19) have described endogenous budding in a large amoeba from liver-abscess pus. This amoeba also was studied on agar media. Liston says that he saw an amoeba develop three or four 'buds' within its body while under observation and that these were liberated. Older and larger amoebae might contain as many as six 'buds' in various stages of development. If this were so, it is unlikely that they were true endogenous buds at all, because endogenous buds are usually formed of a certain definite size which does not increase or change before they are born. Liston also states that the 'buds' became recognizable in the amoebae 'when a larger mass of chromatic material was assembled than could be reasonably explained on the supposition that it was formed from ingested bacteria', that the 'buds' were formed around these masses of chromatic material, and that these masses then became the nuclei of the 'buds'. Martin, in a study of the

stained material, confirms this and says that the nucleus of the 'bud' is formed from 'chromidia contained in it when it is first formed and derived from the chromidia scattered through the cytoplasm of the parent'.

He also says, however, that 'the nucleus of the amoeba takes no direct part in the formation of the bud. There is absolutely no evidence, either from observation on the live amoebae or from the stained films, for any form of nuclear division connected with the bud formation.'

This latter statement might equally well have been made about the spheres described in this paper. When it is remembered that I also, under the influence of the view that the spheres were endogenous buds, found in my amoebae structures which could easily be interpreted as chromidia, the parallel is complete.

Upon re-examination of my preparations, however, I have been unable to convince myself that the fine grains in my amoebae were chromidia at all, and certainly I have never seen anything resembling a collecting together of these grains inside the spheres to form their nuclei. All the spheres had a fully-formed vesicular nucleus. While I must admit, therefore, that Martin may have been dealing with something quite different from my spheres, I still am of the opinion, without desiring to impugn his high reputation as an accurate observer, that his 'buds' were in reality of the same nature as my spheres, that is to say, that they were amoebae of the same or another species which had been ingested.¹ Two types of amoebae were present in the cultures of Liston and Martin, and it is possible that one kind was ingesting the other. The method of cultivation of these organisms upon agar

¹ Dr. H. M. Woodcock, of the Lister Institute, first suggested to me, in 1920, that the 'buds' described by Liston and Martin were probably not true buds at all and thus gave me the clue to the real nature of the spheres in my own cultures. Recently Dobell and O'Connor (8a) have expressed the same opinion. Compare, also, the still more recent remarks of Woodcock (32) with regard to the need for care in the interpretation of cultural forms of Protozoa.

media might be expected to induce them to exhibit abnormal behaviour in this and in other respects.

It is much more probable that Wallich (29) also saw something similar to the observations recorded in this paper, since he figures a small amoeba which he calls a 'gemmule' and believed he had proved the occurrence of 'gemination' and 'viviparous reproduction' in *Amoeba villosa*. His 'viviparous reproduction' seems to rest upon the occurrence of many small amoebae in his cultures, such as also occurred in my own, and it is probable that his 'gemmule' was either an amoeba which had become rounded off or one which had been recently extruded, after having been ingested. He also describes structures which he calls 'nucleated corpuscles' and 'sarcoblasts', and he says that the 'sarcoblasts' are obviously reproductive, because, although he never saw them develop into amoebae while they were yet within an 'amoeba cyst' (a structure which is obviously not a cyst, but a dying amoeba), yet he saw bodies present in the same fluid at the same time, outside and identical in appearance, which did develop into amoebae! Since he made no permanent preparations, it is not possible to know what he really was dealing with, but it is unlikely that either the 'sarcoblasts' or the 'nucleated corpuscles' were in any way similar to my spheres. Wallich, however, further describes what he refers to as 'a process resembling gemination or viviparous reproduction'. His figure of a 'gemmule' is very like the recently extruded sphere of my cultures, but since Wallich says that he never saw his 'gemmule' emerge, and further that he is 'unable to vouch for' the process of gemination 'on his own authority', it is not possible to attach much importance to his observations.

While there are, therefore, several references to the occurrence of endogenous budding in the *Amoebæ*, there seems to be no single record of it which is free from doubt and certainly no record which has been confirmed by subsequent workers. This is a curious fact, when we remember that endogenous budding does occur in forms so closely allied to the *Amoebæ* as *Arcella* and other *Thecamoebida*. It even

suggests that, either some of the cases cited above are correctly interpreted as instances of endogenous budding, or that, alternatively, the Thecamoebidae are not so closely allied to the Amoebae as has been thought.

All these considerations shook my belief in the very attractive view that I was witnessing an epidemic of endogenous budding.

(3) Hypothesis of Cannibalism.

Turning to the third alternative I found that the cannibalism hypothesis not only explained those facts which the other views explained, but explained them much more simply and readily. In addition, it did not fail where the other two views had failed. This hypothesis provides the simplest explanation and it covers all the facts without introducing into the already complicated problem of the life-history of amoeba a new and hitherto unauthenticated process.

Further, it explains simply enough how such structures as those shown in Pl. 28, figs. 1, 2, and 4, and Pl. 29, fig. 7, can arise. These structures are explained in detail in the text explaining the figures. It is sufficient here to say that such structures arise by the ingestion by amoebae of other amoebae which had previously themselves ingested yet other amoebae, a process which can give rise to the most remarkable and complicated structures. Such phenomena must be pathological. Whether cannibalism itself is pathological is a matter of opinion, in the present state of our knowledge. That it is not a frequent occurrence is shown by the paucity of references to it in the literature, although Doflein (9a) says that he has often seen cannibalism, i.e. the eating by amoebae of young forms or of cysts of their own species, and that such occurrences have given rise to statements about internal budding and formation of embryos.

An amoeba, in the absence of its normal diet, will eat almost anything. In my own cultures of *Amoeba proteus*, for example, these organisms, which were thriving upon a diet

of bacteria, became voracious carnivores when they were supplied with *Colpidium colpoda*; and Dofflein has recorded a similar fact (9*b*). It is not surprising, therefore, that an amoeba like *Amoeba vespertilio*, which feeds normally upon diatoms and had been kept for many years in an old hay infusion in which its normal food supply must have been for long scarce, and in which Paramoecium and other Ciliates were present, should have turned, under the stimulus of the change of environment provided by the sub-cultures, to the ingesting, not only of the diatoms which developed in those sub-cultures, but also of other amoebae, both of its own and of other species.

At first I was inclined to think that starvation played a part in causing the amoebae to become cannibalistic. They showed, however, few other signs of starvation. They exhibited normal activity, they multiplied abundantly, and, beyond what was probably a more marked vacuolation than is usual for the species, were in no other way abnormal. They are still living in the same dishes, although they have been practically untouched for two years; but they only occasionally now ingest one another, and are feeding actively upon algae which have developed in the cultures.

It is, moreover, by no means certain that in 1920 they were ingesting their own species alone. Though this probably occurred often, in other cases a comparison of the sizes of the spheres and especially of their nuclei with those of the other amoebae present in the cultures (cf. *supra*, p. 675) suggested that the small spheres were mostly ingested examples of *Amoeba limax*. Many of the medium-sized spheres might equally well have been either large individuals of *A. limax* or small examples of *A. vespertilio*.

In this connexion the interesting question arises as to whether an amoeba, even if it ingest a member of its own species, can digest it. I have only been able to follow, in the living object, one case of what appeared to be the digestion of the ingested sphere (v. also *supra*, p. 686). In the stained preparations spheres were often seen, of all sizes, which took the

stain more feebly than the others on the same slides, the nucleus often not staining at all. These may have been spheres which were undergoing digestion, or they may have been merely dead ones. In the majority of cases the spheres certainly seemed to resist digestion, although it was evident that most of them were killed by their sojourn in the food vacuole or were, at any rate, so much damaged that they were unable to resume their activity after they were extruded. The appearance of a contractile vacuole in them indicated an attempt at the resumption of vitality; but usually the attempt went no further and the extruded spheres disintegrated if they were not again ingested. In a few cases abortive attempts at amoeboid movements occurred; and in fewer still these were successful and the sphere became transformed again into a small amoeba which was apparently little the worse for its experience.

It is evident, therefore, that the amoebae found difficulty, at least, in digesting other amoebae which they took up. They might, therefore, extrude them again, just as they will extrude other indigestible material. If these extruded amoebae had been killed by their sojourn in the food vacuole or died soon after extrusion they might be again ingested by other amoebae; and it is probable, although I can produce no evidence to prove it, that these dead or dying amoebae could be digested. One is reminded here of the fact that, in Vertebrates, the gastric juice does not digest the mucous membrane of the stomach, unless that is damaged or in a pathological condition, but that post-mortem digestion of the stomach can and does occur.

Another reason for the extrusion of the spheres is suggested by the observation of Rhumbler, as quoted by Minchin (19*a*), that amoebae disgorge any food matter that they may contain under the influence of strong light, such as that to which they are subjected when they are brought into the field of the microscope. That this is not the only reason in this case is shown by the frequent occurrence of free spheres in the cultures themselves, before any of the fluid had been examined under the microscope. They could be picked up from the bottom

of the culture dishes with a pipette, and must, therefore, have been extruded in the cultures where the stimulus of strong light did not operate. Drying of the slide might conceivably have caused extrusion, as Wallich also suggested (29). But this factor also would not operate either in the cultures or in the preparations used for observing living specimens.

To return to the question of what caused the amoebæ to become cannibalistic, I am unable to offer any intelligent suggestion. It has already been mentioned that the cultures were not unhealthy, since the amoebæ thrived and multiplied, as did also the Ciliates and other small amoebæ. The balance of evidence showed that the amoebæ were not to be regarded as starved, and certainly not as so starved that they resorted to utilizing their own kind as food, a condition which must be rare in both natural and artificial conditions. Further, we have seen that it is at least very doubtful that they were really feeding at all on the amoebæ which they ingested, since the evidence is that they could only very occasionally digest them. Their condition seems to have been like that of the army recruit, who, when he asked for a drink on the march, was told to suck a stone.

A possible explanation may be sought in the view that the amoebæ had become so numerous in the cultures that the active ones were ingesting the rounded ones and, finding them indigestible, were extruding them again. Schaeffer's work on the feeding habits of amoebæ (23) is interesting in this connexion. He found that the ingestion of particles by amoebæ is not to be explained entirely by chemotaxis, but that other factors operate, especially movement, either natural or mechanical, in the material offered, the nature of the amoeba itself, i. e. whether it were 'raptorial' or not, the physical similarity to or difference from the normal diet of the material offered and the degree of hunger from which the amoebæ were suffering. He found, for example, in his experiments with carmine grains, that the amoebæ got rid of these much more quickly than normal food matter, and generally as soon as possible. Also he thought that the carmine was extruded because it was

actually disagreeable to the endoplasm, though not to the ectoplasm, and not merely because it was indigestible. Further, a piece of carmine was eaten only once if the amoeba was only mildly hungry; several times if it was very hungry; but the amoebae showed less and less inclination to ingest the same grain if it were offered to them several times in succession. The same was true if a number of different grains were offered, each only once.

It is obvious, therefore, that the factors which govern the feeding of amoebae are by no means simple. It is probably for this reason that I have been unable to induce my amoebae to repeat their performance of 1920, either in the old or in fresh cultures, on anything like the same scale. I have also looked carefully for similar phenomena in thick cultures of *Amoeba proteus* obtained by the methods of Taylor (27) and Doflein (9b). But, although these amoebae often exist in such numbers that they are in close contact, and are actively feeding upon *Colpidium* and *Chilomonas*, i. e. upon a carnivorous diet, they have never showed the slightest tendency to ingest one another. Schaeffer (23) also found that his amoebae, although they were eating Ciliates and Flagellates readily, never ingested one another. Further, Doflein (9), in his study of *Amoeba vespertilio*, does not mention any case of their ingesting one another. He used, however, chiefly amoebae containing zoochlorellae, whose metabolism must have been, therefore, abundantly provided for even in the absence of their normal diet; and in my own cultures of *Amoebae vespertilio* containing zoochlorellae, relatively very few of the amoebae contained spheres, and in those which did the spheres also contained zoochlorellae.

It is very likely, therefore, that the epidemic of cannibalism which is described in this paper was an isolated occurrence, dependent for its causation upon the physical and chemical constitution of the culture medium and also, as Schaeffer's work shows, upon the physiological condition of the amoebae themselves. The fact that, in those other cases in which similar phenomena have been observed in other than isolated

individuals and which have been erroneously interpreted as cases of endogenous budding, the amoebae were studied under conditions of artificial cultivation which at least differed widely from the normal environment of the amoebae, is additional evidence in support of this view. Until the methods of cultivating Protozoa are standardized upon the basis of a scientific physical and chemical analysis of the normal environment of these highly sensitive organisms, we must expect that atypical and bizarre phenomena will be witnessed in cultures, and that these will not only be rashly interpreted by the inexperienced, but will also readily mislead even the most careful and conscientious workers.

Reviewing the whole of the facts, I conclude that the hypothesis of cannibalism explains the facts described above readily and simply. It explains the variation in size of the spheres and the similarity of their structure to that of the amoebae which contained them. It explains also their inability to live after extrusion, the presence of food in them while they were still inside the amoebae, and the complete absence of any cytological evidence of the formation of endogenous buds. It affords also an explanation of the ingestion and extrusion and, in some cases, of the re-ingestion of the spheres, and of the remarkable occasional occurrence of several amoebae enclosing one another. I am, however, unfortunately unable to throw any light upon the interesting question as to whether an amoeba can digest individuals of its own species, or to determine what the actual stimulus was which led these amoebae to adopt temporarily the cannibalistic habit.

In conclusion, I am pleased to have the opportunity of recording here my indebtedness to Professor S. J. Hickson, F.R.S., in whose department the work was done, for his kindly interest and help, and to Miss Ann Bishop, B.Sc., and Mr. J. T. Wadsworth, for many very useful suggestions and helpful criticisms.

SUMMARY.

1. This paper describes the temporary adoption by *Amoeba vespertilio* of cannibalistic habits. The amoebae fre-

quently ingested, but in most cases failed to digest, other individuals of their own and also of other species (*A. limax*).

2. In some cases, an amoeba, which had ingested another, might itself then be ingested by a third amoeba; and these three might then be taken up by a fourth amoeba, so that remarkable figures, suggesting concentric fission, resulted.

3. The victims were usually ingested while they were rounded off or sluggish, and, after extrusion, usually failed to resume their activity, although most of them developed contractile vacuoles and some showed tentative amoeboid movements. A few recovered their normal activity and resumed normal life. Amoebae, after extrusion by one amoeba, were often taken up again by other amoebae.

4. In one case an *Amoeba vespertilio* was observed to chase and enclose an *Amoeba limax*, but the *Amoeba limax* subsequently escaped again.

5. The ingested amoebae may easily be mistaken for endogenous buds, but there is less danger of their being mistaken for parasites.

6. No trustworthy evidence was found as to the nature of the stimulus which caused the adoption of these habits, but the question is discussed.

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EXPLANATION OF PLATES 28 AND 29.

PLATE 28.

Fig. 1.—An *Amoeba vespertilio* with vesicular nucleus which shows well the meshwork structure of the endosome (*E*) and the clear halo round it. In the cytoplasm is a large vacuole (*vac.1*) containing another amoeba with its nucleus (*n.1*). This second amoeba contains a vacuole (*vac.2*) which encloses a third amoeba and its nucleus (*n.2*). The third amoeba contains another vacuole (*vac.3*) which encloses a fourth amoeba and its nucleus (*n.3*). *Ect.*, Ectoplasm. *End.*, Endoplasm.

Fig. 2.—An *Amoeba vespertilio* with its nucleus (*N*). The amoeba contains food vacuoles and a vacuole (*vac.1*) in which is a second amoeba with its nucleus (*n.1*) and two food vacuoles containing diatoms. This second amoeba contains in a vacuole (*vac.2*) a third amoeba with its nucleus (*n.2*).

Fig. 3.—A free sphere containing diatoms in food vacuoles (*d*). *n*, nucleus.

Fig. 4.—An amoeba with its nucleus (*N*) and two vacuoles. In one of the latter lies a second amoeba with its nucleus (*n.3*). In the other is a third amoeba with its nucleus (*n.1*), and this again contains a fourth amoeba with its nucleus (*n.2*).

Fig. 5.—An amoeba with its nucleus (*N*) and food vacuoles (*f.b.*), which has ingested one other amoeba with its nucleus (*n*) and food vacuoles (*f.b.1*).

Fig. 6.—A typical free sphere, extruded from an amoeba (compare with the ingested amoeba in fig. 5). The structure of the nucleus is well shown (compare with the nucleus of the outer amoeba in figs. 1, 2, and 4).

PLATE 29.

Fig. 7.—An amoeba with its nucleus (*N*) and a food vacuole (*f.vac.*). It contains three other vacuoles, in two of which two other amoebae lie. One of these, with its nucleus (*n.1*) is free from food bodies; the other, with its nucleus (*n.2*) contains diatoms. The third vacuole contains an amoeba with its nucleus (*n.3*), which itself contains a food vacuole, (*f.vac.1*) and three other amoebae with their nuclei (*n.4*, *n.5*, and *n.6*), the latter being free from food bodies.

Fig. 8.—An amoeba containing two other amoebae in separate vacuoles, one of which is a typical sphere, the other an elongate oval. Both the ingested amoebae contain food bodies.

Fig. 9.—A star-shaped form of *Amoeba vespertilio* with its nucleus (*N*) and food vacuole (*f.b.*). It contains another amoeba with its nucleus (*n*) and food bodies (*f.b.1*) (cf. Pl. 28, fig. 5).

Fig. 10.—An *Amoeba vesperilio* with a large food vacuole (*f.vac.*) and an irregularly-shaped amoeba which it has ingested.

Fig. 11.—An amoeba with its nucleus (*N*), which has ingested five other amoebae, the smallest of which are probably *A. limax*. *n.1-n.5*, nuclei of the ingested amoebae.

Fig. 12.—A binucleate amoeba with its two nuclei (*N,N*), with food bodies and an ingested amoeba with its nucleus (*n*).

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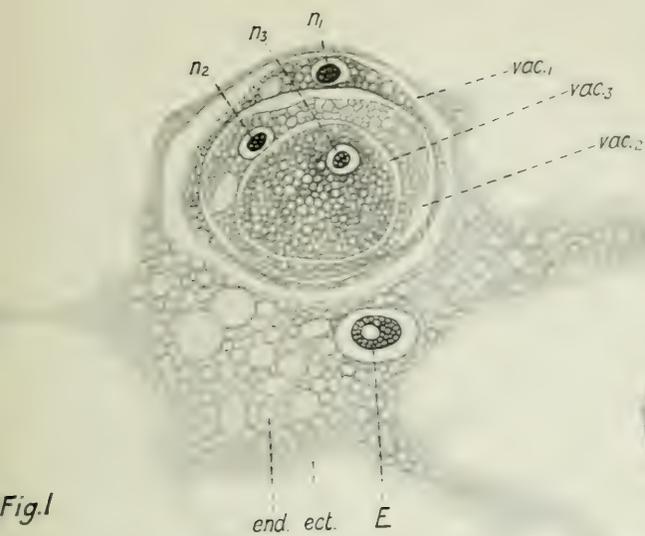


Fig. 1

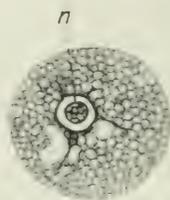


Fig. 6

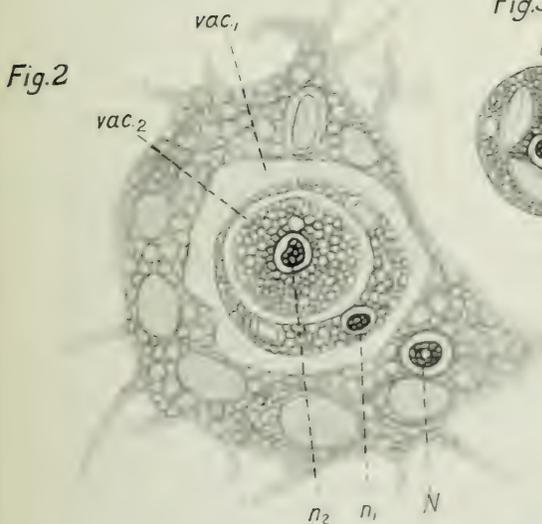


Fig. 2

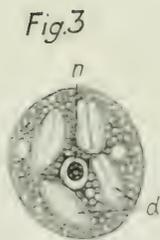


Fig. 3

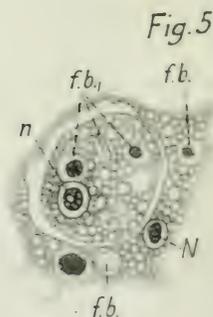


Fig. 5

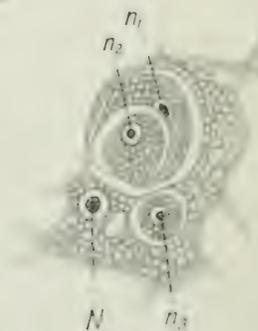


Fig. 4

G.L. del.

Fig.7

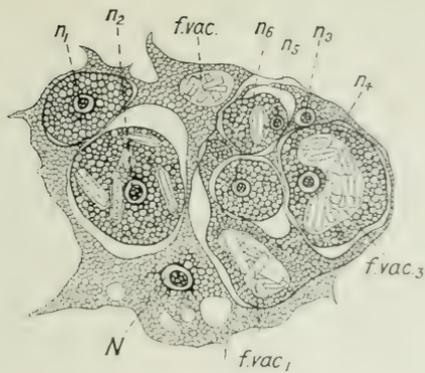


Fig.8

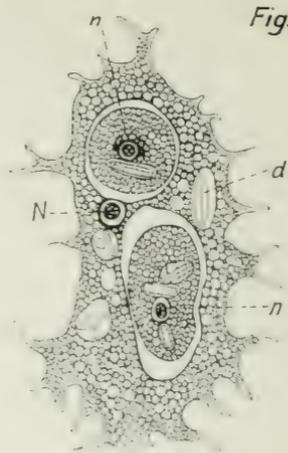


Fig.9

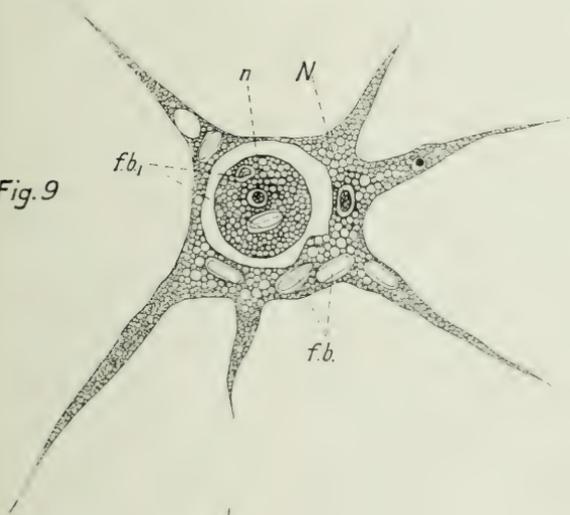


Fig.10

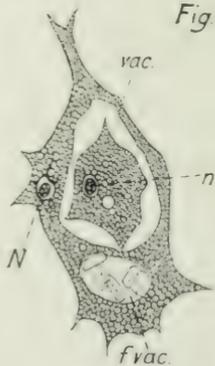


Fig.11

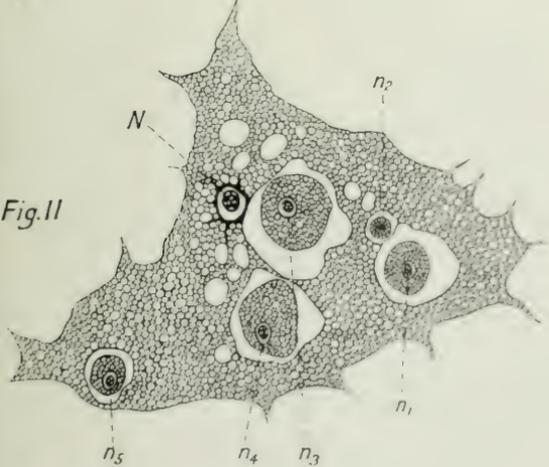
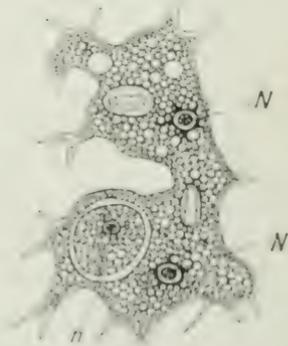


Fig.12



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